

Condition-dependent expression of melanin-based coloration in the Eurasian kestrel

Romain Piault · Valentijn van den Brink ·
Alexandre Roulin

Received: 28 February 2012 / Revised: 2 April 2012 / Accepted: 3 April 2012 / Published online: 22 April 2012
© Springer-Verlag 2012

Abstract Melanin is the most common pigment in animal integuments and is responsible for some of the most striking ornaments. A central tenet of sexual selection theory states that melanin-based traits can signal absolute individual quality in any environment only if their expression is condition-dependent. Significant costs imposed by an ornament would ensure that only the highest quality individuals display the most exaggerated forms of the signal. Firm evidence that melanin-based traits can be condition-dependent is still rare in birds. In an experimental test of this central assumption, we report condition-dependent expression of a melanin-based trait in the Eurasian kestrel (*Falco tinnunculus*). We manipulated nestling body condition by reducing or increasing the number of nestlings soon after hatching. A few days before fledging, we measured the width of sub-terminal black bands on the tail feathers. Compared to nestlings from enlarged broods, individuals raised in reduced broods were in better condition and thereby developed larger sub-terminal bands. Furthermore, in 2 years, first-born nestlings also developed larger sub-terminal bands than their younger siblings that are in poorer condition. This demonstrates that expression of melanin-based traits can be condition-dependent.

Keywords Body condition · Brood size · Handicap models · Melanin · Sexual selection · Signalling

Communicated by: Sven Thatje

R. Piault · V. van den Brink · A. Roulin (✉)
Department of Ecology and Evolution, Biophore,
University of Lausanne,
1015 Lausanne, Switzerland
e-mail: alexandre.roulin@unil.ch

R. Piault
Animal Ecology Group, University of Groningen,
P.O. Box 11103, 9700 CC Groningen, The Netherlands

Introduction

Condition dependence is a form of developmental plasticity whereby the expression of a phenotypic trait is influenced by individual condition, i.e. the amount of resources available for allocation to fitness enhancing traits (Lorch et al. 2003). Although condition dependence is likely to affect most aspects of organismal growth, development and function, interest has focussed on the degree of condition dependence of exaggerated secondary sexual traits (Johnstone 1995; Cotton et al. 2004). Handicap models of sexual selection propose that male ornaments serve as signals of male genetic and/or environmental quality for choosy females. The underlying theory assumes that production and/or display of male ornaments draws away resources from fitness enhancing traits (e.g. viability) and that individuals of higher condition (with a larger pool of resources to allocate among competing demands) incur lower marginal costs per unit elaboration of the ornament (Grafen 1990). Given that phenotypic targets of sexual selection are closely linked to fitness, theory predicts that males of increasing condition will invest increasingly more in their ornaments (Nur and Hasson 1984; Rowe and Houle 1996).

Over the past three decades, birds have emerged as a favourite taxon to examine the relationship between sexual trait expression and measures (or surrogates) of individual condition. Little evidence for condition dependence for melanin-based coloration has been reported so far. Early studies that manipulated condition during moult found no effect on the expression of melanin-based traits (Hill and Brawner 1998; Roulin et al. 1998; Gonzalez et al. 1999; McGraw et al. 2002; Roulin and Dijkstra 2003; Bize et al. 2006), and others showed melanin colorations or patterns to

be under tight genetic control (reviewed in Roulin 2004). However, there is no theoretical reason to believe that the expression of this type of ornaments should never be sensitive to environmental factors, and furthermore, previous tests may not have been appropriate to test for condition dependence (Griffith et al. 2006). Accordingly, there are some experimental studies that have demonstrated significant condition dependence of melanin-based plumage traits in birds (e.g. Griffith 2000). For instance, Fitze and Richner (2002) found that breeding great tits (*Parus major*) exposed to hen fleas (*Ceratophyllus gallinae*) had reduced breast stripe size the following year. This latter finding is consistent with the handicap theory and suggests that, although condition dependence of melanin-based traits may not be a general pattern across avian species, it may have evolved in some bird species.

