JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12553

Genetic basis and fitness correlates of dynamic carotenoid-based ornamental coloration in male and female common kestrels *Falco tinnunculus*

P. VERGARA*†, J. A. FARGALLO* & J. MARTÍNEZ-PADILLA*‡

*Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain
‡Department of Ethology and Biodiversity Conservation, Estación Biológica de Doñana – CSIC, Sevilla, Spain

Keywords:

animal model;
direct benefits;
female ornamentation;
good genes;
heritability;
parental care;
sexual selection.

Abstract

Knowledge of the genetic basis of sexual ornaments is essential to understand their evolution through sexual selection. Although carotenoid-based ornaments have been instrumental in the study of sexual selection, given the inability of animals to synthesize carotenoids *de novo*, they are generally assumed to be influenced solely by environmental variation. However, very few studies have directly estimated the role of genes and the environment in shaping variation in carotenoid-based traits. Using long-term individual-based data, we here explore the evolutionary potential of a dynamic, carotenoid-based ornament (namely skin coloration), in male and female common kestrels. We first estimate the amount of genetic variation underlying variation in hue, chroma and brightness. After correcting for sex differences, the chroma of the orange-yellow eye ring coloration was significantly heritable ($h^2 \pm SE = 0.40 \pm 0.17$), whereas neither hue ($h^2 = 0$) nor brightness ($h^2 = 0.02$) was heritable. Second, we estimate the strength and shape of selection acting upon chromatic (hue and chroma) and achromatic (brightness) variation and show positive and negative directional selection on female but not male chroma and hue, respectively, whereas brightness was unrelated to fitness in both sexes. This suggests that different components of carotenoid-based signals traits may show different evolutionary dynamics. Overall, we show that carotenoid-based coloration is a complex and multifaceted trait. If we are to gain a better understanding of the processes responsible for the generation and maintenance of variation in carotenoid-based coloration, these complexities need to be taken into account.

Introduction

Predicting the evolutionary dynamics of a trait requires knowledge of the relative importance of additive genetic variation in shaping phenotypic variation (i.e. its heritability), as well as of how it is related to fitness. This is particularly relevant within the context of sexual selection, as heritable variation in sexual ornamentation is a prerequisite for models of sexual selection based on indirect (genetic) benefits of mate choice (Qvarnström *et al.*, 2006). Models based on direct (nongenetic) benefits of mate choice, on the other hand, such as an increased parental care or reduced parasite transmission between mates (Martinez-Padilla *et al.*, 2012), do not require additive genetic variation to explain trait variation and may be more applicable to ornaments that are shaped by environmental sources of variation (Evans & Sheldon, 2012).

Among the variety of secondary sexual traits that can be found in nature, ornamental coloration is among the most diverse and well-studied subject in evolutionary ecology (Hill & McGraw, 2006), playing a key role in pre- and post-mating sexual selection (Helfenstein *et al.*, 2010; Mehlis *et al.*, 2013). Although a plethora of studies has suggested a main role for sexual selection in

Correspondence: Jesús Martínez-Padilla, Department of Ethology and Biodiversity Conservation, Estación Biológica de Doñana – CSIC, Américo Vespucio s/n. 41092, Sevilla, Spain. Tel.: +34 954 232 340; fax: +34 954 621 125; e-mail: jmartinezpadilla12@gmail.com
†PV deceased 7th of August 2013.

© 2014 EUROPEAN SOCIETY FOR EVOLUTIONARY BIOLOGY. *J. EVOL. BIOL.* **28** (2015) 146–154
JOURNAL OF EVOLUTIONARY BIOLOGY © 2014 EUROPEAN SOCIETY FOR EVOLUTIONARY BIOLOGY

explaining the evolution and maintenance of ornamental colorations in a wide variety of taxa (Endler, 1995; Boughman, 2001; Hill & McGraw, 2006; Maan & Cummings, 2009; Roulin & Ducrest, 2013), we still know remarkable little about their quantitative genetic basis (Fitze *et al.*, 2003; Mundy, 2006; Evans & Sheldon, 2012). In birds, for example, our knowledge is restricted to a handful of study systems and is strongly biased towards melanin-based coloration and unpigmented white patches, mostly in passerines (reviewed by Mundy, 2006), with some notable exceptions focusing on carotenoid or structural-based colours (Johnsen *et al.*, 2003; Hadfield *et al.*, 2006, 2007; Evans & Sheldon, 2012; Roulin & Ducrest, 2013).

The scarcity of studies addressing the quantitative genetics of carotenoid-based coloration can at least partly be attributed to the fact that they are often assumed to be fully environment dependent (McGraw, 2006). Indeed, as vertebrates cannot synthesize carotenoids themselves, they have to be acquired through their diet (McGraw, 2006). In line with this, a number of studies have shown that the environment is an important source of variation in carotenoid-based coloration (Endler, 1995; Hill, 2006). Importantly however, this does not rule out a role for genes in shaping variation in carotenoid-based coloration (Olson & Owens, 2005; Pérez-Rodríguez, 2008; Evans & Sheldon, 2012). For example, variation in foraging behaviour or in the physiological machinery needed to internally process carotenoids can be heritable traits (see Evans & Sheldon, 2012 and references there-in), generating additive genetic variation in carotenoid-based coloration.

Understanding the evolutionary dynamics of carotenoid-based traits not only requires knowledge of the quantitative genetic architecture of carotenoid-based traits, but also of the form and strength of selection acting upon them. Under natural conditions, it is well established that enhanced expression of carotenoid-based coloration is beneficial on both a behavioural and physiological level. For example, brighter coloration has been shown to be a proxy of enhanced reproductive output and higher survival probability (Horak *et al.*, 2001; Simons *et al.*, 2012). Furthermore, coloration may be associated with different reproductive or mating strategies (Badyaev & Hill, 2002) and maternal allocation (Giraudeau *et al.*, 2011). On a physiological level, the immunostimulant and antioxidant properties of carotenoid have been well established. Therefore, carotenoid-based ornaments may signal an individual's capacity to fight parasites, as well as its immune function and antioxidant capacities (Horak *et al.*, 2001; Martinez-Padilla *et al.*, 2007; Mougeot, 2008; Helfenstein *et al.*, 2010; Mougeot *et al.*, 2010a,b).

