Dynamic signalling using cosmetics may explain the reversed sexual dichromatism in the monogamous greater flamingo

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Abstract

Colourful plumage is typical of males in species with conventional sex roles, in which females care for offspring and males compete for females, as well as in many monogamous species in which both sexes care for offspring. Reversed sexual dichromatism—more colourful females than males—is predominant in species with sex role reversal. In the latter species, males care for offspring and females compete for mates, the mating system is mainly polyandrous and there is reversed size dimorphism—females are larger than males. Here we document a case of reversed dichromatism, in the greater flamingo *Phoenicopterus roseus*, in which there is no sex role reversal and no reversed size dimorphism. Although theoretical models postulate that cases of reversed dichromatism should be rare among monogamous ornamented birds, our findings show that the use of cosmetics might be a mechanism for the occurrence of more ornamented females than males. Indeed, the concentrations of carotenoids in the uropygial secretions used as make-up were higher in females than in males. Apparently, there was a trade-off between coloration and antioxidant defence, as the concentrations of carotenoids in the uropygial secretions were lower during chick provisioning than in other periods, contrary to those in plasma. In this system the application of make-up would act as a dynamic signal, which would allow a rapid reallocation of resources used for signalling among functions depending on needs. Cases like this may have evolved to signal the ability to provide parental care when females are more physiologically stressed than males.

Significance statement

For species in which there is no sex role reversal, but females are ornamented and the resources allocated to ornaments are important for offspring viability, it has even been suggested that females should be less ornamented than males. This may be because for females it would be better to invest directly in fecundity rather than in costly ornaments. We show a case of reversed sexual dichromatism in a monogamous bird with no sex role reversal, the greater flamingo, where females apply make-up over feathers. In the case of this species there could be directional male mate preferences for female plumage coloration because the costs of signalling would not affect breeding investment in females, since cosmetic coloration is not used after it is no longer required, thus allowing the resources used in make-up (carotenoids) to be used in other functions.

Keywords: Carotenoids • Make-up • Plumage coloration • Plumage maintenance • Sexual differences • Uropygial secretions

Introduction

Ever since Darwin (1871) the variation in the patterns of plumage coloration in birds has fascinated evolutionary biologists. Sexual differences in potential reproductive rates have led to gender-biased sexually selected ornamentation and sexual size dimorphism (Andersson 1994). Colourful plumage is usually considered as an ornament in birds, and may signal individual quality (Hill 2002, 2014). Carotenoids are one of the pigments found inside bird feathers, and as they cannot be synthesised by animals, but have to be ingested with food, carotenoid-related coloration has been considered to communicate the individual ability to obtain resources (Endler 1980; Hill 2002; Searcy and Nowicki 2005; McGraw 2006). Flexibility in the expression of plumage coloration could signal the ability to cope with rapidly changing and/or unpredictable environments. Carotenoids are incorporated into the plumage during moult and as feathers are inert tissue, there may be little opportunity for plumage colour to express its signalling function beyond the moulting period. This would be especially critical if the conditions at times when the signal should be functional differ from those when the colour was acquired, sometimes months before (Searcy and Nowicki 2005; Montgomerie 2006; Botero and Rubenstein 2012). A means through which birds may express flexibility in plumage coloration is by the application, as make-up, of pigmented substances over the feathers. This may signal the ability to rapidly track unpredictable environmental changes, as signalling would be dynamic (Montgomerie 2006; Delhey et al. 2007; Amat et al. 2011).

In a review on female ornamentation in birds, Amundsen and Pärn (2006) indicated that the occurrence of cases in which both sexes are colourful, but females more so, is limited to polyandrous species in which there is sex role reversal, such as

greater painted snipe *Rostratula benghalensis*, Eurasian dotterel *Charadrius morinellus* and phalaropes *Phalaropus* spp. For species in which there is no sex role reversal, but females are ornamented and the resources allocated to ornaments are important for offspring viability, it has even been suggested that females should be less ornamented than males because for females it would be better to invest directly in fecundity rather than in costly ornaments (Chenoweth et al. 2006). This, for instance, would occur if females need some resources, as carotenoids, to combat the oxidative stress derived from parental activities. In this case, it would not pay females to invest carotenoids in ornamentation. However, as we will show here, there may be cases of reversed sexual dichromatism among species with no sex role reversal. This should be possible if the signalling of ornaments is dynamic, so that females are able to rapidly reallocate resources for different functions that do not overlap temporally (Amat and Rendón 2017). Among birds, for instance, this is likely when individuals use for signalling bare body parts pigmented with carotenoids, such as caruncles, legs, eye rings or bills. Indeed, such body parts may experience rapid shifts in colour depending on conditions (e.g., food deprivation, immune challenge) (Faivre et al. 2003; Torres and Velando 2003; Pérez-Rodríguez and Viñuela 2008; Rosenthal et al. 2012; Broughton et al. 2017).

