

Stefania Casagrande · David Costantini
James Tagliavini · Giacomo Dell’Omo

Phenotypic, genetic, and environmental causes of variation in yellow skin pigmentation and serum carotenoids in Eurasian kestrel nestlings

Received: 9 November 2007 / Accepted: 26 March 2008 / Published online: 16 May 2008
© The Ecological Society of Japan 2008

Abstract In the context of sexual selection and parent-offspring communication, carotenoid-based coloration operates as a dynamic condition-dependent signal, as pigments stored in the skin and in the bill can be reallocated to other tissues in accordance with physiological needs. We studied the proximate factors affecting the carotenoid-dependent coloration of the Eurasian kestrel (*Falco tinnunculus*). Kestrel nestlings show carotenoid-based coloration at the integument level. Adult males and females share similar characteristics, but to a different extent. By cross-fostering nestlings, we evaluated the importance of the “nest of rearing” and the “nest of origin” to determine variation in skin color and blood carotenoids. The nest of rearing accounted for most of the observed variance in skin color, as well as serum carotenoids, while the nest of origin was not causal to the variability of carotenoids in young kestrels. The study indirectly shows that carotenoid-based color expressed by young kestrels is not affected by pre-laying conditions. Furthermore, we found that carotenoid coloration and blood carotenoid concentration were correlated at phenotypic and environmental levels, while the hereditary component of the carotenoid traits was too low to estimate their correlation at the genetic level.

Keywords Skin coloration · Carotenoids · Heritability · Environment · Cross-fostering · Proximate factors · Variance component · Kestrel · *Falco tinnunculus*

Introduction

The cost associated with the expression of carotenoid-based colorations has recently been hypothesized to increase the honesty of the signal in sexual selection (Hill 1999; Duckworth et al. 2004; reviewed by Olson and Owens 1998) or parent-offspring communication (Götmark and Ahlström 1997; Saino et al. 2000). Indeed, carotenoid-based coloration is costly because carotenoids are limited within the kestrel diet and the animals are unable to synthesize them de novo (e.g., Brush 1990). They are also used for several physiological functions, such as immune stimulation (Blount et al. 2003; Faivre et al. 2003; McGraw and Ardia 2003; Saino et al. 2003). Since carotenoids are used to meet physiological demands they are no longer available for the production of color, thus, only healthy individuals can allocate all the absorbed pigments for the production of a signal (Faivre et al. 2003).

Since carotenoids are absorbed only through the diet, carotenoid-based coloration may vary as a consequence of their availability (Linville and Breitwusch 1997; Bortolotti et al. 2000), as well as the individual’s foraging capability (Casagrande et al. 2006; see Møller et al. 2000 for a review). When looking among vertebrates at intra-specific level, diet accounts for most of the variation in the body concentration of carotenoids. Examples include the guppy *Poecilia reticulata* (Grether et al. 1999) and the Galápagos land iguana *Conolophus subcristatus* (Costantini et al. 2005b). Carotenoid intake can also explain the variation in plumage color in birds, for example in the American goldfinch *Carduelis tristis* (McGraw et al. 2002), or in the house finch *Carpodacus mexicanus* (Hill et al. 2002). However, carotenoid-based coloration is not always primarily explained by

S. Casagrande (✉)
Department of Behavioural Biology,
University of Groningen, PO Box 14,
9750AA Haren, Groningen, The Netherlands
E-mail: casagrande@biol.unipr.it
Tel.: +39-521-637000

D. Costantini
Dipartimento di Biologia Animale e dell’Uomo,
Università La Sapienza, Rome, Italy

J. Tagliavini
Dipartimento di Biologia Evolutiva e Funzionale,
Università di Parma, Parma, Italy

G. Dell’Omo
Ornis italica, Ornithological Association, Rome, Italy

carotenoid intake, as suggested by the color differences recorded in American kestrels *Falco sparverius* kept under the same diet (Negro et al. 2001). On the other hand, a recent survey on carotenoid expression at inter-specific level revealed in birds that the diet explains most of the variance in plumage color but not in the pigmentation of the bare parts, such as legs, lores, cere, combs, bill, or bill-knob (Olson and Owens 2005). The lack of association between diet and carotenoid-based coloration in the bare parts of birds suggests that the skin carotenoid-based coloration might harbor some genetic variance.

