



Pheomelanin-based coloration is related to individual quality and oxidative stress in blue petrels

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Abstract

In several species, the rusty color of hair or feathers is due to pheomelanin pigments, whose adaptive function is unknown. Pheomelanin may be costly because it is photo-toxic and its production consumes a key intracellular antioxidant. Pheomelanin-based traits are, however, positively associated with individual quality in several bird species, where they have thus been suggested to have evolved through sexual selection. Here we investigated the signaling potential of the pheomelanin-based coloration of the crown feathers in the blue petrel. Although this pelagic seabird is nocturnal at the breeding colony and breeds within deep burrows, it might use visual communication when settled on the water during daytime. We tested the correlation between crown color and several fitness-related traits, and we found that higher-quality females displayed less-orange crown than poorer-quality females. This result is inconsistent with an adaptive function of pheomelanin-based coloration in inter-, or intra-, sexual selection in females. We suggest that it might, however, be in line with a signaling function of eumelanin-based coloration, if inter-individual variations in orange coloration are mainly due to eumelanin-to-pheomelanin ratio, rather than to pheomelanin quantity. In contrast to females, we did not find strong evidence for associations between melanin-based coloration and individual quality in males, suggesting sex-specific selective pressures on melanin-based traits in this species.

Keywords Birds · Pheomelanin · Signals · Feathers · Oxidative stress · Procellariidae

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Introduction

Melanin is the most common pigment in higher vertebrates, occurring in two main chemical forms: eumelanin, which produces black, gray and dark brown colors, and pheomelanin, which is responsible for reddish and yellowish colors. Eumelanin-based traits have been suggested to play a role in signaling, thermoregulation, camouflage to predators, and protection from UV radiation, mechanical damage or feather-degrading parasites (Mcgraw 2006; Galván and Solano 2016). In contrast, the adaptive value of pheomelanin remains unclear, as it is phototoxic and its synthesis in melanocytes requires the consumption of cysteine via glutathione (GSH), the most important intracellular antioxidant (Galván et al. 2012b; Panzella et al. 2014). Accordingly, in humans, red-haired individuals are more prone to skin cancers (Gerstenblith et al. 2010), and in Asian barn swallows (*Hirundo rustica gutturalis*), European nuthatches (*Sitta europaea*), boars (*Sus scrofa*) and lab mice, pheomelanin morphs have lower viability and higher oxidative stress (Galván et al. 2012a; Mitra et al. 2012; Galván 2017; Arai et al. 2018). Pheomelanin can confer crypsis (Nachman et al. 2003; Negro et al. 2009; Singaravelan et al. 2010), but not all pheomelanin-based traits are involved in concealment pattern.

Recently, pheomelanin has been suggested to have evolved because of its potential role in removing excess cysteine (Galván et al. 2012b). Although cysteine is an essential component of GSH, thereby being important for antioxidant protection, it can cause a variety of problems when in excess, including poor growth, pregnancy complication and brain damage (Olney and Ho 1970; Orth et al. 1992; El-Khairi et al. 2003; Galván and Alonso-Alvarez 2017). Therefore, under low levels of environmental oxidative stress—i.e., when cysteine is not needed for GSH-mediated antioxidant protection and can potentially be in excess—, pheomelanogenesis may be advantageous as it consumes toxic cysteine (Galván et al. 2012b). In contrast, pheomelanogenesis may represent a physiological cost under high environmental stress, when cysteine is needed for antioxidant protection (Galván and Solano 2009). In line with this hypothesis, recent studies have shown that, in some species, pheomelanin synthesis is increased under exposure to excess cysteine, and decreased under exposure to high environmental oxidative stress (Galván et al. 2017; Rodríguez-Martínez et al. 2019).

According to the handicap principle (Zahavi 1975), the cost associated with pheomelanogenesis in environments with high oxidative stress may have promoted the evolution of pheomelanin-based color as honest signals of quality (Galván and Solano 2009; Galván 2018), because only individuals with a high antioxidant capacity may be able to generate large pheomelanin-based signals. In agreement, in several bird species, pheomelanin-based traits are positively associated with individual quality and may thus play a role in sexual selection (Jawor and Breitwisch 2003). For instance, in eastern bluebirds (*Sialia sialis*), males with larger pheomelanin-based breast patches fledge heavier offspring (Siefferman and Hill 2003), and in American barn swallows (*Hirundo rustica rustica*), males with brighter pheomelanin-based throat have higher reproductive success (Safran and Mcgraw 2004).