Previous studies on sexual dichromatism in birds without reversed size dimorphism have been on species in which females have sex-specific ornaments, as in the eclectus parrot *Eclectus roratus* in which females have red instead of green plumage as males (Heinsohn et al. 2005), or in the upland goose *Chloephaga picta* in which females have yellow-orange legs whereas those of males are black (Glabach et al. 2010). To our knowledge, no study has so far studied the signalling function of reversed dichromatism in any monogamous species without sex-specific ornaments. The evolutionary potential for females being more brightly coloured than males in

monogamous species in which both sexes care for offspring may arise when females are more physiologically stressed than males as a consequence of parental activities (Amat and Rendón 2017). In this case, there may be sexual differences in selection pressures and signalling of parental quality may be more important in females than in males (Clutton-Brock 2007). To assess this we compared sex-related variations in plumage colour in greater flamingos *Phoenicopterus roseus*, and related such variations to attributes of individual quality. A recent study with individuals of this species in captivity showed reversed sexual dichromatism (Freeman et al. 2016), but the mechanism responsible for this was not suggested. Here we show that reversed sexual dichromatism in greater flamingos is due to the application of make-up over feathers. For this we analysed sex-related seasonal variations in the maintenance behaviour of plumage, and compared carotenoid content both in uropygial secretions, which the birds use as make-up, and in plasma, which may be used as anti-oxidants. With this we aimed at ascertaining whether there was a reallocation of pigments among functions depending on needs. This approach provides an opportunity to test the relationship between parental care propensity and female ornamentation, a process for which there is little evidence (Tobias et al. 2012; Bulluck et al. 2017).

Material and methods

Study species

Greater flamingos are long-lived colonial waterbirds with delayed reproduction (mean age of first reproduction is 7 years), and in which there is mutual mate choice (Johnson

and Cézilly 2007; Perrot et al. 2016). Clutch size is one, and the single egg is incubated by both parents during 28 days; after hatching the chick is fed by both parents during 3 months (Johnson and Cézilly 2007). In southern Spain the flamingos are observed displaying in groups mainly during October-April (Amat et al. 2011), there is competition for nesting sites (Rendón et al. 2001), and breeding adults move to distant wetlands for foraging (150-400 km from the breeding colony), visiting the colony to feed their chick every 6-9 days (Amat et al. 2005; Rendón et al. 2012, 2014). The uropygial secretions of greater flamingos contain carotenoids, mainly canthaxanthin, which the birds use as make-up, and the feathers become more colourful with the quantity of pigments applied over them (Amat et al. 2011). To spread the make-up over their plumage, the flamingos rub their cheeks on the uropygial gland, and then rotate the head from side to side on their neck and anterior back (Amat et al. 2011; see photographs in Amat and Rendón 2017, and Electronic Supplementary Material, Fig. S1).

Field observations, plumage colour and body condition

Using telescopes, we recorded visually the neck plumage colour and behaviour of individually banded flamingos in southern Spanish wetlands. We made the observations during the morning (06:00–11:00 h, GMT), and allocated the colour to three categories (Amat et al. 2011): (i) very pale pink that at a distance looks white, (ii) pale pink, and (iii) pink (see Electronic Supplementary Material, Fig. S1). There was a very good agreement between observers in the assignments of plumage colour scores (Amat et al. 2011). In addition, to determine objectively whether flamingos are able of discriminating among colour categories, we recorded reflectance values (300-700 nm)

of neck feathers from adult flamingos using an Ocean Optics, Inc. USB 2000 spectrometer connected to a deuterium-halogen light source with a coaxial reflectance probe (QR-400-7-UV-vis). Reflectance was expressed as percentage relative to that of a WS-2 white reference tile. We assigned those feathers to colour categories as indicated above, and tested whether flamingos may discriminate between colour categories applying the receptor noise model (Vorobyev and Osorio 1998; Vorobyev et al. 1998), which is dependent on both receptor type and abundance on the retina. The visual system of flamingos has been categorized as violet sensitive (Ödeen and Håstad 2003). Therefore, we modelled an average avian violet visual perception system (Endler and Mielke 2005), assuming cone ratios from peafowl *Pavo cristatus* and mallard *Anas platyrhynchos* (Butler and Toomey 2011), and using a Weber fraction of 0.05. Chromatic distances among the three colour categories of neck feathers were estimated in units of “just noticeable difference” (JND).

We distinguished between males and females based on body size, since males are 20% larger than females (Studer-Thiersch 1986; Johnson and Cézilly 2007). The accuracy of sexing based on size was very high, as 97% of males and 96% of females were correctly sexed (Rendón et al. 2014).