Most studies conducted thus far have attempted to explain the causal factors of color variations in carotenoid-based plumage (e.g., Figuerola et al. 1999; Hill 1999; Brawner III et al. 2000; Inouye 2001; Fitze et al. 2003; McGraw et al. 2003; Navara and Hill 2003; Pryke and Andersson 2003; Hill and McGraw 2004; Hōrak et al. 2004; Mays 2004). Less attention has been directed at the sources of color variation in the shield (Fenoglio et al. 2004), bill (Blount et al. 2003; Faivre et al. 2003; McGraw et al. 2003; Alonso-Alvarez et al. 2004; Bright et al. 2004) or skin (Negro et al. 2000; Bortolotti et al. 2003; Casagrande et al. 2006), in spite of the evidence showing that carotenoid pigmentations are more common in the bare parts than in the plumage, especially in non-passerine birds (Prum and Torres 2003).

Studies on skin-pigmentation or on the horny surface of the bill have shown that carotenoids can be assembled from tissues and re-allocated to enhance the activity of the immune system (Faivre et al. 2003), or to replace carotenoids in organs, such as the eyes (Alves-Rodrigues and Shao 2004). These characteristics give coloration a dynamic condition-dependent property.

The Eurasian kestrel *Falco tinnunculus* is a small falcon with yellow tarsi, lores and cere that absorbs lutein and zeaxanthin from prey, such as reptiles, insects, and birds (Costantini et al. 2005a, 2006; Casagrande et al. 2006). The coloration of males is more intense (redder) than that of females, despite similar diets and blood carotenoid concentrations (Casagrande et al. 2006). Nestling kestrels have pale yellow bare parts but similar blood carotenoid concentrations with respect to adults (Casagrande et al. 2007). A study investigating blood carotenoids in the related American kestrel has shown that the environmental component explains most of the variation of the blood carotenoid concentration in nestlings (Bortolotti et al. 2000). However, skin coloration was not considered in that study. Since carotenoids deposited in a signalling trait are affected by different metabolic processes than circulating carotenoids (Casagrande et al. 2007), estimating the components that determine such variance is also valuable. In a previous study, we found that there is significant inter-nest variation in both skin color and blood carotenoid concentration in kestrel nestlings (Casagrande et al. 2007). Since siblings of the same nest share both genetic and environmental characteristics, we investigated the phenotypic, genetic and environmental components of the

variation of carotenoid-based coloration and serum carotenoids in nestlings with a cross-fostering experiment to evaluate (1) whether body carotenoids express a main environmental component in a species having a large food niche and consistent individual feeding preferences (Costantini et al. 2005a); (2) whether the skin color (i.e., a sexually dimorphic trait in this species) shows heritable genetic variance, so as to better understand the role of a carotenoid-based coloration in an evolutionary context; (3) the phenotypic, genetic and environmental correlation between the color and the circulating carotenoids.

Materials and methods

Study area, field work and experimental procedure

The study was carried out during the 2003–2004 breeding seasons in a 1,200-km² area near Rome, where a population of kestrels (*Falco tinnunculus*) breeds in nest boxes mounted on the utility lines of two local electric power companies. The habitat was characterized mainly by cultivated and set-aside fields. The cultivations were predominantly agricultural and included olive plantations, vineyards, and cereal fields, whereas the set-asides were dry pastures interspersed with typical Mediterranean scrub vegetation. We carried out a cross-fostering experiment to evaluate the genetic and environmental components that indicate the variance of skin color and serum carotenoid levels. The nests were visited regularly during the entire breeding season in order to assess occupation and to record clutch size, hatching date, and brood size. Hatching dates were determined by visits to the nests in the pre-hatching period and were supported by growth curves (error ± 1 day). The nestlings were reciprocally cross-fostered when the oldest chick of the brood was 7 days old (the age of the other chicks in the brood ranged from 4 to 7 days). We used the same procedure for all the nests, which assured us also that all nests were in a same developmental phase. Nest pairs were matched according to hatching date and brood size. Exchanged nestlings were of the same age and of similar size. The cross-fostering did not change the original brood sizes. We also manipulated the remaining nestlings of the brood in a similar way to control for potential handling stress. We marked the chicks individually, first with a permanent marker then with aluminum rings, in order to recognize them during subsequent visits.