In the present study, we investigated condition dependence of a melanin-based trait in Eurasian kestrels (*Falco tinnunculus*). Adult females are predominantly reddish-brown with black bars on the back and tail, and males blue-grey on the head, rump, upper tail coverts and tail, pinkish-red with black spots on the back and single large sub-terminal black band on tail feathers (Palokangas et al. 1994). This band is larger in males than females and in adults than juveniles (Glutz von Blotzheim 1987). In an aviary experiment, females preferred to approach males with a more pinkish-red back and greyer tail feathers (Palokangas et al. 1994; but see Zampiga et al. 2008). This suggests that, in kestrels, sexual dimorphism in plumage coloration may have evolved in response to sexual selection on males by choosy females. Here, we examined the degree of condition dependence of a melanin-based plumage trait in nestling kestrels close to fledging. Although the adult sexual dimorphism in plumage coloration is not fully developed in fledglings (both sexes look like females), this version of the plumage is partly retained in the first breeding season (1-year-old individuals show adult characters in back, rump–upper tail coverts and head). We performed a brood size manipulation experiment and examined its effect on an offspring colour trait thought to be under directional sexual selection in adults (width of sub-terminal black bands on tail feathers). Previous studies in kestrels have shown that broods can suffer high nestling mortality even in years of abundant natural prey owing to insufficient food supply (Wiehn and Korpimäki 1997) and that brood size reduction significantly increases nestling food intake (Dijkstra et al. 1990). Brood size manipulation is thus an efficient way to manipulate the quantity of food available (condition) to each nestling. To confirm results obtained from this manipulation, we investigated in 2 years whether the first-born nestlings develop wider black tail bands than their younger siblings that are usually outcompeted in sibling competition over parental resources (Müller et al. 2010).

Material and methods

Brood size manipulation experiment in 2006

The brood size manipulation experiment was carried out in western Switzerland between June and August 2006, in a population of kestrels breeding in nest boxes fastened to barn walls. Twenty experimental nests were regularly visited to determine laying date and clutch size. Nestlings were individually recognised by clipping off different combinations of toenails before being ringed at 12 days with an aluminium-numbered band. All twenty breeding females and 18 breeding males were captured, and we measured the width of black bars on all tail feathers along the calamus to the nearest 0.1 mm using a calliper.

We manipulated brood size by swapping hatchlings among 10 pairs of nests with similar hatching dates (Spearman's correlation, $r_s=0.84$, $P=0.002$). Clutch size and the width of the sub-terminal black bands in breeding adults were similar in broods that would be later experimentally enlarged or reduced (Wilcoxon signed-ranks tests, all P values >0.12). Within each pair, part of the nestlings in both nests was swapped so that, at the same time, one brood was reduced and the other enlarged by one nestling. Prior to manipulation, each nest contained between one and five nestlings and brood size remained within the natural range after the manipulation (two to six nestlings). All eggs hatched except in two nests (in one nest four of the five eggs hatched, and in another nest, only one egg hatched out of five) explaining why one enlarged brood contained only two chicks. Reduced broods contained significantly fewer young than enlarged ones immediately after manipulation (mean \pm SD, 3.40 ± 0.70 nestlings in reduced broods (range 2 to 4) against 4.90 ± 1.45 in enlarged ones (range 2 to 6), one-tailed Wilcoxon matched-pairs signed-rank test $P=0.008$). Brood sizes hence remained within the natural range (i.e. one to seven nestlings) after the manipulation. Among the nests whose brood size was increased, one failed soon after the swap of hatchlings, which resulted in a final sample size of 9 enlarged (36 nestlings) and 10 reduced broods (32 nestlings), after manipulation. We visited nestlings on three occasions: for brood size manipulation and identifying position of each nestling in the within-brood age hierarchy (mean age of nestlings \pm SD, 2.6 ± 2.0 days), for ringing (12.5 ± 2.3 days), and to measure nestling phenotypes (25.6 ± 2.5 days). The older individual was assigned rank 1, the second oldest individual rank 2 and so on. In the case we could not determine if one individual was born before or after a nestmate, we assigned them the same rank. For instance, if these were the first two born nestlings, they obtained rank 1.5. Nestlings were weighed to the nearest 0.1 g and the length of their left wing and central tail feather to the nearest 1 mm and length of the left tarsus to the nearest 0.1 mm, respectively. The width of the sub-terminal black

bands was measured along the calamus on the 12 rectrices to the nearest 0.1 mm using a calliper. The mean width of the tail black bands was calculated over the 12 measures for each nestling and used in statistical analyses. We did not assess the repeatability of measuring this colour trait. However, we are confident that our method was reliable because there is ample inter-individual variation in the size of this large tail band. Indeed, as expected, breeding males had larger tail bands than breeding females (paired t test comparing mean tail band width of breeding partners captured in 2006: $t_{17}=7.26$, $P<0.0001$). Furthermore, breeding females had larger tail bands than their offspring ($t_{18}=5.94$, $P<0.0001$) (Fig. 1). Blood samples were collected from their brachial vein to determine sex (Py et al. 2006).

