



Colouration in Atlantic puffins and blacklegged kittiwakes: monochromatism and links to body condition in both sexes

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Sexual dimorphism is widely used as an indirect measure of the intensity of sexual selection. It is also a way to evaluate whether different selective pressures act on males and females. Dichromatism, defined as a difference in colouration between males and females, may for instance result from selection for crypsis in females and selection for conspicuousness in males. Here, we conducted a study to investigate whether differential sexual selective pressures might act on the colour traits of two colonial seabird species, the Atlantic puffin *Fraterecula artica* and the black-legged kittiwake *Rissa tridactyla*. First, we used spectrophotometry and visual modelling to determine whether these presumed monomorphic birds are really monochromatic from an avian perspective (birds and humans have a different vision). Second, we estimated whether some of their colourations have the potential to be sexually or socially selected by determining whether these colourations were related to body condition in males and females, and whether the yellow, orange and red colourations may contain carotenoid pigments. Our results indicated that both species were fully monochromatic from an avian perspective. Moreover, our preliminary analyses suggested that the yellow, orange and red colours of these birds contained carotenoids. Lastly, some indices of colouration were positively linked to estimates of condition. Birds in better condition had redder gape (both species) and bill (puffins). In puffins, the relation between condition and gape colouration was significantly stronger in females than males. By contrast, the size of the gape rosette was larger in males than females. The positive links we found between colour indices and condition, together with the absence of sexual dichromatism, suggest that mutual sexual selection may act in these two species.

Sexual dimorphism is widely used as an indirect measure of sexual selection in comparative studies (Badyaev and Hill 2000, Morrow and Pitcher 2003, Ord and Stuart-Fox 2006). Dichromatism is a special case of sexual dimorphism whereby males and females differ in colouration. Dichromatism is widespread and highly variable at the interspecific level. Extreme dichromatism can be encountered for instance in the mallard *Anas platyrhynchos*: males and females are so different that they had been identified as two different species when first described (Anderson 1994). By contrast, a highly subtle dichromatism that cannot be detected by human eyes can be found in other species (e.g. black-capped chickadee *Parus atricapillus*: Mennill et al. 2004).

Sexual dichromatism can be explained by differences in the relative strength of selective pressures acting on both sexes: stronger sexual selection can lead to the elaboration of colour traits while a stronger natural selection can lead to crypsis (Badyaev and Hill 2003, Owens 2006, Gomez and Thery 2007). The intensity and diversity of sexual dichromatism in animal signals can be explained by the variation in several major ecological factors and life history traits such as mating systems, social competition in both sexes,

intensity of parental care, extra pair paternity, latitude, predation, habitat, ambient light or diet (Badyaev and Hill 2003, Owens 2006). From comparative and theoretical studies, it can be globally predicted that species with biparental care where both sexes feed and incubate or where one sex incubates in a relatively safe place like a cavity, should be monochromatic (Owens 2006, Kraaijeveld et al. 2007). By contrast, sexual dichromatism can be predicted if only one sex feeds and incubates and if the evolution of cryptic colouration reduces predator detection in the sex that invests most in reproduction. Dichromatism could also be predicted if one sex invests more in territorial defence.

An intense sexual dichromatism is generally used as an indication of an intense sexual selection in males. However, more and more studies are showing that sexual or social selection may be acting equally in both sexes (Roulin et al. 2001, Lebas 2006, Clutton-Brock 2007, Kraaijeveld et al. 2007, Doutrelant et al. 2008, 2012, Rubenstein and Lovette 2009, Midamegbe et al. 2011). Hence, the absence of dichromatism should not preclude that these species bear intensively selected traits in both sexes. To test the potential signalling function of colouration in both sexes, an

important step is to determine whether colouration honestly signals quality in males and females.

Condition-dependence is one of the main mechanisms supposed to insure that individuals reliably signal their quality or condition (Zahavi 1975, Grafen 1990, Iwasa and Pomiankowski 1994, 1999). Condition-dependence suggests that signals have to be costly so that only high-quality individuals in a population can present exaggerated sexual ornaments, armaments or displays and be viable afterwards (i.e. be fecund and survive). It assumes a positive link between the conspicuousness of the ornaments or armaments and indices of conditions such as body condition or immunocompetence. It also assumes that ornaments or armaments are constituted by costly elements.