When the nestlings were 22 days old, skin color measurements and blood sampling were performed. The wing length was used as an index of age, while the skin color of the right tarsus (cleaned with water and air-dried) was measured with a hand-held spectrophotometer operating at wavelengths between 400 and 700 nm (Oracolor Corob, Modena). The color intensity was quantified by the hue value according to our previous studies (Casagrande et al. 2006, 2007; Costantini et al. 2007). Hue is considered to be an informative

colorimetric variable to describe carotenoid-based coloration (Hörak et al. 2001; Saks et al. 2003). Corob spectrophotometers automatically calculate the hue as a unitless value on a 360° color wheel, where the hue score decreases with an increase of redness. We took three consecutive measures per individual and, after checking for repeatability (hue $r = 0.80$, $P < 0.001$; Lessels and Boag 1987), a mean value was determined to be used in subsequent analysis. The data obtained with the spectrophotometer was analyzed with Corob Quality 1.0 software (Corob, Modena). Although, we did not consider the UV component of the color, we think that our findings are reliable since carotenoid pigments have a great subtractive action on the visible part of the spectrum, while the reflectance in the UV part is minimal. The total UV reflectance is usually caused by the pale background of the integument (Mougeot et al. 2007) and is not a product of the carotenoid pigmentation. Given these points, a covariance between some UV component and VIS components is expected, as shown in Mougeot et al. (2007), and the measurement error may have relatively little effect on the conclusions from studies using digital color meters (e.g., Jawor et al. 2004) or scoring the colored trait by reference to color charts (Eraud et al. 2007). Moreover, although kestrels can perceive UV colors (Koivula et al. 1999) and the consideration of the UV spectra is recommended (Montgomerie 2006), the study focused on determining which factors explain carotenoid deposition in the skin and blood and not on understanding the role of the color in the intra-specific communication.

To assess serum carotenoid concentration, a sample of blood (up to 400 μl) was drawn from the brachial vein. The samples were kept cool (0–5°C) until centrifugation, which occurred within a few hours. The serum was stored at –20°C.

Carotenoid analysis

Carotenoids were quantified with a Beckman DU 7400 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA) at 476 nm. The serum was diluted with methanol (1:8) and the flocculent proteins were precipitated by centrifugation to obtain the serum. The carotenoid concentration was estimated as $\mu\text{g mL}^{-1}$ of serum using the standard absorbance curve of lutein or alpha-carotene-3,3'-diol; Sigma-Aldrich, the predominant carotenoid in kestrels (Bortolotti et al. 2000; Casagrande et al. 2006).

Statistical analyses

Since the cross-fostering experiment was conducted during two subsequent breeding seasons, we evaluated the effect of the year on skin color and serum carotenoid concentration (considered in turn as dependent variables) with general linear models (GLMs). A one-way

ANOVA was computed considering nest as a random factor and year as a fixed factor.

The variance components of body carotenoids were estimated using a bivariate restricted maximum likelihood (REML) analysis, as outlined by Kruuk and Hadfield (2007) for the animal model. The genetic component was represented by the nest of origin in the model, which estimated the genetic transmission variance in a given trait, including pre-manipulation parental effects and a quarter of dominance variance. Two explicit environmental components were represented by the duplicate (a pair of nests matched for the cross-fostering of nestlings) and by the nest of rearing. The duplicate reflected the environmental variation between pairs of nests (e.g., time of season), while the nest of rearing reflected the environmental variation within a single nest. The nest of origin and the nest of rearing were nested within the duplicate. Nest of origin, rearing, and duplicate were considered as random factors. Error variance of the model equals all the remaining variation not considered in the model; the random environmental variation and the genetic variation not represented by the nest of origin and three-quarters of dominance variance (see Merila 1996 for further details). In agreement with Kruuk and Hadfield (2007), the phenotypic, genetic and environmental correlation between the color trait and circulating carotenoids were evaluated using the bivariate REML mixed model analysis, which considered the random factors previously described (for further details, see Hadfield et al. (2008)). The analyses were carried out with STATISTICA 6.0 (StatSoft 2001, Tulsa, USA) and ASReml 2.0 (Gilmour et al. 2002).