The blue petrel (*Halobaena caerulea*) is a Procellariidae burrowing seabird that harbors a variable amount of orange feathers in the crown (Fig. 1), which is likely due to variation in the quantity of pheomelanin. Blue petrels being pelagic, the orange coloration is unlikely to play a role in camouflage. Like many other procellariidae, this seabird is nocturnal on the breeding ground (Warham 1996), and breeds within deep burrows, which have led to the suggestion that color signals are not essential to communicate (Bretagnolle 1996). However, petrels are

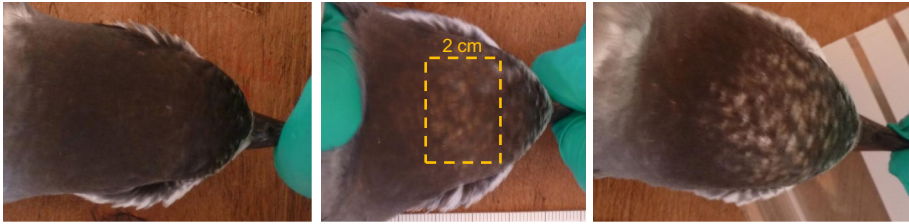


Fig. 1 Pictures of 3 blue petrels with different crown coloration. The yellow rectangle represents the standardized area used for color measurements. (Color figure online)

active during daytime at sea, where they can aggregate in very dense foraging flocks (Van Franeker et al. 2002), or settle in groups on the calm water (Tickell 1962). Little is known about vision in procellariidae. Burrow-nesting petrels do not have exceptional nocturnal vision, and seem to have lower visual acuity than surface-nesting petrels (Brooke 1989; Mitkus et al. 2016). However, in the leach-storm petrel (*Oceanodroma leucorhoa*), a species with similar ecology as the blue petrel, the smallest object that an individual may see in daylight at a distance of 2 m is 16 mm in diameter for low-contrast objects and 2 mm in diameter for high contrast objects (Mitkus et al. 2016). These observations suggest the potential for visual communication among individuals who are settled close to each other on the water, even in burrow-nesting petrels. In addition, males and females do not differ in foraging distribution during breeding and non breeding seasons (Phillips et al. 2009), suggesting that inter-sexual communication might occur.

We investigate the signaling potential of the pheomelanin-based coloration of the crown feathers of blue petrels, by testing the correlation between color expression and individual quality, which we define as a composite measure of multiple phenotypic traits putatively related to fitness (Wilson and Nussey 2010). If orange pheomelanin-based coloration is a quality signal used in sexual selection, we expect good-quality individuals to display more intense orange crown feathers. In contrast, a negative association would preclude a signaling role of pheomelanin color. In this species, males and females provide similar incubation and parental care (Chaurand and Weimerskirch 1994b), thus we predict that crown coloration reflects individual quality in both sexes and that breeding pairs show assortative mating in relation to this trait. We also explore whether variation in crown coloration is potentially linked to oxidative stress, by testing the correlation between color expression and oxidative stress in plasma. We expect blue petrels with high oxidative stress to display less-intense orange crown coloration, because oxidative stress may be a physiological trait related to individual quality (Bize et al. 2008; Cohen Alan et al. 2008; Hill 2014), and because it is suggested to downregulate pheomelanin production, at least in some species (Galván 2018). We measure oxidative stress at the time of breeding and not at the time of molt when pheomelanin is deposited into feathers. However, although oxidative status is known to change over time, a few studies showed that differences in oxidative status among individuals are consistent over a few week period, as well as across breeding seasons (Costantini et al. 2007; Hau et al. 2015; Herborn et al. 2016).

Materials and methods

Study site

A colony of about 80 nest burrows of blue petrels was studied at île Verte in the Kerguelen archipelago (southern Indian Ocean; 49°510 S, 70°050 E). 16 adult males and 23 adult females (including 12 breeding pairs) were included in the analyses. Burrows were checked every 2–4 days to check for bird, egg and chick presence. Birds were captured during incubation, and tarsus length and body mass were recorded. For a subset of birds ($n=4$ males and 11 females), a blood sample was collected from the brachial vein and plasma was stored at $-20\text{ }^{\circ}\text{C}$ until analyses.

