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

3 Sarah Leclaire^{1,2,*}, Samuel Perret², Ismael Galván³, Francesco Bonadonna²

4
5 **Running title:** Pheomelanin coloration and individual quality in petrels

6
7 ¹Laboratoire Evolution & Diversité Biologique, 118 rte de Narbonne, 31062 Toulouse, France

8 ²Centre d'Ecologie Fonctionnelle et Evolutive, CNRS–CEFE, 1919 route de Mende, 34293 Montpellier,
9 France

10 ³Departamento de Ecología Evolutiva, Estación Biológica de Doñana, CSIC, Sevilla, Spain

11
12
13 * Corresponding author: sarah.leclaire@free.fr

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20

21 **Abstract**

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

38

39

40 Introduction

41 Melanin is the most common pigment in higher vertebrates, occurring in two main chemical forms:
42 eumelanin, which produces black, gray and dark brown colors, and pheomelanin, which is
43 responsible for reddish and yellowish colors. Eumelanin-based traits have been suggested to play a
44 role in signaling, thermoregulation, camouflage to predators, and protection from UV radiation,
45 mechanical damage or feather-degrading parasites (Mcgraw 2006; Galván and Solano 2016). In
46 contrast, the adaptive value of pheomelanin remains unclear, as it is phototoxic and its synthesis in
47 melanocytes requires the consumption of cysteine via glutathione (GSH), the most important
48 intracellular antioxidant (Galván et al. 2012b; Panzella et al. 2014). Accordingly, in humans, red-
49 haired individuals are more prone to skin cancers (Gerstenblith et al. 2010), and in Asian barn
50 swallows (*Hirundo rustica gutturalis*), European nuthatches (*Sitta europaea*), boars (*Sus scrofa*) and
51 lab mice, pheomelanin morphs have lower viability and higher oxidative stress (Galván et al. 2012a;
52 Mitra et al. 2012; Galván 2017; Arai et al. 2018). Pheomelanin can confer crypsis (Nachman et al.
53 2003; Negro et al. 2009; Singaravelan et al. 2010), but not all pheomelanin-based traits are involved
54 in concealment pattern.

55 Recently, pheomelanin has been suggested to have evolved because of its potential role in
56 removing excess cysteine (Galván et al. 2012b). Although cysteine is an essential component of GSH,
57 thereby being important for antioxidant protection, it can cause a variety of problems when in
58 excess, including poor growth, pregnancy complication and brain damage (Olney and Ho 1970; Orth
59 et al. 1992; El-Khairi et al. 2003; Galván and Alonso-Alvarez 2017). Therefore, under low levels of
60 environmental oxidative stress - *i.e.*, when cysteine is not needed for GSH-mediated antioxidant
61 protection and can potentially be in excess -, pheomelanogenesis may be advantageous as it
62 consumes toxic cysteine (Galván et al. 2012b). In contrast, pheomelanogenesis may represent a
63 physiological cost under high environmental stress, when cysteine is needed for antioxidant
64 protection (Galván and Solano 2009). In line with this hypothesis, recent studies have shown that, in
65 some species, pheomelanin synthesis is increased under exposure to excess cysteine, and decreased
66 under exposure to high environmental oxidative stress (Galván et al. 2017; Rodríguez-Martínez et al.
67 2019).

68 According to the handicap principle (Zahavi 1975), the cost associated with
69 pheomelanogenesis in environments with high oxidative stress may have promoted the evolution of
70 pheomelanin-based color as honest signals quality (Galván and Solano 2009; Galván 2018), because
71 only individuals with a high antioxidant capacity may be able to generate large pheomelanin-based
72 signals. In agreement, in several bird species, pheomelanin-based traits are positively associated with

73 individual quality and may thus play a role in sexual selection (Jawor and Breitwisch 2003). For
74 instance, eastern bluebird (*Sialia sialis*) males with larger pheomelanin-based breast patches fledge
75 heavier offspring (Siefferman and Hill 2003), and American barn swallow (*Hirundo rustica rustica*),
76 males with brighter pheomelanin-based throat have higher reproductive success (Safran and McGraw
77 2004).