Results

The cross-fostering included 26 nests and 129 nestlings. Since it was not always possible to bleed the nestlings, two nests were excluded from the analyses of serum carotenoid concentration. Data gathered during the 2003 and 2004 breeding seasons did not differ in hue values ($F_{(1,127)} = 0.74$, $P > 0.05$; Table 1) or in serum carotenoid concentration ($F_{(1,110)} = 0.27$, $P > 0.05$; Table 1), thus data from the 2 years were pooled together. The manipulation produced no effect on either variable, as these did not differ between non-fostered and fostered nestlings (hue $t = 0.83$, $P > 0.05$; carotenoid concentration $t = 0.25$, $P > 0.05$).

The experiment showed that the expression of the carotenoid traits considered are greatly influenced by the nest of rearing, thus by the environment (Table 2a, b), while the nest of origin does not explain the variation of the skin hue, nor the carotenoid circulating in the blood (Table 2a, b).

From the bivariate analysis, we discovered that the environmental variance is high, both for the skin hue and the carotenoid concentration, while the genetic variance is very low for the carotenoid concentration

Table 1 Descriptive statistic of the variability of skin hue and of serum carotenoids ($\mu\text{g mL}^{-1}$) in two different breeding seasons from the cross-fostering experiments

Variable	Year	N	Mean	SE	-95%	+95%
Hue	2003	90	1.52	0.005	1.51	1.53
	2004	39	1.53	0.008	1.52	1.55
Serum xanthophylls	2003	75	23.91	0.99	21.96	25.86
	2004	37	22.96	1.53	19.94	25.98

Table 2 Results of the univariate REML analysis to account for the effects of environment (rearing and duplicate) and nest of origin on (a) skin hue and (b) carotenoid concentration as expressed in $\mu\text{g mL}^{-1}$

Source	Asymptotic z	Asymptotic p
a		
Duplicate	0.17	0.86
Origin (duplicate)	0.85	0.40
Rearing (duplicate)	2.01	0.04
Error	6.83	0.000
b		
Duplicate	0.74	0.46
Origin (duplicate)	1.27	0.21
Rearing (duplicate)	1.92	0.05
Error	6.14	0.000

The nest of origin and the nest of rearing were independently nested in the duplicate (the pair of matched nests in the reciprocal cross-fostering)

and not detectable for the skin hue (Table 3). Also, the concentration is greatly influenced by the environmental conditions associated with the season and expressed by the duplicate, while this source of variation is not significant in explaining the carotenoid-based coloration. At the phenotypic level, the variance is very high for the skin hue and less high for the carotenoid concentration (Table 3).

Considering the relationship between the two dependent variables at different levels, we found that the phenotypic correlation between skin hue and blood carotenoids is negative (the hue value decreases with the increasing of carotenoids deposited in the skin), and that this correlation is greatly strengthened at the environmental level (Table 3). We also found a strong positive correlation between the two variables at the duplicate level (Table 3), while the genetic correlation was not significant because of the low variance explained by the nest of origin (Table 3).

Discussion

Although the importance of carotenoid-based colorations in social or sexual signalling is well documented in birds (Kilner and Davies 1998; McGraw and Hill 2000; Pryke et al. 2001; McGraw et al. 2003; Bright et al. 2004), the proximal factors underlying the expression of these colorations in free-living birds is still relatively unknown (Tschirren et al. 2003; reviewed in Olson and Owens 1998). It has been shown that carotenoid-based

Table 3 Results of the bivariate REML analysis

Source	Skin hue	Carotenoid concentration	Correlation
Duplicate	0.05	0.31	0.83**
Origin	0.00	0.09	0.00
Rearing	0.30	0.24	-0.92**
Origin Rearing	0.10	0.0	0.0
Phenotypic	0.55	0.36	-0.23*

Variance components are expressed as the proportion of the total variation observed. The correlations between hue and concentration are expressed at different levels (* $P < 0.05$; ** $P < 0.001$)

colors are genetically determined only when expressed at the plumage level (in the house finch *Carpodacus mexicanus*, Hill 1991; in the great tit *Parus major*, Tschirren et al. 2003; in the blu tit, *Parus caeruleus*, Johnsen et al. 2003), while there is little available data for skin coloration where the source of variation seems to be determined by the environment (Bortolotti et al. 2000; Olson and Owens 2005; Griffith et al. 2006).