In many species, colouration is due to pigments such as carotenoids or melanine and/or to structures (Hill and McGraw 2006). To date, most experimental studies have shown that colouration is condition-dependent because some structures are costly to elaborate, some pigments are costly to acquire or submitted to allocation tradeoff (Mcgraw et al. 2002, Faivre et al. 2003, McGraw and Ardia 2003, Hill et al. 2009, Doutrelant et al. 2012). Given that different colours may signal different aspects of quality, identifying the pigments of ornamental colours is important to determine the type of information contained in coloured traits (Mcgraw et al. 2002, 2005b). For instance, carotenoids are fat soluble pigments that are only acquired by food intake and are often considered as a limited resource (Olson and Owens 1998). They have antioxidant properties (Von Schantz et al. 1999, Svensson and Wong 2011; but see Hartley and Kennedy 2004, Costantini and Møller 2008, Olsson et al. 2008) and are also immunomodulators that regulate and stimulate the immune system (Lozano 1994, Olson and Owens 1998, Møller et al. 2000). As such, carotenoids are often assumed to be relevant indicators of individual health and foraging ability. Identifying the structure or pigments is especially informative when coloration is a dynamic trait that may therefore convey a continual update on individual condition. This is the case of bare part coloration (i.e. the coloration of beaks, legs for instance in birds) that have been shown by several experimental studies to vary within few weeks (Blount et al. 2003, Faivre et al. 2003, McGraw and Ardia 2003) to few days (Velando et al. 2006).

Seabirds stand a particularly interesting group regarding colour ornaments. Most species are black and white and appear monochromatic to humans, but they also often harbour colourful traits (including in bare parts) during the breeding season. Nevertheless, we know very little about the role of colouration in most seabird species (but see Jones and Hunter 1993, Massaro et al. 2003, Nolan et al. 2005, Torres and Velando 2005, Kristiansen et al. 2006).

In the current study, we worked on two seabird species that appear monochromatic to a human eye: the Atlantic puffin *Fratercula artica* and the black-legged kittiwake *Rissa tridactyla*. Our first aim was to determine whether these species were really monochromatic when bird vision was taken into account. We then identified the potential condition dependence of their colouration by examining whether their expression was linked to body condition and whether the yellow, orange, and red bare parts of both species contained

carotenoids. For puffins, we also examined the potential size dimorphism in the gap rosette size.

To explore these questions, we quantified colouration using quantitative spectrometry and avian vision modelling (Endler and Mielke 2005, Gomez and Thery 2007). Birds have a different vision compared to humans, with sensitivity to ultraviolet (UV) radiation (Bennett and Cuthill 1994). Hence, it is crucial to accurately and objectively measure coloration – by using spectrometry – and to model avian vision to understand signals from their perspective, and the role of sexual selection in their evolution. For puffins and kittiwakes, we predicted a slight or an absence of sexual dichromatism. Indeed, both species present life history traits that should be linked to monochromatism (Owens 2006, Kraaijeveld et al. 2007). Both are long-lived monogamous species, with similar survival, nest care, incubation, and chick feeding (Creelman and Storey 1991, Coulson and Johnson 1993, Coulson 2011, Harris and Wanless 2011). In addition, both species present a null or very low level of extra pair paternity-EPP (between 0 and 8%: Helfenstein et al. 2004, Anker-Nilssen et al. 2008) and a moderate level of divorces (less than 7% in puffins and around 20% in kittiwakes: Coulson 1972, Hatch et al. 1993, Naves et al. 2006, Harris and Wanless 2011).

We estimated the potential condition-dependence of coloration by relating colouration to body condition. Puffins and kittiwakes present coloured bare parts and feathers. Given that bare part colouration represents a more dynamic signal of quality than feather colouration (feathers are formed only once or twice earlier in the season, bare part colouration may change in less than 15 d: Faivre et al. 2003), we expected body condition to be more closely associated with bare part colouration than feather colouration. A recent study investigated whether bill and gape colourations are linked to quality indices in a North Pacific population of kittiwakes (Leclaire et al. 2011) and found that their colouration was correlated to quality indices (reproductive success, heterozygosity, antioxidant levels). To our knowledge, no such study has ever been conducted on puffin colouration.

Material and methods

Study species and sampling of individuals

The Atlantic puffin and the black-legged kittiwake are common breeding seabirds of the North Atlantic Ocean and Barents Sea (Coulson 2011, Harris and Wanless 2011). Their breeding season, during which they aggregate on large coastal colonies, span from around early April until August in the southern Barents Sea (Anker-Nilssen et al. 2000). The puffin lays a single egg per season in burrows (Harris and Wanless 2011). The kittiwake lays a clutch ranging from one to three eggs in open nests on vertical cliffs (Barrett 2001a). Both species are long-lived and faithful to their breeding site, with a long incubation period (45 d for puffins, 27 d for kittiwakes). In the study colony, situated on the island of Hornøya (eastern Finnmark, Norway; 70°22'N, 31°10'E), breeding phenology is relatively similar in both species, although kittiwakes lay eggs earlier than puffins (Barrett 2001b).