Color measurements

Crown coloration was measured from digital photographs. Pictures were taken at approximately 40 cm using a digital camera (Panasonic, DMC-TZ30). For each photograph, the same color swatch (QpCARD 201) was placed next to the bird to standardize subsequent measurements. Low quality pictures (due to ambient lighting variations) were removed from the dataset. All pictures were analyzed using the software Adobe Photoshop v7.0 and the CIELAB color space. The CIELAB color space is a perceptually uniform color space designed to provide estimates of human luminance and chromatic perception. It has been suggested to be more appropriate than the RGB color space that is non-uniform and cannot represent all perceivable colors even to humans (Stevens and Cuthill 2005). In the CIELAB color space, L^* represents the achromatic signal, while a^* and b^* represent two chromatic channels representing green–red and blue–yellow respectively. The average components of $L^*a^*b^*$ were recorded in a standardized area of the crown (length of the standardized area: 2 cm measured from where the top of the skull angles to the frontal zone; width of the standardized area: the width of the skull at the angle between the top of the skull and the frontal zone; Fig. 1). The $L^*a^*b^*$ values of each integument were corrected according to the $L^*a^*b^*$ values of the color swatch, by using the residuals of a linear regression between the values of the crown and the values of the color swatch. In our study, color varied from black (lower values of L^* , a^* and b^*) to orange (higher values of L^* , a^* and b^* ; Fig. S1). In addition, we recorded the standard deviation of L^* ($L^*\text{std}$), which represented the patchiness of crown coloration. L^* , a^* , b^* and $L^*\text{std}$ values were highly correlated (Pearson correlation tests: all $r>0.66$, and all $P>0.0001$; except for the correlation between $L^*\text{std}$ and a , and $L^*\text{std}$ and b : $r=0.46$, $P=0.001$ and $r=0.43$, $P=0.003$ respectively). Therefore, the color of the crown was described by the first two principal components of a PCA on these four color variables (hereafter referred to as $\text{PC1}_{\text{color}}$ and $\text{PC2}_{\text{color}}$). $\text{PC1}_{\text{color}}$ accounted for 74% of the variation observed among color variables, and higher $\text{PC1}_{\text{color}}$ indicated lighter, patchier and more orange crown (eigenvectors: L^* : 0.91, a^* : 0.88, b^* : 0.89 and $L^*\text{std}$: 0.76). $\text{PC2}_{\text{color}}$ accounted for 19% of the variation observed among color variables, and higher $\text{PC2}_{\text{color}}$ mainly indicated patchier crown (eigenvectors: L^* : 0.26, a^* : -0.39 , b^* : -0.40 and $L^*\text{std}$: 0.61).

In animal color studies, color scores obtained from digital photographs have been suggested to be less appropriate to than those obtained with a reflectance spectrophotometer and visual models, because they do not consider the perception of color by the receiver's visual system (Stevens et al. 2009). However, information obtained from digital pictures

has revealed patterns and effects of biological meaning in several bird species (Pérez-Rodríguez and Viñuela 2008; Laucht et al. 2010; Leclaire et al. 2011). In addition, in tawny owls (*Strix aluco*), who show variations in pheomelanin coloration in feathers, coloration scores obtained from pictures correlate with those obtained with a spectrophotometer and with the concentration of pheomelanin pigments in feathers (Gasparini et al. 2009).

Pigment analyses

To obtain firm evidence of the pigment nature of the orange crown feathers of blue petrels, we collected feathers from three adult birds. The feathers were analyzed by micro-Raman spectroscopy, as pheomelanin and eumelanin exhibit distinctive Raman signals that can be used to identify them (Galván et al. 2013). We used a Thermo Fisher DXR confocal dispersive Raman microscope (Thermo Fisher Scientific, Madison, WI, USA) with a point-and-shoot Raman capability of 1 μm spatial resolution and using a near-infrared excitation laser of 780 nm. Laser power was set at 7 mW, integration time at 3 s and number of accumulations at 8. The spectra were obtained using a 50 \times confocal objective and a slit aperture of 50 μm . The system was operated with Thermo Fisher OMNIC 8.1 software. Calibration and alignment of the spectrograph were checked using pure polystyrene. We obtained two Raman spectra from the feathers of each individual bird, and then computed the average spectrum. We only obtained Raman signal of pheomelanin, as the spectra showed the three distinctive Raman bands of this pigment at about 500, 1500 and 2000 cm^{-1} (Fig. S2) (Galván et al. 2013), thus confirming that the orange feathers in the crown of blue petrels are colored by pheomelanin.