Whereas male ornamentation has been subject of intense study over the past decades, there has been little interest in female ornamentation. This has recently changed, however, and it has now become an active field of research within evolutionary biology (Roulin *et al.*, 2010; Tobias *et al.*, 2012). So far, much attention has been given to describing the relationship between female showiness or weaponry and components of fitness (Nordeide *et al.*, 2013). However, if the genetic basis of male carotenoid-based traits has so far largely been neglected, the lack of knowledge for females is even more evident (Roulin & Dijkstra, 2003; Potti & Canal, 2010).

The common kestrel is a medium-sized, sexually dimorphic raptor, in which males are smaller and overall brighter than females (Village, 1990). They show a number of ornamental, melanin- and carotenoid-based, colour patches (Palokangas *et al.*, 1994; Fargallo *et al.*, 2007; Vergara & Fargallo, 2011). For example, in both male and female kestrels, their bare parts, including their legs and cere, are conspicuously yellow (Casagrande *et al.*, 2006; Vergara & Fargallo, 2011). This yellow coloration is carotenoid-based and differs between the sexes (males are more colourful) and is associated with body condition and breeding parameters in males (Casagrande *et al.*, 2006; Vergara & Fargallo, 2011). Together, these findings suggest that carotenoid-based ornamental coloration in common kestrels has a signalling function, similar to that found in other raptor species (Blas *et al.*, 2013; Martínez-Padilla *et al.*, 2013). Furthermore, previous studies on other raptor species have highlighted a main role for the environment in mediating carotenoid-based skin coloration (Sternalski *et al.*, 2010), and Casagrande *et al.* (2009) showed that skin carotenoid coloration in kestrel nestlings is strongly environment dependent and found no evidence for a genetic component. However, patterns of additive variation in sexual traits can change with age (Robinson *et al.*, 2008), and further work focusing on adult coloration is needed to better understand how sexual selection shapes carotenoid-based coloration.

Here, we provide insight into the quantitative genetic basis of a dynamic carotenoid-based ornament (namely skin coloration) in breeding adult male and female common kestrels, as well as quantify the strength and shape of selection acting upon this trait. First, by applying an animal model approach to data from a long-term individual-based study, we estimate the additive genetic variance component for the chromatic (hue and chroma) and achromatic (brightness) components of colour (Kruuk, 2004). Second, we use regression-based methods to explore the relationship between hue, chroma and brightness of carotenoid-pigmented traits with reproductive proxies of fitness.

Materials and methods

Study area and general procedures

The study was conducted in the Campo Azálvaro region (central Spain), which is characterized by homogenous

and extensive grasslands where trees and tall bushes are scarce and most kestrels breed in nest boxes (Fargallo *et al.*, 2009). The study area, covering an area of 22 km^2 contains 62 nest boxes in which a total of about 30–45 kestrel pairs breed each year (Fargallo *et al.*, 2009; Vergara *et al.*, 2009). From 1994 onwards, breeders and nestlings have been individually marked (see below for more details) and breeding parameters, including laying date, clutch size (CS) and the number of nestlings (at the age of 26 days, shortly before fledging), have been recorded. Adults were captured in the box during incubation (females) or during feeding when nestlings were 10–13 days old (both males and females) and body measurements (body mass and tarsus length) were recorded. At the age of 26 days, we recorded body mass and tarsus length for all nestlings, and we collected a blood sample for molecular sexing (see Fargallo *et al.*, 2002 for procedures). The age of unringed individuals born outside the study area was estimated from plumage characteristics (1 vs. > 1 year old, Vergara *et al.*, 2009).

Colour measurements

Following procedures previously described in Vergara & Fargallo (2011), we measured carotenoid-based eye ring coloration, which correlates with the coloration of other bare parts (i.e. cere and tarsi) in this species (Vergara & Fargallo, 2011). In 2005, only males were measured, whereas from 2007 until 2012, we measured both sexes. In short, we used a Nikon D70 digital camera (Nikon, Tokyo, Japan) (objective: 18–70 mm AF-S Nikkor DX) to photograph the eye ring, aiming the camera directly at the eye (i.e. at an angle of 90° between the objective and the surface of the eye), while keeping a distance of 50 cm. To keep variation in light conditions to a minimum, photos were taken under a sunshade in the morning. Digital images were further standardized with respect to light conditions using a grey scale (Kodak, New York, NY, USA) that was placed close to the trait. Using Photoshop CS3 (Adobe Systems Incorporated, California, USA), we selected the eye ring and determined hue (calculated in degrees around a 360° colour wheel, with red set at 0°), chroma (colour purity) and brightness (both on a scale from 0 to 100). Mean hue ± SE was 44.11 ± 0.18 (males: 43.55 ± 0.25, range: 38–49; females: 44.42 ± 0.24, range: 36–53), mean chroma was 69.21 ± 0.64 (males: 76.58 ± 0.74, range: 57–93; females: 65.32 ± 0.76, range: 32–87) and mean brightness was 71.08 ± 0.53 (males: 71.84± 0.93, range: 55–95; females: 70.68 ± 0.64, range: 40–88). All colour measurements have been shown to be highly repeatable (see Vergara & Fargallo, 2011 for further details and repeatability).

Selection analyses

We used standard methods to estimate selection gradients, regressing relative fitness, based on either CS or number of fledging (NF), against chroma, hue or brightness (Lande & Arnold, 1983; Arnold & Wade, 1984). We calculated relative fitness by dividing an individual's CS or NF by the mean for that year. We used both CS and NF as proxies for fitness because they may help distinguishing between the different selective forces acting on components of coloration.

In common kestrels, CS is influenced by female condition, which as females are fed by their mates from courtship until halfway through of the nestling period (Village, 1990), and is highly dependent on characteristics of her partner (Aparicio, 1994; Martinez-Padilla *et al.*, 2010a). Whereas CS largely captures variation in female fecundity, NF also captures variation in offspring survival between hatching and fledging. This variation may be substantial, as it will be shaped by the environmental condition after clutch completion, parental effort and ability, as well as predation.