78 The blue petrel (*Halobaena caerulea*) is a Procellariidae burrowing seabird that harbors a
79 variable amount of orange feathers in the crown (Fig. 1), which is likely due to variation in the
80 quantity of pheomelanin. Blue petrels being pelagic, the orange coloration is unlikely to play a role in
81 camouflage. Like many other procellariidae, this seabird is nocturnal on the breeding ground
82 (Warham 1996), and breeds within deep burrows, which have led to the suggestion that color
83 signals are not essential to communicate (Bretagnolle 1996). However, petrels are active during
84 daytime at sea, where they can aggregate in very dense foraging flocks (Van Franeker et al. 2002), or
85 settle in groups on the calm water (Tickell 1962). Little is known about vision in procellariidae.
86 Burrow-nesting petrels do not have exceptional nocturnal vision, and seem to have lower visual
87 acuity than surface-nesting petrels (Brooke 1989; Mitkus et al. 2016). However, in the leach-storm
88 petrel (*Oceanodroma leucorhoa*), a species with similar ecology as the blue petrel, the smallest
89 object that an individual may see in daylight at a distance of 2m is 16mm in diameter for low-contrast
90 objects and 2mm in diameter for high contrast objects (Mitkus et al. 2016). These observations
91 suggest the potential for visual communication among individuals who are settled close to each
92 other on the water, even in burrow-nesting petrels. In addition, males and females do not differ in
93 foraging distribution during breeding and non breeding seasons (Phillips et al. 2009), suggesting that
94 inter-sexual communication might occur.

95 We investigate the signaling potential of the pheomelanin-based coloration of the crown
96 feathers of blue petrels, by testing the correlation between color expression and individual quality,
97 which we define as a composite measure of multiple phenotypic traits putatively related to fitness
98 (Wilson and Nussey 2010). If orange pheomelanin-based coloration is a quality signal used in sexual
99 selection, we expect good-quality individuals to display more intense orange crown feathers. In
100 contrast, a negative association would preclude a signaling role of pheomelanin color. In this species,
101 males and females provide similar incubation and parental care (Chaurand and Weimerskirch 1994b),
102 thus we predict that crown coloration reflects individual quality in both sexes and that breeding pairs
103 show assortative mating in relation to this trait. We also explore whether variation in crown
104 coloration is potentially linked to oxidative stress, by testing the correlation between color
105 expression and oxidative stress in plasma. We expect blue petrels with high oxidative stress to
106 display less-intense orange crown coloration, because oxidative stress may be a physiological trait

107 related to individual quality (Bize et al. 2008; Cohen et al. 2008; Hill 2014), and because it is
108 suggested to downregulate pheomelanin production, at least in some species (Galván 2018). We
109 measure oxidative stress at the time of breeding and not at the time of molt when pheomelanin is
110 deposited into feathers. However, although oxidative status is known to change over time, a few
111 studies showed that differences in oxidative status among individuals are consistent over a few week
112 period, as well as across breeding seasons (Costantini et al. 2007; Hau et al. 2015; Herborn et al.
113 2016).

114

115 **Material and methods**

116 *Study site*

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

123

124 *Color measurements*

125 Crown coloration was measured from digital photographs. Pictures were taken at approximately 40
126 cm using a digital camera (Panasonic, DMC-TZ30). For each photograph, the same color swatch
127 (QpCARD 201) was placed next to the bird to standardize subsequent measurements. Low quality
128 pictures (due to ambient lighting variations) were removed from the dataset. All pictures were
129 analyzed using the software Adobe Photoshop v7.0 and the CIELAB color space. The CIELAB color
130 space is a perceptually uniform color space designed to provide estimates of human luminance and
131 chromatic perception. It has been suggested to be more appropriate than the RGB color space that is
132 non-uniform and cannot represent all perceivable colors even to humans (Stevens and Cuthill 2005).
133 In the CIELAB color space, L* represents the achromatic signal, while a* and b* represent two
134 chromatic channels representing green-red and blue-yellow respectively. The average components of
135 L*a*b* were recorded in a standardized area of the crown (length of the standardized area: 2 cm
136 measured from where the top of the skull angles to the frontal zone; width of the standardized area:
137 the width of the skull at the angle between the top of the skull and the frontal zone; Fig. 1). The
138 L*a*b* values of each integument were corrected according to the L*a*b* values of the color
139 swatch, by using the residuals of a linear regression between the values of the crown and the values
140 of the color swatch. In our study, color varied from black (lower values of L*, a* and b*) to orange

