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JOURNAL OF AVIAN BIOLOGY 37: 6–12, 2006

Carotenoid coloration in great black-backed gull *Larus marinus* reflects individual quality

Kai O. Kristiansen, Jan O. Bustnes, Ivar Folstad and Morten Helberg

Kristiansen, K. O., Bustnes, J. O., Folstad, I. and Helberg, M. 2006. Carotenoid coloration in great black-backed gull *Larus marinus* reflects individual quality. – J. Avian. Biol. 37: 6–12.

Carotenoids are a large group of biochemicals, with similar properties, synthesised by bacteria, fungi, algae and plants. Vertebrates obtain these biologically active pigments through the diet, and they are a disproportionately common component of animal colour signals and play important roles in immune functions and as antioxidants. Carotenoids are believed to be a limited resource and because of the trade-off between allocation of carotenoids to signals and to other functions, carotenoid based signals are often thought to be handicap signals. The purpose of this study was to investigate the signalling potential of carotenoid-based tissue coloration in the great black-backed gull *Larus marinus*. The intensity of carotenoid-based coloration in bill, gape and eye-ring coloration was investigated in relation to body condition, reproductive parameters, levels of immune activity, and sexual dimorphism. In males there was a positive relationship between colour intensity and body condition, but in females no such relationship was found. However, females with high colour intensity had larger eggs and clutches. Additionally, females with high red scores tended to have high density of circulating lymphocytes. There was no sexual dimorphism in coloration and there was a negative relationship between colour intensity and sampling time, which indicates that this coloration is most intensely expressed early in the breeding season. The results in this study suggest that carotenoid-based coloration in great black-backed gull are partly condition dependent and reveal information about individual quality in both males an females. Hence, it might have evolved as an important signal for assessing the quality of potential mates.

K. O. Kristiansen, I. Folstad, and M. Helberg, Faculty of Science, Department of Biology, University of Tromsø, 9037 Tromsø, Norway. J. O. Bustnes (correspondence), Norwegian Institute for Nature Research, Unit for Arctic Ecology, The Polar Environmental Centre, N-9296 Tromsø, Norway. E-mail: Jan.O.Bustnes@nina.no

During the last decades much research has focused on carotenoid-based signals. Carotenoids are disproportionately common components of animal colour signals such as those used in sexual communication, signalling between offspring and their parents and in warning colours (Møller et al. 2000). They are a large group of more than 600 different biochemicals, with similar properties, synthesised by bacteria, fungi, algae and plants. The signalling functions of carotenoids are reflecting other physiological properties of these biochemicals. They play important roles in immunoregulation in vertebrates by stimulating the production, capacities and functions of different immunological components (Bendich 1989, Chew 1993, Olson and

Owens 1998, van Poppel et al. 1993). A second major function of carotenoids, not independent of their effect on immune function, is detoxification. They act as antioxidants and protectors of biologically important molecules from the damaging potential of free radicals and products of their metabolism (Edge et al. 1997, Krinsky 1998). Since carotenoids are used for protection against free radicals and different mutagenic and toxic compounds, as well as in immune system activation, there may be a trade-off between allocation of carotenoids to signals and to other functions. The honesty of these signals can therefore potentially be explained by the handicap model (Zahavi 1975, 1977, Grafen 1990, Maynard Smith and Harper 1995, Hasson 1997, Getty 1998). The production costs of carotenoid-based signals do however assume that carotenoids are a limiting resource (Møller et al. 2000).

One way to determine whether carotenoids are a limited resource is to provide animals with extra carotenoids and measure to what extent signals can become more exaggerated. A number of different experimental studies have shown, through such supplementary feeding that carotenoid-based signals in some species are below maximum possible expression (Hill 1992, Grether et al. 1999, Saino et al. 2000). For example, adult lesser black-backed gulls *Larus fuscus* provided with a carotenoid-rich diet had more brightly coloured secondary sexual signals (bill, leg, gape and eye-ring) than control birds not receiving carotenoid supplementation (Blount et al. 2002a). Moreover, chickens experimentally infected with *Coccidia* may during a short period lose up to 80% of their body carotenoids (Allen 1997). Thus, carotenoids may also be scarce because their turnover may reach very high levels under immune activity or during scavenging of free radicals.

The great black-backed gull *Larus marinus* is a monogamous seabird with sexual size dimorphism in which both sexes exhibit similar parental behaviour (Butler and Butler 1983). Moreover, both sexes show intense carotenoid-based coloration during the breeding season, including red eye-rings, gape flanges, gape and bill spots. The bill is pale yellow. Adult non-breeders have pale red or orange eye-rings and gape flanges. The gape is flesh coloured and the bill paler (Cramp and Simmons 1983). These differences between breeders and none-breeders may indicate that the carotenoid-based coloration is condition dependent and involved in sexual communication.