As CS and NF are proxies of fitness related to the pair, colour traits (hue, saturation or brightness) of both the male and female were included as explanatory covariates in the model. As shown below (Results – Selection Analyses), colour traits were not correlated between males and females. Colour traits were z-transformed to have a mean of 0 and a standard deviation of 1 for both sexes separately. Standardized selection gradients (β) were estimated as the slope of the regression of relative fitness against the standardized trait values. To obtain nonlinear selection gradients (γ), squared trait values were included in addition to the linear term. The coefficients for the squared terms were doubled to calculate nonlinear selection gradients (Stinchcombe *et al.*, 2008). Both directional and nonlinear selection gradients were estimated using linear mixed effect models with male and female identity included as random effects to account for repeated measures on the same individual (Husby *et al.*, 2011).

Quantitative genetic analyses

To estimate genetic and environmental variance components for the chromatic (hue and chroma) and achromatic (brightness) variation in eye ring coloration, we first fitted a set of univariate animal models using restricted maximum likelihood (REML) in ASReml-R v3.0 (Butler, 2009). The estimation of the additive genetic variance made use of pedigree information for more than 2700 marked individuals. Siblings from the same brood for which paternal and/or maternal identity was unknown were assigned a common 'dummy' parental identity to preserve sibship information (Kim

et al., 2013). Mean maternal and paternal sibship sizes in the pedigree were 6.41 and 4.96, respectively. Full details of the pedigree statistics can be found in Kim *et al.* (2013). The rate of extra-pair paternity in our study population as well as in other kestrel populations is below 5% (J.A. Fargallo, J. Martínez-Padilla & P. Vergara unpublished data; Korpimäki *et al.*, 1996), suggesting that extra-pair paternity is unlikely to influence our estimates of additive genetic variance of the traits considered (Charmantier & Réale, 2005).

The univariate animal models for each trait (hue, chroma and brightness) included age class as a fixed effect with two levels to account for a difference in eye ring coloration between 1- and > 1-year-old individuals (see Results). Similarly, the sex of the individual was fitted as a fixed effect with two levels. Finally, date of capture was fitted as a covariate, expressed as the number of days since the 1st of March. In addition to the random additive genetic animal effect (a_i), which uses the phenotypic resemblance among related individuals to provide an estimate of the additive genetic variance, we included a permanent environment (pe_i), which accounts for the nonindependence of repeated measures made on the same individual in different years due to constant differences in the environmental conditions they experience throughout their lives (Kruuk, 2004). Finally, a year effect (y_i) quantifies the covariance among individuals living in the same year and experiencing similar environmental conditions (e.g. food abundance, weather conditions, etc.). As detailed previously, some females were captured twice within the same year. In those cases, we only included data from the first capture (i.e. coloration during incubation).

The variance explained by the additive genetic (a_i), permanent environment (pe_i), year (y_i) and residual (e_i) terms (V_A, V_{PE}, V_Y and V_R, respectively) were estimated using REML. Total phenotypic variance (V_P) was calculated as: $V_P = V_A + V_{PE} + V_Y + V_R$. Heritability was calculated as: $h^2 = V_A/V_P = V_A/(V_A + V_{PE} + V_Y + V_R)$. The statistical significance of each variance component was assessed using a likelihood ratio test (LRT), which assumes −2 times the difference in REML log-likelihood scores between a model with and without the random effect of interest following a χ^2 distribution with one degree of freedom.

Finally, we estimated phenotypic and genetic correlations (r_P and r_A, respectively) among colour components, as well as genetic correlations between male and female coloration. For the latter, we fixed the residual covariation between the male and the female trait to 0. In a last step, we explored genetic correlations between the three colour traits on the one hand and the two fitness-related traits on the other. Covariances among traits with near-zero variances were fixed at zero to facilitate model convergence.

Results

Phenotypic relationships among colour components

In males, chroma correlated negatively with hue ($r_P = -0.349$, $P < 0.001$, $n = 104$) and brightness ($r_P = -0.201$, $P = 0.004$, $n = 104$). Hue and brightness were not significantly correlated ($r_P = 0.168$, $P = 0.097$, $n = 104$). In females, hue and chroma were negatively correlated as well ($r_P = -0.274$, $P = 0.003$, $n = 100$), but neither hue nor brightness ($r_P = -0.060$, $P = 0.377$, $n = 100$) nor chroma and brightness ($r = -0.029$, $P = 0.573$, $n = 100$) were correlated.

Age- and sex-dependence of carotenoid-based coloration

Chroma differed between the sexes ($F_{1,176} = 65.76$, $P < 0.001$) and between age classes ($F_{1,63} = 21.09$, $P < 0.001$) and decreased with capture date ($b = -0.019 \pm 0.004$, $F_{1,62} = 23.38$, $P < 0.001$). Interactions among these variables were not statistically significant (all $P > 0.325$). Specifically, males had higher chroma (76.57 ± 0.74) than females (65.59 ± 0.88). Also, old individuals showed higher chroma (71.92 ± 0.67; males: 77.23 ± 0.77, females: 67.50 ± 0.85) than yearlings (64.84 ± 1.79; males: 73.63 ± 2.02, females: 60.32 ± 2.18).

Hue did not differ between sexes ($F_{1,176} = 0.11$, $P = 0.738$), but did differ among age classes ($F_{1,63} = 9.36$, $P = 0.003$). It also increased during the course of the season (estimate: 0.017 ± 0.005, $F_{1,63} = 13.10$, $P < 0.001$). Old individuals had lower hue (43.56 ± 0.22) than 1-year-old birds (44.82 ± 0.43). Finally, brightness was not influenced by sex, age or capture date (all $P > 0.323$).