For this study, a total of 36 puffins and 55 kittiwakes were captured using a nose pole. These captures were performed in June 2006 (12–24 June for puffins and 7–22 June for kittiwakes), when breeders of both species were incubating (Barrett 2001a). Puffins were caught on the grassy slopes outside burrows and kittiwakes were caught on their nests. Captured birds were marked with a pen for puffins and with a metal ring and combination of colour rings for kittiwakes (as a part of a long term study: Boulinier et al. 2008).

For each bird, we measured body mass (± 1 g using a spring balance), wing length and head-and-bill length (± 0.5 mm), and we measured the colouration of different body parts using a spectrophotometer. A blood sample was taken from each individual, which was used for sex determination. In addition, each puffin had its head photographed with a digital camera equipped with a macro lens and a ring flash on a black stand next to a ruler (Supplementary material Appendix A1, Fig. A1). Photographs were analysed using ImageJ Software (<http://rsb.info.nih.gov/ij/>) to measure gape rosette area.

Body parts measured in spectrophotometry

Puffin colouration was measured on five body parts which are potentially involved in sexual selection as their size or colour changes between the winter and the beginning of the reproductive season (Harris and Wanless 2011). These parts are the black part of the bill, the red part of the bill, the yellow-orange 'gape rosette', the orange legs and the white-grey cheeks (Supplementary material Appendix A1, Fig. A1).

For kittiwakes, we measured the colour of the yellow bill, the orange gape, the black legs, the white head, the grey mantle and the black of the wings (Supplementary material Appendix A1, Fig. A1). We did not measure the eye-ring coloration because the light of the spectrophotometer could injure the eyes.

We took three or four measurements for each colour patch, depending of the location. Performing several replicates per colour patch allowed us to estimate the repeatability of colour measurements (Lessells and Boag 1987).

Spectrometry

Colour was measured with a spectrometer, a xenon light source (covering the range 300–700 nm) and a 200 μ m optic probe. All measurements were taken perpendicular to the surface of the colour patch, the probe being mounted with a back rubber cap to exclude ambient light, in which the probe was held at a fixed distance of 2 mm to guarantee a constant distance between light and sample surface. We generated reflectance data relative to a white standard (WS1 ocean optics) and the dark reference (black felt background).

Colorimetric variables

We analysed spectral data using two complementary methods: 1) we analysed colorimetric variables characterising spectral shape to assess whether bird colours were dimorphic and linked to individual condition. 2) We modelled avian vision to test whether colouration appeared dimorphic from an avian visual perspective.

Colorimetric variables based on the shape of reflectance spectra

We extracted classic achromatic (brightness) and chromatic (chroma, hue) descriptors (Andersson et al. 1998, 2002, Montgomerie 2006, Doutrelant et al. 2008). For all patches, brightness was computed as the average reflectance over the total range of bird sensitivity 300–700 nm. For red, orange and yellow chromatic colouration, we also extracted chromatic descriptors of hue and chroma (Andersson et al. 1998, Delhey et al. 2003, Griffith et al. 2003, Korsten et al. 2006). Hue was computed as the wavelength at which reflectance was halfway between its minimum and maximum (L_{R50} measured). Chroma was computed as $(R_{700}-R_{450})/R_{700}$, an index which is particularly suited to carotenoid-based colours (Andersson and Prager 2006). All measurements but the brightness of the puffin gape rosette were significantly repeatable (significant repeatabilities are ranging from 40 to 90% all $p < 0.05$).

For puffins' and kittiwakes' bill, leg and gape, the three colour descriptors were significantly correlated (all $p < 0.05$). We thus ran PCAs on each colour patch independently to avoid colinearity in further statistical analyses (Sheldon et al. 1999, Siefferman and Hill 2005, Doutrelant et al. 2008). For each of these colour patches, we kept the first two principal components (PC), which we present in Table 1 and 2, along with average colorimetric values. These two PC explained more than 85% of the variation and were useful to keep both chromatic and achromatic informations. For the bill and the rosette of the puffins, PC1 described chromatic aspects, whereas PC2 described more the achromatic aspects of the colour (Table 1). For the leg of the puffins and the gape of the kittiwakes, PC1 mostly reflected chroma and PC2 hue. For the bill of kittiwakes, PC1 mostly reflected hue and PC2 chroma. Higher scores of hue indicate redder colorations and lower more yellow or orange colorations.