We distinguished rubbing behaviour, in which the flamingos spread make-up over feathers, from normal preening, in which individuals use lateral strokes of the bill to preen their feathers (Amat et al. 2011; Amat and Rendón 2017), and recorded plumage maintenance behaviour (either rubbing or preening) in relation to neck plumage colour and sex of birds by scan-sampling flocks of flamingos in southern Spanish wetlands, during both the displaying period (Oct.-Apr.) and outside that period (May-Sep.). We recorded proportionally more males than females during the displaying period than during the rest of the year. This was because of the smaller size of females,

so that the bands of females, placed on tibia, were under water more frequently than those of males, due to the greater water depth during the displaying period than during the rest of the year. We recorded simultaneously neck plumage colour, sex and behaviour in 2497 occasions (see Electronic Supplementary Material Table S1). We made 18-405 observations per month, and although 11.4% of individuals were resampled more than once, the observations may be considered as independent, as they were made in different months or even years (Amat et al. 2011).

To relate neck plumage colour to body condition, we made observations at the Fuente de Piedra (S. Spain) breeding colony, and used the abdominal profile as an index of individual quality. The shapes of some body traits (e. g., crop in eagles, white rump in antelopes, abdomen in spiders) have been frequently used as indices of individual quality in studies of signalling (Maynard Smith and Harper 2003). The abdominal profile has been found to be significantly correlated with the quantity of body reserves in waterbirds (Bowler 1994; Zillich and Black 2002), and ultimately with breeding success (Johnson and Sibly 1993). In addition, the abdominal profile may be related to the quantity of food ingested during a recent foraging bout (Rendón et al. 2009). The observations were conducted using a telescope, and we allocated the abdominal profile to six ordinal scales, from concave (i) to hanging (vi) (Bowler 1994; Rendón et al. 2009; Electronic Supplementary Material Fig. S2, Fig. S3). Most observations (> 80%) on the abdominal profiles were conducted by a single observer; anyway, there was a very good concordance among observers in the assignments of scores (Electronic Supplementary Material Fig. S4). We recorded the date, sex, neck colour and abdominal profile the first time that we observed an individually marked flamingo in the breeding island. Such date was considered as a surrogate of laying date (Amat et al. 2011). The abdominal profiles of some individuals could not be recorded in all occasions, as they were

occluded from view in the dense aggregation of the colony. So, sample sizes for abdominal profiles are smaller (87 females and 67 males) than for plumage coloration (126 females and 101 males) of flamingos in the breeding colony. To check for individual sex-related temporal changes in plumage colour and/or condition of breeding birds, we recorded plumage colour and abdominal profile when the same individuals were observed on average 52 days later after they were observed the first time, i. e., when they were provisioning chicks (Amat et al. 2011).

It was not possible to record data blindly because our study involved focal birds in the field.

Samples of uropygial secretions and blood

We collected samples of uropygial secretions from captive greater flamingos to study sex-related variations in the concentrations of carotenoids in those secretions, mainly canthaxanthin, following procedures in Amat et al. (2011). We took 1 mL of blood from the tarsal vein to sex flamingos using molecular methods (Griffiths et al. 1998), as well as to determine plasma concentrations of carotenoids as indicated in Negro et al. (2000). Not all individuals were sampled simultaneously for blood and uropygial secretions on all occasions, neither were the same individuals sampled every time. The samples were collected during months corresponding to displaying (October [$n = 18$ for secretions and 5 for plasma] and February [$n = 13$ for secretions and 13 for plasma]) and non-displaying (July [$n = 8$ for secretions and 17 for plasma]) periods, and were kept at about 4 °C until transportation to a laboratory, within the next 5 h after collection, where they were frozen until analyses. Prior to this, blood samples were centrifuged at 13,000 g during 10 min to separate the plasma. Birds in captivity came from the same

population where the behavioural observations were conducted, and they fed in a wetland where no food was provided. To capture captive birds we baited a cage with an automatic closure door using rice grain, from which husk had been removed. Rice grains with husk removed do not contain carotenoids (Ye et al. 2000).

In addition, we collected blood samples from 14 wild flamingos while they were provisioning chicks (July; captured as indicated in Amat et al. 2005), to sex them and determine plasma concentrations of carotenoids.

Statistical procedures

Values greater than 1 JND indicate that birds are able of discriminating among colour categories (Osorio and Vorobyev 1996). Moreover, to determine if average distance between colour samples is both statistically and biologically meaningful, we applied the approach suggested by Maia and White (2018). To test for statistical differences between groups we applied distance-based PERMANOVA ($\alpha = 0.05$, 999 permutations). Then, we tested if colour groups were perceptually distinct using a bootstrap procedure to generate a distribution of mean colour distances and estimated a confidence interval. In the case that the colour categories are statistically different and the confidence interval determined by resampling does not include the 1 JND threshold, we considered that flamingos can discriminate between neck colour categories.