Selection analyses

We first explored the correlation between male and female colour traits, which would be suggestive of assortative mating with respect to ornament coloration. However, we did not find evidence for a correlation between male and female hue, chroma or brightness (Hue, estimate: 0.173 ± 0.129, $R^2 = 0.229$, $P = 0.187$; Chroma, estimate: 0.224 ± 0.119, $R^2 = 0.094$, $P = 0.073$; Brightness, estimate: 0.083 ± 0.108, $R^2 = 0.068$, $P = 0.187$; $P > 0.443$, $n = 74$ in all cases). We found a significantly negative directional selection gradient for female hue when using either CS or NF as a proxy of fitness, whereas there was no effect of male hue. Whereas there was also no association between male chroma and either relative CS or NF, we found positive directional selection on female chroma. Finally, there was no association between either of the two fitness components and brightness, in either males or females. For more details, see Table 1 and Fig. 1.

Table 1 Selection on carotenoid-pigmented traits in breeding common kestrels. Results of general linear mixed models estimating directional (β) and nonlinear selection (γ) between coloration components (i.e. hue, chroma and brightness) and fitness components (response variables). Individual identity was included as random factor.

	Clutch size		Number of fledglings	
Trait	$\beta \pm$ SE	$\gamma \pm$ SE	$\beta \pm$ SE	$\gamma \pm$ SE
Hue male	0.003 ± 0.016	0.004 ± 0.014	0.024 ± 0.052	0.051 ± 0.043
Hue female	−0.071 ± 0.018**	−0.020 ± 0.016	−0.124 ± 0.057*	−0.059 ± 0.052
Chroma male	−0.004 ± 0.018	−0.021 ± 0.015	−0.048 ± 0.054	−0.009 ± 0.047
Chroma female	0.043 ± 0.019*	0.021 ± 0.015	0.129 ± 0.058*	0.018 ± 0.047
Brightness male	−0.015 ± 0.018	−0.012 ± 0.018	0.049 ± 0.053	−0.018 ± 0.054
Brightness female	−0.008 ± 0.017	−0.008 ± 0.012	−0.066 ± 0.049	−0.027 ± 0.035

**$P < 0.001$ and *$P < 0.01$.

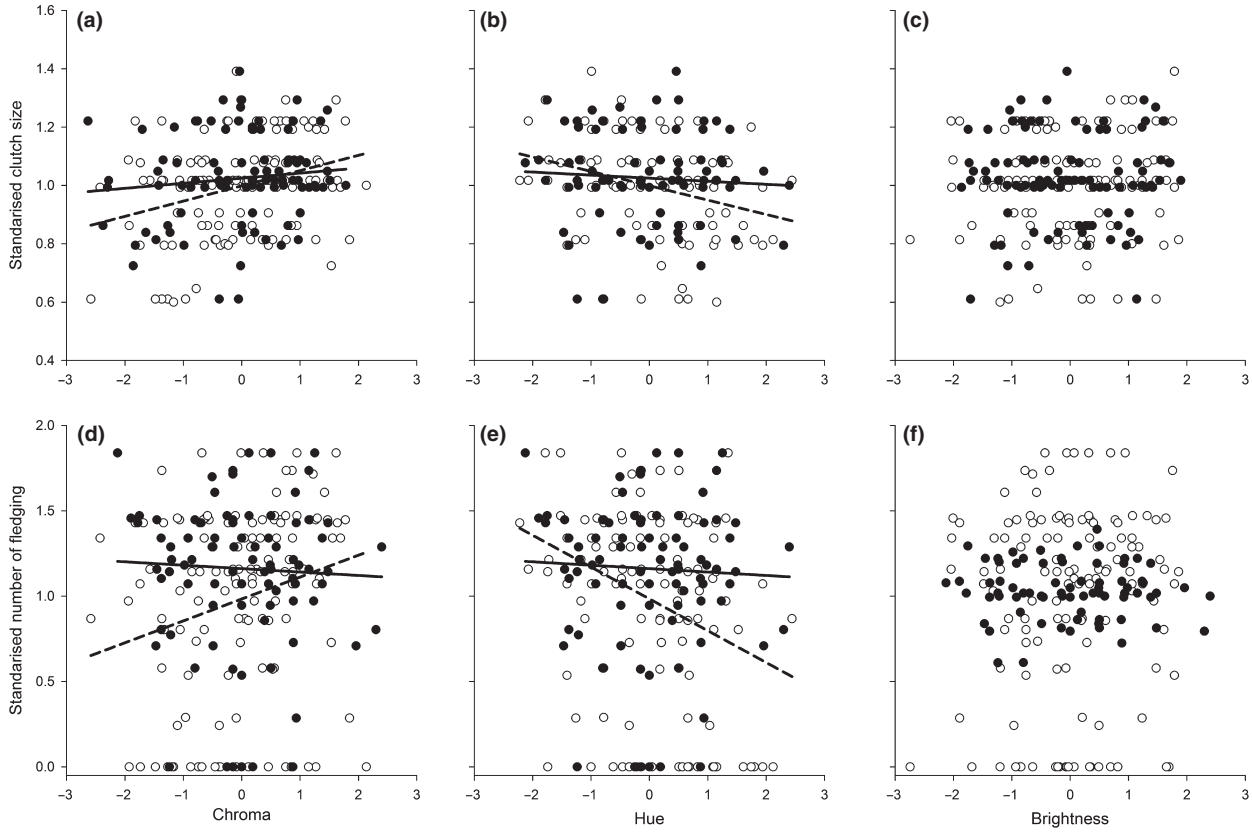

Fig. 1 Selection on components of eye ring coloration in breeding adult Eurasian kestrels. Broken lines and empty dots denote females, and black dots and continuous lines denote males. We find positive directional selection on female chroma using clutch size (CS) (a) and number of fledglings (d) as proxies of fitness. Similarly, we find negative directional selection on hue using CS (b) and number of fledging (e) as proxies of fitness only in females. Finally, we did not find any association between clutch size (c) or number of fledglings (f) and brightness.

Quantitative genetics of carotenoid-based coloration

Univariate animal models combining coloration for both sexes showed significant additive variance for some components of eye ring coloration (Table 2). The h^2 of chroma, after correcting for fixed effects, was 0.40 ± 0.17, whereas hue and brightness showed lower or nonestimable additive genetic variance (Table 2). We found that V_Y was statistically significant for hue and

Table 2 Quantitative genetics of eye ring coloration in male and female adults of kestrels from univariate animal models. The models were constructed as follows: $trait_i = \mu + \text{fixed effects}_i + a_i + pe_i + y_i + e_i$ (see text for more details). Models included males ($n_{individuals}$ = 78, $n_{records}$ = 104) and females ($n_{individuals}$ = 100, $n_{records}$ = 139). Significant terms are highlighted in bold. Column 'Test' denotes the ratio between the estimates of a given variable to its standard error.