The cross-fostering experiment allowed us to partition brood variance into genetic and environmental effects. The results show that environmental factors, such as the quantity and quality of food consumed by nestlings, determine the expression of carotenoid traits, and that the influence of the genetic component is of limited importance. The effect of the nest of rearing on skin color and plasma carotenoid concentration agrees with results found by Bortolotti et al. (2000) in the American kestrel for the blood carotenoid content. The results are also consistent with Hōrak et al. (2000) in the great tit (*Parus major*) and Hadfield and Owens (2006) in the blue tit (*Parus caeruleus*), both for the carotenoid-based plumage color. The low heritability of a trait is usually related to its positive contribution to fitness. Indeed, strong directional and constant selection is supposed to decrease the genetic variation, with all remaining variations being environmentally determined (Mousseau and Ruff 1987). This is because directional selection decreases the additive genetic variance of a trait, since the alleles conferring the highest fitness are expected to be driven quickly to fixation by natural selection (e.g., Kimura 1958). However, fitness traits can also harbor elevated levels of additive genetic variation, probably as a consequence of the acquisition of genetic variation and mutations from the many loci involved in the trait expression (Merilä 1996; Merilä and Sheldon 1999; Costantini and Dell'Omo 2006). In fact, it has been suggested that the genetic component can be masked by

a higher environmental variance (Merilä and Sheldon 1999), leading to inter-year differences in the heritability estimate.

The expression of carotenoid-based coloration can also be influenced by a direct transfer of pigments from the mother to the yolk, and in turn to the nestlings. Maternal effects were found both in skin (Koutsos et al. 2003) and plumage (Biard et al. 2007) colorations. Our results are not consistent with those findings since we have not found any effect of the nest of origin as source of variation for body carotenoids. This result indirectly suggests that the potential maternal effect is no longer appreciable in young kestrels close to fledge.

Finally, as far as we know, this is the first study to test whether significant differences exist between phenotypic, genetic and environmental correlations of carotenoid traits. Our findings show a correspondence between the association of the traits observed at phenotypic and environmental level, although the association was much stronger considering the environmental variance component correlation. It should be noted that blood carotenoid concentration can vary quite rapidly in consequence of many physiological processes and the overall condition of the bird. A strong association between the expression of the trait, such as the skin color, and circulating carotenoids is not expected when the carotenoids acquired with food do not occur in consistent and abundant quantities, as in the case of a predator like the kestrel. The correlation between skin color and blood carotenoid concentration is much stronger considering the environmental components of variation because carotenoids are acquired with the diet. Although kestrel pairs nested in similar environments, they may show different feeding habits (Costantini et al. 2005a), which can explain the strong correlation between hue and concentration observed at the nest of rearing level. The positive and strong correlation observed at the duplicate level is unexpected since the duplicate should account for the environmental variation of the pair of nests involved in the reciprocal cross-fostering. Since nestlings were not cross-fostered all in the same day, but rather in accordance with their hatching date, the pairs of nests shared the environmental condition associated with the season. At the duplicate level, we have seen high carotenoid concentration variations and low skin hue variations, a phenomenon that is very different from what has been observed at the environmental or phenotypic level. We can hypothesize that the concentration of serum carotenoids varies with environmental factors related to seasonal changes. Examples include variation in food availability and changes in physiological demands throughout the season. Conversely, the deposition of the pigments in the skin was probably affected in other ways by environmental factors related to seasonal changes. This could explain the disentanglement of the two variables, although further investigation is needed.

Acknowledgments We thank Fiona Grant for revising the English version of the manuscript, Fernando Costantini for helping in data collection during the 2004 breeding season, Alberto Fanfani and Gianfranco Brambilla for their support of this research, two anonymous reviewers for valuable comments on the manuscript. Part of this work was sponsored by the scientific association *Ornis italica*. David Costantini was supported by a PhD fellowship issued from the University of Rome *La Sapienza*.