Tetrachromatic colour variables were calculated using the R package *pavo* (Maia et al. 2013) and JND values were tested following Supplementary Material in Maia and White (2018).

We created with the data a multidimensional contingency table (Electronic Supplementary Material Table S1), on which we performed a log-linear analysis to

uncover relationships among categorical variables. We considered plumage colour as the major response variable, and all other factors (behaviour, period, and sex) as design factors. To determine more specifically the nature of the relationships (either positive or negative) between colour categories and sex, behaviour and period, we applied ordinal logistic cumulative regression models (Agresti 2002) with the independent variables and functional relationships determined by log-linear model. Ordinal models are applied to J responses measured on an ordinal scale. These models make use of cumulative logits, producing $J - 1$ probability functions with different independent terms and a common slope. We used *polr* function in the MASS package (Venables and Ripley 2002) of R 2.15.3 (R Core Team 2013) to fit ordinal regression models. Because of small sample sizes and unequal variances between groups, we applied robust statistical methods (Wilcox 2016) using WRS2 package (Mair et al 2017) in R. Robust ANOVA was performed to test monthly and sex-related variations in the amount of carotenoids in both the uropygial secretions and plasma (raw data in Electronic Supplementary Material Tables S2 and S3). Robust t -test for independent samples (“yuen” function) was used to compare plasma carotenoids in wild and captive individuals, and a measure of effect size ξ were provided, with values of $\xi = 0.1, 0.3$, and 0.5 corresponding to small, medium, and large effect size (Mair et al 2017). Sexual and temporal variations in neck plumage colour and abdominal profile ordinal scores were tested using Mann-Whitney U test, Wilcoxon matched-pair test, or Spearman correlation using the STATISTICA software (Dell Inc. 2015).

Results

Noise-corrected colour distances between plumage colour categories were statistically significant (PERMANOVA: $F_{2,25} = 7.07$, $P = 0.001$; $R^2 = 0.32$). Furthermore, the bootstrapped confidence intervals for mean distances between colour categories did not include the 1 JND threshold (Electronic Supplementary Material Fig. S5). These results indicate that flamingos are able of distinguishing among colour categories according to their visual capabilities.

We tested simultaneously all four-factor (sex, behaviour, plumage colour, and period) interactions and all marginal and partial association models in a log-linear analysis. The least complex model did not need to contain any three-way association (Pearson $\chi^2_7 = 10.94$, $P = 0.141$), but should contain two-way associations (Pearson $\chi^2_9 = 2078.86$, $P < 0.001$). The two-way associations that should be included in the model (Table 1) were behaviour and plumage colour (redder plumage was associated with rubbing, ordinal logistic regression: $\beta_{[\text{rubbing}]} = 1.616 \pm 1.109$ (SE), $P < 0.001$; Fig. 1), behaviour and period (rubbing was mainly used during displaying period; Amat et al. 2011), plumage colour and period (redder plumage was mainly recorded during the displaying period, $\beta_{[\text{displaying}]} = 3.157 \pm 1.151$, $P < 0.001$; Fig. 1), plumage colour and sex (females were redder than males, $\beta_{[\text{female}]} = 1.107 \pm 0.100$, $P < 0.001$; Fig. 1), and period and sex (see Methods) (partial associations χ^2 with P 's ≤ 0.013 , Table 1). The partial associations that produced effects with $P \approx 0$ were ranked in terms of their relative importance according to their standardised residual values. This indicated that the association plumage colour and period was the most important one, followed by plumage colour and behaviour, plumage colour and sex, and behaviour and period (Table 2).

Since there were no sexual differences in the frequency of rubbing behaviour (Table 1), the difference in plumage colour may be linked to sexual differences in the

amount of pigments in the uropygial secretions, which the birds use as make-up and are known to make the feathers redder with the quantity of pigments applied over them (Amat et al. 2011). Indeed, the concentration of carotenoids in the uropygial secretions was higher in females than in males (robust ANOVA with 10% trimmed means: $Q = 5.81$, $P = 0.026$), and varied across months ($Q = 46.92$, $P < 0.001$). There was no month $\times$ sex interaction ($Q = 2.42$, $P = 0.342$) in the concentration of carotenoids in the uropygial secretions (Fig. 2). However, there were not sex-related differences in the concentrations of plasma carotenoids ($Q = 1.29$, $P = 0.281$), and these concentrations were higher in July than in the other months ($Q = 4.61$, $P = 0.039$), i.e., contrary to those in the uropygial secretions (Fig. 3).