Variable	h^2	Estimate ± SE	Test	d.f.	*F*	*P*
Hue	n.e.					
Fixed effects ($n_{records}$ = 243; $n_{individuals}$ = 178)						
Intercept		**37.92 ± 1.63**	23.16	1,5.7	801.40	<0.001
Age		**−1.00 ± 0.42**	−2.41	1,235.1	5.832	0.002
Sex		**−1.15 ± 0.47**	−2.48	1,177.6	6.167	0.001
Capture date		**0.07 ± 0.016**	4.38	1,237.2	19.20	<0.001
Random effects						
V_A		$2.61 \times e^{-6} \pm 4.0 \times e^{-7}$	6.54			n.e.
V_{PE}		0.19 ± 0.84	0.22			0.832
V_Y		**2.88 ± 1.84**	**1.56**			**<0.001**
V_R		6.74 ± 1.03	6.54			
Chroma	**0.40 ± 0.17**					
Fixed effects ($n_{records}$ = 243; $n_{individuals}$ = 178)						
Intercept		**84.08 ± 4.116**	**20.42**	**1,11.6**	**406.60**	**<0.001**
Age		**4.65 ± 1.197**	**3.88**	**1,215.9**	**15.09**	**<0.001**
Sex		**15.66 ± 1.475**	**10.62**	**1,187.3**	**112.80**	**<0.001**
Capture date		**−0.25 ± 0.043**	**−6.04**	**1,112.9**	**36.51**	**<0.001**
Random effects						
V_A		**29.44 ± 13.81**	**2.13**			**0.018**
V_{PE}		9.29 ± 12.58	0.74			0.411
V_Y		1.42 ± 1.88	0.76			0.264
V_R		33.26 ± 5.89	5.64			
Brightness	0.02 ± 0.05					
Fixed effects ($n_{records}$ = 243; $n_{individuals}$ = 178)						
Intercept		**68.30 ± 5.14**	**13.28**	**1,6.0**	**219.00**	**<0.001**
Age		1.09 ± 1.22	0.88	1,234.2	0.782	0.377
Sex		**2.94 ± 1.38**	**2.13**	**1,204.9**	**4.546**	**0.003**
Capture date		−0.01 ± 0.04	−0.30	1,238.7	0.093	0.761
Random effects						
V_A		2.34 ± 5.06	0.46			0.594
V_{PE}		$2.41 \times e^{-5} \pm 2.97 \times e^{-06}$	8.10			n.e
V_Y		**43.79 ± 26.69**	**1.64**			**<0.001**
V_R		58.35 ± 7.20	8.10			

n.e., nonestimable.

brightness, but not for chroma (Table 2). In all cases, V_{PE} was low (average number of repeated measures per individual is shown in Table 2).

Intersexual and fitness-based genetic correlations

We found that the intersexual genetic covariation of chroma between males and females ($COV_A \pm SE$) was 15.387 ± 17.214 (r_A = 0.461 ± 0.508) and not statistically significant (Z_{ratio} = 0.882, P = 0.998). Similarly, the intersexual genetic covariation for brightness was 0.545 ± 12.973 (r_A = 0.316 ± 8.058) and again not statistically significant (Z_{ratio} = 0.042, P = 0.771). The intersexual correlation for hue could not be estimated. Finally, we did not find a genetic correlation between brightness or chroma and any proxy of fitness considered. However, it should be noted that all these analyses suffer from low power due to the relatively small sample sizes. Indeed, we were not able to estimate intersexual genetic correlations of hue or genetic correlations between hue and fitness because models did not converge. For all details, see Table 3.

Table 3 Genetic correlations and covariances between colour traits (chroma and brightness of the eye ring) and fitness components in adults of common kestrels from bivariate animal models.

	$Cov_A \pm SE$	Genetic correlation	*P*
Chroma and CS	−1.120 ± 0.719	−0.673 ± 0.581	1
Chroma and NF	−1.171 ± 1.401	−0.952 ± 4.055	0.125
Brightness and CS	n.e.	n.e	n.e
Brightness and NF	−1.234 ± 1.460	−0.429 ± 0.591	0.059

CS, clutch size; NF, number of fledglings; n.e.: nonestimable.

Discussion

Combining colour measures of adult common kestrels, collected over 7 years, with a social pedigree constructed over 19 years of monitoring, we report a case of significant additive genetic variance in a dynamic, carotenoid-based ornament in a wild bird. In particular, we show that heritability of the chroma (i.e. purity) of the orange-yellow skin coloration is moderately high (i.e. $h^2 = 0.40 \pm 0.17$). On the other hand, the other two components of skin coloration, hue and brightness, showed very low levels of additive variance, suggesting that their expression is mostly environmentally mediated. As it has previously been shown in other bird species that chroma can be considered a proxy of carotenoid content in yellow traits (Peters *et al.*, 2012; Freeman-Gallant *et al.*, 2014; Tonra *et al.*, 2014), our results reinforce the idea that despite their environmental dependency (Martinez-Padilla *et al.*, 2010b), dynamic, carotenoid-based signals may have a significant genetic component (Velando *et al.*, 2006; Evans & Sheldon, 2012). This is likely to be mediated by additive genetic variation in those aspects of behaviour (e.g. foraging) and/or physiology (e.g. transportation and or deposition into tissues) associated with the expression of carotenoid-based coloration (Pérez-Rodríguez, 2008).

As the models used to estimate additive genetic variances included sex as fixed effect, estimates of additive genetic variance of colour traits are corrected for differences in the mean between the sexes. However, although we lack the statistical power to obtain a meaningful estimate of the intersexual genetic correlation for the three colour components, it is possible that the genetic architecture of male and female yellow coloration differs to some degree. Further studies analysing intersexual relationships (Potti & Canal, 2010), as well as a deeper understanding of the genetic architecture of the trait within each sex (Wright *et al.*, 2007) is needed.