References

- Alonso-Alvarez C, Bertrand S, Devevey G, Gaillard M, Prost J, Faivre B, Sorci G (2004) An experimental test of the dose-dependent effect of carotenoids and immune activation on sexual signals and antioxidant activity. *Am Nat* 164:651–659
- Alves-Rodrigues A, Shao A (2004) The science behind lutein. *Toxicol Lett* 150:57–83
- Biard C, Surai PF, Møller AP (2007) An analysis of pre- and post-hatching maternal effects mediated by carotenoids in the blue tit. *J Evol Biol* 20:326–339
- Blount JD, Metcalf NB, Birkhead R, Surai PF (2003) Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science* 300:125–127
- Bortolotti GR, Tella JL, Forero MG, Dawson RD, Negro JJ (2000) Genetics local environment and health as factors influencing plasma carotenoids in wild American kestrels (*F. sparverius*). *Proc R Soc Lond B* 267:1433–1438
- Bortolotti G, Fernie K, Smits JE (2003) Carotenoid concentration and coloration of American kestrels (*Falco sparverius*) disrupted by experimental exposure to PCBs. *Funct Ecol* 17:651–657
- Brawner WR III, Hill GE, Sundermann CA (2000) Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. *Auk* 117:952–963
- Bright A, Waas JR, King CM, Cuming PD (2004) Bill colour and correlates of male quality in blackbirds: an analysis using canonical ordination. *Behav Proc* 65:123–132
- Brush AH (1990) Metabolism of carotenoid pigments in birds. *FASEB* 4:2969–2977
- 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, Fanfani A, Tagliavini J, Dell’Omo G (2007) Patterns of plasma carotenoid accumulation and skin colour variation in nestling kestrels in relation to breeding conditions and different terms of carotenoid supplementation. *J Comp Physiol B* 177:237–245
- Costantini D, Dell’Omo G (2006) Environmental and genetic components of oxidative stress in wild kestrel nestlings (*Falco tinnunculus*). *J Comp Physiol B* 176:575–579
- Costantini D, Casagrande S, Di Lieto G, Fanfani A, Dell’Omo G (2005a) Consistent differences in feeding habits between neighbouring breeding kestrels. *Behaviour* 142:1403–1415
- Costantini D, Dell’Omo G, Casagrande S, Fabiani A, Carosi M, Bertacche V, Marquez C, Snell H, Snell H, Tapia W, Gentile G (2005b) Inter-population variation of carotenoids in Galapagos land iguanas (*Conolophus suberistatus*). *Comp Biochem Physiol Part B* 142:239–244
- Costantini D, Casagrande S, De Filippis S, Brambilla G, Fanfani A, Tagliavini J, Dell’Omo G (2006) Correlates of oxidative stress in wild kestrel nestlings (*Falco tinnunculus*). *J Comp Physiol B* 176:329–337
- Costantini D, Coluzza C, Fanfani A, Dell’Omo G (2007) Effects of carotenoid supplementation on colour expression oxidative stress and body mass in rehabilitated captive adult kestrels (*Falco tinnunculus*). *J Comp Physiol B* 177:723–731
- Duckworth RA, Mendonc MT, Hill GE (2004) Condition-dependent sexual traits and social dominance in the house finch. *Behav Ecol* 15:779–784

