

Assortative mating by multiple skin color traits in a seabird with cryptic sexual dichromatism

Isabel López Rull^{1,2,4} · Leticia Nicolás¹ · Nadia Neri-Vera² · Víctor Argáez² · Margarita Martínez^{1,3} · Roxana Torres²

Received: 27 August 2015 / Revised: 19 April 2016 / Accepted: 3 May 2016
© Dt. Ornithologen-Gesellschaft e.V. 2016

Abstract Most seabirds are monochromatic in plumage, yet many of them show multiple colored integuments, typically modulated by sex steroids such as testosterone, which can reflect individual quality and may be under mutual sexual selection. In the Masked Booby *Sula dactylatra*, both sexes exhibit multiple color traits that vary in their expression either in color (feet ranging from olive to orange, yellowish bill and black mask) or size (mask). Here, we report sexual differences in skin color traits and evaluate whether their expression is related to individual body condition and plasma testosterone, whether different color traits co-vary, and whether Masked Boobies mate assortatively by color. Our results show that Masked Boobies are sexually dichromatic in foot color and monochromatic in bill and mask color. After controlling for body size and testosterone levels, mask size was larger in females than in males. We found a positive relationship between body condition and plasma testosterone in males and females, suggesting a condition-dependence of plasma

androgen levels. Interestingly, foot color covaries positively with testosterone levels in individuals with good body condition and negatively in individuals with poor body condition, whereas mask size was positively related with body condition. Taken together, these results suggest that only individuals in good condition could produce more conspicuous traits. Also, we found a negative relationship between mask color and condition, probably due to the fact that melanin-based traits can pleiotropically vary with other functions, including food intake patterns. Finally, within-individual foot color and mask size were positively correlated and pairs mated assortatively with respect to foot color and mask color and size. We suggest that such a mating pattern may result from a scenario where both sexes choose mates based on traits that convey reliable information of individual quality.

Keywords Condition-dependent traits · Multiple ornaments · Mutual choice

Communicated by C. Barbraud.

✉ Isabel López Rull
isalorull@gmail.com

¹ Centro Tlaxcala de Biología de la Conducta, Universidad Autónoma de Tlaxcala, Tlaxcala, Mexico

² Instituto de Ecología, Departamento de Ecología Evolutiva, Universidad Nacional Autónoma de México, México, DF 4510, Mexico

³ Instituto de Investigaciones Biomédicas, Departamento de Biología Celular y Fisiología, Universidad Nacional Autónoma de México, México, DF 4510, Mexico

⁴ Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, C/José Gutiérrez Abascal, 2, Madrid 28006, Spain

Zusammenfassung

Assortative Paarung für unterschiedliche Hautfarben bei Seevögeln mit nicht eindeutigem Geschlechtsdimorphismus

Die meisten Seevögel haben einfarbiges Gefieder, viele von ihnen aber Haut in unterschiedlichen Färbungen, die in der Regel durch Geschlechtshormone wie z.B. Testosteron bestimmt werden. Diese Färbungen könnten Hinweise auf individuelle Qualität geben und stünden dann vermutlich unter Selektionsdruck. Beim Maskentölpel (*Sula dactylatra*) entwickeln beide Geschlechter Färbungen, die in Größe (Maske) oder Farbton variieren können (die Farbe der Füße reicht von oliv bis orange, bei gelben Schnäbeln

und schwarzen Masken). Wir berichten hier über Geschlechtsunterschiede in der Hautfärbung und untersuchen, ob deren Ausprägung mit der physischen Verfassung der einzelnen Individuen und ihrem Plasma-Testosteron zusammenhängt, ob unterschiedliche Färbungen kovariieren, und ob sich die Maskentöpel bei den Farben assortativ verpaaren. Unsere Ergebnisse zeigen, dass bei Maskentöpel die Füße geschlechtsabhängig in zwei Farben und die Schnäbel und Masken nur in einer Farbe vorkommen. Nach Feststellen des Geschlechts anhand von Körpergröße und Testosteron-Spiegel, zeigte es sich, dass die Weibchen größere Masken als die Männchen hatten. Wir fanden für beide Geschlechter eine positive Korrelation zwischen physischer Verfassung und Plasma-Testosteron, was auf eine Abhängigkeit der Verfassung vom Androgen-Spiegel hinweist. Interessanterweise kovariiert die Fuß-Färbung bei Individuen in guter physischer Verfassung positiv mit dem Testosteron-Spiegel, aber negativ bei Individuen in schlechter Verfassung, wohingegen die Größe der Maske stets positiv mit der physischen Verfassung korrelierte. Aus diesen Ergebnissen kann geschlossen werden, dass möglicherweise nur Individuen in guter Verfassung stärker auffallende Merkmale zeigen. Wir fanden außerdem eine negative Korrelation zwischen Masken-Färbung und physischer Verfassung, was wahrscheinlich daran liegt, dass auf Melanin basierende Merkmale pleiotropisch mit anderen Funktionen wie z. B. Mustern in der Nahrungsaufnahme zusammenhängen können. Bei Einzelindividuen waren Fuß-Färbung und Masken-Größe positiv korreliert, und in Hinblick auf Fuß- und Masken-Färbung und Masken-Größe verlief die Paarbildung assortativ. Wir denken, dass solch ein Paarbildungsmuster auf ein Szenario zurückgeführt werden kann, in dem beide Geschlechter ihre Partner anhand von Merkmalen auswählen, die verlässliche Information über die individuelle Qualität geben.

Introduction

In many animals, individuals display extravagant traits and conspicuous colorations that honestly reflect their genetic quality or condition, and enhance their mating success through mate choice (hereafter ornaments; Andersson 1994). This communication system of ornaments and associated preferences is based on the benefits that ornamentation confers to both displayers and choosers. The benefits to the displayer lie in a net reproductive advantage through higher mating success, in spite of the costs of producing the signal (Darwin 1871; Zahavi 1975; Maynard Smith and Harper 2003). Benefits to choosers derive from obtaining reliable information on the quality of potential

mates, which is important since mating with a high-quality partner may confer direct benefits in the form of parental care and territorial resources (Kirkpatrick and Ryan 1991), or indirect benefits such as the inheritance of good genes for attractiveness (Fisher 1930) and viability (Zahavi 1975).

Sexual displays are often complex exhibitions involving the simultaneous expression of multiple cues rather than a single trait. Multiple ornaments may be arbitrary by-products of runaway sexual selection (“unreliable signal hypothesis”; Møller and Pomiankowski 1993); alternatively, they may function as honest indicators of quality (reviewed in Candolin 2003; Lozano 2009). Among the different hypotheses proposed to explain the adaptiveness of multiple ornaments (reviewed in Candolin 2003; Lozano 2009), the “redundancy hypothesis” and the “multiple-messages hypothesis” have received growing interest (Møller and Pomiankowski 1993; Candolin 2003). While the former posits that each ornament conveys similar information about a single aspect of quality, the latter states that each ornament conveys complementary information about different attributes of quality. For colorful ornaments, both hypotheses might explain the expression of multiple color traits. The two most prevalent pigments in animal coloration are carotenoids (responsible for the production of most of the red, orange and yellow colors) and melanins (responsible for the production of black and brown colors). Carotenoids must be acquired through diet and, beyond their role as pigments, they have diverse biological functions due to their immunostimulant and antioxidant properties (Chew and Park 2004; Simons et al. 2012). Therefore, carotenoid-based traits may function as reliable indicators of quality because they are limited resources that are traded-off against other important physiological functions. In contrast, melanins can be synthesized *de novo* from the amino acid tyrosine. Importantly, genes responsible for the expression of melanin-based traits can have pleiotropic effects on immune function, metabolism, glucocorticoid stress responses, or aggressiveness (Ducrest et al. 2008), thus linking melanin-based traits to individual variation in condition and hormonal profiles. So, based on the biochemical differences between melanin and carotenoids, together with their different physiological roles, the display of different color traits may convey multiple messages of quality with carotenoid-dependent traits reflecting foraging ability, nutritional condition and immunological state (Hill and Montgomerie 1994; Linville and Breitwisch 1997; McGraw and Hill 2000; reviewed in Hill 2006), and melanin-dependent traits reflecting hormonal profiles, social status and nutritional condition (Jawor and Breitwisch 2003; Bókony et al. 2008; Alonso-Alvarez and Velando 2001; Evans et al. 2000; Fargallo et al. 2007; D’Alba et al.

2014). On the other hand, the allocation of both pigments to ornaments may be under genetic control (Mundy 2006; Evans and Sheldon 2012; Vergara et al. 2015), and modulated by sex steroids such as estrogen and testosterone (Kimball 2006). Given that maintaining high testosterone levels may impose costs in terms of immune function (Folstad and Karter 1992; Roberts et al. 2004), metabolic rate (Wikelski et al. 1999; Buchanan et al. 2001), and oxidative stress (Alonso-Alvarez et al. 2007), multiple ornaments based on different pigments could also convey similar information about physiological condition (e.g., Griffith et al. 2006; Vergara and Fargallo 2011). Thus, either through multiple messages or redundancy, the expression of multiple colored ornaments mediated by testosterone may function as handicaps that, when considered together, allow individuals a more reliable evaluation of mate quality than by considering a single trait (Møller and Pomiankowski 1993).