As carotenoids can be metabolized and their biochemical properties can be modified when deposited in fleshy or feather-based ornaments, we cannot quantify the selective forces acting on carotenoid type or level. Instead, we estimated the strength and shape of selection acting upon the expression of carotenoid-based sexual signals. We found that patterns of selection differed not only with respect to which aspect of colour we looked at, but also with respect to the sex of the bird. Specifically, there was negative directional selection on hue and positive directional selection on chroma when using either number of fledglings or CS as a proxy of fitness, but only in females. This suggests that natural selection favours females showing low hue and high chroma. Indeed, these two colour components are negatively correlated at the phenotypic level.

Contributing to the long-lasting debate over the function and evolution of female ornamentation (Amundsen, 2000; Tobias *et al.*, 2012), our results suggest that hue and chroma are reliable indices of individual fitness and thus quality in females, but not in males. This might be explained by a fine-tuned trade-off between allocating carotenoids to ornament expression and reproduction (Blount *et al.*, 2003, 2004; Morales *et al.*, 2009). However, when fecundity trades off with ornament expression, theory predicts stabilizing rather than directional selection on the expression of sexual displays in females, for which we find no evidence here (Fitzpatrick *et al.*, 1995; Chenoweth *et al.*, 2006; Wheeler *et al.*, 2012). Irrespective of the underlying mechanism, our results suggest that in a mate choice context, males may benefit from choosing females with lower hue and higher chroma to increase their fitness. The latter is in agreement with the idea that mate choice is more symmetrical than is often believed (Martinez-Padilla *et al.*, 2012).

Our findings are also relevant to the controversy surrounding the relative importance of genetic vs. nongenetic models of sexual selection (Qvarnström *et al.*, 2006). We found that eye ring chroma was heritable and that females showing a more saturated colour (chroma) on average produce more offspring. Hence, choosing a partner with high chroma could provide indirect benefits. Although this would require a genetic correlation between ornamentation and fitness (Qvarnström *et al.*, 2006), for which we find no evidence, the latter may be due to small sample sizes and hence very low statistical power. On the other hand, we did find an association between hue and fitness, but hue is not heritable. This suggests that different characteristics (chroma and hue) of the same signal may support both genetic and nongenetic models of sexual selection.

Conclusion

Exploring the evolutionary meaning of sexual signals requires insight into their potential to evolve and thus the quantification of additive genetic variances. This has rarely been performed for carotenoid-pigmented traits. Our study reinforces the idea that despite their tight environmental dependence, some components (i.e. chroma) of dynamic carotenoid-based coloration show heritable variation, whereas others do not (hue and brightness). Furthermore, our findings highlight the complexity of the evolutionary meaning of a single carotenoid-pigmented trait when hue, brightness and chroma are studied separately, providing support for both direct and indirect models of sexual selection regarding carotenoid-based coloration. We argue that for full comprehension of the evolutionary meaning of carotenoid-based sexual traits, we need more studies exploring their evolutionary potential in wild conditions.

Acknowledgments

Thanks to the Finat family and Juan San Teodoro for their collaboration in our studies. The authors deeply thank Dr. E. Postma and Prof. M. Jennions for their support, help and collaboration to greatly improve the very first version of the manuscript. I. López-Rull, J. Navarro and D. Lopez-Idiaquez helped in the field. PV and JMP were supported by a Juan de la Cierva and a JAE-Doc fellowships, respectively. This study was financed by the Spanish Ministerio de Ciencia e Innovación (Projects: CGL2004-04479/BOS; CGL2010-15726/BOS). This is a contribution of the El Ventorrillo Field Station (CSIC).

References

Amundsen, T. 2000. Why are female birds ornamented? *Trends Ecol. Evol.* **15**: 149–155.

Aparicio, J.M. 1994. The seasonal decline in clutch size: an experiment with supplementary food in the Kestrel, *Falco tinnunculus*. *Oikos* **25**: 451–458.

Arnold, S.J. & Wade, M.J. 1984. On the measurement of natural and sexual selection: theory. *Evolution* **38**: 709–719.

Badyaev, A.V. & Hill, G.E. 2002. Paternal care as a conditional strategy: distinct reproductive tactics associated with elaboration of plumage ornamentation in the house finch. *Behav. Ecol.* **13**: 591–597.

Blas, J., Cabezas, S., Figuerola, J., López, L., Tanferna, A., Hiraldo, F. *et al.* 2013. Carotenoids and skin coloration in a social raptor. *J. Raptor Res.* **47**: 174–184.

Blount, J.D., Metcalfe, N.B., Birkhead, T.R. & Surai, P.F. 2003. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science* **300**: 125–127.

Blount, J.D., Houston, D.C., Surai, P.F. & Møller, A.P. 2004. Egg-laying capacity is limited by carotenoid pigment availability in wild gulls *Larus fuscus*. *Proc. R. Soc. Lond. B* **271**: S79–S81.

Boughman, J.W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* **411**: 944–948.

Butler, D. 2009. Asreml fits the linear mixed model. R package version 3.0.

Casagrande, S., Csermely, D., Pini, E., Bertacche, V. & Tagliavini, J. 2006. Skin carotenoid concentration correlates with male hunting skill and territory quality in the kestrel *Falco tinnunculus*. *J. Avian Biol.* **37**: 190–196.

Casagrande, S., Costantini, D., Tagliavini, J. & Dell'Omo, G. 2009. Phenotypic, genetic, and environmental causes of variation in yellow skin pigmentation and serum carotenoids in Eurasian kestrel nestlings. *Ecol. Res.* **24**: 273–279.

Charmantier, A. & Réale, D. 2005. How do misassigned paternities affect the estimation of heritability in the wild? *Mol. Ecol.* **14**: 2839–2850.

Chenoweth, S.F., Doughty, P. & Kokko, H. 2006. Can non-directional male mating preferences facilitate honest female ornamentation? *Ecol. Lett.* **9**: 179–184.

Endler, J.A. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol. Evol.* **10**: 22–29.

Evans, S.R. & Sheldon, B.C. 2012. Quantitative genetics of a carotenoid-based color: heritability and persistent natal environmental effects in the great tit. *Am. Nat.* **179**: 79–94.