- Eraud C, Devevey G, Gaillard M, Prost J, Sorci G, Faivre B (2007) Environmental stress affects the expression of a carotenoid-based sexual trait in male zebra finches. *J Exp Biol* 210:3571–3578
- Faivre B, Grégoire A, Prévault M, Cézilly F, Sorci S (2003) Immune activation rapidly mirrored in a secondary sexual trait. *Science* 300:103
- Falconer DS (1989) Introduction to quantitative genetics. Longman Scientific and Technical, Essex, England
- Fenoglio S, Cucco M, Fracchia L, Martinotti MG, Malacarne G (2004) Shield colours of the Moorhen are differently related to bacterial presence and health parameters. *Ethol Ecol Evol* 16:149–156
- Figuerola J, Muños E, Gutiérrez R, Ferrer D (1999) Blood parasites leucocytes and plumage brightness in the Cirl bunting *Emberiza cirulus*. *Funct Ecol* 13:594–601
- Fitze PS, Tschirren B, Richner H (2003) Carotenoid-based colour expression is determined early in nestling life. *Oecologia* 137:148–152
- Gilmour AR, Gogel BJ, Cullis BR, Welham SJ, Thompson R (2002) ASReml user guide release 10. VSN International, Hemel, Hempstead
- Götmark F, Ahlström M (1997) Parental preference for red mouth of chicks in a songbird. *Proc R Soc Lond B* 264:959–962
- Grether GF, Hudon J, Millie DF (1999) Carotenoid limitation of sexual coloration along an environmental gradient in guppies. *Proc R Soc Lond B* 266:1317–1322
- 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
- Hadfield JD, Owens IPF (2006) Strong environmental determination of a carotenoid-based plumage trait is not mediated by carotenoid availability. *J Evol Biol* 19:1104–1114
- Hadfield JD, Nutall A, Osorio D, Owens IPF (2008) Testing the phenotypic gambit: phenotypic genetic and environmental correlations of colour *J Evol Biol* (in press). doi: 10.1111/j.1420-9101.2006.01262.x
- Hill GE (1991) Plumage coloration is a sexually selected indicator of male quality. *Nature* 350:337–339
- Hill GE (1999) Is there an immunological cost to carotenoid-based ornamental coloration? *Am Nat* 154:589–595
- Hill GE, McGraw KJ (2004) Correlated changes in male plumage coloration and female mate choice in cardueline finches. *Anim Behav* 67:27–35
- Hill GE, Inouye CY, Montgomerie R (2002) Dietary carotenoid predicts plumage coloration in wild house finches. *Proc R Soc Lond B* 269:1119–1124
- Hörak P, Vellau H, Ots I, Møller AP (2000) Growth conditions affect carotenoid-based plumage coloration of great tit nestlings. *Naturwissenschaften* 87:460–464
- Hörak PL, Ots I, Vellau H, Spottiswoode C, Møller AP (2001) Carotenoid-based plumage coloration reflects hemoparasites infection and local survival in breeding great tits. *Oecologia* 126:166–173
- Hörak P, Surai PF, Ots I, Møller AP (2004) Fat soluble antioxidants in brood-rearing great tits *Parus major*: relations to health and appearance. *J Avian Biol* 35:63–70
- Inouye CY, Hill GE, Stradi RD, Montgomerie R (2001) Carotenoid pigments in male house finch plumage in relation to age subspecies and ornamental coloration. *Auk* 118:900–915
- Jawor JMN, Gray N, Beall SM, Breitwisch R (2004) Multiple ornaments correlates with aspects of condition and behaviour in female Northern Cardinals *Cardinalis cardinalis*. *Anim Behav* 67:875–882
- Johnsen A, Delhey K, Andersson S, Kempnaers B (2003) Plumage colour in nestling blue tits: sexual dichromatism condition dependence and genetic effects. *Proc R Soc Lond B* 270:1263–1270
- Kilner R, Davies NB (1998) Nestling mouth colour: ecological correlates of a begging signal. *Anim Behav* 56:705–712
- Kimura M (1958) On the change of population fitness by natural selection. *Heredity* 12:145–167
- Koivula M, Viitala J, Korpimäki E (1999) Common kestrels prefer scent marks according to species and reproductive status of voles. *Ecoscience* 6:415–420
- Koutsos EA, Clifford AJ, Calvert CC, Klasing KC (2003) Maternal carotenoid status modifies the incorporation of dietary carotenoids into immune tissues of growing chickens (*Gallus gallus domesticus*). *J Nutr* 133:1132–1138
- Kruuk LEB, Hadfield JD (2007) How to separate genetic and environmental causes of similarity between relatives. *J Evol Biol* 20:1890–1903