Index of individual quality

We assessed individual quality by conducting a principal components analysis (PCA) on several life-history traits commonly related to individual fitness: tarsus length, body mass index, date of laying, egg volume, number of days spent incubating the egg over the whole incubating period and hatching success. Four females and one male, for whom we did not have the incubation duration, were excluded from these analyses (sample size for the analyses on individual quality: $n = 19$ females and 15 males).

In blue petrels, body mass and number of days spent incubating the egg have been shown to be related to reproductive success (Chaurand and Weimerskirch 1994a). Once the single egg is laid, males and females take individual incubating bouts in the nest, that can last up to 12 days and during which parents lose body mass (Chaurand and Weimerskirch 1994a). Individuals in higher body condition at the start of the incubation bout are able to take longer incubation bouts, thereby increasing hatching success (Chaurand and Weimerskirch 1994a). We therefore calculated a body mass index as the residual of a linear regression between body mass and duration of the incubating bout until capture. Because this correlation tended to depend on sex (interaction between sex and bout duration until capture: $F_{1,35} = 3.72$, $P = 0.06$), we performed the linear regression for each sex separately (correlation between body mass at date of capture and bout duration until capture: males: $r = -0.82$, $P = 0.0001$; females: $r = -0.78$, $P < 0.0001$). In a high number of studies, body condition is calculated by controlling body mass for body size effects (Schulte-Hostedde et al. 2005; Peig and Green 2009). However, in our study, body mass and corrected body mass index were not related to tarsus length (females: $r = 0.10$, $P = 0.65$ and $r = 0.14$, $P = 0.53$; males: $r = -0.43$, $P = 0.10$ and $r = -0.20$, $P = 0.46$). The other measures included

in the PCA (i.e., tarsus length, date of laying and egg volume) have not yet been associated with reproductive success in blue petrels, but they are related to fitness in numerous other bird species (Kingsolver and Pfennig 2004; Verhulst and Nilsson 2008; Krist 2011).

Individual quality was described by the first three principal components of a PCA on traits related to individual fitness (hereafter referred to as $PC1_{\text{quality}}$, $PC2_{\text{quality}}$ and $PC3_{\text{quality}}$). $PC1_{\text{quality}}$, $PC2_{\text{quality}}$ and $PC3_{\text{quality}}$ accounted for 32%, 23% and 15% of the variation observed among life-history traits. Individuals with higher $PC1_{\text{quality}}$ had higher body condition, spent more days incubating their egg and had higher hatching success (eigenvectors: 0.66, 0.84 and 0.68 respectively). Individuals with higher $PC2_{\text{quality}}$ had longer tarsus and larger egg (eigenvectors: 0.72 and 0.74 respectively). The egg of individuals with higher $PC3_{\text{quality}}$ was laid earlier (eigenvectors related to laying date: -0.79).

Oxidative stress analyses

Oxidative stress was measured in plasma samples using the d-reactive oxygen metabolites (d-ROM) and the oxy-adsorbent tests (Diacron International, Grosseto, Italy) as previously described in birds including blue petrels (Costantini and Bonadonna 2010). The d-ROM test measures plasmatic hydroperoxydes, a reactive oxygen metabolite (ROM) resulting from the attack of reactive oxygen species on organic substrates (carbohydrates, lipids, amino acids, proteins, nucleotides), while the oxy-adsorbent test measures the total plasma anti-oxidant capacity.

Statistical analyses

To test the relationships between color expression in crown feathers ($PC1_{\text{color}}$ and $PC2_{\text{color}}$) and individual quality ($PC1_{\text{quality}}$, $PC2_{\text{quality}}$ and $PC3_{\text{quality}}$), we used linear mixed models (package lme4, version 1.1-21, in R; Bates et al. 2014). $PC1_{\text{quality}}$, $PC2_{\text{quality}}$, $PC3_{\text{quality}}$, sex, and the two-way interactions between sex and each PC_{quality} were included in the models as fixed effects. Because pheomelanin color can fade with time (Arai et al. 2015), we included date of capture as a fixed effect also. Burrow identity was included as a random factor.