Frequently, ornaments are present in both sexes (mutual ornamentation; Kraaijeveld et al. 2007; Clutton-Brock 2009). Mutual signaling of quality via ornaments is expected when reproductive rates of males and females are similar, and when the benefits of choice are likely to be large for both sexes and strongly related to mate quality (Trivers 1972; Johnstone et al. 1996). For instance, selection should promote mutual ornamentation and mutual mate choice when mating is highly costly to males, when female quality is highly variable, or when males provide a substantial amount of parental care (e.g., Jones and Hunter 1993; Romero-Pujante et al. 2002; Kraaijeveld et al. 2004). Under any of these scenarios, positive assortative mating may arise. Assortative mating, defined as a particular pattern of mate choice in which individuals of similar phenotype or quality mate together more often than is expected by chance (Burley 1983), can result from selection acting directly or indirectly on mate choice, or when fitness of the offspring depends on the similarity of mate partners (Jiang et al. 2013). Alternatively, assortative mating may be non-adaptive and may arise as a consequence of other aspects of the mating system; for instance, when assortment results from age-specific access to reproduction and there is strong mate fidelity, or when color traits are associated with timing of breeding or territory acquisition (Cézilly 2004). In birds, assortative mating in relation to body measurements, body condition, age and visual signals has been reported (review in Jiang et al. 2013).

Seabirds are a particularly interesting group for research on mutual signaling because (1) they are long-lived species that vary substantially in individual quality, (2) their reproduction takes place in dense colonies that promotes exercising mate choice, and (3) they exhibit obligate bi-parental care. Studies of sexual selection in relation to color traits in this group are few compared to terrestrial

birds, possibly due to the fact that most seabirds appear monochromatic to humans and display simple plumage coloration which is mainly black and/or white (Bretagnolle 1993). Interestingly, in many seabird species, individuals show colored integuments. The information expressed in integuments differs from plumage colors since pigments present in integuments can be mobilized facultatively (Lozano 1994), may reflect more recent physiological events, and hence have the potential to serve as dynamic signals of current condition (Lozano 1994; Negro et al. 1998; Faivre et al. 2003; Torres and Velando 2003; Velando et al. 2006; Martínez-Padilla et al. 2007; Pérez-Rodríguez 2008; Pérez-Rodríguez and Viñuela 2008; Gladbach et al. 2010; Doutrelant et al. 2013), thus allowing individuals to continuously evaluate their mate. Indeed, several studies have shown that colored integuments reflect individual quality and may be under current sexual selection (Massaro et al. 2003; Torres and Velando 2003, 2005; Kristiansen et al. 2006; Velando et al. 2006, 2014; Cuervo et al. 2009; Nolan et al. 2010; Ismar et al. 2010; Leclaire et al. 2011; Doutrelant et al. 2013; Montoya and Torres 2015).

The Masked Booby (*Sula dactylatra*) is a socially monogamous seabird with an extended period of pair courtship (around 3–6 weeks) and obligate bi-parental care (Nelson 1978). Both parents incubate a modal clutch size of two eggs for around 6 weeks, and feed the chicks during a period of 23–26 weeks (Nelson 1978, 2005). Although apparently monomorphic in plumage (white except black primaries, secondaries, humerals, and tail), Masked Boobies are dimorphic in size (females are roughly 14 % heavier), and both sexes display multiple skin color traits (Nelson 1978). The extent of size dimorphism in boobies and their relatives is well documented (Nelson 2005), yet their dichromatism has so far rarely been investigated, particularly in the apparently more monomorphic taxa (but see Childress and Bennun 2002; Torres and Velando 2003, 2005; Velando et al. 2006, 2014; Ismar et al. 2014). Male and female Masked Boobies show variably colored feet ranging from olive to orange, yellowish bills (both of these traits presumably pigmented by carotenoids), and a mask of bare dark skin (presumably pigmented by melanins) that comprises the orbital ring eyelids, the facial skin running onto the bill and the gular skin (Fig. 1). The feet, bill and mask are exhibited prominently during mutual courtship displays (Nelson 1978), yet little is known on the extent of sexual dichromatism on these traits, and its possible signaling function has not been investigated. Here, we report sexual differences in skin color traits and evaluate whether their expression is related to circulating testosterone and reflects individual quality in terms of body condition, and whether within individuals different color traits covary. Finally, we assessed whether Masked Boobies mate

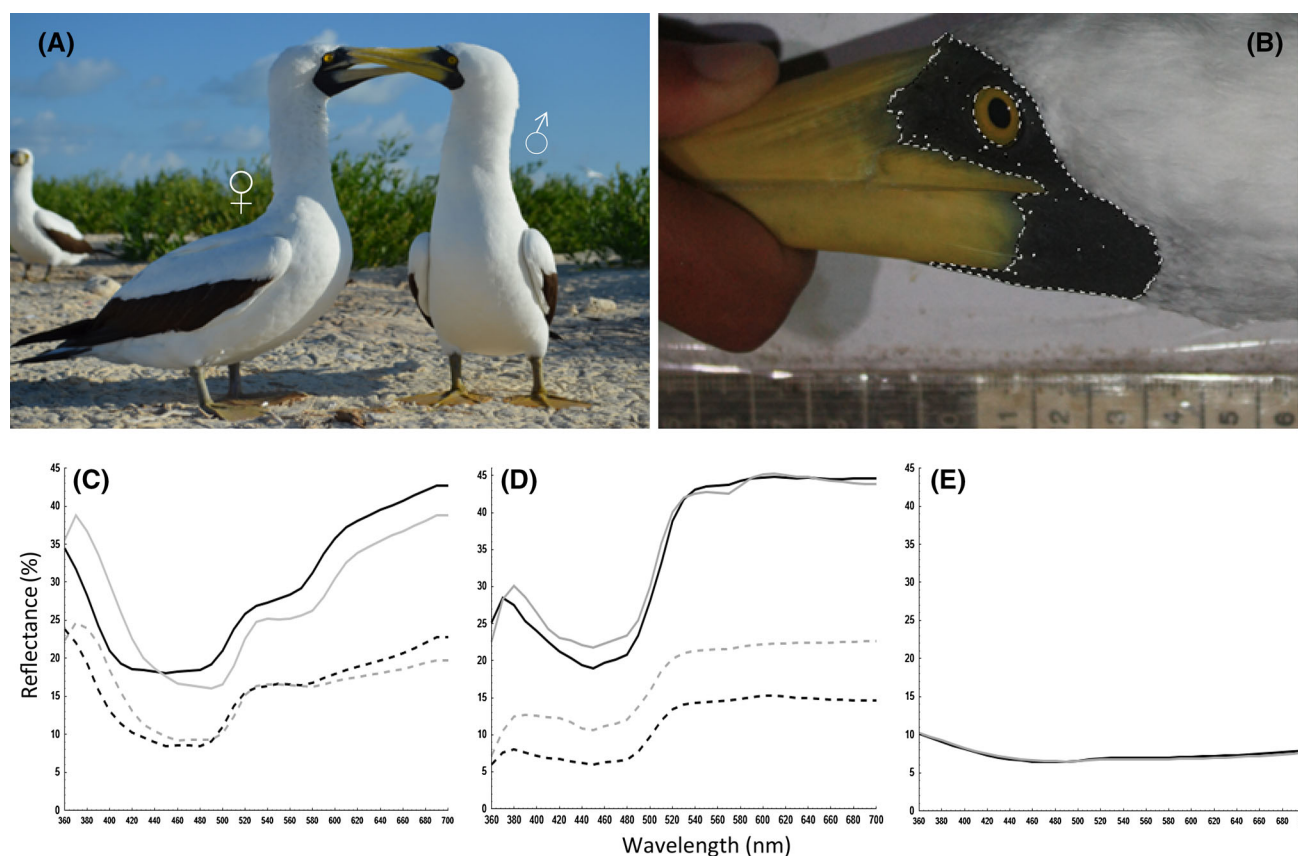


Fig. 1 **a** Multiple ornaments of adult Masked Boobies (*Sula dactylatra*) during courtship. **b** Mask size was measured from digital photographs. Reflectance spectra of **c** foot color, **d** bill color, and

e mask color of males (black lines) and females (gray lines). For foot and bill color, sample reflectance spectra from intense (solid line) and drab (dotted line) coloration are shown

assortatively by skin color traits. If multiple skin color traits represent dynamic signals of quality in this species, we expect them to be inter-correlated and to convey reliable information of individual quality. If there is mutual sexual selection by color traits, we expect assortative mating by skin color.

