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Skin carotenoid concentration correlates with male hunting skill and territory quality in the kestrel *Falco tinnunculus*

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In the context of sexual selection carotenoid based signals are candidates for indicator traits: they have to be taken up in the diet by animals, they can indicate the ability of the bearer to accumulate a limited resource, and they help in maintaining the health status. We investigated the yellow-orange colouration of the tarsi of the kestrel *Falco tinnunculus* in relation to sex, diet and to different aspects of male reproductive behaviour. The colouration of the tarsi (estimated as hue) was more intense in males than in females. Among males, the tarsi hue was associated with the intake of invertebrates; this was true also if the population diet was based mainly on voles. Carotenoid based colouration was positively associated with the number of vertebrate preys delivered to the nest per time unit and with territory quality (calculated on the basis of home-range size, habitat extension and prey availability). These results are consistent with predictions derived from good-parent models of sexual selection, suggesting that in the common kestrel carotenoid based colouration is important as an indicator of male quality.

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In species where individuals provide material resources, such as food, to their mates, secondary sexual characters that are phenotypically correlated with male quality (indicator traits) can be of great importance in mate choice (Wolf et al. 1997, Kokko 1998). Thus, an indicator trait may testify in a reliable way to a female the associated material resources or genetic benefits that increase the fitness of her offspring (Zahavi 1975). The trait can indicate the condition of the bearer when the production of the character arises from a trade-off between health maintenance or access to a limited resource and trait production (McGraw and Hill 2000a). If the trait is represented by brilliant colouration, the origin of the colour can be important, as it can be either structural or based on pigments. Among pigments, carotenoid based colorations are probable candidates for indicator traits, since they can not be synthesized by birds (Brush 1990). Therefore, they can indicate the ability of the bearer to accumulate a limited resource (Grether et al. 1999, Negro et al. 2002), and play an important role in health maintenance (Møller et al. 2000). Several studies have reported a negative association between immune challenges and carotenoid based colourations (Saino et al. 1999, Brawner III et al. 2000, Faivre et al. 2003). A positive association between carotenoid level and an increase of cell-mediated immune function has been found in the zebra finch *Taeniopygia guttata* (Blount et al. 2003).

In biparental species, such as the kestrel *Falco tinnunculus*, females can gain substantial benefits from mating with males with good hunting skills. In this species, the main material resource is the food provided by the male to the female during the pre-laying and the incubation periods and to the offspring during the

rearing period (Village 1990). Another important material resource is the male's territory which is shared by the female from pair formation, in spring. More generally, the female can gain both direct and indirect benefits from mating with a male in good health (Hamilton and Zuk 1982, Village 1990, Folstad and Karter 1992). No studies appear to have been carried out on the visual signalling function of common kestrel skin colour. It is plausible that the skin colour may convey information about the current or the recent past of an individual's physical condition, as it can change in a few days (Negro et al. 1998). This contrasts with the colour of plumage, since a colourful feather reflects the condition of an individual at moult. In species that moult only once per year, such as the kestrel, it is possible that ornamental plumage plays a major role during the non-breeding season, for example, in intra-sexual competition (Badyaev and Hill 2000, McGraw and Hill 2000b).

The first aim of this study was to investigate whether the bare skin coloration could be considered as a sexually dimorphic trait. We studied tarsi colouration of the sexes to determine whether any differences existed between males and females in the ability to store carotenoids in the skin to produce a signal. If the bare skin colouration was a dimorphic trait evolved by sexual selection, male skin would be expected to be brighter than female skin. Second, we investigated the possible associations between the expression of secondary sexual traits in an individual and its diet. Carotenoids cannot be synthesized by animals and they are rare in the diet of birds at the top of the food web (Gray 1996, Olson and Owens 1998). Hence, we hypothesized that the diet could directly affect the expression of carotenoid based colouration. Third, we studied male skin colouration relative to hunting skills and to the quality of the territory. Since carotenoid based colourations may function as an indicator of the individual foraging ability and resistance to parasites or diseases, we predicted that this trait would be associated with the hunting skills of males (brighter males bring more food to incubating females) and with the quality of their territory (brighter males have better territory), in accordance with the good parent model (Heywood 1989, Hoelzer 1989).