141 (higher values of L^* , a^* and b^* ; Fig. S1). In addition, we recorded the standard deviation of L^* (L^* std),
142 which represented the patchiness of crown coloration. L^* , a^* , b^* and L^* std values were highly
143 correlated (Pearson correlation tests: all $r > 0.66$, and all $P > 0.0001$; except for the correlation
144 between L^* std and a , and L^* std and b : $r = 0.46$, $P = 0.001$ and $r = 0.43$, $P = 0.003$ respectively).
145 Therefore, the color of the crown was described by the first two principal components of a PCA on
146 these four color variables (hereafter referred to as $PC1_{color}$ and $PC2_{color}$). $PC1_{color}$ accounted for 74% of
147 the variation observed among color variables, and higher $PC1_{color}$ indicated lighter, patchier and more
148 orange crown (eigenvectors: L^* : 0.91, a^* : 0.88, b^* : 0.89 and L^* std: 0.76). $PC2_{color}$ accounted for 19%
149 of the variation observed among color variables, and higher $PC2_{color}$ mainly indicated patchier crown
150 (eigenvectors: L^* : 0.26, a^* : -0.39, b^* : -0.40 and L^* std: 0.61).

151 In animal color studies, color scores obtained from digital photographs have been suggested
152 to be less appropriate to than those obtained with a reflectance spectrophotometer and visual
153 models, because they do not consider the perception of color by the receiver's visual system (Stevens
154 et al. 2009). However, information obtained from digital pictures has revealed patterns and effects of
155 biological meaning in several bird species (Pérez-Rodríguez and Viñuela 2008; Laucht et al. 2010;
156 Leclaire et al. 2011). In addition, in tawny owls (*Strix aluco*), who show variations in pheomelanin
157 coloration in feathers, coloration scores obtained from pictures correlate with those obtained with a
158 spectrophotometer and with the concentration of pheomelanin pigments in feathers (Gasparini et al.
159 2009).

160

161 *Pigment analyses*

162 To obtain firm evidence of the pigment nature of the orange crown feathers of blue petrels, we
163 collected feathers from three adult birds. The feathers were analyzed by micro-Raman spectroscopy,
164 as pheomelanin and eumelanin exhibit distinctive Raman signals that can be used to identify them
165 (Galván et al. 2013). We used a Thermo Fisher DXR confocal dispersive Raman microscope (Thermo
166 Fisher Scientific, Madison, WI, USA) with a point-and-shoot Raman capability of 1 μm spatial
167 resolution and using a near-infrared excitation laser of 780 nm. Laser power was set at 7 mW,
168 integration time at 3 s and number of accumulations at 8. The spectra were obtained using a 50x
169 confocal objective and a slit aperture of 50 μm . The system was operated with Thermo Fisher OMNIC
170 8.1 software. Calibration and alignment of the spectrograph were checked using pure polystyrene.
171 We obtained two Raman spectra from the feathers of each individual bird, and then computed the
172 average spectrum. We only obtained Raman signal of pheomelanin, as the spectra showed the three
173 distinctive Raman bands of this pigment at about 500, 1500 and 2000 cm^{-1} (Fig. S2) (Galván et al.

174 2013), thus confirming that the orange feathers in the crown of blue petrels are colored by
175 pheomelanin.