The purpose of this study was to investigate the signalling potential of carotenoid-based tissue coloration in relation to body condition and reproduction in great black-backed gulls. Additionally, we examined how coloration is expressed under different levels of immune activation. Our hypothesis was that coloration is condition depended and reflects individual quality. We had three *a priori* predictions: 1) the intensity of carotenoid-based coloration was expected to be positively correlated with body condition, 2) individual reproduction is quality related so birds with intense coloration will show a better reproductive performance compared to individuals with less intense coloration, and 3) because of the trade-off between carotenoids to immune functions and to signals, immune parameters will show a negative relationship with colour intensity.

Materials and methods

Study area

The fieldwork was carried out at Loppa (70° 21′ N, 21° 25′ E) from late April to middle June 2001. Loppa is an island situated at the coast in western part of Finnmark, Northern Norway. It is 7.6 km long and 2.5 km at the widest and has a large topographical diversity. The breeding population of great black-backed gulls at Loppa in 2001 was approximately 150 pairs, and the nests were located in different habitats all over the island. The clutch, which normally consists of three eggs, is laid in the end of April to early May, and the incubation period lasts for approximately 28 days. Both parents participate in the incubation, brooding and feeding of the chicks (Cramp and Simmons 1983).

Data collection

All nests were checked each day beginning in late April. The eggs were marked with a marker pen, nests were numbered and the axial length and breadth of each egg were measured with a digital calliper to the nearest 0.005 mm. These measurements were taken twice for repeatability test and the egg volume was estimated by the formula; volume $= 0.476 \times \text{length} \times \text{breath}^2$ (Harris 1964). Repeatability of egg volume measurements was estimated to: $r^2 = 0.999$, $n = 267$.

All gulls in this study were caught between 19th of May and 11th of June, using a nest trap (Helberg et al. 2005). The trapped birds were equipped with numbered steel bands and PVC colour bands for identification, weighted to the nearest 10 g by a Pesola spring balance and the length of the skull and bill was measured with a sliding calliper (± 0.05 mm). Colour measurements were recorded (see below) and a blood sample was taken from the wing vein. The time from the trap was triggered to the blood sample was taken was measured. This measure was recorded to identify potential effects of individual handling time.

Analysis of the carotenoid-based tissue colour at the eye-ring, gape and bill was taken using a portable spectrometer (Avantes). Measurements were taken with a 2.5 mm reflection probe (FCR-7UV200) connected to

a halogen light source (HL-2000) and a spectrometer (AVS-USB2000) by fibre optic cables. The light from the light source was coupled via a standard SMA905 connector into a fibre bundle consisting of six fibres and carried to the probe end. The surface reflected light back into a seventh fibre. This fibre transferred the data to the output SMA905 connector, which was coupled to the spectrometer. The data from the spectrometer was loaded into a computer with Spectrawin 4.2 software. The measurements were relative and referred to a standard white reference tile (WS-2) and to dark. The colour measurements hue, saturation and darkness were recorded. Hue is given in inverse scale, that is, lower values of hue means redder coloration. To avoid misunderstandings we have therefore inverted the scale so higher values of hue mean redder coloration. Hue represents the wavelength of the colour, while saturation and darkness can respectively be understood as the density of pigmentation and the amount of black pigmentation. The colours were measured at 12 standard points; two points at each eye ring, four points in the gape and four points at the bill (two at the top of the bill and one at each bill spot). One of the points was measured twice and used for estimating the repeatability (hue: $r^2 = 0.94$, saturation: $r^2 = 0.93$, darkness: $r^2 = 0.94$, $n = 50$). The mean value of hue, saturation and darkness for these points were used in statistical analyses. We will use two different mean values in the following; 'soft tissues' is the mean colour value of soft tissues (eye ring and gape) and 'bill' is the mean colour value of points taken at the bill. We will focus on hue and saturation in further analyses because they represent respectively the type and amount of carotenoid pigmentation. Consequently, they are respectively a qualitative and quantitative measure of carotenoids and therefore the most relevant parameters for testing our predictions. The term 'colour intensity' refers to both parameters in the following.