Study site and methods

The study was carried out near Parma (Po Valley, northern Italy) during the 2001 and 2002 kestrel breeding seasons, in an area of approximately 30 km^2. The area, which held 24–25 kestrel pairs, was characterized by agriculture and grasslands. Fifteen kestrels were trapped with bal-chatri traps during the each mating period, from the beginning of March to the beginning of April. This lowered the possible seasonal variation of carotenoid based colouration and blood carotenoid concentration (Negro et al. 1998).

Blood carotenoid concentration and skin colour measurement

One blood sample from each bird (100 µl) was taken from the tarsal vein and kept on ice (0.5–5.0°C). The blood was centrifuged within 10 h from sampling for 2 min at 12000 × g, and the plasma was separated and stored at −20°C for a maximum of two months before carotenoid analyses. Subsequently, tarsus colour was measured with a hand-held spectrophotometer (Oracolor Corob, Modena, Italy), which operates over the wavelengths 400–700 nm. Colour was described by the colorimetric measure of hue of the tarsus skin, the best parameter to measure carotenoid colouration (Hõrak et al. 2001). We took three consecutive measurements for each individual and, after checking for repeatability (hue: $r = 0.797$, $P < 0.001$) (Lessels and Boag 1987), the mean value of these was considered for the analyses. The spectrophotometric data were analysed with the Corob Quality 1.0 software (Corob, Modena, Italy). Before the measurements, the skin of the tarsus was cleaned with water. The body condition index was calculated by the ratio of the body weight (10 g approximation) and the cube of the wing cord (1 mm approximation). The manipulation and the bleeding of the birds were authorized by INFS (Istituto Nazionale Fauna Selvatica, Bologna).

In order to evaluate the presence of carotenoids in the back plumage, which is reddish in males, two feathers were plucked from each bird about midway between the two scapulas and analysed by HPLC.

Hunting skills and territory quality

The hunting behaviour of fifteen male kestrels that were equipped with 3.5 g tail-mount radio-tags (Biotrack, Wareham, Dorset, UK) was recorded from the beginning of incubation until fledging. Each observation session lasted three consecutive hours and there were three sessions per week. The three sessions started one hour after sunrise, one hour before noon and four hours before sunset. The duration and location of the behavioural categories observed were described as reported in Masman et al. (1988).

As there were many observations for each individual, we were able to calculate the home-range size of each individual by joining the outer points of the localization reported on a 1:10,000 map. For the analyses, the size, shape and habitat composition of each territory were also considered. We grouped the habitats used by the kestrels into four classes: (1) Short vegetation (lawns, natural and cultivated grasslands that were periodically

cut). (2) Tall vegetation (uncultivated field, field margins, road margins). (3) Waterside banks (artificial and natural banks of rivers and irrigation channels characterized by short and tall vegetation and scattered trees). (4) Cultivated and urbanized areas (cultivated and ploughed fields, sand and asphalted roads, farmyards). The population of small mammals was studied by counts conducted four times a year (February, April, June and October during 2001 and 2002), using Long-worth traps and in few cases snap traps. The traps were positioned along a transect every 10 m within the four habitat types of each kestrel home-range, and they were inspected two times per day (in the morning and in the evening) for four consecutive days.

A territory quality index (tq) was calculated from the formula (modified from Komdeur et al. 1997):

$$tq = a[\Sigma_{x=1}^{4}(c_x i_x)]/100$$

where a is the home-range area (ha), x is one of the four habitat types, c_x is the area (ha) of the habitat x and i_x is the number of small mammals (number of mammals trapped/100 trap nights) trapped in the habitat x.

Diet analysis

Diet analysis was based on the observations of hunting activity and on regurgitated pellets. All strikes made by males against a potential prey were recorded and the prey were classified as mammal, bird, reptile or invertebrate. We also recorded whether the prey was eaten entirely or partially by the male or brought to the nest.

Food remains and pellets were gathered from under the perching sites and from within the nest during the breeding season. Pellets were carefully examined and all bones, feathers and parts of insects were separated and identified. The environmental availability of carotenoids was determined by measuring the amount of carotenoids in the blood of short-tailed voles *Microtus savii* and wood mice *Apodemus sylvaticus*, both of which were present within the diet of the observed kestrel population. The individuals of each species were trapped during the small mammal count and a blood sample of 50 µl was taken from the caudal vein.