176

177 *Index of individual quality*

178 We assessed individual quality by conducting a principal components analysis (PCA) on several life-
179 history traits commonly related to individual fitness: tarsus length, body mass index, date of laying,
180 egg volume, number of days spent incubating the egg over the whole incubating period and hatching
181 success. Four females and one male, for whom we did not have the incubation duration, were
182 excluded from these analyses (sample size for the analyses on individual quality: $n = 19$ females and
183 15 males).

184 In blue petrels, body mass and number of days spent incubating the egg have been shown to
185 be related to reproductive success (Chaurand and Weimerskirch 1994a). Once the single egg is laid,
186 males and females take individual incubating bouts in the nest, that can last up to 12 days and during
187 which parents lose body mass (Chaurand and Weimerskirch 1994a). Individuals in higher body
188 condition at the start of the incubation bout are able to take longer incubation bouts, thereby
189 increasing hatching success (Chaurand and Weimerskirch 1994a). We therefore calculated a body
190 mass index as the residual of a linear regression between body mass and duration of the incubating
191 bout until capture. Because this correlation tended to depend on sex (interaction between sex and
192 bout duration until capture: $F_{1,35} = 3.72$, $P = 0.06$), we performed the linear regression for each sex
193 separately (correlation between body mass at date of capture and bout duration until capture:
194 males: $r = -0.82$, $P = 0.0001$; females: $r = -0.78$, $P < 0.0001$). In a high number of studies, body
195 condition is calculated by controlling body mass for body size effects (Schulte-Hostedde et al. 2005;
196 Peig and Green 2009). However, in our study, body mass and corrected body mass index were not
197 related to tarsus length (females: $r = 0.10$, $P = 0.65$ and $r = 0.14$, $P = 0.53$; males: $r = -0.43$, $P = 0.10$
198 and $r = -0.20$, $P = 0.46$). The other measures included in the PCA (*i.e.*, tarsus length, date of laying and
199 egg volume) have not yet been associated with reproductive success in blue petrels, but they are
200 related to fitness in numerous other bird species (Kingsolver and Pfennig 2004; Verhulst and Nilsson
201 2008; Krist 2011).

202 Individual quality was described by the first three principal components of a PCA on traits
203 related to individual fitness (hereafter referred to as $PC1_{\text{quality}}$, $PC2_{\text{quality}}$ and $PC3_{\text{quality}}$). $PC1_{\text{quality}}$,
204 $PC2_{\text{quality}}$ and $PC3_{\text{quality}}$ accounted for 32%, 23% and 15% of the variation observed among life-history
205 traits. Individuals with higher $PC1_{\text{quality}}$ had higher body condition, spent more days incubating their
206 egg and had higher hatching success (eigenvectors: 0.66, 0.84 and 0.68 respectively). Individuals with

207 higher PC2_{quality} had longer tarsus and larger egg (eigenvectors: 0.72 and 0.74 respectively). The egg
208 of individuals with higher PC3_{quality} was laid earlier (eigenvectors related to laying date: -0.79).

209

210 *Oxidative stress analyses*

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

217

218 *Statistical analyses*

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

225 We tested the association between color scores and ROM and OXY levels using linear
226 models. Since ROM and OXY levels were not correlated within individuals (Pearson correlation test: r
227 = 0.002, $P = 0.99$), they were both included as fixed effects in the models. In addition, because the
228 sample size was very low for males ($n = 4$ males), we restricted these analyses on females.

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

232

233 **Results**

234 *Color and individual quality*

235 Plasma ROM levels were positively related to PC2_{quality} in females ($F_{1,6} = 6.92$, $P = 0.039$, $n = 8$ females
236 for whom we had ROM levels and all quality-related variables; Fig. S3), but not to PC1_{quality} and
237 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**

238 related to some aspects of individual quality in females, namely larger eggs and longer **tarsi**. Plasma
239 OXY levels were related to none of the PC_{quality} (all P > 0.10).