We tested the association between color scores and ROM and OXY levels using linear models. Since ROM and OXY levels were not correlated within individuals (Pearson correlation test: $r=0.002$, $P=0.99$), they were both included as fixed effects in the models. In addition, because the sample size was very low for males ($n=4$ males), we restricted these analyses on females.

To test for assortative mating according to crown feather coloration, we used Pearson correlation tests to correlate female coloration with feather color expression of the breeding partner. All statistical analyses were carried out with the R software (R version: 3.6.0; R Core Team 2017).

Results

Color and individual quality

Plasma ROM levels were positively related to $PC2_{\text{quality}}$ in females ($F_{1,6}=6.92$, $P=0.039$, $n=8$ females for whom we had ROM levels and all quality-related variables; Fig. S3), but

not to $PC1_{quality}$ and $PC3_{quality}$ ($F_{1,5}=2.21, P=0.20$ and $F_{1,5}=0.00, P=0.97$). This result suggests that oxidative damage is related to some aspects of individual quality in females, namely larger eggs and longer tarsi. Plasma OXY levels were related to none of the $PC_{quality}$ (all $P > 0.10$).

$PC1_{color}$ varied with the interactions between sex and $PC1_{quality}$ ($\chi^2_1=4.29, P=0.038$; Fig. 2), and sex and $PC2_{quality}$ ($\chi^2_1=4.15, P=0.042$; Fig. 2). In females, $PC1_{color}$ decreased