Carotenoid measurement

Feather carotenoids were analysed by high-performance liquid chromatography (HPLC; cf. Stradi et al. 1995, for a detailed description of the method). HPLC was also used to ascertain whether the carotenoids deposited in the bare skin and those in the blood were the same. For qualitative analysis of skin carotenoids small pieces of the integument were excised from the tarsus, cere and lores of two dead adult males belonging to the Raptor Rehabilitation Centre of Parma, where they were kept at −20°C for 2 days. Qualitative analysis of blood carotenoids was conducted by sonicating weighed amounts of plasma for 3 min in acetone (1.5 ml). The suspension obtained was centrifuged for 5 min at 14,000 rpm and the supernatant was analysed by HPLC (lutein standard) in the following conditions, mobile phase: acetonitrile–methanol (70:30), flow: 0.6 ml/min column: Purosphere RP18e, Purosphere RP18e, 250 × 4.6 mm, 5 µm; 37°C. Carotenoids were extracted from a weighed amount of skin with the same protocol used for the other organic complex matrix, as described in Schiedt et al. (1995) and Saino et al. (2002) for egg yolk analysis. The chromatographic analyses were prolonged (75 min) to evaluate the presence of esters.

Total carotenoid content was estimated spectrophotometrically in a supernatant obtained by centrifugation for 5 min at 14,000 × g of plasma diluted with acetone (1:40 plasma: acetone). The absorbance was measured at 476 nm wavelength with a spectrophotometer (Pharmacia Biotech Ultrospec 2000, Cambridge, UK); the spectrophotometric chamber temperature was regulated (0–4°C) to minimize evaporation during the measure. Carotenoids concentration was calculated according to the Lambert-Beer formula:

$$C = A_{476}/(E_{476} \times L)$$

where C is the percentage concentration of solute, A is the spectrophotometric reading absorbance at the peak of lutein (476 nm), L is the path length of the light trough the sample (1 cm), E_{476} is the percentage extinction coefficient. For arbitrary carotenoids E_{476} corresponds to 2500 (Britton 1985). This value is usually taken when no experimentally determined value has been reported for an unknown compound or to give an estimate of total carotenoids in an extract (Britton, 1985).

Data analysis

The data were analysed using SPSS 10.0 for Windows (SPSS 2000) software. Comparison of tarsus colorimetric variable between males and females and of carotenoid concentration, respectively, were analysed with the Mann-Whitney U Test (U) and the association between variables with the Spearman's Rank Correlation (r_s). The number of variables considered increased the likelihood of significance arising by chance, so we applied the sequential Bonferroni procedure to evaluate the significance of correlation when multiple comparisons were used to test the same hypothesis. Since the spectrophotometer assigned lower values with the increasing of red (brighter individuals had lower hue value), the hue data were transformed with the inverse ratio. Since it was not always possible to collect the full set of information for each bird trapped, the sample size

has been specified in each test. The median is indicated with the first and third quartile (lower, median, upper quartile) and the probability is two-tailed.

Results

Twenty-one adult kestrels (15 males and 6 females) were captured. The analysis of tissues (blood and skin) revealed the presence of two xanthophylls: zeaxanthin, and particularly lutein (~90% of the total xanthophylls) (Fig. 1a, b). Fig. 1a and b show also that carotenoids deposited in the skin and in the blood were the same.

In contrast, the reddish back plumage colouration of the male was not carotenoids dependent, since no carotenoid pigments were found in the feathers.

Differences between males and females

The hue of the tarsus of males was redder than that of females (0.81, 0.82, 0.85 vs. 0.76, 0.769, 0.77; Mann-Whitney U Test: $U = 2.970$, $N_1 = 9$, $N_2 = 6$, $P = 0.03$). There was no significant difference in carotenoid concentration between males and females (males: 25.10, 34.78, 46.47 μg/g, $N = 13$; females: 21.64, 32.51, 40.94 μg/g, $N = 6$). Additionally, no correlation was found between blood carotenoid concentration and skin hue in males and females (Spearman Rank Correlation: hue: $r_s = 0.272$, $N = 15$, $P = 0.327$).