Observation in 2009

In 2009, we visited 24 nests with a total of 81 nestlings. When nestlings were 15 ± 0.2 days old, we determined their rank in the within-brood age hierarchy as explained above. Just before they took their first flight, we visited nests to measure the width of sub-terminal black tail bands in nestlings aged 26.7 ± 0.2 days. Brood size was not manipulated in 2009.

Statistical analyses

Although the design of our brood size manipulation experiment in 2006 involved a partial cross-fostering, and thus the presence of nestlings from two origins within the nests of rearing, five nests (two from the reduced group and three from the enlarged group) ended up with offspring of only one origin due to nestling mortality. Therefore, our dataset did not enable us to estimate the influence of common origin (i.e. inherited genes and maternal effects) on the width of the sub-terminal tail band via the inclusion of the term “nest of origin” in the analyses. Instead, we included the term “nest

of rearing” as a random factor to account for the non-independence of measurements collected within the same nest. To evaluate whether position in the within-brood age hierarchy could affect the expression of melanin-based coloration, we standardised nestling rank in the within-brood age hierarchy as a covariate (i.e. mean centred and divided by its standard deviation within each brood size treatment). We used standardised ranks to ensure that mean ranks do not differ between enlarged and reduced broods. For the 2009 data, we ran a linear mixed model with nest as a random variable and hatching ranks and sex as two dependent variables. All statistical tests were performed with the software program JMP 9.01. Tests were two-tailed and P values smaller than 0.05 were considered significant.

Results

Brood size manipulation experiment in 2006

Enlarged broods suffered higher nestling mortality than reduced broods (nine vs. two nestlings died, respectively, over the whole experiment). Nestlings from the enlarged treatment had longer rectrices (a key trait in the kestrel to forage) than those raised in reduced broods (linear mixed model with nest of rearing as random variable, treatment: $F_{1, 17.29}=4.90$, $P=0.04$; wing length as an index of age: $F_{1, 62.9}=1001.47$, $P<0.0001$; sex was not significant and hence removed from the model). Although similar analyses on tarsus length and body mass showed no significant differences between the two brood size treatments (P values >0.69), we can conclude that the manipulation of brood size affected some aspects of nestling condition.

Nestlings from the enlarged treatment produced significantly shorter black tail bands than those raised in the reduced treatment (linear mixed model with nest of rearing as random variable, treatment: $F_{1, 15.16}=5.95$, $P=0.027$; Fig. 2); in this model, we also introduced standardised ranks in the within-brood age hierarchy ($F_{1, 54.17}=8.70$, $P=0.0047$). Figure 3 shows that first-born nestlings (i.e. seniors) developed larger black tail bands than their younger nestmates (i.e. juniors). Sex (Fig. 1) and interaction between brood size treatments and rank were not significant (P values >0.38) and hence removed from the final model.

Observation in 2009

As in 2006, the width of the sub-terminal black tail bands was shorter in junior compared to senior nestling kestrels (linear mixed model with site as a random variable: $F_{1, 61.2}=4.41$, $P=0.04$; Fig. 4). Sex was not significant ($F_{1, 61.32}=0.30$, $P=0.75$) and hence removed from the final model.

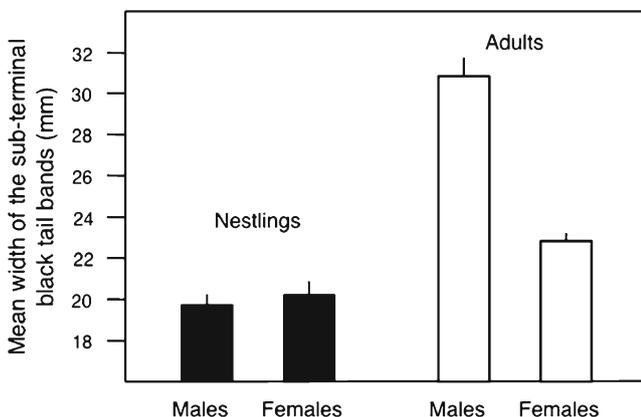


Fig. 1 Mean \pm SE width of the sub-terminal black tail bands in male and female kestrels at the nestling stage and adulthood in 2006