Materials and methods

The study was carried out in August 2011 during the reproductive season of the Masked Booby colony at Isla Muertos, located at the National Park Arrecife Alacranes (Gulf of Mexico, 22°25'11"N, 89°42'56"W). During the courtship period, both members of 40 pairs were captured at night by directing a light into the bird's eyes and catching hold of the bird. Pairs were identified as birds that were standing 20 cm or less from each other during capture and were observed performing sexual displays during the subsequent days. Immediately after capture, a blood sample was taken for testosterone determination. Each bird was marked at the breast with a non-toxic marker, and body

mass (± 5 g), ulna, wing chord and culmen lengths (± 1 mm) were measured. Feet, bill, and mask color were measured using a spectrophotometer (see below). Bill coloration was only measured in 30 pairs. Also, to estimate the area of the mask (hereafter mask size), digital photographs were taken (see below).

Color measurements

The color of the feet, bill and mask was measured using a portable spectrophotometer (Minolta CM-2600d; Minolta, Osaka, Japan) which measures the reflectance from 360 to 740 nm in intervals of 10 nm. Data was truncated at 700 nm since this is the upper limit of the avian visible spectrum (Montgomerie 2006). Reflectance spectra were automatically produced as the mean of three sequential measurements of each individual by changing its position with respect to the apparatus. From the reflectance spectra of feet and bill, lightness (the sum of reflectances from 360 to 700 nm) and chroma saturation were calculated (Montgomerie 2006). Since feet and bill of Masked Boobies reflect light maximally in the ultraviolet (UV) and yellow–

red (YR) regions of the spectrum (Fig. 1), UV and YR chroma saturations were calculated (UV: R360–400/R360–700; and YR: R560–700/R360–700). Although there are no available data for Masked Boobies, in the Australasian gannet (*Morus serratur*), another member of the sulid family, it has been found to be violet sensitive but not ultraviolet sensitive (Machovsky Capuska et al. 2011; Håstad et al. 2005; Ismar et al. 2014). From the reflectance spectra of the mask (Fig. 1), only lightness (the sum of reflectances from 360 to 700 nm) was calculated.

To estimate mask size, digital photographs were taken from the right side of each bird while they were held with an angle of 90° between the objective of the camera (camera Canon Rebel EOS xsi, objective: 18–55 mm) and the surface of the head. Lighting was provided by two manual lamps in order to avoid flashguns. Mask size was estimated using the Adobe Photoshop program (Fig. 1). Before measurements, a metric scale was set in all pictures by equaling a pixel distance to the measurement unit (cm²). All measurements were performed at real scale. The dark mask is easily distinguishable from the yellow background of the bill; hence, the mask was outlined using the “lasso tool”, its area was determined as the number of pixels occupied, and finally the number of pixels was translated to cm². To estimate repeatability, the mask size of 15 individuals selected at random was measured by ILR three times with an interval of 8 days between measurements. Repeatability of mask size was high ($r = 0.85$, $F_{1,44} = 18.09$, $P < 0.0001$; Lessells and Boag 1987).

Testosterone measurement

Immediately after capture, 1 ml of blood was collected from the brachial vein with a heparinized syringe to avoid clotting. Time from capture to bleeding never exceeded 3 min. We were able to bleed 71 individuals (37 males and 34 females). Samples were kept cool for the rest of the night (maximum 6 h) and subsequently centrifuged at 10,000 rpm for 10 min. The separated fraction of plasma was stored in microtubes at –80 °C in a liquid nitrogen tank until analyses in the laboratory. Testosterone was extracted twice with a volume of 10× diethyl ether from 100-μl plasma samples and resuspended in assay buffer (Cayman Chemicals, Ann Arbor, MI, USA). We conducted pilot analyses in order to determine the optimal dilution for each sex. Since males showed higher testosterone levels than females, the dried extract of male plasma was resuspended in a 1:10 dilution to fit the central part of the standard curve, while the dried extract of female plasma was resuspended in a 1:3 dilution. Testosterone concentration for each sample was then determined using the equation obtained from the standard curve plot and accounting for the dilution factor of each sample, as is

indicated in the manufacturer’s protocol. Hormone concentrations were determined in duplicate using a commercially available enzyme immunoassay (Cayman Chemicals) following the manufacturer’s protocol, and a set of identical internal controls was run in each assay. The assay is 100 % specific for testosterone, 27.4 % for 5α-dihydro-testosterone (5α-DHT), and 3.7 % for androstenedione. The intra-assay coefficient of variation was 9.23 %, and the inter-assay coefficient of variation was 3.19 %.

Statistical procedures

Foot color variables were combined in a principal components analysis (PCA) that resulted in only one main PC axis showing an eigenvalue >1 and explaining 69 % of the variance. From this analysis, the PC (hereafter foot color) represented a gradient of foot color varying from low lightness, low YR saturation and high UV saturation in the negative extreme, to high lightness, high YR saturation and low UV saturation in the positive extreme (factor loadings: lightness = 0.65, UV = –0.94, YR = 0.88). Similarly, bill color variables were combined in a PCA that resulted in only one main PC axis (hereafter bill color) showing an eigenvalue >1 and explaining 51 % of the variance and representing a gradient of bill color varying from high lightness, high UV saturation and low YR in the negative extreme, to low lightness, low UV saturation and high YR saturation in the positive extreme (factor loadings: lightness = –0.67, UV = –0.87, YR = 0.56). Feet and bill PC scores, as well as mask size, were normally distributed (Kolmogorov–Smirnov, all $p > 0.20$). Mask lightness (hereafter mask color) was log-transformed to approach normality (mask lightness after transformation, Kolmogorov–Smirnov, $p > 0.20$).

As an index of body condition, we calculated the scaled mass index (SMI) following Peig and Green (2009, 2010). SMI adjusts the mass of all individuals to the mass they would have if they had the same body size, using the equation of the linear regression of log₁₀ body mass on log₁₀ ulna length, estimated by type-2 (standardized major axis; SMA) regression. Because females are heavier and have longer ulnas than males, SMI was calculated separately for males and females. The SMI was computed for each individual as follows: $SMI = M_i \times (L_0/L_i)^b$, where M_i and L_i are, respectively, the body mass and the ulna length of the individual, L_0 is the arithmetic mean value of ulna length for the whole study population (males: $L_0 = 20.4$ cm; females: $L_0 = 21.1$ cm) and b is the slope estimate of a standardized major axis (SMA) regression of log-transformed body mass on log-transformed tarsus length (males: $b = 3.54$; females: $b = 2.38$). Sexual differences in size (body mass, ulna length, wing chord length

and culmen length), color traits (foot, bill and mask) and testosterone levels were compared using general linear models (GLMs) that included sex as a fixed factor. In all analyses of mask size, ulna length was included in the models as a covariate to control for allometric effects. In the analysis of testosterone, body condition was included as a covariate, and the interaction between sex and body condition was tested.

GLMs with a normal distribution and an identity-link function were used to evaluate the potential correlations between ornamental traits, body condition and testosterone levels in both sexes. Initial models included each ornamental trait as the response variable, sex as a fixed factor, body condition and testosterone levels as covariates and the second-level interactions between these variables. Before performing all analyses, variance inflation factors (VIF) were calculated to ensure that our data met independence (foot color: testosterone VIF = 1.39, body condition VIF = 1.63 and sex VIF = 1.96; bill color: testosterone VIF = 1.27, body condition VIF = 1.49 and sex VIF = 1.77; mask color: testosterone VIF = 0.72, body condition VIF = 0.61 and sex VIF = 0.51; mask size: testosterone VIF = 0.71, body condition VIF = 0.44, ulna VIF = 0.47 and sex VIF = 0.27), normality of residuals (Kolmogorov–Smirnov, all $p > 0.20$) and homogeneity of variances (Levene F, all $p > 0.37$) were verified. We also calculated Cook's distance to identify possible influential points and found that none of our data showed a Cook's distance greater than 1, indicating that there is no reason to believe that we have outliers. We used Akaike's information criterion corrected for small sample size (AICc) for model selection following a backward stepwise procedure. The best model was the one with the lowest AICc value with a difference >2 from the second best model (Burnham and Anderson 2002). Best final models were tested against null models to evaluate the importance of explanatory variables. Finally, Pearson's correlations were used to evaluate within sex correlations between color traits and assortative mating by color traits, morphometry, body condition and testosterone levels. A significance level of $\alpha < 0.05$ was used for all tests. Mean values \pm standard deviations are shown throughout the text. Analyses were performed in SAS (SAS 1989-297 96 Institute, Cary, NC, USA) and STATISTICA software.

Results

Differences between sexes

Masked Boobies showed sexual differences in body mass, and ulna and wing length. Females were on average 11 % heavier, and had ulnas and wing chords 4 and 2 % longer than males, respectively (Table 1). No differences in

culmen length of males and females were detected (Table 1). There was sexual difference for foot color with foot integuments of males showing higher lightness and higher reflectance in the yellow–orange chroma (higher PC values; Table 1). No difference in bill color or in the mask size and color were found (Table 1).

Circulating testosterone was on average 1.5 times higher in males (130.75 ± 111.75 pg/ml) than in females (52.58 ± 46.79 pg/ml), and in both sexes it was positively related to body condition (sex: $F_{1,68} = 39.56$, $P < 0.0001$, condition: $F_{1,68} = 10.75$, $P = 0.002$; sex \times condition $F_{1,67} = 0.22$, $P = 0.64$).