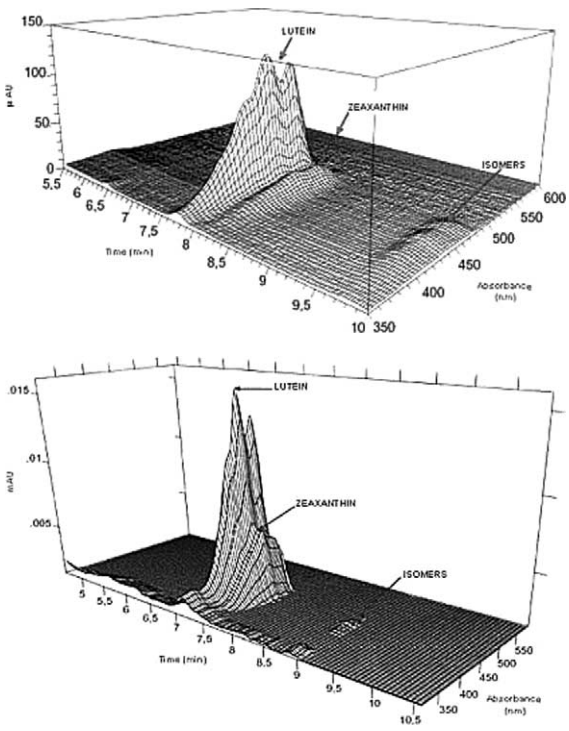

Fig. 1. Three-dimensional absorption spectrum showing the carotenoid pattern of kestrel. The chromatograms of HPLC results for integument tissue (a) and blood (b) carotenoids show that only two kinds of xanthophylls were present both in skin and blood: lutein and zeaxanthin. Isomers are by-products of methodology analysis.

Diet and carotenoids

We recorded and identified 729 prey items captured by males: 434 (59.5%) of these were vertebrates and 40.5% invertebrates. Of the 377 pellets analysed mammals were found in 341 (90.4%) and invertebrates occurred in 149 (39.6%). The skin hue was found to be associated with the frequency of invertebrates eaten per hour by males (Spearman Rank Correlation: $r_s = 0.778$, $N = 9$, $P = 0.014$; Fig. 2a). No relationship was found between hue and vertebrates. The blood xanthophyll concentration of *Microtus savii* and *Apodemus sylvaticus* (both found in the diet of kestrels) was 0.35 μg/g ($N = 2$) and 0.28 μg/g ($N = 2$), respectively. This was almost 100 fold

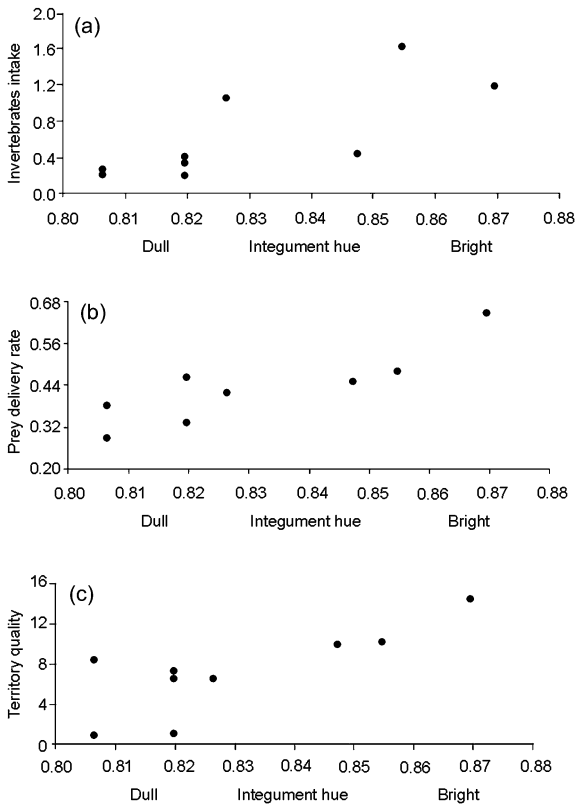

Fig. 2. Relationship between the integument hue of the carotenoid based colouration plotted against (a) the frequency of invertebrates eaten by males in one hour; (b) the frequency of vertebrates delivered to the nest in one hour; (c) the male territory quality.

less than the xanthophylls concentrations found in kestrel blood.