240 PC_{1color} varied with the interactions between sex and PC_{1quality} ($\chi^2_1 = 4.29$, P = 0.038; Fig. 2),
241 and sex and PC_{2quality} ($\chi^2_1 = 4.15$, P = 0.042; Fig. 2). In females, PC_{1color} decreased with both PC_{1quality}
242 and PC_{2quality} ($F_{1,15} = 8.14$, P = 0.012 and $F_{1,15} = 4.94$, P = 0.042; Fig. 2). In contrast, in males, PC_{1color} did
243 not vary with PC_{1quality} ($F_{1,13} = 0.00$, P = 0.98; Fig. 2), and tended to increase with PC_{2quality} ($F_{1,13} = 3.46$,
244 P = 0.085; Fig. 2). PC_{1color} decreased with PC_{3quality} in both sexes ($\chi^2_1 = 4.63$, P = 0.031; Fig. 3). PC_{1color}
245 tended to decrease with date ($\chi^2_1 = 2.75$, P = 0.097). In females, PC_{1color} decreased with ROM levels in
246 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 =
247 0.91). PC_{1color} therefore decreased with oxidative stress, which was calculated as ROM levels / OXY
248 levels ($F_{1,9} = 5.65$, P = 0.041).

249 PC_{2color} varied with none of the variables included in the models related to individual quality
250 (*i.e.*, the three PC_{quality} and date: all P > 0.10), and neither with ROM nor OXY levels (all P > 0.15).

251

252 *Sexual dichromatism and assortative mating*

253 On average, males had higher PC_{1color} than females ($\chi^2_1 = 5.82$, P = 0.016). No sex-differences in
254 PC_{2color} were detected ($\chi^2_1 = 0.18$, P = 0.89). PC_{1color} was positively correlated among breeding pairs
255 ($r = 0.57$, P = 0.047; Fig. 5), while PC_{2color} was not ($r = -0.36$, P = 0.23).

256

257 **Discussion**

258 The orange color of crown feathers, which is produced by pheomelanin, was related to fitness traits
259 and oxidative stress in incubating blue petrels, especially in females. Females with more-intense
260 orange coloration (*i.e.*, higher PC_{1color}) were poor-quality females (*i.e.*, **lower** PC_{quality} **being** related to
261 **lower** body mass, **shorter tarsi**, **smaller egg**, **shorter** time spent incubating the egg, **lower**
262 reproductive success and **delayed** laying date). Similar negative relationships between pheomelanin-
263 based coloration and individual-quality traits were found in nestling Eurasian nuthatches (*Sitta*
264 *europaea*) and tawny owls (*Strix aluco*), where individuals with more intense pheomelanin-based
265 feathers are in poorer condition (Galván 2017) and have lower viability during adverse environmental
266 conditions (Karell et al. 2011), respectively. Pheomelanin production has been suggested to be costly
267 because it is phototoxic and depletes glutathione stores in melanocytes (Kinnaert et al. 2004;
268 Napolitano et al. 2014). It thereby increases oxidative stress (Roulin et al. 2011; Mitra et al. 2012;

269 Napolitano et al. 2014), which is known to impair numerous cell functions and reduce survival and
270 fecundity (Bize et al. 2008). Thus the negative association between pheomelanin-based coloration
271 and individual quality in blue petrel females may be due to the important physiological costs
272 associated with pheomelanin expression. Non exclusively, pheomelanin-based coloration might be
273 related to individual quality because of the functional role of pheomelanogenesis in cysteine
274 detoxification (Galván et al. 2012b). Poor-quality females may develop more intense pheomelanin-
275 based coloration of the crown if they have higher need for cysteine detoxification, due for instance
276 to higher cysteine levels in the diet or higher susceptibility to cysteine toxicity. To further evaluate
277 this hypothesis, studies are needed to determine whether excess cysteine can occur in blue petrels
278 and cause health problems.

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

299 We found that, during incubation, high-quality females (and thus females with less-orange
300 **crowns**) had higher oxidative stress, as a result of higher oxidative damage, than poor-quality
301 females. This positive association between oxidative damage and individual quality in females seems
302 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.