Visual models

To assess whether birds appeared sexually dimorphic to their conspecifics, we computed photoreceptor responses taking into account 1) the ambient light illuminating a colour patch, 2) the reflectance spectrum of the colour patch, and 3) the spectral sensitivity of the photoreceptors (Endler and Mielke 2005). We chose the standard illuminants D65 (CIE) as a representative spectrum for open habitat midday ambient light. Concerning vision, diurnal birds have a tetrachromatic vision, with a sensitivity to short wavelengths maximally sensitive in the ultraviolet and violet range defining UVS and VS vision respectively (Hart 2001, Ödeen and Håstad 2003, Håstad et al. 2005, Ödeen et al. 2010). Kittiwakes have a UVS vision (Håstad et al. 2005) and we thus chose the blue tit *Cyanistes caeruleus* to model kittiwake vision as blue tits are commonly taken as a representative of UVS vision (Håstad et al. 2005). All alcid species investigated so far have a VS vision, with a peak at 406 nm (Ödeen et al. 2010). We thus chose the wedge-tail shearwater *Puffinus pacificus*, a close relative with VS vision and a sensitivity peak at 406 nm to model puffin vision. In both cases, we considered that brightness was processed by double cones, as suggested by previous studies (Campenhausen and Kirschfeld 1998, Osorio et al. 1999).

Table 1. Average values (Av.), and standard errors (SE) of colorimetric variables in puffins and results of PCA for orange colouration. A total of 17 females (F) and 19 males (M) was measured.

Patch	Objective colorimetrics	M (Av. \pm SE)	F (Av. \pm SE)	Pc1: % of variation explained/eigenvalue	Pc2: % of variation explained/eigenvalue
Black bill	brightness	18.3 \pm 0.94	17.6 \pm 0.97		
Orange/red bill				58%	35%
	brightness	28.0 \pm 1.69	28.0 \pm 1.5	-0.35	0.85
	hue	578.6 \pm 2.92	571.3 \pm 2.90	0.71	-0.029
	chroma	0.54 \pm 0.019	0.51 \pm 0.028	0.60	0.53
Orange gape rosette				55%	30%
	brightness	14.4 \pm 0.78	14.2 \pm 0.87	-0.44	0.85
	hue	526.7 \pm 1.78	524.4 \pm 1.91	0.67	0.096
	chroma	0.79 \pm 0.008	0.77 \pm 0.01	0.59	0.51
Orange leg				60%	34%
	brightness	23.0 \pm 1.04	22.2 \pm 1.48	-0.52	0.67
	hue	576.1 \pm 3.31	563.5 \pm 2.41	0.47	0.74
	chroma	0.63 \pm 0.015	0.62 \pm 0.016	0.70	0.001
White cheek	brightness	26.3 \pm 1.97	27.9 \pm 1.21		

We computed the responses of photoreceptors using Endler and Mielke's model (2005) and further analysed them in relation to sex. All spectral data analyses were conducted using Avicol ver. 5 (Gomez 2010).

Sexing methods

All puffins were sexed using genetics. Genetic sexing was performed using polymerase chain reaction (PCR) amplification of an intron in the sex-linked chromobox-helicase- DNA-binding gene (Fridolfsson and Ellegren 1999).

For kittiwakes, sex determination was determined with morphological measures (head-and-bill size) for large and small birds and with genetic sexing for individuals of intermediate sizes. Twenty-three individuals with head-and-bill values above 93 mm were classified as males, 15 individuals with head-and-bill values below 89.5 were classified as females, and the remaining 17 individuals (with head-and-bill values ranging between 89.5 mm and 93 mm) were sexed genetically. It has been shown by earlier studies conducted in various kittiwake populations, notably the studied population, that kittiwakes can reliably be sexed based on this approach (Barret et al. 1985, Jodice et al. 2000, Gasparini et al. 2002, Coulson 2009). For instance Coulson 2009 studied a sample of more than 600 individuals and found that individuals measuring below 89 mm or above 92 mm were respectively all males and females or Gasparini et al. (2002) found in our study

population that the lower limit of the 95% confidence interval drawn from 19 previously sexed males is 92.7 and the upper limit of the 95% confidence interval drawn from 19 previously sexed females is 90.2.

Carotenoid analyses

To examine the biochemical basis of the yellow, red and orange bare part colourations in puffins (leg and bill) and kittiwakes (bill, gape, red eye ring), we performed an exploratory analysis. We collected the body parts of a freshly dead individual (one per species) found on the ground at our study site and kept frozen until the analysis.

Carotenoid preparation was performed following the protocol of McGraw et al. (2005a) for extraction and analysis using high performance liquid chromatography (HPLC: Isaksson and Andersson 2007, Gauthier et al. 2008, Leclaire et al. 2011) for saponification. Saponification is required to rid samples of fatty-acid esters and other lipids that disrupt HPLC elution.

Peaks were identified by comparing samples to simultaneously analysed standards of lutein, β -carotene, canthaxanthin, zeaxanthin, astaxanthin, β -cryptoxanthin and by visual inspection of the spectral absorbance peaks (Britton et al. 1995). We quantified the total carotenoid concentration for each tegument by comparison with standard curves. This measure was expressed in micrograms of carotenoids per milligram of tegument.