Finally, we investigated whether there were changes in plumage colour after the chicks hatched, and whether the magnitude of those changes was related to individual variations in body condition. Because the probability of renesting after chick loss in greater flamingos is low (Johnson and Cézilly 2007), we expected that after hatching there would be no need to further make the plumage more colourful by using make-up if plumage colour is intended to signal the parental quality of individuals (Amat et al., 2011), and ultimately related to mate choice (Freeman et al. 2016). Given that carotenoids bleach rather quickly when exposed to ambient conditions (Woodall et al. 1997), plumage colour should fade when no make-up is applied over feathers. Indeed, we found that fading of colour after the chicks hatched, but it was more pronounced in individual females (mean $\pm$ SD difference in plumage colour scores between laying and chick provisioning: 0.30 ± 0.79 , $n = 126$) than in males (0.05 ± 0.68 , $n = 101$) (Mann-Whitney U test, $z = 2.23$, $P = 0.026$). This change in colour could be related to a reallocation of carotenoids among functions once the chicks hatched, likely to combat the oxidative stress resulting from commuting between the breeding site and foraging

wetlands (see “Study species”). In support of this, we found that in July the concentrations of carotenoids in the blood of flamingos were higher in captive individuals, which were not provisioning chicks, than in wild ones, which were provisioning chicks (Fig. 3; robust analysis with 10% trimmed means: $t(21.67) = 2.26$, $P = 0.035$, $\xi = 0.61$).

The abdominal profile and plumage colour at the start of incubation were correlated in females (Spearman correlation: $r_s = 0.26$, $n = 87$, $P = 0.007$), but not in males ($r_s = 0.01$, $n = 67$, $P = 0.956$), indicating that females in better condition had redder plumage. There were no differences in the abdominal profile of males ($N = 67$) between the incubation (3.74 ± 0.59) and the chick provisioning (3.67 ± 0.61) periods (Wilcoxon test, $Z = 0.69$, $P = 0.489$). However, females ($n = 87$) had a lower abdominal profile during the chick provisioning period (3.99 ± 0.83) than during incubation (4.36 ± 0.83) ($Z = 2.63$, $P = 0.009$). Moreover, the relationship between the difference in colour between incubation and the chick provisioning periods and the difference in abdominal profile between such periods was significant for females (Spearman correlation: $r_s = 0.32$, $n = 87$, $P = 0.003$), but not for males ($r_s = 0.20$, $n = 67$, $P = 0.107$).

Discussion

Our results show that plumage coloration in the greater flamingo has a strong seasonal component that is due to the application of make-up, and that females are more colourful than males. The more colourful individuals are preferred as mates (Freeman et al. 2016) and also start breeding earlier than the less colourful ones (Amat et al. 2011). In addition, as in other species with carotenoid-based plumage, colour may be related to

females' provisioning rates (Bulluck et al. 2017), and also signal the ability to manage oxidative stress (Henschen et al. 2016), which in flamingos may be important during chick provisioning (see below).

We scored plumage coloration from the human perspective. The differences in plumage coloration, as determined in this study, could be appreciated by flamingos because the vision of birds is rather acute with respect to hue (Hodos 1993). Indeed, flamingos are able of discriminating among plumage colour categories according to their visual capabilities.

Female flamingos experiencing a lower degree of fading in plumage colour between laying and chick provisioning were those that maintained a better body condition, which suggests that plumage colour signals individual quality. Because the body condition of males was not similarly affected between breeding phases, this suggests that there would be more selection pressures on female than male flamingos to signal their individual quality. In the American flamingo *Phoenicopterus ruber* the displaying bouts of females apparently contain more detailed information about reproductive condition than those of males (Powell 1997). In the greater flamingo the variance in fitness may be higher in females than in males (see Cézilly 1993; Tavecchia et al. 2001), so that the opportunity for selection would be stronger in females than in males (Shuster and Wade 2003). In line with this, Delhey et al. (2006) showed that between-individual temporal variation in the decline in coloration in male blue tits *Cyanistes caeruleus* was related to attributes of male (in this case the sex experiencing stronger opportunity for selection), but not female, quality, such as condition.

In the greater flamingo pair bonds usually last for a single breeding attempt, and may be established a few months before breeding, but there is continuous partner assessment during the long displaying period, as suggested by frequent mate changes