Color traits, testosterone and body condition

The model selection procedure for foot color resulted in one best model (Table 2) showing that foot color of both males and females was influenced by the interaction between plasma levels of testosterone and body condition (Table 3). Increased levels of plasma testosterone had a positive effect on foot color of individuals in good body condition, and a negative effect on foot color of individuals in poor condition (Fig. 2). Although foot color of males and females differed (Table 3), the effect of testosterone and body condition on foot color did not differ between sexes (both interactions, $P > 0.32$).

For mask color, the model selection procedure resulted in two best models with similar AICc values (Table 2). In both models, mask color was positively related to body condition (Table 3) with individuals in better body condition displaying lighter masks (Fig. 3a). Mask color did not differ between sexes (Table 3) and was unrelated to plasma testosterone levels (main terms and interactions, all $P > 0.31$). The interaction between sex and body condition was not significant ($P = 0.84$).

The model selection procedure for mask size also yielded two best models with similar AICc values (Table 2). Both models showed that, after controlling for body size and testosterone levels, mask size was influenced by sex with females showing larger masks than males (Table 3). The second final model showed that in both sexes mask size was positively related to body condition (Table 3; Fig. 3b). The interaction between sex and body condition was not significant (Table 3). Mask size was unrelated to plasma testosterone levels (Table 3; interaction sex \times T: $P = 0.25$). The interaction between testosterone and condition was not significant (Table 3).

For foot color, mask color and mask size, differences between best final models and null models were significant (all $P > 0.01$). The model selection procedure for bill color yielded one best model (Table 2) showing that bill color did not differ between sexes ($F_{1,52} = 0.01$, $P = 0.92$) and was unrelated to plasma testosterone and marginally

Table 1 Sexual differences in color traits and morphology of adult Masked Boobies (*Sula dactylatra*) during courtship

	Females			Males			Comparison		
	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>	<i>F</i>	<i>P</i>	<i>df</i>
Color traits									
Foot color (PC)	−0.44	0.14	40	0.44	0.14	40	19.07	<0.0001	1,78
Bill color (PC)	−0.23	0.96	30	0.23	1.00	30	3.28	0.07	1,58
Mask color	2.4	0.09	40	2.4	0.12	40	0.00	0.95	1,78
Mask size (cm ²)	4.22	0.63	40	3.98	0.62	40	0.45	0.50	1,78
Morphology									
Body mass (g)	1699	120.74	40	1526	105.36	40	46.62	<0.0001	1,78
Ulna length (mm)	211.18	6.28	40	203.5	4.09	40	41.85	<0.0001	1,78
Wing cord length (mm)	426.25	9.13	40	416.2	11.07	40	19.61	<0.0001	1,78
Culmen length (mm)	103.65	2.44	40	102.51	3.28	39	3.05	0.08	1,78

To control for allometric effects ulna length was included in the analysis of mask size, therefore the *F* and *p* values for ulna length shown in the table correspond to the interaction of ulna length × sex

Significant differences are shown in bold. The first principal components (PC) from a principal component analysis of foot and bill color are shown

Table 2 Ranking of the most probable linear models testing the potential relationships between color traits, body condition (BC) and testosterone levels (T) in both sexes

Dependent variable	Terms included in the model	AICc	ΔAICc
Foot color	Sex, T, BC, sex × T, sex × BC, T × BC	194.2	−14.3
	Sex, T, BC, sex × T, T × BC	192.6	−15.9
	Sex, T, B C, T × B C	190.6	−17.9
	Null model	208.5	
Bill color	Sex, T, BC, sex × T, sex × BC, T × BC	172	8.3
	Sex, T, BC, sex × T, T × BC	169.8	6.1
	Sex, T, BC, T × BC	167.2	3.5
	Sex, T, B	164.8	1.1
	T, B C	162.4	−1.3
	BC	171.6	7.9
	Null model	163.7	
Mask color	Sex, T, BC, sex × T, sex × BC, T × BC	−126.2	−2.5
	Sex, T, BC, sex × T, T × BC	−128.7	−5
	Sex, T, BC, sex × T	−130.1	−6.4
	Sex, T, BC	−131	−7.3
	Se x, BC	−137.4	−13.7
	BC	−136.8	−13.1
	Null model	−123.7	
Mask size	Sex, T, BC, UL, sex × T, sex × BC, T × BC	129.1	0.4
	Sex, T, BC, UL, sex × BC, T × BC	126.4	−2.3
	Se x, T, B C, UL, T × B C	124.4	−4.3
	Se x, T, B C, UL	124.9	−3.8
	Sex, BC, UL	150.4	21.7
	BC, UL	151	22.3
	Null model	128.7	

To control for allometric effects ulna length (UL) was included in the analysis of mask size. Models were selected using Akaike's information criterion corrected for small sample size (AICc). Model selection was performed through a backward stepwise procedure. The ΔAICc values of all models with covariates were calculated using the AICc values of the null models as references. Final models with the lowest AICc value with a difference >2 from the second best model are highlighted

Table 3 Color traits in female and male Masked Boobies in relation to plasma testosterone (*T*) and body condition (*BC*)

Trait	Effect	Estimate	Lower 95 % CI	Upper 95 % CI	<i>F</i>	<i>df</i>	<i>P</i>
Foot color	Sex	1.1264	0.2875	0.8398	15.95	1,66	0.0002
	T	-0.0340	-0.0604	-0.0075	6.34	1,66	0.01
	BC	-0.0016	-0.0038	0.0007	1.92	1,66	0.17
	BC × T	0.0000	0.0000	0.0000	5.44	1,66	0.02
Bill color	T	0.0021	-0.0008	0.0050	1.94	1,53	0.17
	BC	-0.0018	-0.0035	-0.0001	4.12	1,52	0.05
Mask color 1	Sex	0.0438	-2.2445	29.1800	2.7	1,77	0.1
	BC	0.0003	0.0407	0.2460	9.59	1,77	0.003
Mask color 2	BC	0.0002	0.0075	0.1805	6.58	1,78	0.01
Mask size 1	Sex	0.5649	0.0456	0.5218	5.4	1,65	0.02
	T	-0.0151	-0.0315	0.0012	3.28	1,65	0.07
	BC	0.0013	-0.0002	0.0029	2.78	1,65	0.1
	BC × T	0.0000	0.0000	0.0000	2.93	1,65	0.09
Mask size 2	Ulna	0.3741	0.1079	0.6437	7.48	1,65	0.01
	Sex	0.6200	0.0705	0.5521	6.36	1,66	0.01
	T	-0.0009	-0.0025	0.0007	1.27	1,66	0.26
	BC	0.0022	0.0009	0.0034	12.08	1,66	0.001
	Ulna	0.3938	0.1231	0.6679	8.02	1,66	0.006

Linear models shown are those with the lowest Akaike's information criterion corrected for small sample size. For mask color and size the model selection procedure yielded two best models, respectively (with similar AICc), the table reports both. Estimates, 95 % confidence intervals (95 % CI), *F* degrees of freedom (*df*) and *P* values are shown. Significant effects are shown in bold

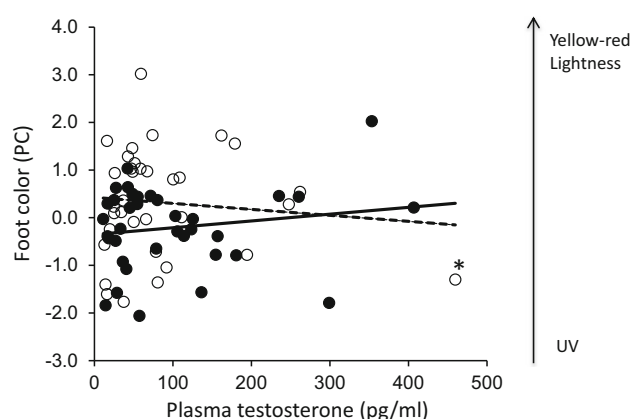


Fig. 2 Effect of plasma testosterone (pg/ml) on foot color of individuals in good condition (those whose condition was higher than the mean condition from their sex; filled circles and solid line), and in poor condition (those whose condition was lower than the mean condition from their sex; open circles and dashed line). Foot color was analyzed as the first principal component (PC) from principal component analyses: greater positive values indicate a more yellow-orange coloration. For visual clarity, body condition in the figure was treated as a two-level factor (good condition individuals versus poor condition individuals), but note that the analysis was performed including this variable as a continuous covariate

related to body condition (Table 3; interactions all $P > 0.59$). Yet differences between the final model and the Null model were not significant ($P = 0.18$).

Relationships between color traits

Mask color was positively correlated with mask size and foot color in males, and negatively correlated with bill color in females (Table 4). All other color traits in both sexes were not correlated (Table 4).