Table 2. Average values (Av.) and standard errors (SE) in kittiwakes, and results of PCA in yellow and red colouration. A total of 32 females (F) and 23 males (M) was measured.

Patch	Objective colorimetrics	M (Av. \pm SE)	F (Av. \pm SE)	Pc1: % of variation explained/eigenvalue	Pc2: % of variation explained/eigenvalue
Yellow bill				51.6%	39.2%
	brightness	33.0 \pm 1.80	32.3 \pm 1.39	-0.50	0.67
	hue	491 \pm 3.12	488 \pm 2.34	0.75	0.025
	chroma	0.49 \pm 0.02	0.49 \pm 0.01	0.43	0.74
Red gape				57.5%	36.5%
	brightness	9.0 \pm 0.61	9.2 \pm 0.56	-0.601	0.534
	hue	571 \pm 2.86	568 \pm 2.06	0.3319	0.842
	chroma	0.76 \pm 0.02	0.75 \pm 0.75	0.727	0.057
White head	brightness	57.3 \pm 2.43	55.6 \pm 1.73		
Black leg	brightness	11.3 \pm 1.169	11.0 \pm 0.81		
Grey mantle	brightness	24.9 \pm 0.94	23.8 \pm 1.05		
Black wing	brightness	5.0 \pm 0.396	5.3 \pm 0.40		

Statistical methods

We analysed colour variations in relation to sex and condition with generalized linear models (GLM) using SAS (ver. 9.1.3). Models included the interaction between sex and body condition. We computed two different body condition indexes: 1) the residuals of the regression between body weight and wing length, 2) body mass as a factor and wing length as a covariate. As results were similar for the two indexes, we only presented here the results corresponding to the first index that allows better visual representation of the statistical associations. For model selection, we used backward selection procedures and type III errors. Minimal models included all explanatory variables that showed a *p* value below 0.10. Hereafter, av., SE and est. respectively refer to average, standard error and model estimate. Model estimates were drawn from the final models.

We explored whether birds appeared sexually dimorphic in the eyes of their conspecifics by computing two complementary analyses. First, we used LSED–MRPP, a non-parametric multivariate model elaborated by Endler and Mielke (2005). This model allows to analyse colour coordinates in a sensory space and compare male and female colour patterns in their entirety, as clouds of points (comparison of the location of cloud barycentres, density of cloud points, volume occupied by the cloud, all tests spotting any possible difference in coloration between males and females). As a complement, we explored dichromatism following Eaton's method (2005): the four photoreceptor outputs were used as predictors of sex, which was coded as a binary response variable in generalised linear models. A full model was tested and a backward selection procedure was run using proc genmod in SAS.

Results

We measured 17 females and 19 males in puffins and 32 females and 23 males in kittiwakes. The Supplementary material Appendix A1, Fig. A3 and A4 show the spectra of colour patches measured in puffins and kittiwakes. The values of the colorimetric variables obtained for these patches are presented in Table 1 and 2.

Puffin colouration: sexual dichromatism and association with body condition

Using colorimetric variables computed based on the shape of the reflectance spectra, puffins presented a rather restricted or null dichromatism. Only the legs seemed different between sexes, with female colouration being less bright and chromatic than male colouration (PC2, $F_{1,34} = 5.91$, $p = 0.02$). None of the colorimetric variables

of the other four colour patches was significantly different between males and females (*p*-values were between 0.25 and 0.79 for all colorimetric variables). In addition, puffins presented no dichromatism when considering avian perspective. Male and female puffins had similar colour patterns (LSED–MRPP: $K = -0.135$, $p = 0.30$) and considering each patch separately, sex was not predicted by any photoreceptor response, either isolated or in combination (all $p > 0.10$).

Puffin condition was correlated to the colouration of two bare parts. Bill colouration (PC1, Table 3) was redder (higher value of hue and chroma, lower brightness) when males and females were in better condition (Fig. 1 – the interaction between sex and condition was not significant: $p = 0.75$). Gape rosette colouration (PC1, Table 3) was affected by the interaction between sex and condition (Table 3). Colouration was related to body condition in females but not in males, with females in better condition showing a more orange gape rosette (Fig. 2). Lastly, the colouration of the orange legs, the black part of bill and the white grey cheeks was not significantly linked to condition, either alone or in interaction with sex ($p > 0.12$ for all cases).

Puffin rosette size: sexual dimorphism

Puffin rosette size was significantly larger in males than in females, in average 19% higher in males than in females (av. \pm SD = 0.65 ± 0.0169 in females and 0.79 ± 0.02 in males, $F_{1,33} = 21.62$, $p < 0.0001$). This difference remained significant even if we entered bill size in the GLM model showing that this difference was not only due to a higher male bill size (bill size $F_{1,32} = 0.96$, $p = 0.33$; sex $F_{1,32} = 11.22$ $p = 0.002$). Rosette size was not linked to our index of condition (condition: $p = 0.52$, condition \times sex: $p = 0.87$).