even just until laying (Jonhson and Cézilly 2007). Signalling of individual quality in females may be beneficial when males make repeated choices and mate splitting rates before laying are high (Kraaijeveld 2003; Kraaijeveld et al. 2007; Clutton-Brock 2009), as in the greater flamingo. The provisioning rate of female flamingos is higher than that of males (Rendón et al. 2014). Because of this, during the chick provisioning phase the need of carotenoids to respond to the oxidative stress likely produced during commuting between the breeding colony and long-distant feeding areas (Simons et al. 2012; Jenni-Eiermann et al. 2014) may be higher in females than males. This may be exacerbated because greater flamingo females are 20% smaller than males (Studer-Thiersch 1986; Johnson and Cézilly 2007), so that during breeding they would be more energetically stressed than males (Cézilly 1993). Therefore, females would experience a greater need than males in investing carotenoids in their antioxidant capacity when they are feeding chicks, which would explain the fading in plumage colour following egg hatching. In support of this we found that during non-displaying periods the concentrations of carotenoids in the plasma of captive flamingos were higher than during displaying periods, a pattern contrary to that found for uropygial carotenoids. However, in wild flamingos the concentrations of plasma carotenoids were lower than in captive flamingos during non-displaying periods. In spite that sample sizes are not too high, our results suggest that wild (provisioning) birds were using carotenoids to combat oxidative stress, and also support a trade-off between plumage pigmentation and antioxidant defence (but see Isaksson and Andersson 2008). Although the captive and wild flamingos may obtain different quantities of carotenoids from their diets, we think our results are conservative, as wild birds were more colourful than captive ones (data not shown), indicating that wild birds ingested more carotenoids. As indicated by Amat and Rendón (2017), the reassignment of pigments to different functions would be

facilitated because the allocation of pigments from uropygial secretions to feathers has a strong seasonal component, and after egg hatching there would be no need to further signal individual quality.

Amat and Rendón (2017) suggested that a secondary function of the use of cosmetic coloration would be to signal status at foraging sites, which may explain why greater flamingos also use make-up during non-displaying periods, though much less frequently than during displaying periods. This, however, would not explain satisfactorily why females were more coloured than males, neither the strong seasonal component in the coloration of plumage.

It has been hypothesised that under male choice, the best strategy for males is to develop stabilizing preferences to avoid females that invest too many or too few resources in ornamentation, when such resources are essential for reproduction (Chenoweth et al. 2006). However, when signalling is dynamic, so that the resources assigned to signalling may rapidly be reallocated for different functions, and there is no temporal overlap among such functions—as it may be for the carotenoids that female flamingos use as make-up for their feathers and likely to combat oxidative stress—male mate preferences may be directional (Svensson et al. 2005; Nordeide et al. 2013). In other words, in the case of greater flamingos there could be directional male mate preferences for female plumage coloration because the costs of signalling would not affect breeding investment in females, since cosmetic coloration is not used after it is no longer required. Although there is a limited number of studies on dynamic signalling showing how the expression of ornaments fluctuates over the short term to mirror environmental and/or physiological changes (Faivre et al. 2003; Torres and Velando 2003; Pérez-Rodríguez and Viñuela 2008; Rosenthal et al. 2012; Broughton et al. 2017), cases demonstrating rapid reallocation among functions of resources used in

ornamentation, when there is no temporal overlap in the requirements of such resources, are lacking.

In conclusion, our findings provide support for a mechanism by which there may be directional selection on the expression of female ornaments that lead to reversed sexual dichromatism in a monogamous species. Our findings also have implications for the understanding of dynamic signalling, by providing insights into the use of resources needed for signalling.

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Compliance with ethical standards

Ethical approval

International, national, and institutional guidelines for the use and capture of animals were followed. Consejería de Medio Ambiente from the Junta de Andalucía (Regional Government) gave permission to conduct the study. An approval from an ethics committee was not needed.

Conflict of interest The authors declare no conflict of interest.

Data availability The data associated with this article are in the Electronic Supplementary Material.

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Table 1 Simultaneous tests for all 4-factor interactions, and tests of all marginal and partial association models in a log-linear analysis of the frequency table (see Electronic Supplementary Material Table S1) (1 = behaviour, 2 = plumage colour, 3 = period, 4 = sex)

			Partial association		Marginal association	
			χ^2	<i>P</i>	χ^2	<i>P</i>
1	1	1	624.5	<0.001	624.5	<0.001
2	2	2	924.0	<0.001	924.0	<0.001
3	1	1	177.3	<0.001	177.3	<0.001
4	1	1	44.3	<0.001	44.3	<0.001
12	2	2	246.9	<0.001	599.2	<0.001
13	1	1	55.3	<0.001	393.3	<0.001
14	1	1	1.7	0.191	14.0	<0.001
23	2	2	672.1	<0.001	1008.6	<0.001
24	2	2	138.4	<0.001	149.2	<0.001
34	1	1	6.2	0.013	12.7	<0.001
123	2	2	0.8	0.670	0.8	0.681
124	2	2	2.0	0.369	2.8	0.245
134	1	1	0.2	0.696	0.0	0.926
234	2	2	5.1	0.076	6.4	0.041

Table 2 Interactions where $P \approx 0$ from the saturated model using the data of simultaneous tests for all 4-factor interactions (see Table 1)