Hunting skill and carotenoids

In order to eliminate brood size effects, we considered the males' hunting efficiency by evaluating the number of vertebrates brought to the female by the male in one hour during incubation. During the observation period no invertebrates were delivered to the nest. The tarsus hue of the male correlated with the number of vertebrates delivered to the nest (Spearman Rank Correlation: $r_s = 0.843$, $N = 8$, $P = 0.009$; Fig. 2b). Although the number of invertebrates eaten per hour and the vertebrates delivered to the nest both depend on male foraging ability, there was no correlation found between these two variables (Spearman Rank Correlation: $r_s = 0.400$, $N = 13$, $P = 0.176$).

Territory quality was found to be associated with the male bare skin hue (Spearman Rank Correlation: $r_s = 0.707$, $N = 9$, $P = 0.033$; Fig. 2c). Among males the hue of skin colouration was not correlated with the body condition index.

Discussion

Xanthophylls were the only carotenoids absorbed by the common kestrel. This is similar to what has been observed in others birds (Bortolotti et al. 1996, Møller et al. 2000, Stradi et al. 2001). Compared to birds that have different diets, carotenoid concentration in the kestrel blood was either similar (e.g. zebra finch, Blount et al. 2003), or in some cases greater (e.g. barn swallow *Hirundo rustica*, Saino et al. 1999; red-legged partridge *Alectoris rufa*, Negro et al. 2001). Since the diet of the study population consisted almost entirely of small mammals, the access to carotenoids was limited (Olson and Owens, 1998, Bortolotti et al. 2000). For this reason this species can be classified as a selective accumulator of xanthophylls (Slifka et al. 1999). Moreover, we could exclude that the cost to accumulate lutein and zeaxanthin in the integument is represented by metabolic processes to transform carotenoids, since the carotenoids deposited were the same as those absorbed.

Both male and female kestrels have yellow-orange tarsi, cere and lores. We found that the colouration of skin was brighter (hue more red) in males than in females. It is interesting to note that the carotenoid concentration in the blood was actually similar in both sexes, suggesting that some physiological mechanisms and not the diet are likely to be responsible for the different accumulation of carotenoids in the integument.

The colour intensity of the integument and the carotenoid concentration in the blood were not correlated with each other. This outcome differs from findings of the American kestrel (Bortolotti et al. 1996), possibly due to our study being conducted in the wild as opposed to the American kestrel study, conducted in captivity. Within the presented study it was not possible to control the timing and the type of the last meal. It is likely that the carotenoid intake can affect the carotenoid concentration detectable in the blood over a short period (Casagrande et al. unpubl. data). Carotenoids are used in many physiological processes for maintaining the health status and therefore a direct relationship between ingestion and external colouration is unlikely. It has been shown that there is a clear seasonal change of American kestrel integument colour (Negro et al. 1998), which suggests that there is a complex physiological regulation of carotenoid expression. Finally, the difference in skin colour between this study and that of the American kestrel could also be a result of the different methodologies used. The skin colour of the American kestrel was evaluated by colour chart comparison, whilst this study incorporated spectrophotometric analysis.

We found that carotenoids produce only the bare skin colouration in the kestrel while in the plumage carotenoids were absent. The colour of the integument can vary over a short period (Negro et al. 1998) and, since carotenoids produce condition dependent signals, this carotenoid based character can be considered as a honest signal of the carrier's quality.

The coloration of the tarsus was correlated with the number of invertebrates eaten per hour. Although we did not measure the carotenoid content of invertebrates, it was assumed that they possibly represent the main source of xanthophylls in the diet, as small mammals are a poor source of these pigments. It has been shown that the blood concentration of carotenoids in the wild American kestrel was inversely associated with the vole abundance within the bird territory (Bortolotti et. al. 2000). Our results agree with the findings of Witmer (1996) and Hill et al. (2002), that the expression of carotenoid based colouration in wild vertebrates is affected by the amount and types of carotenoid pigments that are absorbed with the diet.