Blood smears were made from the blood collected. These were fixed, stained and later used to determine the lymphocyte/erythrocyte ratio by taking the average of lymphocyte and erythrocyte counts from six independent areas on the smear. Hematocrit (i.e., the percentage of red blood cells in a blood sample) for each bird was determined by centrifuging a portion of the blood sample in a capillary tube for 195 s at 11,500 rpm with a Compur Mini Centrifuge. Then, by multiplying hematocrit value by the lymphocyte/erythrocyte ratio, an estimate for the level of circulating lymphocytes in each bird was obtained. Both lymphocyte densities and hematocrit were estimated twice (repeatability between the two estimates: $r^2 = 0.54$ and $r^2 = 0.98$, respectively; $n = 20$ in both cases).

In gulls the best morphological measurement reflecting body size is skull length (Coulson et al. 1983). Thus, skull length was used as a measure of body size. Body condition was estimated as body weight controlled statistically for body size (Garcia-Bethou 2001). However, to illustrate the relationship between body condition and colour measurements (Fig. 1), we used the residuals between body weight and body size as a measure of body condition (see Sedinger et al. 1997). In gulls males are normally larger than females (Coulson et al. 1983) and sex was therefore determined by size. In this study there was no overlap in skull length between birds classified as males and females (Table 1), but because only one bird from each pair was caught, the accuracy of the sex determinations was checked by visually comparing the caught and colour-ringed bird with the other bird in the pair. Males were larger and had a different head profile with more prominent eyebrows and larger and higher bills than females (K. O. Kristiansen and M. Helberg pers. obs.).

Statistical analysis

Statistical analyses were performed with the programs StatView (SAS 1998), Statistica (Statsoft 2001) and SAS System software (SAS 1990). Frequency distributions were tested using Kolmogorov-Smirnov one-sample tests. The distribution of all parameters fulfilled the assumptions of normality and linear regressions, logistic regressions and unpaired *t*-tests were consequently used in the statistical analyses. In the logistic regression, the criteria for assessing goodness-of-fit indicated underdispersion in the data (Pearson chisquare/df < 1). We

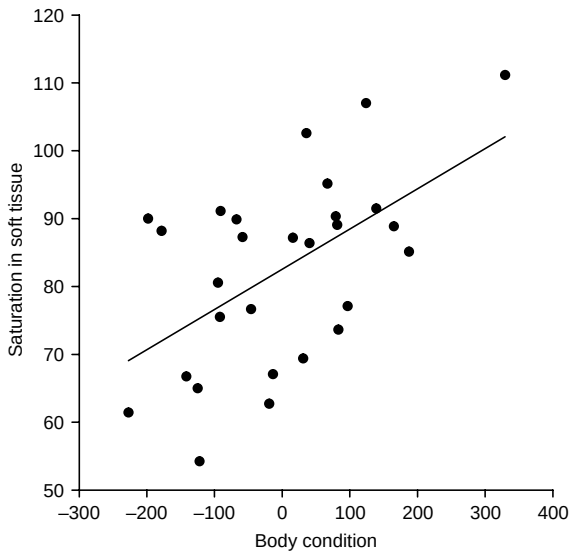

Fig. 1. Relationship between saturation in 'soft tissues' and body condition in male great black-backed gull. Data from Loppa in 2001. To control for body size in the figure, body condition is represented as the residuals from the regression between body weight and body size (head and bill length; see methods).

Table 1. Comparisons (unpaired t –tests) of colour parameters, body weight (kg), body size (head and bill: mm), lymphocyte counts and heamatocrit between males and females in great black-backed gulls from Loppa, Norway, 2001. Due to small numbers, lymphocyte counts are given as: lymphocyte counts $\times 100$.

	Males (n = 28)		Females (n = 22)			
Variable	$\bar{x}$	SE	$\bar{x}$	SE	t –value	P-value
Hue soft	− 72.17	1.69	− 74.37	1.73	− 0.90	0.38
Hue bill	− 76.40	0.94	− 77.45	0.97	− 0.77	0.45
Saturation soft	82.53	2.65	81.19	3.51	− 0.31	0.76
Saturation bill	95.57	3.34	92.78	4.08	− 0.53	0.60
Darkness soft	44.11	1.70	44.84	2.18	0.27	0.79
Darkness bill	54.87	1.50	56.71	1.77	0.80	0.43
Body weight	1.73	0.03	1.32	0.03	− 10.48	< 0.0001
Body size	149.95	0.66	136.07	0.73	− 14.09	< 0.0001
Lymphocyte counts	0.54	0.08	0.58	0.10	0.36	0.72
Hematocrit	44.15	1.22	44.63	0.68	0.10	0.75

therefore used quasi-likelihood estimation (SAS 1993, McCullagh and Nelder 1989). In studies with multiple comparisons one might adjust P-values, but we did not conduct such adjustments. A central argument for not adjusting the P values is that doing so would disqualify further examination of potentially important relationship (Rothman 1990, Nakagawa 2004). All statistical tests are however two-tailed and P-values < 0.05 were considered statistically significant. In regression analysis the best statistical models were chosen by backward selections, with a colour property, a reproductive trait or lymphocyte counts as the response variable and body condition, a colour property or lymphocyte counts and a set of potential confounding variables as independent variables. We started with a full model, removing independent variables one at the time if they were not significant. Males and females were analysed separately.