Interaction	Partial χ^2	df	Standardised residual rank (partial χ^2/df)
Plumage colour $\times$ period	672.1	2	1 (336.1)
Plumage colour $\times$ behaviour	256.9	2	2 (128.5)
Plumage colour $\times$ sex	138.4	2	3 (69.2)
Behaviour $\times$ period	55.4	1	4 (55.4)

LEGENDS TO FIGURES

Fig. 1 Predicted probabilities of each neck colour category of greater flamingos according to behaviour, period and sex from the selected ordinal regression model. Neck plumage colour categories were as follows: (1) very pale pink that at a distance looks white, (2) pale pink, and (3) pink

Fig. 2 Monthly variations (mean $\pm$ SE, sample sizes in parentheses) in the concentration of canthaxanthin in uropygial secretions of male and female greater flamingos

Fig. 3 Monthly variations (mean $\pm$ SE, sample sizes in parentheses) in the concentration of canthaxanthin in plasma of captive (open symbols) and wild (closed symbols) male and female greater flamingos. Samples from wild birds were taken only in July

Figure 1

Figure 2

Figure 3

SUPPLEMENTARY ONLINE MATERIAL TO

**Dynamic signalling using cosmetics may explain the
reversed sexual dichromatism in the monogamous
greater flamingo**

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Fig S1 Individual variations in neck colour of greater flamingos and scores of plumage coloration

Table S1 Table of frequencies of data of greater flamingos on which statistical analyses were performed, according to plumage maintenance behaviour, neck plumage colour, period and sex

Table S2 Monthly concentrations of canthaxanthin in uropygial secretions of male and female greater flamingos

Table S3 Monthly concentrations of canthaxanthin in the plasma of male and female greater flamingos

Inter-rater reliability in the allocations of abdominal profile categories to greater flamingos

Fig S2 Abdominal profile scores for greater flamingo

Fig S3 Images of greater flamingos used to record concordance among observers in the allocation of abdominal profile categories

Fig S4 Frequency distribution in the allocation of abdominal profile scores by four raters

Fig S5 Spectral reflectance values for three colour categories of neck feathers of greater flamingos, and colour distances between neck colour categories in the colour space

Fig S1 Individual variations in neck colour of greater flamingos and assigned scores of plumage coloration. Note an individual on the right that is rubbing its cheeks on the uropygial gland to obtain the secretions used as make-up

Table S1 Table of frequencies of data of greater flamingos on which statistical analyses were performed, according to plumage maintenance behaviour, neck plumage colour, period and sex. Neck plumage colour categories were as follows: (1) very pale pink that at a distance looks white, (2) pale pink, and (3) pink (after Amat et al. 2011)

BEHAVIOUR	COLOUR	PERIOD	SEX	FREQUENCY
Rubbing	1	Displaying	Male	34
Preening	1	Displaying	Male	249
Rubbing	2	Displaying	Male	169
Preening	2	Displaying	Male	163
Rubbing	3	Displaying	Male	15
Preening	3	Displaying	Male	12
Rubbing	1	NonDisplaying	Male	16
Preening	1	NonDisplaying	Male	412
Rubbing	2	NonDisplaying	Male	1
Preening	2	NonDisplaying	Male	11
Rubbing	3	NonDisplaying	Male	0
Preening	3	NonDisplaying	Male	0
Rubbing	1	Displaying	Female	29
Preening	1	Displaying	Female	203
Rubbing	2	Displaying	Female	225
Preening	2	Displaying	Female	285
Rubbing	3	Displaying	Female	122
Preening	3	Displaying	Female	74
Rubbing	1	NonDisplaying	Female	17
Preening	1	NonDisplaying	Female	413
Rubbing	2	NonDisplaying	Female	8
Preening	2	NonDisplaying	Female	39
Rubbing	3	NonDisplaying	Female	0
Preening	3	NonDisplaying	Female	0

Reference

Amat JA, Rendón MA, Garrido-Fernández J, Garrido A, Rendón-Martos M, Pérez-Gálvez A (2011) Greater flamingos *Phoenicopterus roseus* use uropygial secretions as make-up. Behav Ecol Sociobiol 65: 665-673

Table S2 Monthly concentrations of canthaxanthin in uropygial secretions of male and female greater flamingos