- Lessels CM, Boag PT (1987) Unrepeatable repeatabilities: a common mistake? *Auk* 104:116–121
- Linville SU, Breitwusch R (1997) Carotenoid availability and plumage coloration in a wild population or northern cardinals. *Auk* 114:796–800
- Mays HL, McGraw KJ, Ritchison G, Cooper S, Rush V, Parker RS (2004) Sexual dichromatism in the yellow-breasted chat *Icteria virens*: spectrophotometric analysis and biochemical basis. *J Avian Biol* 35:125–134
- McGraw KJ, Hill GE (2000) Plumage brightness and breeding-season dominance in the house finch: a negatively correlated handicap? *Condor* 102:456–461
- McGraw KJ, Ardia DR (2003) Carotenoids immunocompetence and the information content of sexual colors: an experimental test. *Am Nat* 162:704–712
- McGraw KJ, Gregory AJ, Parker RS, Adkins-Regan E (2003) Diet plasma carotenoids and sexual coloration in the zebra finch (*Taeniopygia guttata*). *Auk* 120:400–410
- McGraw KJ, Hill GE, Stradi R, Parker RS (2002) The effect of dietary carotenoid access on sexual dichromatism and plumage pigment composition in the American goldfinch. *Comp Biochem Physiol B* 131:261–269
- Merilä J (1996) Genetic variation in offspring condition: an experiment. *Funct Ecol* 10:465–474
- Merilä J, Sheldon BC (1999) Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. *Heredity* 83:103–109
- Møller AP, Biard C, Blount JD, Houston DC, Ninni P, Surai PF (2000) Carotenoid-dependent signals: indicators of foraging efficiency immunocompetence or detoxification ability? *Avian Poultry Biol Rev* 11:137–159
- Montgomerie R (2006) Analyzing colors In: Hill GE, McGraw KJ (eds) Bird coloration mechanisms and measurements, vol 1. Harvard University Press, Cambridge, pp 109
- Mougeot F, Martínez-Padilla J, Pérez-Rodríguez L, Bortolotti G (2007) Carotenoid-based coloration and ultraviolet reflectance of the sexual ornaments of grouse. *Behav Ecol Sociobiol* 61:741–751
- Mousseau TA, DA Roff (1987) Natural selection and the heritability of fitness components. *Heredity* 59:181–197
- Navara KJ, Hill GE (2003) Dietary carotenoid pigments and immune function in a songbird with extensive carotenoid-based plumage coloration. *Behav Ecol* 14:909–916
- Negro JJ, Tella JL, Blanco G, Forero MG, Garrido-Fernández J (2000) Diet explains interpopulation variation of plasma carotenoids and skin pigmentation in nestling white storks. *Physiol Biochem Zool* 73:97–101
- Negro JN, Tella JL, Hiraldo F, Bortolotti GR, Prieto P (2001) Sex and age related variation in plasma carotenoids despite a constant diet in the red-legged partridge *Alectoris rufa*. *Ardea* 89:275–280
- Olson VA, Owens IPF (1998) Costly sexual signals: are carotenoids rare risky or required? *TREE* 13:510–514
- Olson VA, Owens IPF (2005) Interspecific variation in the use of carotenoid-based coloration in birds: diet life history and phylogeny. *J Evol Biol* 18:1534–1546
- Prum RO, Torres R (2003) Structural coloration of avian skin: convergent evolution or coherently scattering dermal collagen arrays. *J Exp Biol* 206:2409–2429
- Pryke SR, Andersson S (2003) Carotenoid-based epaulettes reveal male competitive ability: experiments with resident and floater red-shouldered widowbirds. *Anim Behav* 66:217–224

- Pryke SR, Lawes MJ, Andersson S (2001) Agonistic carotenoid signalling in male red-collared widowbirds: aggression related to the colour signal of both the territory owner and model intruder. *Anim Behav* 62:695–704
- Saino N, Ninni P, Calza S, Martinelli R, De Bernardi F, Møller AP (2000) Better red than dead: carotenoid-based mouth coloration reveals infection in barn swallow nestlings. *Proc R Soc Lond B* 267:57–61
- Saino N, Ferrari R, Romano M, Martinelli R, Møller AP (2003) Experimental manipulation of egg quality affects immunity of barn swallow nestlings. *Proc R Soc Lond B* 270:2485–2489
- Saks L, McGraw KJ, Hõrak P (2003) How feather colour reflects its carotenoid content. *Func Ecol* 17:555–561
- Tschirren B, Fitze PS, Richner H (2003) Proximate mechanisms of variation in the carotenoid-based plumage coloration of nestling great tits (*Parus major* L.). *J Evol Biol* 16:91–100