Results

Effects of handling and sampling time

No significant relationship was found between intensity of the colour parameters and handling time (P-values between 0.41 and 0.99) or between lymphocyte levels and handling time ($P = 0.34$), suggesting that handling time did not influence these labile traits in either males or females. In males, sampling date (the day birds were caught and measured) was negatively correlated with hue in 'soft tissues' and saturation in both 'soft tissues' and 'bill' (Table 2). In females, sampling date was not correlated with saturation (P-values between 0.88 and 0.68), but there was a negative relationship between hue in 'soft tissues' and sampling date (Table 3).

Sexual dimorphism

There were no significant differences between males and females in neither of the colour parameters. There was however a significant difference between males and females in body mass (Table 1).

Body condition

A positive relationship was found between saturation in both 'bill' and 'soft tissues' and body condition (body weight controlled for body size) in males (Table 2 and Fig. 1). Hue was, on the other hand, not correlated with body condition in males (P-values between 0.12 and 0.64) and there was additionally no relationship between colour parameters and body condition in females (P-values between 0.31 and 0.58).

Reproductive parameters and ornamentation

Females with three eggs had redder hue in 'soft tissues' than females with two eggs ($F_{1,\ 19} = 10.37$, $P < 0.005$, Estimate = 0.25, SE = 0.098, n = 21), but no association between saturation and clutch size was found (P-values between 0.14 and 0.65). Furthermore, a positive relationship was found between mean egg volume and hue in both 'bill' and 'soft tissues' controlled for body size in females (Table 3). Total clutch volume was also

Table 2. Relationships between colour parameters and sampling date, and saturation and body condition (body weight controlled for body size) in male great black-backed gulls.

Response	Parameter	df	P-value	Estimate	SE	R^2
Hue soft	Sampling date	1, 26	0.02	− 0.58	0.23	0.19
Saturation soft	Sampling date	1, 26	0.01	− 0.98	0.36	0.22
Saturation bill	Sampling date	1, 26	0.059	− 0.94	0.48	0.13
Saturation soft	Body condition	1, 25	0.0025	59.25	17.67	0.33
Saturation bill	Body condition	1, 25	0.014	61.68	23.36	0.27

Table 3. Relationships between hue in 'soft tissues' and sampling date, mean egg-volume and hue in both 'soft tissues' and 'bill' (controlled for body size) and total clutch volume and hue in both 'soft tissues' and 'bill' (controlled for body size) in female great black-backed gulls. Partial-R^2 in multiple regressions is given as R^2 in the table.

Response	Parameter	df	P-value	Estimate	SE	R^2
Hue soft	Sampling date	1, 20	0.046	−0.53	0.25	0.18
Mean egg-volume	Hue bill	2, 19	0.0046	1.32	0.41	0.38
	Body size	2, 19	0.0037	1.76	0.52	0.40
Mean egg-volume	Hue soft	2, 19	0.0015	0.82	0.22	0.46
	Body size	2, 19	0.0028	1.59	0.46	0.42
Total clutch volume	Hue soft	2, 13	0.0002	3.65	0.72	0.66
	Body size	2, 13	0.0003	5.67	1.15	0.65
Total clutch volume	Hue bill	2, 13	0.018	3.71	1.37	0.36
	Body size	2, 13	0.009	5.06	1.65	0.42

positively correlated with hue in both 'bill' and 'soft tissues' controlled for body size in females with three eggs (Table 3). However, no relationships between saturation and egg volume were found (P-values between 0.67 and 0.89), and there was no significant relationship between laying date of the first egg and any of the colour parameters in neither males nor females (P-values between 0.07 and 0.86).

Lymphocytes and ornamentation

Males did not differ from females in lymphocyte counts (Table 1) and no significant relationships were found between lymphocyte counts and colour parameters. Yet, females with high lymphocyte counts had a tendency to have redder hue in 'soft tissues' ($r^2 = 0.14$, $P = 0.08$, $n = 22$).