Assortative mating within color traits

Masked Boobies paired assortatively by foot color ($r^2 = 0.21$, $P = 0.003$, $n = 40$; Fig. 4a), mask color ($r^2 = 0.29$, $P < 0.001$, $n = 40$; Fig. 4b), and mask size ($r^2 = 0.11$, $P = 0.04$, $n = 40$; Fig. 4c). No correlation in bill color between male and female partners was found ($r^2 = 0.02$, $P = 0.91$, $n = 30$). Masked boobies did not pair assortatively by condition ($P = 0.38$), size ($P = 0.23$), or T level ($P = 0.48$).

Discussion

Although apparently monochromatic in their feather coloration, Masked Boobies show sexual dichromatism in their feet with males expressing more yellow-red reflectance than females. Masked Boobies show reversed sexual size dimorphism; females are larger and heavier than males, although culmen length did not differ between the

sexes. Overall, no difference between the sexes in bill color and mask color and size were found; however, after controlling for testosterone levels and body size, mask size differed between sexes with females showing larger masks. Mask color and size were correlated to body condition, while foot color was influenced by plasma testosterone and body condition. Interestingly, we found assortative mating by foot color, mask color, and mask size, but not by bill color, thus suggesting that the color of the feet and the size

and color of the mask in the Masked Booby may function as sexual signals in both sexes.

Color traits as indicators of quality

One mechanism to ensure that ornaments honestly reflect quality is that their development and maintenance must depend on the phenotypic condition of the bearer (Zahavi 1975; Hill 2011), thus leading to a positive correlation between trait conspicuousness and indices of condition. Body condition, which can be viewed as the size of an individual's energy reserves relative to its body size (Schulte-Hostedde et al. 2005), is often regarded as indicator of quality as it is linked to individual fitness by affecting survival and reproduction in many species, including seabirds (e.g., Blums et al. 2005; Monticelli and Ramos 2012). In the Masked Booby, mask color and size were associated with body condition in both sexes, thus providing a good indication of individual quality. Furthermore, we found a positive relationship between body condition and plasma testosterone in males and females, suggesting that plasma androgen levels may depend on individuals' body condition. Interestingly, the interaction between these two variables influenced foot color: the influence of testosterone levels on ornament expression tended to be positive in individuals with good body condition, and negative in individuals with poor body condition. These results are in agreement with the idea that, in addition to signal health status (see below), androgen-mediated traits could also indicate general nutritional state due to their condition-dependence (Pérez-Rodríguez et al. 2006). Given that body condition plays an important role in testosterone production (Wingfield 1987; Duckworth et al. 2001), differences in body condition may influence the expression of testosterone-dependent ornamental traits (Pérez-Rodríguez et al. 2006). Hence, our results suggest that foot color and mask color and size are indicators of the individual current condition.

In the Masked Booby, the mechanisms controlling foot color and the size and color of the mask are unknown.

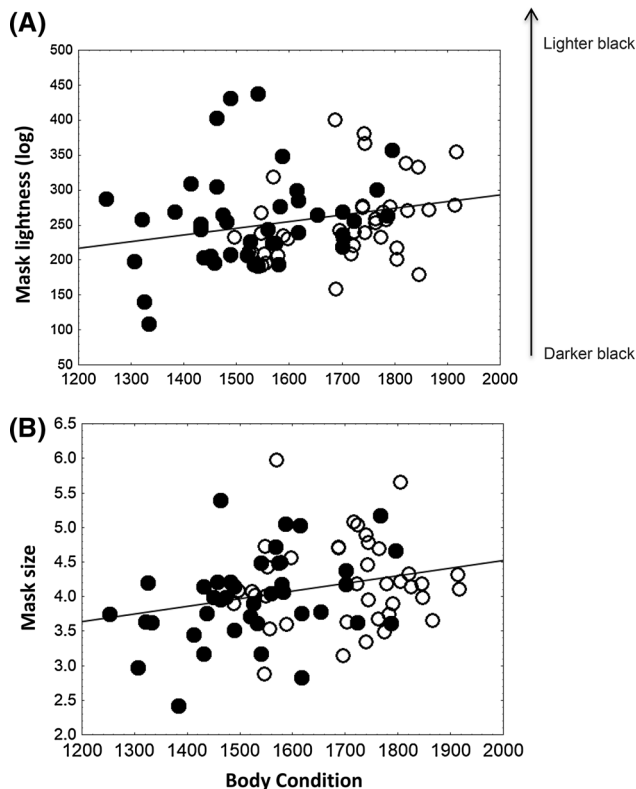


Fig. 3 Mask color and size in relation to body condition in males (solid circles) and females (open circles). Mask color was analyzed as lightness (sum of reflectances from 360 to 700 nm) and was log-transformed to approach normality. Body condition was calculated as the scaled mass index

Table 4 Correlations between color traits of courting Masked Boobies

	Bill color (<i>n</i> = 30 pairs)		Mask color (<i>n</i> = 40 pairs)		Mask size (<i>n</i> = 40 pairs)	
	Males	Females	Males	Females	Males	Females
Foot color (PC)	0.27, 0.13	0.19, 0.32	0.47, 0.001	0.04, 0.79	0.28, 0.07	−0.14, 0.39
Bill color (PC)	–	–	0.07, 0.69	−0.38, 0.04	−0.14, 0.47	0.03, 0.85
Mask color	–	–	–	–	0.34, 0.03	0.1, 0.54

Values are Pearson correlation coefficients and *p* values (Pearsons *R*, *p* value). Significant correlations (*P* < 0.05) are shown in bold. The first principal components (PC) from principal component analyses of foot and bill color were used for analyses

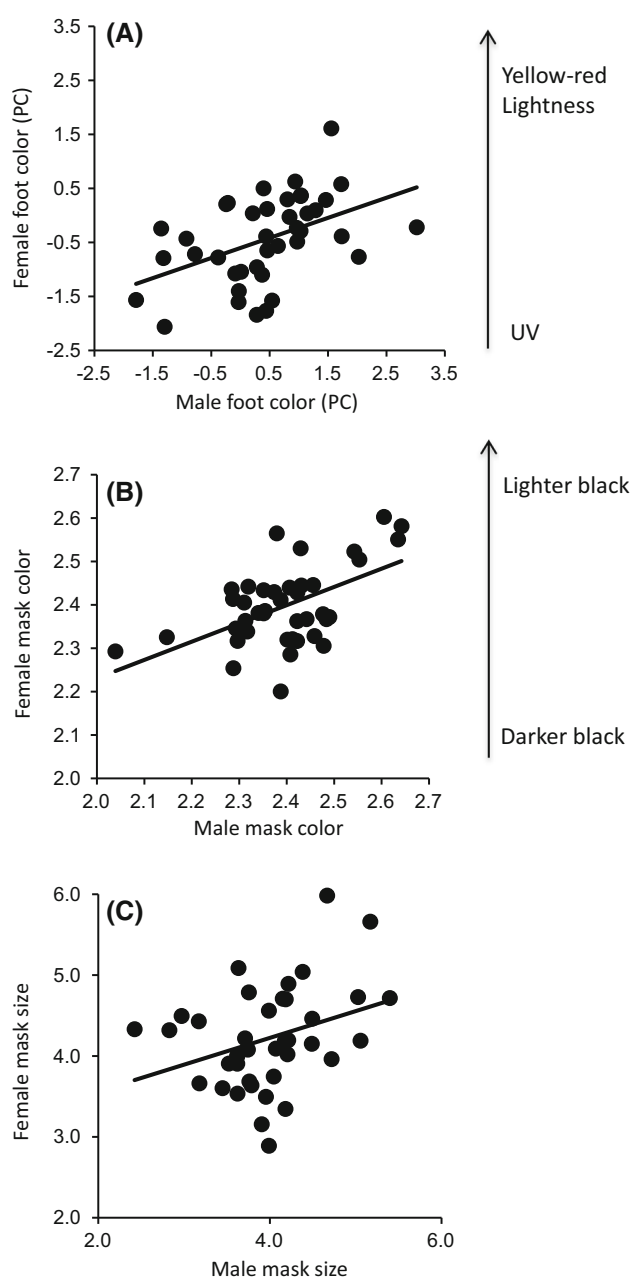


Fig. 4 Assortative mating by condition-dependent color traits. Within-pairs correlations between **a** foot color, **b** mask color and **c** mask size (cm^2). Foot color was analyzed as the first principal component (PC) from principal component analyses: greater positive values indicate a more yellow–orange coloration. For mask color, the log-transformed total lightness is shown: greater values indicate a lighter *black color*