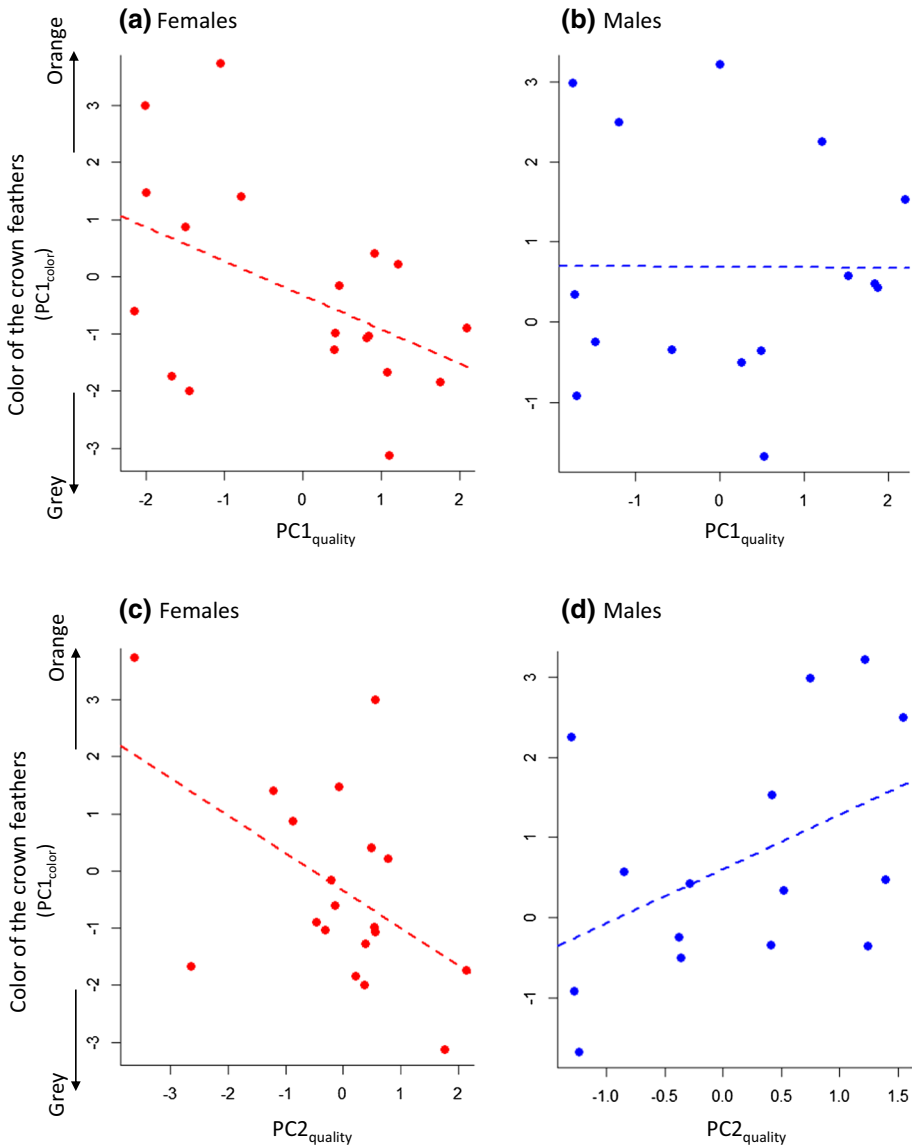


Fig. 2 Color of the crown feathers (as described by $PC1_{color}$) in relation to individual quality as measured by the first (a, b) or second (c, d) axis of a PCA analysis on several fitness-related traits. Females are in red (a, c), while males are in blue (b, d). (Color figure online)

Fig. 3 Color of the crown feathers (as described by $PC1_{color}$) in relation to individual quality as measured by the third axis of a PCA analysis on several fitness-related traits. Females are in red, while males are in blue. (Color figure online)

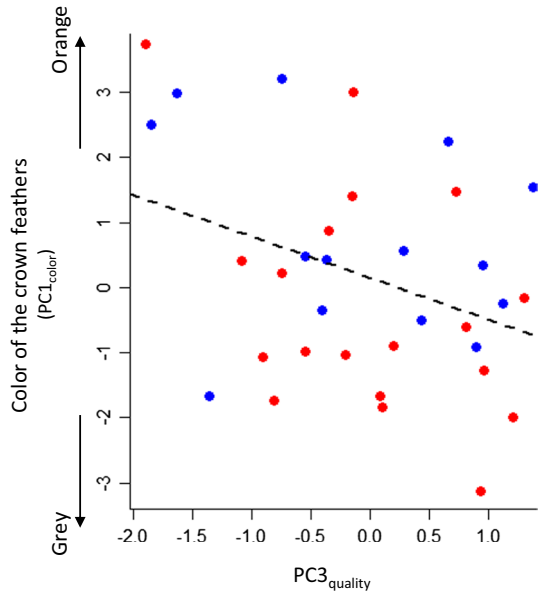
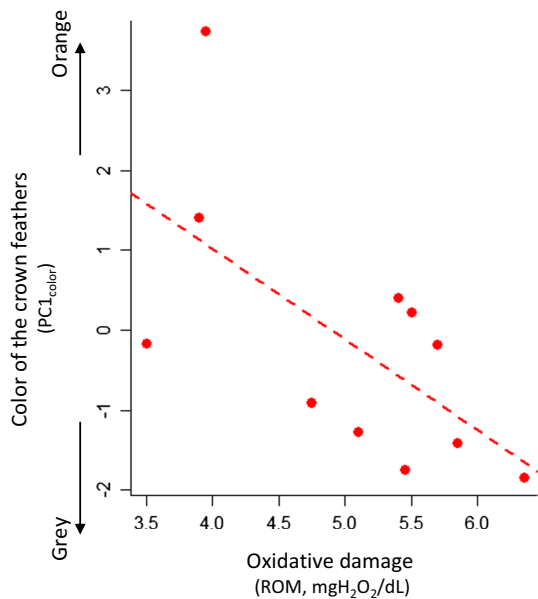


Fig. 4 Color of the crown feathers (as described by $PC1_{color}$) in relation to oxidative damage in plasma, in females



with both $PC1_{quality}$ and $PC2_{quality}$ ($F_{1,15}=8.14$, $P=0.012$ and $F_{1,15}=4.94$, $P=0.042$; Fig. 2). In contrast, in males, $PC1_{color}$ did not vary with $PC1_{quality}$ ($F_{1,13}=0.00$, $P=0.98$; Fig. 2), and tended to increase with $PC2_{quality}$ ($F_{1,13}=3.46$, $P=0.085$; Fig. 2). $PC1_{color}$ decreased with $PC3_{quality}$ in both sexes ($\chi^2_1=4.63$, $P=0.031$; Fig. 3). $PC1_{color}$ tended to decrease with date ($\chi^2_1=2.75$, $P=0.097$). In females, $PC1_{color}$ decreased with ROM levels in plasma ($F_{1,9}=5.89$, $P=0.038$; Fig. 4), but it did not vary with OXY levels in plasma

($F_{1,8} = 0.015$, $P = 0.91$). $PC1_{\text{color}}$ therefore decreased with oxidative stress, which was calculated as ROM levels/OXY levels ($F_{1,9} = 5.65$, $P = 0.041$).