Males with brighter carotenoid based colouration had better hunting skill as well as a higher-quality territory. Since the sexual dichromatism of this species is visually expressed at the integument level, our findings support the hypothesis that the skin colouration is a reliable signal of male quality. This finding supported our hypothesis that carotenoid based colouration is in accordance with the good parent model. Male kestrels in general feed females before the laying date and during incubation; reproductive success is affected by male nest attentiveness both during incubation and the rearing of young. Choosing an inattentive male is costly for a female and, considering her fitness, it could be profitable for her to mate with brighter males. Although at present

there are no data showing that female kestrels prefer males having brighter yellow-orange skin, in other species a significant role for carotenoid based colouration in female mate choice has been suggested (Hill 1991, Zuk 1992, Linville et al. 1998, Senar et al. 2002), in relation with the good-parent process of sexual selection (Hoelzer 1989) or also with the parasite avoidance hypothesis (Hamilton and Zuck 1982).

In conclusion, our study reveals that the yellow-orange integument colouration of male kestrels is associated with the diet and that it signals the paternal quality of the bearer. It would be of interest to investigate the proximate mechanisms that regulate the production of the carotenoid based signal in the kestrel in order to understand how this colour correlates with male skill.

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References

Badyaev, A. V. and Hill, G. E. 2000. Evolution of sexual dichromatism: contribution of carotenoid-versus melanin-based coloration. – Biol. J. Linn. Soc. 69: 153–172.

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

Bortolotti, G. R., Negro, J. J., Tella, J. L., Marchant, T. A. and Bird, D. M. 1996. Sexual dichromatism in birds independent of diet, parasites and androgens. – Proc. R. Soc. B 263: 1171–1176.

Bortolotti, G. R., Tella, J. L., Forero, M. G., Dawson, R. D. and Negro, J. J. 2000. Genetics, local environment and health as factors influencing plasma carotenoids in wild American kestrels (*Falco sparverius*). – Proc. R. Soc. B 267: 1433–1438.

Brawner III, W. R., Hill, G. E. and Sundermann, C. A. 2000. Effects of coccidial and mycoplasmal infection on carotenoid-based plumage pigmentation in male house finches. – Auk 117: 952–963.

Britton, G. 1985. General carotenoid methods. – Meth. Enzymol. 111: 113–149.

Brush, A. H. 1990. Metabolism of carotenoid pigments in birds. – FASEB J. 4: 2969–2977.

Corob, S.p.A. 2001. Corob Quality 1.0. – Corob S.p.A., Modena, Italy.

Faivre, B., Grégoire, A., Préault, M., Cézilli, F. and Sorci, G. 2003. Immune activation rapidly mirrored in a secondary sexual trait. – Science 300: 103.

Folstad, I. and Karter, A. J. 1992. Parasites, bright males, and the immunocompetence handicap. – Am. Nat. 139: 603–622.

Gray, D. A. 1996. Carotenoids and sexual dichromatism in North American passerine birds. – Am. Nat. 148: 453–480.

Grether, G. F., Hudon, J. and Millie, D. F. 1999. Carotenoid limitation of sexual coloration along an environmental gradient in guppies. – Proc. R. Soc. B 266: 1317–1322.

Hamilton, W. D. and Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? – Science 218: 384–387.

Heywood, J. S. 1989. Sexual selection by the handicap mechanism. – Evolution 43: 1387–1397.

Hill, G. E. 1991. Plumage coloration is a sexually selected indicator of male quality. – Nature 350: 337–339.

Hill, G. E., Inouye, C. Y. and Montgomerie, R. 2002. Dietary carotenoids predict plumage coloration in wild house finches. – Proc. R. Soc. B 269: 1119–1124.

Hoelzer, G. A. 1989. The good parent process of sexual selection theory. – Anim. Behav. 38: 1067–1078.

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

Kokko, H. 1998. Should advertising parental care be honest? – Proc. R. Soc. B 265: 1871–1878.

Komdeur, J., Daan, S., Tinbergen, J. and Mateman, C. 1997. Extreme adaptive modification in sex ratio of the Seychelles warbler's eggs. – Nature 385: 522–525.

Lessels, C. M. and Boag, P. T. 1987. Unrepeatable repeatabilities: a common mistake. – Auk 104: 116–121.

Linville, S. U., Breitwusch, R. and Schilling, A. J. 1998. Plumage brightness as an indicator of parental care in northern cardinals. – Anim. Behav. 55: 119–127.