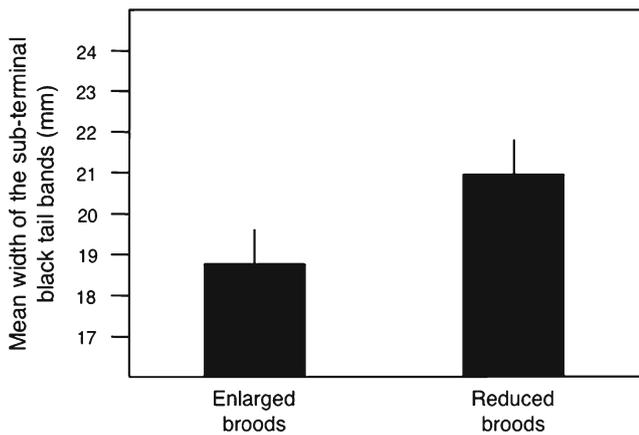


Fig. 2 Mean \pm SE width of the sub-terminal black tail bands in nestling kestrels in relation to the brood size manipulation experiment. This figure is based on mean nest values (Student's t test: $t_{17}=2.72$, $P=0.015$)

Discussion

Eurasian kestrels show a pronounced sexual dimorphism in melanin-based plumage coloration, and based on handicap models of sexual selection, this colourful trait could exhibit sensitivity to condition if it signals absolute individual quality. To date, however, experimental support for condition-dependent expression of melanin-based traits is weak (but see Griffith 2000; Fitze and Richner 2002; Griffith et al. 2006). By manipulating the brood size of breeding kestrels, and thereby the amount of resources available to each nestling, we have shown experimentally that, compared to nestling kestrels raised in enlarged broods, those raised in

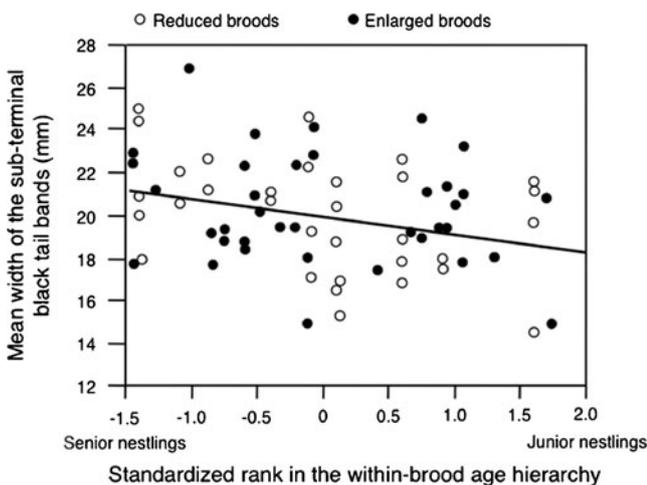


Fig. 3 Relationship between the width of the sub-terminal black tail bands and the position of each nestling kestrel in the within-brood age hierarchy. Data from 2006 representing individuals raised in enlarged (full dots) and reduced (open dots) broods. The regression line is reported for illustrative purpose. This figure presents leverages from the statistical model presented in the results

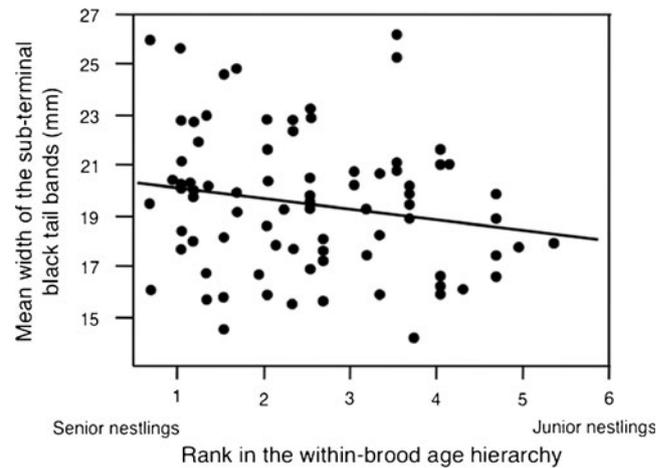


Fig. 4 Relationship between the width of the sub-terminal black tail bands and the position of each nestling kestrel in the within-brood age hierarchy in 2009. The regression line is reported for illustrative purpose. This figure presents leverages from the statistical model presented in the results

reduced broods developed larger sub-terminal black bands on the tail feathers (Fig. 2). This result, although based on a relatively low number of pairs, was confirmed in 2 years by comparing coloration of early and late-hatched nestmates that are in prime and poor conditions, respectively (Figs. 3 and 4).