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

341

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352

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556

557 **Figure 1:** Pictures of 3 blue petrels with different crown coloration. The yellow rectangle represents
558 the standardized area used for color measurements.

559

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

563

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

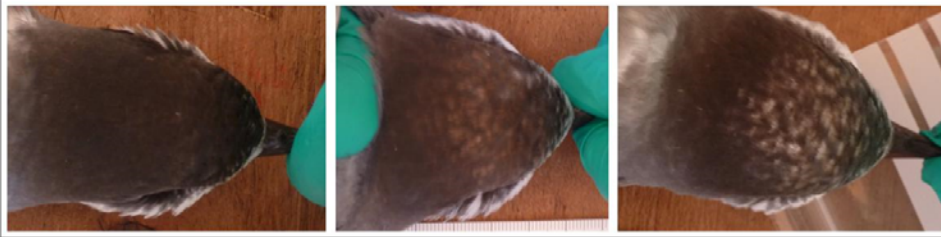
567

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

570

571 **Figure 5:** Color of the crown feather ($PC1_{color}$) in females in relation to that in their male breeding
572 partner.

416 **Figure 1:** Pictures of 3 blue petrels with different crown coloration.



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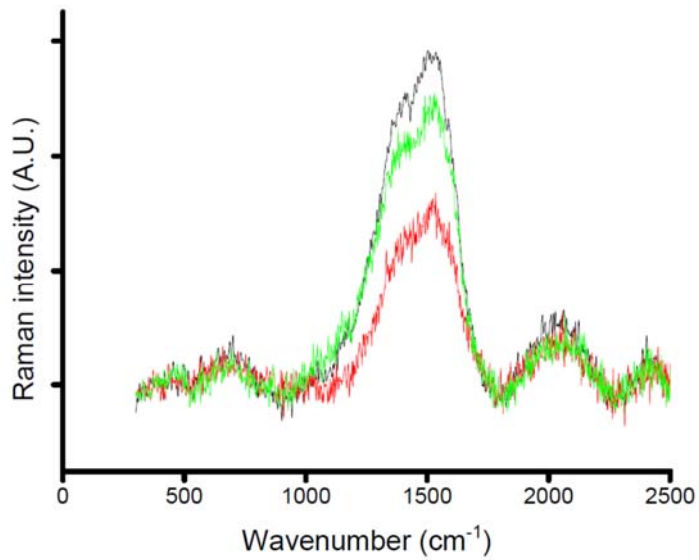
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420 **Figure 2:** Raman spectra of blue petrel orange crown feathers. Each color represents the average of
421 two Raman spectra obtained from a different bird. The spectra show the three diagnostic
422 Raman bands of pheomelanin at about 500, 1500 and 2000 cm^{-1} , indicating that the orange color is
423 caused by the presence of this pigment. The curves were smoothed by the adjacent averaging
424 technique using ORIGIN v.7 software (OriginLab Corporation, Northampton, MA, USA).