However, carotenoid pigments are involved in the production of the green–turquoise and green–yellow foot color of two sister species, the Blue footed Booby (*Sula nebouxi*; Velando et al. 2006) and the Brown Booby (*Sula leucogaster*; Montoya B. and Torres R. unpublished data). Hence, assuming that the yellow–orange foot color in Masked Boobies is produced by carotenoid pigments, our

results agree with other studies in seabirds in which individuals in better condition display more vivid skin colors than individuals in poor condition (e.g., Blount et al. 2002; Kristiansen et al. 2006; Velando et al. 2006; Cuervo et al. 2009; Leclaire et al. 2011; Doutrelant et al. 2013; Blévin et al. 2014). On the other hand, dark melanin coloration is generally positively associated with dominance and aggressiveness via testosterone effects (“T-regulation hypothesis”; Jawor and Breitwisch 2003; Bókonyi et al. 2008), as testosterone favors aggressive behavior (Wingfield et al. 1990; Alonso-Alvarez and Velando 2001), and regulates melanin production (Evans et al. 2000; Fargallo et al. 2007). In the Masked Booby, we found that mask size and lightness are positively related to body condition (assortative mating occurs for both mask size and color). Interestingly, in males but not in females, these two traits are positively correlated, suggesting that individuals may face a trade-off between allocating melanin pigments to either size or color, with individuals in better condition showing larger masks. We found that individuals in poorer condition show a darker black mask than individuals in better condition, and both testosterone and body condition influenced mask size. Although some studies have shown a positive link between plumage melanin content and body condition (review in Roulin 2015), others have found the opposite, including one study in the Masked Booby (e.g., Schroeder et al. 2009; Fargallo et al. 2014). Because genes involved in the expression of melanin-based traits regulate many physiological and behavioral functions in a complex way (Ducrest et al. 2008), the benefits of the pleiotropic effects may differ between species, implying that different degrees of melanin-based coloration may be indirectly selected in different species (and therefore different degrees of melanin-based coloration may convey different information of condition among different species). Genes involved in melanogenesis can pleiotropically regulate other functions, including food intake patterns (Ducrest et al. 2008). According to this idea, higher levels or activity of melanocortins promote the darkening of coloration by binding melanocortin receptors MCR1 in the melanocytes, hence increasing the production of eumelanin. In addition, high activity of these melanocortin hormones promotes a reduction of food intake by binding to MCR3 and MCR4 receptors placed in the central nervous system and adipose tissue (Ducrest et al. 2008). Our results agree with this hypothesis, as darker individuals showed poor body condition. Similar results have been reported in a recent study with juveniles of Masked Boobies in which paler individuals showed higher body condition than darker individuals (Fargallo et al. 2014).

Bill color was unrelated to testosterone and marginally related to body condition. Contrary to fleshy integuments, such as the membrane of feet or the skin of the mask, the

bill is a keratinized structure and the turnover of carotenoids deposited within it may take place more slowly (Leclaire et al. 2011), and hence will be independent of current body condition or testosterone levels (but see Pham et al. 2014; Dey et al. 2015). Although changes in bill color have been shown to mirror an infection within 7 days (Faivre et al. 2003), a food restriction experiment (Piault et al. 2008) did not significantly affect bill coloration, thus suggesting that such a keratinized trait may not be as informative as fleshy traits, which can change within a few hours (Velando et al. 2006). Therefore, bill color may not indicate current condition in Masked Boobies, while whether it may reflect individual quality in a longer time-scale remains to be explored.

In the Masked Booby, the condition-dependence of plasma androgen levels and its influence in the expression of color traits highlights the importance of the individual's nutritional status in the trade-off between testosterone-dependent trait expression and health. According to the immunocompetence handicap hypothesis, testosterone-dependent ornaments signal individual quality, since only high-quality individuals can afford the immunosuppressive effect of testosterone-dependent ornamentation (Folstad and Karter 1992). While in males there is considerable evidence that testosterone levels are under strong direct selection, and that males with high levels of testosterone have a selective advantage in terms of increased reproductive success (Møller et al. 2005), there is less information on the costs and benefits of high testosterone levels in females (Møller et al. 2005). It has been proposed that circulating levels of testosterone in females may be an indirect consequence of selection acting on males (Møller et al. 2005). However, our study does not support this idea since, as in males, female testosterone levels are related to body condition in Masked Boobies. Moreover, the effects of testosterone and body condition on the expression of traits that signal quality might suggest that female traits mediated by testosterone should function as handicaps in mate choice processes.

Multiple ornaments and assortative mating

In Masked Boobies, foot and mask skin color might convey similar messages of quality, both reflecting body condition and androgen-dependence. Moreover, we found that foot and mask color were correlated between 34 and 47 %. The fact that the signaling content of these color traits was similar in both sexes suggests that color traits in Masked Boobies may be favored by mutual sexual selection. Whereas male ornaments have long been ascribed to sexual selection (Andersson 1994), female ornamentation remains an enduring challenge to evolutionary biologists (Nordeide et al. 2013). The original view posits that female ornaments

evolved as the consequence of a genetic correlation with male ornaments on which selection acts (Darwin 1871; Lande 1980; Lande and Arnold 1985). Alternatively, female ornaments are proposed to be themselves under selection, either through female–female competition, male choice (Jones and Hunter 1999; Amundsen 2000; Griggio et al. 2005; Torres and Velando 2005); or through social competition out of a sexual context (Kraaijeveld et al. 2007; LeBas 2006; Tobias et al. 2011, 2012). In Masked Boobies, we have shown that foot color and mask color and size convey information on condition in both sexes. Thus, our results add to the growing body of evidence that female ornamentation reflects individual quality (Jones and Montgomerie 1992; Johnsen et al. 1996; Potti and Merino 1996; Amundsen et al. 1997; Linville et al. 1998; Kraaijeveld et al. 2007), and suggest that in Masked Boobies mask and foot skin color in females might be selected through a signaling function to attract mates.

Interestingly, Masked Boobies paired assortatively by foot color and mask size and color, but not by bill color. Even though the correlations are weak, the observed mating pattern in Masked Booby may arise as a result of both sexes choosing mates based on phenotypic characteristics that convey reliable information on individual condition, suggesting that foot color and mask color and size may be favored by mutual sexual selection (e.g., Velando et al. 2001). Alternatively, intrasexual competition for non-sexual or sexual resources, or other aspects of the mating system independent from sexual selection, may result in correlations of male and female color traits (Jiang et al. 2013). We have no direct evidence in the Masked Booby that skin color traits are favored by mutual choice; however, in the closely related species, the Blue footed Booby, experimental evidence indicates that mutual mate choice for foot color influences courtship behavior, the propensity to copulate, and breeding decisions (Torres and Velando 2003, 2005; Velando et al. 2006). Furthermore, in seabirds, mutual sexual selection is expected because parental roles of males and females are similar (Johnstone et al. 1996; Jones and Hunter 1999). Indeed, mutual ornamentation and mutual sexual selection has been found in other seabird species, such as the Crested Auklets (*Aethia cristatella*), Yellow-eyed Penguins (*Megadyptes antipodes*), Great back-backed Gull (*Larus marinus*), King Penguins (*Aptenodytes patagonicus*), Black-legged Kittiwakes (*Rissa tridactyla*), and Atlantic Puffins (*Fratercula artica*; Jones and Hunter 1993; Massaro et al. 2003; Kristiansen et al. 2006; Nolan et al. 2010; Doutrelant et al. 2013). In the Masked Booby, male and female preferences and the fitness consequences of assortative mating by condition-dependent color traits need to be studied in the future. Nevertheless, given the large parental investment that both sexes provide (Nelson 1978, 2005), it is likely that mutual

mate choice might be favoring similar mask and foot colorations in males and females.

To conclude, our results show that Masked Boobies are sexually dichromatic in foot color but monochromatic in mask and bill. Feet and mask traits honestly reflect quality in terms of body condition and plasma testosterone. In addition, our study constitutes one of the few cases providing a positive relationship between female natural testosterone levels and body condition. The finding of assortative mating by multiple condition-dependent ornaments suggest that such a mating pattern may result from a scenario where both sexes choose mates based on traits that convey reliable information of individual condition, yet an experimental approach is needed to confirm the findings reported here. Although the fitness consequences of assortative mating by condition-dependent color traits need to be studied in the future, it is likely that mutual mate choice might be favoring mask and foot colorations in males and females due to the large parental investment that both sexes provide.

Acknowledgments We thank J.A. Fargallo for his valuable comments on the manuscript, L.M. Bautista and O. Gordo for statistical advice and L. García and E. Ávila for their kind help in the laboratory and logistics. Research was founded by Consejo Nacional de Ciencia y Tecnología (CONACYT 129774), Universidad Nacional Autónoma de México (UNAM, PAPIIT-IN206713) and Posgrado en Ciencias Biológicas, Universidad Autónoma de Tlaxcala (UATx). I.L.R. was supported by a postdoctoral fellowship from UNAM (DGAPA), and a postdoctoral fellowship from CONACYT at the UATx. Permissions to carry out the research were granted by Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT). Logistical facilities were provided by the Armada de México and the staff of Parque Nacional Arrecife Alacranes. The work was carried out in accordance with the legal and ethical standards of México.