Condition-dependent expression of black tail bands

The condition dependence of melanin-based traits in nestlings can be a by-product of sexual selection exerted on adult males or, alternatively, the outcome of selection on yearlings to express colour traits that signal dominance (Vergara and Fargallo 2008). However, the sub-terminal black bands on the tail feathers in young kestrels are retained for a year after fledging, and both males and females can breed in this time lapse (Village 1990). Hence, the condition dependence of this colour trait might result from directional selection generated by episodes of social competition and mate choice during the first year of life (Vergara and Fargallo 2007, 2008). It remains unclear whether condition dependence of plumage traits in female kestrels is a by-product of sexual selection exerted on males or whether females are also selected to display melanin traits (e.g. Vergara et al. 2009). Given that at adulthood females display smaller tail bands than males (Fig. 1), the primary function of large tail bands should be to signal aspects of phenotypic quality in males rather than in females. As suggested by Vergara and Fargallo (2007), sexual dimorphism in melanin-based coloration may be reduced at the nestling stage because in the first year of life selection favours males that signal a subordinate female-like plumage. Once males become competitively more competent at a latter

stage, they may then fully express melanin-based traits. This appears to be adaptive, as females seem to prefer the brightest males that have a higher reproductive success (Palokangas et al. 1994). This statement can explain why this trait is sexually dimorphic at adulthood but not at the nestling stage, as it is the case for other melanin-based traits in this species (Vergara and Fargallo 2007). Whatever the exact signalling function of melanin-based coloration has in male and female kestrels, our results suggest that male and female fledglings with larger sub-terminal black bands are of better quality than young males with narrower bands.

Two alternative proximate scenarios can be proposed to explain for our results. First, the total amount of eumelanins produced is condition-dependent. Second, the amount of pigments and the duration during which they are produced are not condition-dependent but genetically fixed. If the tail of individuals in poorer condition grows at a slower rate, their black tail band would be, as a consequence, shorter but the concentration in black pigments per square centimetre would be higher. We believe that in future studies testing the condition-dependent expression of pigmentary colour traits, a distinction should be made between whether the production of pigments is condition-dependent or whether this is the display of the colour trait itself which is condition-dependent. For instance, in the study by Fitze and Richner (2002), great tits parasitised by hen fleas may have produced the same number of black feathers as non-parasitised individuals but the surface over which they were spread may have been reduced in parasitised individuals. This is of interest because under the first scenario we assume that the expression of some genes involved in melanogenesis is condition-dependent, whereas in the second scenario another mechanism needs to be invoked. This is particularly interesting given that some of the genes underlying the production of pigments can have pleiotropic effects on other physiological and behavioural functions (Ducrest et al. 2008).

Comparison with other studies on the kestrel

Previous studies on melanin-based coloration in the kestrel focussed on other plumage traits than the size of the terminal black tail band. Correlative studies by Fargallo et al. (2007) showed that the proportion of male nestlings displaying a grey eumelanic rather than reddish pheomelanic rump is higher in years with abundant prey compared to years with poor food supply. This observation suggests that condition-dependent expression of melanin-based coloration is not restricted to the black tail band but may concern other parts of the body. Accordingly, Vergara and Fargallo (2011) found that three grey melanic traits measured on the head, rump and tail were positively correlated, whereas Parejo et al. (2011) did not find evidence for correlation between the size of the black sub-terminal black tail bands and other melanin-

based traits. Furthermore, within breeding adults, these traits were differentially associated with body mass and laying date. Thus, in the kestrel, melanin-based traits can be considered as indexes of quality (Vergara and Fargallo 2008; Vergara et al. 2009, 2010) and it would of interest to examine whether eumelanin- and pheomelanin-based colour traits are differentially costly to produce. If evidence for condition-dependent expression of melanin-based coloration in the kestrel is accumulating, little is known about the extent to which coloration is genetically inherited. This is an important issue to consider if we want to understand whether the association between coloration and quality is only environmentally mediated or whether it has a genetic component. We can safely assume that in the kestrel the expression of melanin-based coloration has a genetic component, but our results and those of other authors suggest that the environment has a significant effect.

Acknowledgments The Swiss National Science Foundation supported this study (grant nos. PPOA-102913 and 31003A_120517 to A. Roulin and PBLAP3-124279 to R. Piau) and the Roche Research Foundation (grant no. Mkl/stm 14-2008 to R. Piau). We are grateful to the three anonymous reviewers who helped us improve our manuscript. The experiment was approved by the veterinary services of Canton de Vaud (licence no. 1736) and birds were ringed under the legal authorisation of the Swiss Agency for the Environment, Forests and Landscape.