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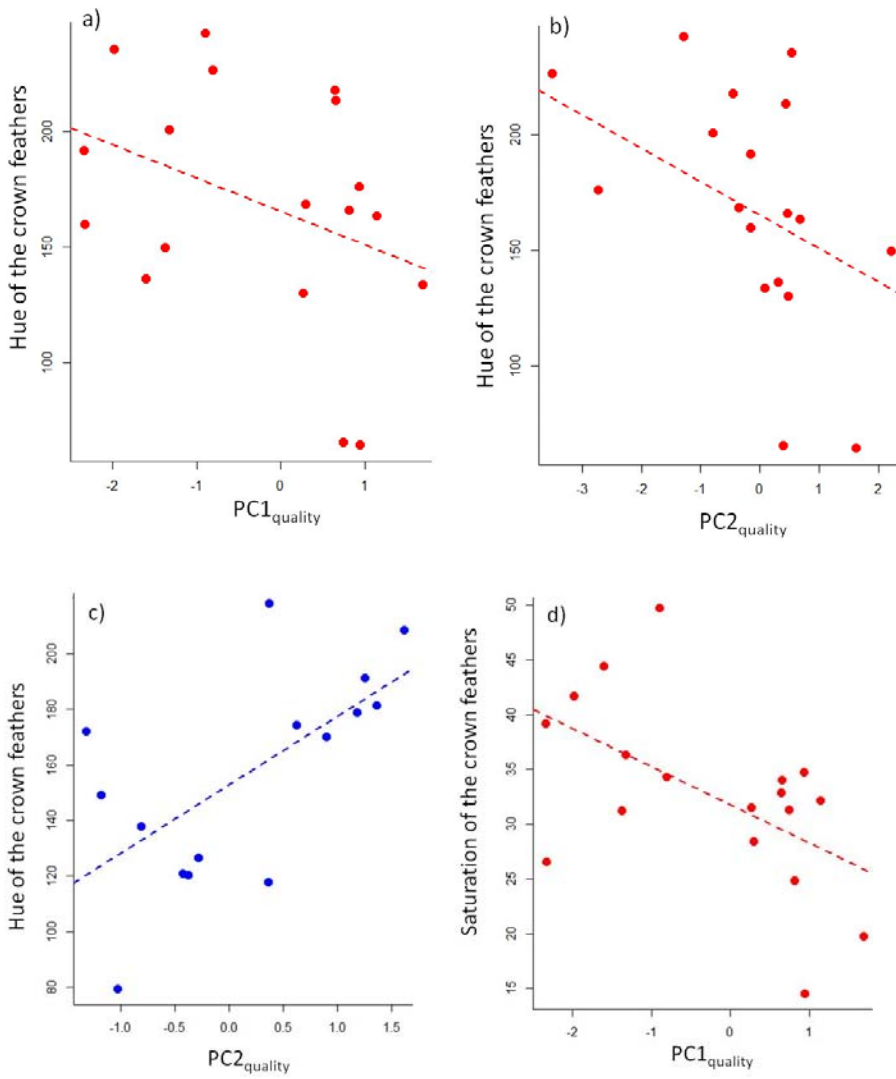
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429 **Figure 3:** Hue (a, b and c) and saturation (d) of crown feathers in relation to individual quality as
430 measured by the first or second axis of a PCA analyses on several fitness-related traits. Females are in
431 red, males are in blue.



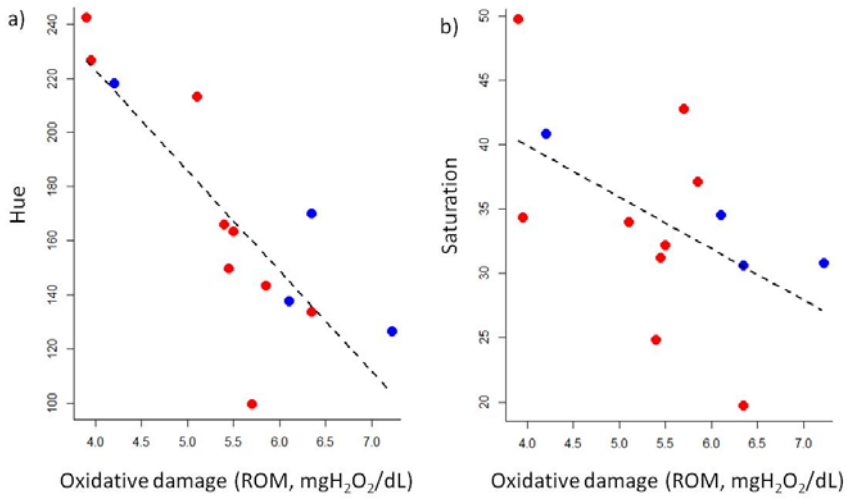
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436 **Figure 4:** (a) Hue and (b) saturation of the crown feathers in relation to oxidative damage in plasma.
437 Females are in red, while males are in blue.



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