Kittiwake colouration: sexual dichromatism and association to body condition

Kittiwakes appeared fully monochromatic when considering colour variables based on the shape of reflectance spectra (*p*-values between 0.31 and 0.98 for all colorimetric variables). Similarly, considering colouration seen by birds, males and females did not differ in colouration (LSED–MRPP: $K = 0.893$, $p = 1.0$) and considering each patch separately, sex was not predicted by any photoreceptor response, either isolated or in combination (all $p > 0.10$).

Gape colouration was significantly correlated to body condition, in both males and females (PC2, Table 4). In addition, head and mantle brightness tended to be correlated to condition (Table 4). Birds in better condition had redder and brighter gape (Fig. 3), and tended to have

Table 3. Factors affecting the variation in puffin colouration. F = females, M = males.

Patch	Dependent variables	Explanatory variables	F	DF	p	Estimate
Orange/red bill	PC1	body condition	3.55	1,34	0.006	0.01 ± 0.007
Orange gape rosette	PC1	body condition	1.65	1.32	0.20	
		sex	0.06		0.8	
		body condition \times sex	3.43		0.07	-0.11 ± 0.45 (F < M)

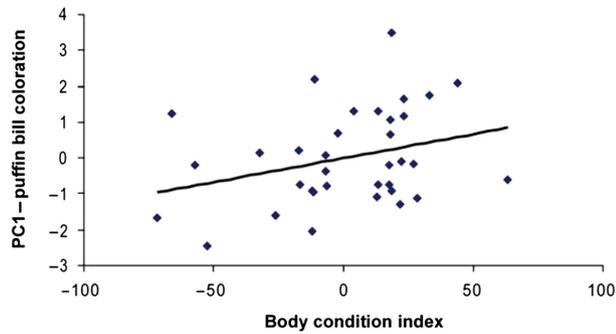


Figure 1. Link between condition and red bill colouration in puffins. Higher PC1 values indicate higher hue and chroma values, so that birds show a redder bill.

brighter head and grey mantle (Table 4). The interaction between sex and condition did not affect these correlations ($p > 0.23$ for the three variables). Condition was not correlated with the colouration of the other coloured patches (all $p > 0.50$).

Biochemical analyses of bare part colouration in puffins and kittiwakes

Chemical tests indicated that carotenoids were present in all the tissues analysed of both puffins and kittiwakes (Table 5). In puffins, all of the pigments identified were carotenoids. The main pigments identified in the orange leg were lutein, zeaxanthin, β -carotene and unknown ketocarotenes. The main pigments identified in the red bill were β -carotene and unknown ketocarotenes (Table 5).

In kittiwakes, the main pigments were β -carotene and unknown ketocarotenes in the three body parts analysed: yellow bill, red gape and red eye-ring. Differences in colouration between these three colour parts were quantitative, with the red eye-ring presenting higher quantity of carotenoids (Table 5).

Discussion

This study first investigated whether puffins and kittiwakes, apparently monochromatic to a human eye, were also monochromatic when bird visual range and sensitivity were taken into account. Using bird vision, we did not detect any sexual dichromatism for the two species. In puffins, however, gape rosette size was sexually dimorphic, being larger in males. We then investigated whether the colouration of both puffins and kittiwakes is correlated with individual condition and thus had the potential to be used as social or secondary sexual signals. In puffins, birds in better condition had more chromatic and redder bills (both males and females), as well as more chromatic and more

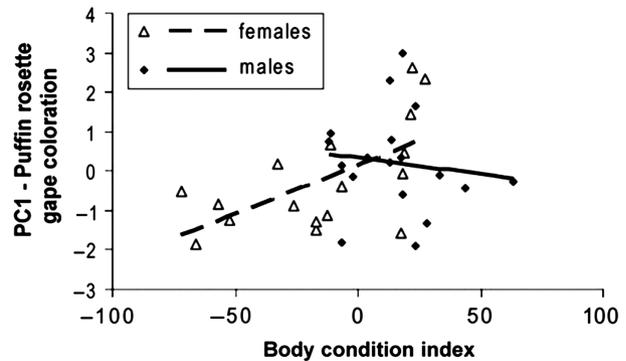


Figure 2. Link between condition and orange rosette gape colouration in puffins. Higher PC1 values indicate higher hue and chroma values, so that birds show a more orange gape rosette.

orange gape rosettes (females only). In kittiwakes, both males and females had brighter and redder gapes when in better condition. Lastly, as far as our exploratory analysis provided generalizable results it seems that yellow, orange, and red bare parts contained carotenoids in both species. Taken together, these results suggest that in these two species, bare part colouration has a signalling potential and may be subjected to mutual social and/or sexual selection. This hypothesis deserves to be experimentally tested in the future.