Fargallo, J.A., Laaksonen, T., Pöyri, V. & Korpimäki, E. 2002. Inter-sexual differences in the immune response of Eurasian kestrel nestlings under food shortage. *Ecol. Lett.* **5**: 95–101.

Fargallo, J.A., Laaksonen, T., Korpimäki, E. & Wakamatsu, K. 2007. A melanin-based trait reflects environmental growth conditions of nestling male Eurasian kestrels. *Evol. Ecol.* **21**: 157–171.

Fargallo, J.A., Martinez-Padilla, J., Vinuela, J., Blanco, G., Torre, I., Vergara, P. *et al.* 2009. Kestrel-prey dynamic in a Mediterranean region: the effect of generalist predation and climatic factors. *PLoS ONE* **4**: e4311.

Fitze, P.S., Kolliker, M. & Richner, H. 2003. Effects of common origin and common environment on nestling plumage coloration in the great tit (*Parus major*). *Evolution* **57**: 144–150.

Fitzpatrick, S., Berglund, A. & Rosenqvist, G. 1995. Ornaments or offspring: costs to reproductive success restrict sexual selection processes. *Biol. J. Linn. Soc.* **55**: 251–260.

Freeman-Gallant, C.R., Schneider, R.L., Taff, C.C., Dunn, P.O. & Whittingham, L.A. 2014. Contrasting patterns of selection on the size and coloration of a female plumage ornament in common yellowthroats. *J. Evol. Biol.* **27**: 982–991.

Giraudeau, M., Duval, C., Czirják, G.Á., Bretagnolle, V., Eraud, C., McGraw, K.J. *et al.* 2011. Maternal investment of female mallards is influenced by male carotenoid-based coloration. *Proc. R. Soc. B Biol. Sci.* **278**: 781–788.

Hadfield, J.D., Burgess, M.D., Lord, A., Phillimore, A.B., Cleg, S.M. & Ownes, P.F. 2006. Direct versus indirect sexual selection: genetic basis of colour, size and recruitment in a wild bird. *Proc. R. Soc. B Biol. Sci.* **273**: 1347–1353.

Hadfield, J.D., Nutall, A., Osorio, D. & Owens, I.P.F. 2007. Testing the phenotypic gambit: phenotypic, genetic and environmental correlations of colour. *J. Evol. Biol.* **20**: 549–557.

Helfenstein, F., Losdat, S., Møller, A.P., Blount, J.D. & Richner, H. 2010. Sperm of colourful males are better protected against oxidative stress. *Ecol. Lett.* **13**: 213–222.

Hill, G.E. 2006. Environmental regulation of ornamental coloration. In: *Bird Coloration*, Vol. **I** (G.E. Hill & K.J. McGraw, eds), pp. 507–560. Harvard University Press, Cambridge.

Hill, G.E. & McGraw, K.J. 2006. *Bird Coloration. Function and Evolution*. Harvard University Press, London.

Horak, P., Ots, I., Vellau, H., Spottiswoode, C. & Møller, A.P. 2001. Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding great tits. *Oecologia* **126**: 166–173.

Husby, A., Visser, M.E. & Kruuk, L.E. 2011. Speeding up microevolution: the effects of increasing temperature on selection and genetic variance in a wild bird population. *PLoS Biol.* **9**: e1000585.

Johnsen, A., Delhey, K., Andersson, S. & Kempenaers, B. 2003. Plumage colour in nestling blue tits: sexual dichromatism, condition dependence and genetic effects. *Proc. R. Soc. Lond. B Biol. Sci.* **270**: 1263–1270.

Kim, S.Y., Fargallo, J.A., Vergara, P. & Martinez-Padilla, J. 2013. Multivariate heredity of melanin-based coloration, body mass and immunity. *Heredity* **111**: 139–146.

Korpimäki, E., Lahti, K., May, C.A., Parkin, D.T., Powell, G.B., Tolonen, P. *et al.* 1996. Copulatory behaviour and paternity determined by DNA fingerprinting in kestrels: effects of cyclic food abundance. *Anim. Behav.* **51**: 945–955.

Kruuk, L.E.B. 2004. Estimating genetic parameters in natural populations using the 'animal model'. *Philos. Trans. R. Soc. B Biol. Sci.* **359**: 873–890.

Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.

Maan, M.E. & Cummings, M.E. 2009. Sexual dimorphism and directional sexual selection on aposematic signals in a poison frog. *Proc. Natl. Acad. Sci. USA* **106**: 19072–19077.

Martinez-Padilla, J., Mougeot, F., Perez-Rodriguez, L. & Bortolotti, G.R. 2007. Nematode parasites reduce carotenoid-based signalling in male red grouse. *Biol. Lett.* **3**: 161–164.

Martinez-Padilla, J., Dixon, H., Vergara, P., Perez-Rodriguez, L. & Fargallo, J.A. 2010a. Does egg colouration reflect male condition in birds? *Naturwissenschaften* **97**: 469–477.

Martinez-Padilla, J., Mougeot, F., Webster, L.M.I., Perez-Rodriguez, L. & Piertney, S.B. 2010b. Testing the interactive effects of testosterone and parasites on carotenoid-based ornamentation in a wild bird. *J. Evol. Biol.* **23**: 902–913.

Martinez-Padilla, J., Vergara, P., Mougeot, F. & Redpath, S.M. 2012. Parasitized mates increase infection risk for partners. *Am. Nat.* **179**: 811–820.

Martínez-Padilla, J., Mougeot, F., García, J.T., Arroyo, B. & Bortolotti, G.R. 2013. Feather corticosterone levels and carotenoid-based coloration in Common Buzzard (*Buteo buteo*) nestlings. *J. Raptor Res.* **47**: 161–173.

McGraw, K.J. 2006. Mechanics of carotenoid-based coloration. In: *Bird Coloration*, Vol. **I** (G.E. Hill & K.J. McGraw, eds), pp. 177–242. Harvard University Press, Cambridge.