References

- Alonso-Alvarez C, Velando A (2001) Effect of testosterone on behaviour of yellow legged gulls in a high density colony during the courtship period. *Ethol Sociobiol* 13:343–351
- Alonso-Alvarez C, Bertrand S, Faivre B, Chastel O, Sorci G (2007) Testosterone and oxidative stress: the oxidation handicap hypothesis. *Proc R Soc Lond B* 274:819–825
- Amundsen T (2000) Why are female birds ornamented? *Trends Ecol Evol* 15:149–155
- Amundsen T, Forsgren E, Hansen LTT (1997) On the function of female ornaments: male bluethroats prefer colorful females. *Proc R Soc Lond B* 264:1579–1586
- Andersson M (1994) Sexual selection. Princeton University Press, Princeton
- Blévin P, Tartu S, Angelier F, Leclaire S, Bustnes JO, Moe B, Herzke D, Gabrielsen GW, Chastel O (2014) Integument coloration in relation to persistent organic pollutants and body condition in arctic breeding black-legged kittiwakes (*Rissa tridactyla*). *Sci Total Environ* 470–471:248–254
- Blount JD, Surai PF, Nager RG, Houston DC, Møller AP, Trewby ML, Kennedy MW (2002) Carotenoids and egg quality in the lesser black-backed gull *Larus fuscus*: a supplemental feeding study of maternal effects. *Proc R Soc Lond B* 269:29–36
- Blums P, Nichols JD, Hines JE, Lindberg MS, Mednis A (2005) Individual quality, survival variation and patterns of phenotypic selection on body condition and timing of nesting in birds. *Oecologia* 143:365–376
- Bókony V, Garamszegi LZ, Hirschenhauser K, Liker A (2008) Testosterone and melanin-based black plumage coloration: a comparative study. *Behav Ecol Sociobiol* 62:1229–1238
- Bretagnolle V (1993) Adaptive significance of seabird coloration: the case of procellariiforms. *Am Nat* 142(1):141–173
- Buchanan KL, Evans M, Goldsmith AR, Bryan DM, Rowe LV (2001) Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signalling? *Proc R Soc Lond B* 268:1337–1344
- Burley N (1983) The meaning of assortative mating. *Ethol Sociobiol* 4:191–203
- Burnham KP, Anderson DR (2002) Model selection and multimodel inference. A practical information theoretic approach. Springer, New York
- Candolin U (2003) The use of multiple cues in mate choice. *Biol Rev* 78:575–595
- Cézilly F (2004) Assortative mating. In *Encyclopedia of Animal Behavior* 876–881 (M. Bekoff, Editor). Greenwood, Westport
- Chew BP, Park JS (2004) Carotenoid action on the immune response. *J Nutr* 134:257S–261S
- Childress RB, Bennun LA (2002) Sexual character intensity and its relationship to breeding timing, fecundity and mate choice in the great cormorant *Phalacrocorax carbo lucidus*. *J Avian Biol* 33:23–30
- Clutton-Brock T (2009) Sexual selection in females. *Anim Behav* 77:3–11
- Cuervo JJ, Palacios MJ, Barbosa A (2009) Beak coloration as a possible sexual ornament in gentoo penguins: sexual dichromatism and relationship to body condition. *Polar Biol* 32:1305–1314
- D’Alba L, Van Hemert C, Spencer KA, Heidinger BJ, Gill L, Evans NP, Monaghan P, Handel CM, Shawkey MD (2014) Condition dependence of melanin-based plumage color through feather microstructure. *Integr Comp Biol* 54:633–644
- Darwin C (1871) The descent of man, and selection in relation to sex. John Murray, London
- Dey CJ, Valcu M, Kempenaers B, Dale J (2015) Carotenoid-based bill coloration functions as a social, not sexual, signal in songbirds (Aves: Passeriformes). *J Evol Biol* 28:250–258
- Doutrelant C, Grégoire A, Gomez D, Staszewski V, Arnoux E, Tveraa T, Faivre B, Boulinier T (2013) Coloration in Atlantic puffins and blacklegged kittiwakes: monochromatism and links to body condition in both sexes. *J Avian Biol* 44:451–460
- Duckworth RE, Medonça MT, Hill GE (2001) A condition dependent link between testosterone and disease resistance in the house finch. *Proc R Soc Lond B* 268:2467–2472
- Ducrest AL, Keller L, Roulin A (2008) Pleiotropy in the melanocortin system, coloration and behavioral syndromes. *Trends Ecol Evol* 23:502–510
- Evans SR, Sheldon BC (2012) Quantitative genetics of a carotenoid-based color: heritability and persistent natal environmental effects in the great tit. *Am Nat* 179:79–94
- Evans MR, Goldsmith AR, Norris SRA (2000) The effects of testosterone on antibody production and plumage coloration in male house sparrows (*Passer domesticus*). *Behav Ecol Sociobiol* 47:156–163
- Faivre B, Grégoire A, Préault M, Cézilly F, Sorci G (2003) Immune activation rapidly mirrored in a secondary sexual trait. *Science* 300:103
- Fargallo JA, Martínez-Padilla J, Toledano-Díaz A, Santiago-Moreno J, Dávila JA (2007) Sex and testosterone effects on growth,

- immunity and melanin coloration of nestling Eurasian kestrels. *J Anim Ecol* 76:201–209
- Fargallo JA, Velando A, López-Rull I, Gañán N, Lifshitz N, Wakamatsu K, Torres R (2014) Sex-specific phenotypic integration: endocrine profile, coloration and behavior in fledgling boobies. *Behav Ecol* 25:76–87
- Fisher RA (1930) *The Genetical Theory of Natural Selection*. Oxford University Press, Oxford
- Folstad I, Karter AJ (1992) Parasites, bright males, and the immunocompetence handicap. *Am Nat* 139:603–622
- Gladbach A, Gladbach DJ, Kempenaers B, Quillfeldt P (2010) Female-specific colouration, carotenoids and reproductive investment in a dichromatic species, the upland goose *Chloephaga picta leucoptera*. *Behav Ecol Sociobiol* 64:1779–1789
- Griffith SC, Parker TH, Olson VA (2006) Melanin- versus carotenoid-based sexual signals: is the difference really so black and red? *Anim Behav* 71:749–763
- Griggio M, Valera F, Casas A, Pilastro A (2005) Males prefer ornamented females: a field experiment of male choice in the rock sparrow. *Anim Behav* 69:1243–1250
- Hästad O, Ernstdotter E, Ödeen A (2005) Ultraviolet vision and foraging in dip and plunge diving birds. *Biol Lett* 1:306–309
- Hill GE (2006) Female mate choice for ornamental coloration. In: Hill G, McGraw K (eds.) *Bird coloration*. Harvard University Press, Cambridge, pp 137–200
- Hill GE (2011) Condition-dependent traits as signals of the functionality of vital cellular processes. *Ecol Lett* 14:625–634
- Hill GE, Montgomerie R (1994) Plumage color signals nutritional condition in the house finch. *Proc R Soc Lond B* 258:47–52
- Ismar SM, Baird K, Patel S, Millar CD, Hauber ME (2010) Morphology of the recently re-classified Tasman masked booby (*Sula dactylatra tasmani*) breeding on the Kermadec Islands. *Mar Ornithol* 38:105–109
- Ismar SM, Daniel C, Iqic B, Morrison-Whittle PK, Ballard G, Millar CD et al (2014) Sexual plumage dichromatism in a size monomorphic seabird. *Wilson J Ornithol* 126:417–428
- Jawor JM, Breitwisch R (2003) Melanin ornaments, honesty, and sexual selection. *Auk* 120:249–265
- Jiang Y, Bolnick DI, Kirkpatrick M (2013) Assortative Mating in Animals. *Am Nat* 181:E125–E138
- Johnsen TS, Hengeveld JD, Blank JL, Yasukawa K, Nolan V Jr (1996) Epaulet brightness and condition in female red-winged blackbirds. *Auk* 113:356–362
- Johnstone RA, Reynolds JD, Deutsch JC (1996) Mutual mate choice and sex differences in choosiness. *Evolution* 50:1382–1391
- Jones IL, Hunter FM (1993) Mutual sexual selection in a monogamous seabird. *Nature* 362:238–239
- Jones IL, Hunter FM (1999) Experimental evidence for mutual inter and intrasexual selection favouring a crested auklet ornament. *Anim Behav* 57:521–528
- Jones IL, Montgomerie R (1992) Least auklet ornaments: do they function as quality indicators? *Behav Ecol Sociobiol* 30:43–52
- Kimball R (2006) Hormonal control of coloration. In: Hill G, McGraw K (eds.) *Bird coloration*. Harvard University Press, Cambridge, pp 431–468
- Kirkpatrick M, Ryan MJ (1991) The evolution of mating preferences and the paradox of the lek. *Nature* 350:33–38
- Kraaijeveld K, Gregurke J, Hall C, Komdeur J, Mulder RA (2004) Mutual ornamentation, sexual selection, and social dominance in the Black Swan. *Behav Ecol* 15:380–389
- Kraaijeveld K, Kraaijeveld-Smit FJL, Komdeur J (2007) The evolution of mutual ornamentation. *Anim Behav* 74:657–677
- Kristiansen KO, Bustnes JO, Folstad I, Helberg M (2006) Carotenoid coloration in great black-backed gull *Larus marinus* reflects individual quality. *J Avian Biol* 37:6–12
- Lande R (1980) Sexual dimorphism sexual selection and adaptation in polygenic characters. *Evolution* 34:292–305
- Lande R, Arnold SJ (1985) Evolution of mating preference and sexual dimorphism. *J Theor Biol* 117:651–664
- LeBas NR (2006) Female finery is not for males. *Trends Ecol Evol* 21:170–173
- Leclaire S, White J, Arnoux E, Faivre B, Vetter N, Hatch SA, Danchin E (2011) Integument coloration signals reproductive success, heterozygosity, and antioxidant levels in chick-rearing black-legged kittiwakes. *Naturwissenschaften* 98:773–782
- Lessells CM, Boag PT (1987) Unrepeatable repeatabilities: a common mistake. *Auk* 104:116–121
- Linville SU, Breitwisch R (1997) Carotenoid availability and plumage coloration in wild populations of northern cardinals. *Auk* 114:796–800
- Linville SU, Breitwisch R, Schilling AJ (1998) Plumage brightness as an indicator of parental care in northern cardinals. *Anim Behav* 55:119–127
- Lozano GA (1994) Carotenoids, parasites and sexual selection. *Oikos* 70:309–311
- Lozano GA (2009) Multiple cues in mate selection: the sexual interference hypothesis. *Biosci Hypotheses* 2:37–42
- Machovsky Capuska GE, Huynen L, Lambert D, Raubenheimer D (2011) UVS is rare in seabirds. *Vision Res* 51:1333–1337
- Martínez-Padilla J, Mougeot F, Pérez-Rodríguez L, Bortolotti GR (2007) Nematode parasites reduce carotenoid-based signalling in male red grouse. *Biol Lett* 3:161–164
- Massaro M, Davis LS, Darby JT (2003) Carotenoid derived ornaments reflect parental quality in male and female yellow-eyed penguins (*Megadyptes antipodes*). *Behav Ecol Sociobiol* 55:169–175
- Maynard Smith J, Harper D (2003) *Animal Signals*. Oxford University Press, Oxford
- McGraw KJ, Hill GE (2000) Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. *Proc R Soc Lond B* 267:1525–1531
- Møller AP, Pomiankowski A (1993) Why have birds got multiple sexual ornaments? *Behav Ecol Sociobiol* 32:167–176
- Møller AP, Garamszegi LZ, Gil D, Hurtrez-Bousses S, Eens M (2005) Correlated evolution of male and female testosterone profiles in birds and its consequences. *Behav Ecol Sociobiol* 58:534–544
- Montgomerie R (2006) Analyzing colors. In: Hill G, McGraw K (eds.) *Bird coloration*. Harvard University Press, Cambridge, pp 90–147
- Monticelli D, Ramos J (2012) Laying date, body mass and tick infestation of nestling tropical Roseate Terns *Sterna dougalii* predict fledging success, first-year survival and age at first return to the natal colony. *Ibis* 154:825–837
- Montoya B, Torres R (2015) Male skin color signals direct and indirect benefits in a species with biparental care. *Behav Ecol* 26:425–434. doi:10.1093/beheco/aru204
- Mundy NI (2006) Genetic basis of color variation in wild birds. In: Hill G, McGraw K (eds.) *Bird coloration*. Harvard University Press, Cambridge, pp 469–506
- Negro JJ, Bortolotti GR, Tella JL, Fernier KJ, Bird DM (1998) Regulation of integumentary colour and plasma carotenoids in American Kestrels consistent with sexual selection theory. *Funct Ecol* 12:307–312
- Nelson JB (1978) *The Sulidae: gannets and boobies*. Oxford University Press, Oxford
- Nelson JB (2005) *Pelicans, cormorants, and their relatives: the Pelecaniformes*. Oxford University Press, Oxford
- Nolan PM, Dobson FS, Nicolaus M, Karels TJ, McGraw KJ, Jouventin P (2010) Mutual mate choice for colorful traits in king penguins. *Ethology* 116:635–644