MONTH	CONCENTRATION (mg/Kg)	SEX
Feb	0.4451	M
Feb	0.4238	M
Feb	0.2936	M
Feb	0.2523	M
Feb	1.1043	F
Feb	1.5158	F
Feb	0.851	M
Feb	0.2867	F
Feb	1.0996	F
Feb	0.6243	M
Feb	0.7872	M
Feb	0.5939	F
Feb	1.0056	F
Jul	0.0336	M
Jul	0.0369	M
Jul	0.2634	F
Jul	0.1160	F
Jul	0.0960	M
Jul	0.2030	M
Jul	0.0871	M
Jul	0.1737	F
Oct	0.7797	M
Oct	0.3742	M
Oct	0.0574	F
Oct	0.3164	M
Oct	0.1603	M
Oct	0.4418	M
Oct	0.9264	F
Oct	0.2334	F
Oct	0.6884	M
Oct	0.0000	M
Oct	0.1816	M
Oct	0.8285	M
Oct	0.8966	F
Oct	0.6075	F
Oct	0.9949	F
Oct	0.5345	M
Oct	0.8408	F
Oct	0.0625	F

Table S3 Monthly concentrations of canthaxanthin in the plasma of wild and captive greater flamingos according to sex

MONTH	CONCENTRATION (g/L)	SEX
Feb-captive	4.5331	M
Feb-captive	4.8785	M
Feb-captive	4.5331	M
Feb-captive	3.6351	M
Feb-captive	11.9975	F
Feb-captive	8.0018	F
Feb-captive	4.227	M
Feb-captive	9.2989	F
Feb-captive	9.1891	M
Feb-captive	6,8984	M
Feb-captive	3.8199	F
Feb-captive	5.6578	F
Feb-captive	1.0056	F
Jul-captive	26.143	M
Jul-captive	12.444	M
Jul-captive	2.206	F
Jul-captive	5.305	M
Jul-captive	4.671	M
Jul-captive	6.337	M
Jul-captive	33.019	F
Jul-captive	8,653	F
Jul-captive	24.236	M
Jul-captive	8.287	M
Jul-captive	21.248	M
Jul-captive	49.574	F
Jul-captive	10.166	F
Jul-captive	11.676	F
Jul-captive	18.120	M
Jul-captive	60.080	F
Jul-captive	2.322	M
Oct-captive	4.2744	F
Oct-captive	2.7183	M
Oct-captive	11,026	M
Oct-captive	6.2682	F
Oct-captive	3.800	M
Oct-captive	2.9931	M
Jul-wild	0.3987	F
Jul-wild	0.6448	F
Jul-wild	21.9286	F
Jul-wild	3.3731	F
Jul-wild	3.4419	F
Jul-wild	8.3641	F

Jul-wild	17.6708	F
Jul-wild	4.0021	F
Jul-wild	1.8452	M
Jul-wild	11.1411	F
Jul-wild	14.0119	M
Jul-wild	2.788	F
Jul-wild	2.6639	F
Jul-wild	0.5988	F

Table S3 (Cont.)

Inter-rater reliability in the allocations of abdominal profile categories to greater flamingos

Interrater reliability in the allocation of the abdominal profile index (API) to greater flamingo (Fig. S2) was tested to assess the extent to which repeated measurements of abdominal roundness scores by different observers get similar values. Intraclass correlation coefficient (ICC) was used as a measure of agreement between multiple raters. The ICC is based on repeated measures of ANOVA models and relates to the proportion of variance that is attributable to objects of measurement (McGraw and Wong 1996; Shrout and Fleiss 1979). The selection of an appropriate ICC model depends on the nature of the study (Shrout and Fleiss 1979). A two-way model (Model 2) was selected, where the same flamingo pictures ($n = 148$, of the 151 individuals depicted in Fig. S3) were used by four raters to assess individual APIs. Because we were interested in assessing the degree in which the coders were consistent in the absolute value of the rating, the ICC model assessed the individual results of each rater and the absolute agreement between raters. ICC values ranges from 0 (random agreement) to 1 (perfect agreement). ICC values < 0.4 suggest interrater reliability, between 0.40 – 0.59 moderate, between 0.60 – 0.74 good, and between 0.75 – 1.0 excellent (Cicchetti 1994). ICC was performed using *irr* package in R (Gamer et al. 2012).

The distribution of API scores from all the four raters is shown in Fig. S4. The results for the ICC is excellent ($ICC = 0.814$; 95% C.I. = 0.768–0.854), suggesting minimal measurement error between coders when APIs were assigned.

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Fig S2 Abdominal profile scores for greater flamingo (1: concave, 2: slightly concave, 3: plain, 4: slightly convex, 5: convex, and 6: hanging). Modified from C. Barbraud and A.R. Johnson (pers.com.). See further explanations in Rendón et al. (2009)

Fig S3 Images of greater flamingos used to record concordance among observers in the allocation of abdominal profile categories

Fig S4 Frequency distribution in the allocation of abdominal profile (API) scores by four raters

Fig S5 (A) Mean ($\pm$ SD, in grey) spectral reflectance values for three colour categories of neck feathers of greater flamingos. (B) Bootstrapped 95% CIs for the mean colour distances between neck colour categories in the colour space. Values above 1 JND indicate that flamingos are able of discriminating among colour categories