Masman, D., Daan, S. and Dijkstra, C. 1988. Time allocation in the kestrel, *Falco tinnunculus*, and the principle of energy minimization. – J. Anim. Ecol. 57: 411–432.

McGraw, K. J. and Hill, G. E. 2000a. Differential effects of endoparasitism on the expression of carotenoid- and melanin-based coloration. – Proc. R. Soc. B 267: 1252–1531.

McGraw, K. J. and Hill, G. E. 2000b. Plumage brightness and breeding-season dominance in the house finch: a negatively correlated handicap? – Condor 102: 456–461.

Møller, A. P., Biard, C., Blount, J. D., Houston, D. C., Ninni, P. and Surai, P. F. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? – Avian. Poult. Biol. Rev. 11: 137–159.

Negro, J. J., Bortolotti, G. R., Tella, J. L., Fernie, K. J. and Bird, D. M. 1998. Regulation of integumentary colour and plasma carotenoids in American kestrel consistent with sexual selection theory. – Funct. Ecol. 12: 307–312.

Negro, J. J., Tella, J. L., Hiraldo, F., Bortolotti, G. R. and Prieto, P. 2001. Sex and age related variation in plasma carotenoids despite a constant diet in the red-legged partridge *Alectorisrufa*. – Ardea 89: 275–280.

Negro, J. J., Grande, J. M., Tella, J. A., Garrido, J., Hornero, D., Donázar, J. A., Sanchez-Zapata, J. A., Benítez, J. R. and Barcell, M. 2002. An unusual source of essential carotenoids. – Nature 416: 807.

Olson, V. A. and Owens, I. P. F. 1998. Are carotenoids rare risky or required? – Trends Ecol. Evol. 13: 510–514.

Saino, N., Ninni, P. and Møller, A. P. 1999. Carotenoid plasma concentration, immune profile and plumage ornamentation of male barn swallows (*Hirundo rustica*). – Am. Nat. 154: 441–448.

Saino, N., Bertacche, V., Ferrari, R. P., Martinelli, R. and Møller, A. P. 2002. Carotenoid concentration in barn swallow eggs is influenced by laying order, maternal infection and paternal ornamentation. – Proc. R. Soc. B 269: 1729–1733.

Schiedt, K., Bischof, S. and Glintz, E. 1995. Example 6: egg yolk. – In: Britton, G., Liaaen-Jensen, S. and Pfander, H. (eds). In Carotenoids. Vol. IA. Birkhäuser, Basel, pp. 253–260.

Senar, J. C., Figuerola, J. and Pascual, J. 2002. Brighter yellow blue tits make better parents. – Proc. R. Soc. B 269: 257–261.
Slifka, K. A., Bowen, P. E., Stacewicz-Sapuntzakis, M. and Crissey, S. D. 1999. A survey of serum and dietary carotenoids in captive wild animals. – J. Nutr. 129: 380–390.
SPSS, Inc. 2000. SPSS 10.0 for Windows: base, professional statistics and advanced statistics. – Chicago, SPSS Inc.
Stradi, R., Celentano, G. and Nava, D. 1995. Separation and identification of carotenoids in bird's plumage by high-performance liquid chromatography-diode-array detection. – J. Chromatog. B 670: 337–348.
Stradi, R., Pini, E. and Celentano, G. 2001. Carotenoids in bird plumage: the complement of red pigments in the plumage of wild and captive bullfinch (*Pyrrhula pyrrhula*). – Comp. Biochem. Physiol. B 128: 529–535.
Village, A. 1990. The kestrel. – T & AD Poyser, London, UK.
Witmer, M. C. 1996. Consequences of an alien shrub on the plumage coloration and ecology of cedar waxwings. – Auk 113: 735–743.
Wolf, J. B., Moore, A. J. and Brodie III, E. D. 1997. The evolution of indicator traits for parental quality: the role of maternal and paternal effects. – Am. Nat. 150: 639–649.
Zahavi, A. 1975. Mate selection – a selection for a handicap. – J. Theor. Biol. 53: 205–214.
Zuk, M. 1992. The role of parasites in sexual selection: current evidence and future directions. – Adv. Stud. Behav. 21: 39–68.

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