Although dichromatism has traditionally been estimated through human vision, recent studies raise some concerns about the relevance of such scoring. Birds and humans have a different vision. Humans have three colour cones while birds have four, this additional cone enabling them to perceive UV wavelengths to which humans are blind (reviewed by Bennett and Cuthill 1994). Moreover, birds have oil droplets that enhance colour discrimination (Vorobyev 2003). In agreement with these doubts concerning human capacity to correctly evaluate bird dichromatism, a reassessment of bird dichromatism in passerine revealed high level of dichromatism when bird vision is taken into account (Eaton 2005, Håstad and Ödeen 2008). Nevertheless, studies including non passerine birds concludes that human capacity to detect bird dichromatism is acceptable when birds do not present any UV-reflecting colouration (Armenta et al. 2008, Seddon et al. 2010).

In kittiwakes, none of our analyses detected a sexual difference in colouration. Our study thus confirmed the results of a recent study conducted on tegument colouration (bill, tongue and gape), during chick rearing period, in an Alaska population of black-legged kittiwakes (Leclaire et al. 2011). It also extended the absence of dichromatism to all colour traits (i.e. tegument and plumage colouration) and to the incubation period. Our study on black-legged kittiwakes is performed with a mix of genetic and morphological sexing. Because in this species morphological sexing is unambiguous for small and large individuals (Barret et al.

Table 4. Factors affecting the variation in kittiwake colouration.

Patch	Dependent variables	Explanatory variables	$F_{1,52}$	p	Estimate
Red gape	PC2	body condition	7.83	0.0072	0.067 ± 0.024
White head	brightness	body condition	3.77	0.06	0.49 ± 0.250
Grey mantle	brightness	body condition	2.92	0.09	0.22 ± 0.129

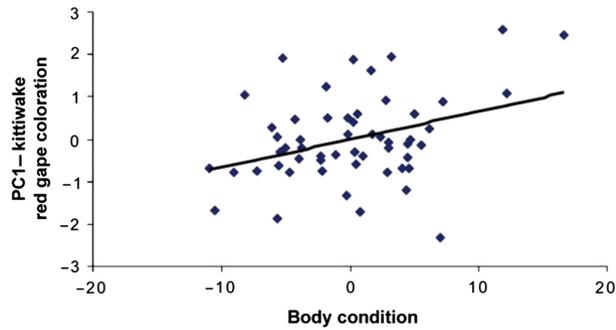


Figure 3. Link between condition and red gape colouration in kittiwakes. Higher PC2 values indicate higher hue and brightness, and so that birds show a brightly red gape.

1985, Jodice et al. 2000, Coulson 2009), we think the conclusions we provided are reliable.

For puffins, both visual modelling and calculation based on spectral shape showed that bill, gape and cheeks were monochromatic. For the orange legs, classic brightness, chroma and hue descriptors suggested a sexual dichromatism while visual models suggested a monochromatism. Differences in results can be explained by the fact that differences between male and female spectra occur in the long wavelength range. Although endowed with a tetrachromatic vision, birds have a relatively poor visual performance at the edges of their sensitivity domain, as shown by vision experiments in chicks (Osorio et al. 1999). Hence, our results confirm that both puffins and kittiwakes are fully monochromatic when taking into account bird visual system, a result that would not be ascertained without spectrophotometry.

Integument colouration has been shown to undergo rapid changes when bird condition varies (e.g. 15 d in blackbirds *Turdus merula*: Faivre et al. 2003, and much more quickly – 48 h – for the feet of the blue-footed bobby *Sula nebouxii*: Velando et al. 2006). Our work has been conducted during incubation. We cannot exclude that the level of monomorphism in bare parts would have been different if measured earlier in the season, before laying for example. More data are thus needed to ensure that monomorphism in bare parts is maintained all year round.

In comparative analyses, monochromatism is often interpreted as a low level of sexual selection and the share of common ornaments in males and females is often assumed

to be the result of a genetic correlation between male and female ornaments when ornaments are not too costly (Lande 1980, Kraaijeveld et al. 2007). However monochromatism might alternatively suggest that sexual or social selection is equally high in both sexes. For instance mutual inter and intra sexual selection has been suggested correlatively or demonstrated experimentally in other seabird species, like crested auklets *Aethia cristatella* (Jones and Hunter 1993), yellow-eyed penguins *Megadyptes antipodes* (Massaro et al. 2003), great back-backed gull *Larus marinus* (Kristiansen et al. 2006), blue-footed bobbies *Sula nebouxii* (Torres and Velando 2005) or king penguins *Aptenodytes patagonicus* (Nolan et al. 2010). For kittiwakes and puffins, the hypothesis of mutual sexual selection is suggested by our results showing a correlation between bare part colouration and body condition in both sexes.