$PC2_{\text{color}}$ varied with none of the variables included in the models related to individual quality (i.e., the three PC_{quality} and date: all $P > 0.10$), and neither with ROM nor OXY levels (all $P > 0.15$).

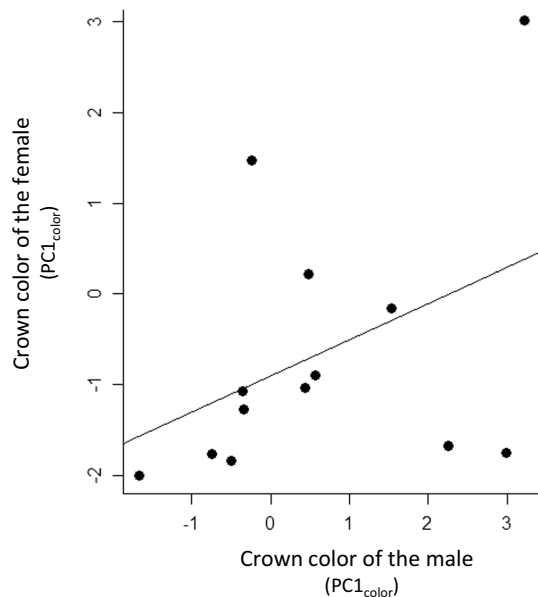
Sexual dichromatism and assortative mating

On average, males had higher $PC1_{\text{color}}$ than females ($\chi_1^2 = 5.82$, $P = 0.016$). No sex-differences in $PC2_{\text{color}}$ were detected ($\chi_1^2 = 0.18$, $P = 0.89$). $PC1_{\text{color}}$ was positively correlated among breeding pairs ($r = 0.57$, $P = 0.047$; Fig. 5), while $PC2_{\text{color}}$ was not ($r = -0.36$, $P = 0.23$).

Discussion

The orange color of crown feathers, which is produced by pheomelanin, was related to fitness traits and oxidative stress in incubating blue petrels, especially in females. Females with more-intense orange coloration (i.e., higher $PC1_{\text{color}}$) were poor-quality females (i.e., lower PC_{quality} being related to lower body mass, shorter tarsi, smaller egg, shorter time spent incubating the egg, lower reproductive success and delayed laying date). Similar negative relationships between pheomelanin-based coloration and individual-quality traits were found in nestling Eurasian nuthatches (*Sitta europaea*) and tawny owls (*Strix aluco*), where individuals with more intense pheomelanin-based feathers are in poorer condition (Galván 2017) and have lower viability during adverse environmental conditions (Karell et al. 2011), respectively. Pheomelanin production has been suggested to be

Fig. 5 Color of the crown feather ($PC1_{\text{color}}$) in females in relation to that in their male breeding partner



costly because it is phototoxic and depletes glutathione stores in melanocytes (Kinnaert et al. 2004; Napolitano et al. 2014). It thereby increases oxidative stress (Roulin et al. 2011; Mitra et al. 2012; Napolitano et al. 2014), which is known to impair numerous cell functions and reduce survival and fecundity (Bize et al. 2008). Thus the negative association between pheomelanin-based coloration and individual quality in blue petrel females may be due to the important physiological costs associated with pheomelanin expression. Non exclusively, pheomelanin-based coloration might be related to individual quality because of the functional role of pheomelanogenesis in cysteine detoxification (Galván et al. 2012b). Poor-quality females may develop more intense pheomelanin-based coloration of the crown if they have higher need for cysteine detoxification, due for instance to higher cysteine levels in the diet or higher susceptibility to cysteine toxicity. To further evaluate this hypothesis, studies are needed to determine whether excess cysteine can occur in blue petrels and cause health problems.