Discussion

We hypothesised that coloration in great black-backed gulls would be condition dependent and that it reflects individual quality, and predicted that coloration should correlate with body condition, reproductive parameters and immune parameters which are phenotypic traits reflecting individual quality. The results indicated that saturation in males during incubation reflects body condition, a frequently used index of individual quality. Individuals in good condition are, for example, not forced to allocate much of their resources to immune defence because they have lower levels of infections, and low immune activity leaves more carotenoids to signalling. Additionally, it has been suggested that there are energetic costs associated with metabolic conversion of carotenoids before they are deposited in ornamental structures (Hill 2000). Cost associated with this process cannot fully explain the relationship between body condition and saturation because saturation is a quantitative property. However, metabolic conversion is a component of 'carotenoid utilisation', which also involves absorption, transport and deposition of carotenoids and there may also be energetic costs associated with these other components (Hill 2000). Consequently, individuals in good condition may not only have more carotenoids but also more resources available to 'carotenoid utilisation' and therefore have more saturated coloration. The lack of relationships between colour intensity and body condition in females, on the other hand, may have several explanations. Females experience a different drainage of carotenoids than males because of egg formation. Additionally, they experience energetic costs during egg formation. The costs of these investments may have confounded the relationship between body condition and colour intensity in females. Yet, the positive association between hue and both clutch and egg size suggest that colour intensity reflect individual reproductive ability in females. That is, avian egg size is commonly held to be an important index of egg quality, because it reflects the quantity of yolk reserves available to the chick during embryonic development and hatching (Bolton 1991), and several studies have shown that hatchability, growth and survival of offspring correlate positively with egg size (reviewed by Williams 1994). But how can female colour intensity reflect egg and clutch size? Positive correlations between maternal condition and egg size have also been reported (reviewed by Williams 1994), and clutch size is expected to be influenced by female body condition (Bolton et al. 1992). Consequently, if colour intensity reflects female condition before egg laying, colour intensity would be positively correlated with egg and clutch size. However, the absence of a relationship between saturation, the other component of colour intensity, and egg size, which is documented in the Antarctic petrel *Thalassoica antarctica* (Birkeland 2003), might be explained by females transferring carotenoids into their eggs. Since embryonic development and the hatching process is believed to involve high levels of oxidative stress, antioxidant protection is vital at this stage of development (Surai et al. 2001). Females therefore invests in their progeny by transferring carotenoids and other antioxidants into their eggs (Saino et al. 2002) and a decline in female integument pigmentation and levels of circulating carotenoids at the time of egg

productions has been reported in various wild bird species (Burley et al. 1992, Negro et al. 1998, Blount et al. 2002b). A trade-off between allocations of carotenoids between the females own needs and the needs of her offspring, may have forced females in poor condition to allocate fewer carotenoids into eggs. Saturation reflects the amount of pigments and therefore the amount of carotenoids available. Thus, if females producing larger eggs, and also transfer a larger amount of carotenoids into the eggs, the relationship between egg size and saturation may be confounded after egg laying.

The negative relationship between colour intensity and sampling date indicates that colour intensity is reduced during the progress of the breeding season, suggesting that the coloration are at the most intense early in the reproductive season. This is in line with findings in other studies. For example, in American kestrel *Falco sparverius*, the most intense coloration occurs during the mating season (Negro et al. 1998).

Although it must be viewed with caution, females with high lymphocyte counts tended to have redder hue. In contrast, several studies have reported negative relationships between intensity of ornamental expression and levels of circulating lymphocytes (Gustafson et al. 1994, Saino and Møller 1994, Zuk et al. 1995, Skarstein and Folstad 1996). Admittedly, if high lymphocyte levels only reflect high levels of current infections, a negative association between immune functions and ornamental expression would indeed be expected. However, high levels of lymphocytes may also be indicative of high immunocompetence (Hanssen et al. 2003). Our results thus may indicate that high quality females with higher hue values are better in dealing with stress and have more resources available, as they seem to afford higher levels of immune activation during reproduction.

In conclusion, the results in this study indicates that carotenoid based-coloration in great black-backed gulls is condition dependent and reveals information about individual quality in both males (i.e., condition) and in females (i.e., reproductive performance). Hence, this coloration might have evolved as an important signal to individuals assessing the quality of potential mates.

Acknowledgements – We thank Dr. Kevin Omland and one anonymous referee for comments that improved an earlier draft of the manuscript. The study was funded by the Norwegian Research Council.

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(*Received 15 March 2005, revised 26 August 2005, accepted 31 August 2005*.)