- Nordeide JT, Kekäläinen J, Janhuhen M, Kortet R (2013) Female ornaments revisited – are they correlated with offspring quality? *J Anim Ecol* 82:26–38
- Peig J, Green AJ (2009) New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* 118:1883–1891
- Peig J, Green AJ (2010) The paradigm of body condition: a critical reappraisal of current methods based on mass and length. *Funct Ecol* 24:1323–1332
- Pérez-Rodríguez L (2008) Carotenoid-based ornamentation as a dynamic but consistent individual trait. *Behav Ecol Sociobiol* 62:995–1005
- Pérez-Rodríguez L, Viñuela J (2008) Carotenoid-based bill and eye ring coloration as honest signals of condition: an experimental test in the red-legged partridge (*Alectoris rufa*). *Naturwissenschaften* 95:821–830
- Pérez-Rodríguez L, Blas J, Viñuela J, Marchant TA, Bortolotti GR (2006) Condition and androgen levels: are condition-dependent and testosterone-mediated traits two sides of the same coin? *Anim Behav* 72:97–103
- Pham TT, Queller PS, Tarvin KA, Murphy TG (2014) Honesty of a dynamic female aggressive status signal: baseline testosterone relates to bill color in female American goldfinches. *J Avian Biol* 45:22–28
- Piault R, Gasparini J, Bize P, Paulet M, McGraw KJ, Roulin A (2008) Experimental support for the makeup hypothesis in nestling tawny owls (*Strix aluco*). *Behav Ecol* 19:703–709
- Potti J, Merino S (1996) Decreased levels of blood trypanosome infection correlate with female expression of male secondary sexual trait: implications for sexual selection. *Proc R Soc Lond B* 263:1199–1204
- Roberts ML, Buchanan KL, Evans MR (2004) Testing the immunocompetence handicap hypothesis: a review of the evidence. *Anim Behav* 68:227–239
- Romero-Pujante M, Hoi H, Blomqvist D, Valera F (2002) Tail length and mutual mate choice in Bearded Tits (*Panurus biarmicus*). *Ethology* 108:885–895
- Roulin A (2015) Condition-dependence, pleiotropy and the handicap principle of sexual selection in melanin-based colouration. *Biol Rev* 91(2):328–348
- Schroeder J, Lourenç PM, Hooijmeijer JC, Both C, Piersma T (2009) A possible case of contemporary selection leading to a decrease in sexual plumage dimorphism in a grassland-breeding shorebird. *Behav Ecol* 20:797–807
- Schulte-Hostedde AI, Zinner B, Millar JS, Graham JH (2005) Restitution of mass–size residuals: validating body condition indices. *Ecology* 86:155–163
- Simons MJP, Cohen AA, Verhulst S (2012) What does carotenoid dependent coloration tell? Plasma carotenoid level signals immunocompetence and oxidative stress state in birds—a meta-analysis. *PLoS ONE* 7:e43088
- Tobias JA, Gamarra-Toledo V, García-Olaechea D, Pulgarin PC, Seddon N (2011) Year-round resource defence and the evolution of male and female song in suboscine birds: social armaments are mutual ornaments. *J Evol Biol* 24:2118–2138
- Tobias JA, Montgomerie R, Lyon BE (2012) The evolution of female ornaments and weaponry: social selection sexual selection and ecological competition. *Philos Trans R Soc Lond B* 367:2274–2293
- Torres R, Velando A (2003) A dynamic trait affects continuous pair assessment in the blue-footed booby *Sula nebouxii*. *Behav Ecol Sociobiol* 55:65–72
- Torres R, Velando A (2005) Male preference for female foot color in the socially monogamous blue-footed booby, *Sula nebouxii*. *Anim Behav* 69:59–65
- Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed) *Sexual selection and the descent of man*. Aldine, Chicago, pp 136–179
- Velando A, Lessells CM, Márquez JC (2001) The function of female and male ornaments in the Inca tern: evidence for links between ornament expression and both adult condition and reproductive performance. *J Avian Biol* 32:311–318
- Velando A, Beamonte-Barrientos R, Torres R (2006) Pigment based skin color in the blue-footed booby: an honest signal of current condition used by females to adjust reproductive investment. *Oecologia* 149:535–542
- Velando A, Beamonte-Barrientos R, Torres R (2014) Enhanced male coloration after immune challenge increases reproductive potential. *J Evol Biol* 27:1582–1589
- Vergara P, Fargallo JA (2011) Multiple colored ornaments in male common kestrels: different mechanisms to convey quality. *Naturwissenschaften* 98:289–298
- Vergara P, Fargallo JA, Martínez-Padilla J (2015) Genetic basis and fitness correlates of dynamic carotenoid-based ornamental coloration in male and female common kestrels *Falco tinnunculus*. *J Evol Biol* 28:146–152
- Wikelski M, Lynn S, Breuner C, Wingfield JC, Kenagy GJ (1999) Energy metabolism, testosterone and corticosterone in white-crowned sparrows. *J Comp Physiol A* 185:463–470
- Wingfield JC (1987) Changes in reproductive function in free-living birds in response to environmental perturbations. In: Stetson M (ed.) *Processing of environmental information in vertebrates*. Springer, New York, pp 121–148
- Wingfield JC, Hegner RE, Dufty AM, Ball GF (1990) The ‘challenge hypothesis’: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am Nat* 136:829–846
- Zahavi A (1975) Mate selection—a selection for a handicap. *J Theor Biol* 67:205–214