Mehlis, M., Hilke, L.K. & Bakker, T.C.M. 2013. Attractive males have faster sperm in three-spined sticklebacks *Gasterosteus aculeatus*. *Curr. Zool.* **59**: 761–768.

Morales, J., Velando, A., Torres, R., Animal, L.D.C. & Vigo, U.D. 2009. Fecundity compromises attractiveness when pigments are scarce. *Behav. Ecol.* **20**: 117–123.

Mougeot, F. 2008. Ornamental comb colour predicts T-cell-mediated immunity in male red grouse *Lagopus lagopus* scoticus. *Naturwissenschaften* **95**: 125–132.

Mougeot, F., Martinez-Padilla, J., Blount, J.D., Perez-Rodriguez, L., Webster, L.M.I. & Piertney, S.B. 2010a. Oxidative stress and the effect of parasites on a carotenoid-based ornament. *J. Exp. Biol.* **213**: 400–407.

Mougeot, F., Martinez-Padilla, J., Bortolotti, G.R., Webster, L.M.I. & Piertney, S.B. 2010b. Physiological stress links parasites to carotenoid-based colour signals. *J. Evol. Biol.* **23**: 643–650.

Mundy, N.I. 2006. Genetic basis of color variation in wild birds. In: *Bird Coloration: Mechanisms and Measurements* (G.E. Hill & K.J. McGraw, eds), pp. 469–506. Harvard University Press, Cambridge.

Nordeide, J.T., Kekäläinen, J., Janhunen, M. & Kortet, R. 2013. Female ornaments revisited – are they correlated with offspring quality? *J. Anim. Ecol.* **82**: 26–38.

Olson, V.A. & Owens, I.P.F. 2005. Interspecific variation in the use of carotenoid-based coloration in birds: diet, life history and phylogeny. *J. Evol. Biol.* **18**: 1534–1546.

Palokangas, P., Korpimäki, E., Hakkarainen, H., Huhta, E., Tolonen, P. & Alatalo, R.V. 1994. Female kestrels gain reproductive success by choosing brightly ornamented males. *Anim. Behav.* **47**: 443–448.

Pérez-Rodríguez, L. 2008. Carotenoid-based ornamentation as a dynamic but consistent individual trait. *Behav. Ecol. Sociobiol.* **62**: 995–1005.

Peters, A., Roberts, M.L., Kurvers, R.H.J.M. & Delhey, K. 2012. Testosterone treatment can increase circulating carotenoids but does not affect yellow carotenoid-based plumage colour in blue tits *Cyanistes caeruleus*. *J. Avian Biol.* **43**: 1–7.

Potti, J. & Canal, D. 2010. Heritability and genetic correlation between the sexes in a songbird sexual ornament. *Heredity* **106**: 945–954.

Qvarnström, A., Brommer, J.E. & Gustafsson, L. 2006. Testing the genetics underlying the co-evolution of mate choice and ornament in the wild. *Nature* **441**: 84–86.

Robinson, M.R., Pilkington, J.G., Clutton-Brock, T.H., Pemberton, J.M. & Kruuk, L.E.B. 2008. Environmental heterogeneity generates fluctuating selection on a secondary sexual trait. *Curr. Biol.* **18**: 751–757.

Roulin, A. & Dijkstra, C. 2003. Genetic and environmental components of variation in eumelanin and phaeomelanin sex-traits in the barn owl. *Heredity* **90**: 359–364.

Roulin, A. & Ducrest, A.-L. 2013. Genetics of colouration in birds. *Semin. Cell Dev. Biol.* **24**: 594–608.

Roulin, A., Altwegg, R., Jensen, H., Steinsland, I. & Schaub, M. 2010. Sex-dependent selection on an autosomal melanic female ornament promotes the evolution of sex ratio bias. *Ecol. Lett.* **13**: 616–626.

Simons, M.J.P., Briga, M., Koetsier, E., Folkertsma, R., Wubs, M.D., Dijkstra, C. *et al.* 2012. Bill redness is positively associated with reproduction and survival in male and female zebra finches. *PLoS ONE* **7**: e40721.

Sternalski, A., Mougeot, F., Eraud, C., Gangloff, B., Villers, A. & Bretagnolle, V. 2010. Carotenoids in nestling Montagu's harriers: variations according to age, sex, body condition and evidence for diet-related limitations. *J. Comp. Physiol. B* **180**: 33–43.

Stinchcombe, J.R., Agrawal, A.F., Hohenlohe, P.A., Arnold, S.J. & Blows, M.W. 2008. Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? *Evolution* **62**: 2435–2440.

Tobias, J.A., Montgomerie, R. & Lyon, B.E. 2012. The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Philos. Trans. R. Soc. B Biol. Sci.* **367**: 2274–2293.

Tonra, C.M., Marini, K.L.D., Marra, P.P., Germain, R.R., Holberton, R.L. & Reudink, M.W. 2014. Color expression in experimentally regrown feathers of an overwintering migratory bird: implications for signaling and seasonal interactions. *Ecol. Evol.* **4**: 1222–1232.

Velando, A., Beamonte-Barrientos, R. & Torres, R. 2006. Pigment-based skin colour in the blue-footed booby: an honest signal of current condition used by females to adjust reproductive investment. *Oecologia* **149**: 535–542.

Vergara, P. & Fargallo, J.A. 2011. Multiple coloured ornaments in male common kestrels: different mechanisms to convey quality. *Naturwissenschaften* **98**: 289–298.

Vergara, P., Fargallo, J.A., Martinez-Padilla, J. & Lemus, J.A. 2009. Inter-annual variation and information content of melanin-based coloration in female Eurasian kestrels. *Biol. J. Linn. Soc.* **97**: 781–790.

Village, A. 1990. *The Kestrel*. T & AD Poyser, London.

Wheeler, J., Gwynne, D.T. & Bussière, L.F. 2012. Stabilizing sexual selection for female ornaments in a dance fly. *J. Evol. Biol.* **25**: 1233–1242.

Wright, D., Kerje, S., Brandstrom, H., Schutz, K., Kindmark, A., Andersson, L. *et al.* 2007. The genetic architecture of a female sexual ornament. *Evolution* **62**: 86–98.

Received 12 November 2014; accepted 12 November 2014