In puffins, two results nonetheless also suggested a potential difference between males and females. First, we found that gape rosette size was sexually dimorphic, being about 20% larger in males. This may reflect a stronger sexual or social selection on this trait if ornament size signals male quality. Second, we found a stronger association between gape rosette colouration and body condition in females than in males. This condition dependence is particularly interesting and can be explained by the fact that under good conditions all individuals may be able to present large signals and that under harsher reproductive conditions, only the good quality individuals may be able to present developed such signals (Van Noordwijk et al. 1986, Doutrelant et al. 2008, Morales et al. 2008, Midamegbe et al. 2013). When coloration was measured, reproductive conditions have been harsher for females than male because females had just finished investing in egg laying. Egg laying can be extremely costly for females (Visser and Lessells 2001, Hanssen et al. 2005, De Heij et al. 2006). In agreement, females presented a lower body condition than males at the time of measurements (condition respectively varied from –32 to 63 in males and –72 to 27 in females: Fig. 2). So, this might explain why we found a relationship in females, but not in males.

It is highly interesting that in puffins, female gape rosette colouration was linked to body condition. As suggested by correlative (Boulet et al. 2010, Huchard et al. 2010) and experimental studies (Roulin et al. 2000, 2001, Smiseth and Amundsen 2000, Siefferman and Hill 2005, Doutrelant et al. 2008), colouration of female ornaments can be highly informative for males. Female colouration could predict maternal investment in eggs, as shown in lesser black-backed gulls (*Larus fuscus*: Blount et al. 2002) or blue tits (Midamegbe et al. 2013). In lesser black-backed gulls for instance, females with duller carotenoid-based integument colouration (bill, eye ring, gape flange and legs) produce clutches which exhibit a strong decline in yolk carotenoid levels over the laying sequence (Blount et al. 2002). During incubation, carotenoid-based colouration can indicate to males female potential capacity to support laying costs (Visser and Lessells 2001, Hanssen et al. 2005, De Heij et al. 2006) and thus to further invest in chicks.

A last argument in favour of the hypothesis that bare part colour traits have the potential to be sexually or socially selected in the two species is that they seem at least partially

Table 5. Description of pigments identified in the teguments. Total carotenoid concentration is expressed in micrograms of carotenoids by mg of tegument.

Body parts	Bird	Pigments	Total carotenoid concentration
Orange leg	puffin	lutein, zeaxanthin, β -caroten, unknown ketocarotenes	657.7
Orange red bill	puffin	β -caroten, unknown ketocarotenes	304.7
Yellow bill	kittiwake	β -caroten, unknown ketocarotenes	44.8
Red gape	kittiwake	β -caroten, unknown ketocarotenes	368
Red-eyed ring	kittiwake	β -caroten, unknown ketocarotenes	546.24

constituted by carotenoids. Although we sampled one individual per species, we can reasonably assume that this individual is representative of its species and that if carotenoids are detected in one, they are present in the other individuals of the same species as well (even if different carotenoids may vary in proportion among sampled individuals). Carotenoids are interesting in the context of sexual selection for at least the two following reasons. 1) They cannot be synthesized *de novo* by animals; they must be acquired by food intake and they may reveal individual capacity to acquire food and assimilate the nutrients. 2) They are concurrently needed by the signalling, immune and detoxification systems (Lozano 1994, Olson and Owens 1998, Von Schantz et al. 1999, Møller et al. 2000, Blount et al. 2003, Faivre et al. 2003, McGraw and Ardia 2003) although arguments against the antioxidant function of carotenoids in adults can be found in other studies (Costantini 2008, Vinkler and Albrecht 2010). In addition, they are protected from oxidation by vitamins (e.g. C, E, A) and enzymes with antioxidant powers. Hence, carotenoid based colouration might signal the availability of other non-pigmentary antioxidant molecules that might protect carotenoids from free radical attacks (Hartley and Kennedy 2004, Bertrand et al. 2006, Navarro et al. 2010).

To conclude, our results show a full monochromatism in puffins and kittiwakes colouration and suggest that mutual sexual selection might act on both kittiwakes and puffins bare part colourations. Bare part colourations are linked to estimators of condition in both sexes and seem to be based on carotenoids. Further descriptive investigation is required on samples coming from several individuals to confirm the carotenoid basis of the bare part colorations we studied here. In addition, experimental studies are needed to test the function of these colourations and the respective role of intra and intersexual and social selection in the evolution of these signals.

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Supplementary material (Appendix JAV-00098 at <www.oikosoffice.lu.se/appendix >). Appendix A1.