Whatever the physiological mechanisms linking pheomelanin expression to individual quality, the negative association between these two traits suggests that, in blue petrels, pheomelanin-based coloration may not have evolved as a social or sexual signal of individual quality in females. In several species, melanin-based coloration is determined not only by the total amount of pigments, but also by the eumelanin-to-pheomelanin ratio (Ito and Wakamatsu 2003; Singaravelan et al. 2010; Morales-Guerrero et al. 2019). Several molecules such as agouti and glutathione that trigger the production of pheomelanin have an inhibitory effect on eumelanin production, whereas other hormones, such as melancortins, have the opposite effect (Benedetto et al. 1982; Lu et al. 1994; Fumura et al. 1996; Le Pape et al. 2008). It is therefore possible that blue petrel females with more-intense orange coloration have lower eumelanin expression in the crown. Under this hypothesis, good-quality females may be able to deposit more eumelanin into feathers than poor-quality females. Eumelanin traits are associated with variation in physiological and behavioral traits in several species (Vágási et al. 2010; Jacquin et al. 2011; Roulin et al. 2011; Arai et al. 2019). In particular, eumelanin is associated with higher fitness in females in barn owls (Roulin and Altwegg 2007), tawny owls (*Strix aluco*) (Roulin et al. 2003) and Eurasian kestrels (*Falco tinnunculus*) (Vergara et al. 2009). Interestingly, similar to barn owls (Roulin 2003), where eumelanin-based coloration is a sexual signal of quality in females only (Roulin and Altwegg 2007), blue petrel females are on average less orange-colored than blue petrel males. To determine whether eumelanin-based coloration of females' crown can signal individual quality, studies incorporating analyses of eumelanin and pheomelanin quantities in feathers are necessary.

We found that, during incubation, high-quality females (and thus females with less-orange crowns) had higher oxidative stress, as a result of higher oxidative damage, than poor-quality females. This positive association between oxidative damage and individual quality in females seems surprising, as oxidative stress, leading to a plethora of deleterious effects on homeostasis (Jones 2006), is generally thought to be negatively related to individual quality and fitness (Bize et al. 2008). Oxidative stress and its sensitivity are known, however, to vary with several ecological factors and life-history traits, including reproductive effort (Costantini 2008; Metcalfe and Alonso-Alvarez 2010). In addition, long periods of starvation lead to elevated oxidative stress in several species (Pascual et al. 2003; Wasselin et al. 2014). The higher oxidative stress observed in good-quality females may thus be due to their increased reproductive effort and longer time spent fasting in the nest. As a result, we cannot exclude that, outside the breeding period, e.g., at the time of molt when pheomelanin pigments are deposited into feathers, high-quality females face lower oxidative stress than poor-quality females. Further studies investigating oxidative stress levels

during crown-feather formation are needed to further evaluate the potential for a mechanism associated with oxidative stress to be at the bases of crown color in blue petrels.

We did not find strong evidence for associations between crown color and individual quality in males. Sex-specific selective pressures on melanin-based traits may be due to differences between sexes in their sensitivity to environmental stress or to excess cysteine (Galván and Alonso-Alvarez 2009; Galván et al. 2012b). However, the sample size of our study is relatively low ($n=15$ males) and further studies should include a higher sample size to draw firm conclusion on the potential association between melanin-based crown coloration and individual quality in males.

In blue petrels, males and females seem to pair assortatively by crown coloration. This type of pairing has been observed in other melanin-colored species (Bortolotti et al. 2008; Rowe and Weatherhead 2011; Indykiewicz et al. 2017). Assortative pairing can occur when there is mutual mate choice for similar ornaments (Holbeck and Riebel 2009). However, the lack of association between color and individual quality in male blue petrels suggests that directional preference is not a plausible mechanism in this species. In numerous species, the behavioral compatibility of mates is a major factor determining reproductive success (Spoon et al. 2006; Ariyomo and Watt 2013; Mariette and Griffith 2015). Because of the pleiotropic effects of genes regulating melanogenesis, variation in behavior is often associated with melanin-based coloration (reviewed in Ducrest et al. 2008). Therefore, homotypic preference for behavioral traits might result in an apparent assortative mating by crown coloration in blue petrels. Assortative mating by age has also been recorded in a wide range of avian species (reviewed in Jiang et al. 2013), and it is widely acknowledged that melanin-based coloration varies with age (e.g., Potti and Montalvo 1991; Budden and Dickinson 2009; Galván and Møller 2009). Assortative mating by age might thus drive, at least to some extent, positive correlation in crown color among mates in blue petrels.

In conclusion, our result provides evidence for an association between individual quality and melanin-based color of crown feathers in female blue petrels. However, females with less-orange crowns were higher-quality females, which is inconsistent with an adaptive function of pheomelanin coloration in inter-, or intra-, sexual selection in this species. Our findings therefore open new doors for further studies that may comprehensively investigate how pheomelanin expression is adaptive in light of natural selection in petrel species.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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