References

- Bize P, Gasparini J, Klopfenstein A, Altwegg R, Roulin A (2006) Melanin-based coloration is a nondirectionally selected sex-specific signal of offspring development in the Alpine swift. *Evolution* 60:2370–2380
- Cotton S, Fowler K, Pomiankowski A (2004) Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc R Soc Lond* 271:771–783
- Dijkstra C, Bult A, Bijlsma S, Daan S, Meijer T, Zijlstra M (1990) Brood size manipulations in the Kestrel (*Falco tinnunculus*)—effects on offspring and parental survival. *J Anim Ecol* 59:269–285
- Ducrest A-L, Keller L, Roulin A (2008) Pleiotropy in the melanocortin system, coloration and behavioural syndromes. *Trends Ecol Evol* 23:502–510
- Fargallo JA, 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
- Fitze PS, Richner H (2002) Differential effects of a parasite on ornamental structures based on melanins and carotenoids. *Behav Ecol* 13:401–407
- Glutz von Blotzheim U (1987) *Handbuch der Vogel Mitteleuropas*. Aulag-Verlag, Wiesbaden
- Gonzalez G, Sorci G, Moller AP, Ninni P, Haussy C, De Lope F (1999) Immunocompetence and condition-dependent sexual advertisement in male house sparrows (*Passer domesticus*). *J Anim Ecol* 68:1225–1234
- Grafen A (1990) Biological signals as handicaps. *J Theor Biol* 144:517–546

- Griffith SC (2000) A trade-off between reproduction and a condition-dependent sexually selected ornament in the house sparrow *Passer domesticus*. *Proc R Soc Lond* 267:1115–1119
- 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
- Hill GE, Brawner WR (1998) Melanin-based plumage coloration in the house finch is unaffected by coccidial infection. *Proc R Soc Lond* 265:1105–1109
- Johnstone RA (1995) Sexual selection, honest advertisement and the handicap principle—reviewing the evidence. *Biol Rev* 70:1–65
- Lorch PD, Proulx S, Rowe L, Day T (2003) Condition-dependent sexual selection can accelerate adaptation. *Evol Ecol Res* 5:867–881
- McGraw KJ, Mackillop EA, Dale J, Hauber ME (2002) Different colors reveal different information: how nutritional stress affects the expression of melanin- and structurally based ornamental plumage. *J Exp Biol* 205:3747–3755
- Müller C, Jenni-Eiermann S, Jenni L (2010) Development of the adrenocortical response to stress in Eurasian kestrel nestlings: defence ability, age, brood hierarch and condition. *Gen Comp Endocrinol* 168:474–483
- Nur N, Hasson O (1984) Phenotypic plasticity and the handicap principle. *J Theor Biol* 110:275–297
- Palokangas P, Korpimäki E, Hakkarainen H, Huhta E, Tolonen P, Alatalo RV (1994) Female kestrels gain reproductive success by choosing brightly ornamented males. *Anim Behav* 47:443–448
- Parejo D, Silva N, Danchin E, Avilés JM (2011) Informative content of melanin-based plumage colour in adult Eurasian kestrels. *J Avian Biol* 42:49–60
- Py I, Ducrest AL, Duvoisin N, Fumagalli L, Roulin A (2006) Ultraviolet reflectance in a melanin-based plumage trait is heritable. *Evol Ecol Res* 8:483–491
- Roulin A (2004) The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biol Rev* 79:815–848
- Roulin A, Dijkstra C (2003) Genetic and environmental components of variation in eumelanin and pheomelanin sex-traits in the barn owl. *Heredity* 90:359–364
- Roulin A, Richner H, Ducrest A-L (1998) Genetic, environmental, and condition-dependent effects on female and male ornamentation in the barn owl *Tyto alba*. *Evolution* 52:1451–1460
- Rowe L, Houle D (1996) The lek paradox and the capture of genetic variance by condition dependent traits. *Proc R Soc Lond* 263:1415–1421
- Vergara P, Fargallo JA (2007) Delayed plumage maturation in Eurasian kestrels: female mimicry, subordination signalling or both? *Anim Behav* 74:1505–1513
- Vergara P, Fargallo JA (2008) Sex, melanic coloration, and sibling competition during the postfledging dependence period. *Behav Ecol* 19:847–853
- Vergara P, Fargallo JA, Martínez-Padilla J, Lemus JA (2009) Inter-annual variation and information content of melanin-based coloration in female Eurasian kestrels. *Biol J Linn Soc* 97:781–790
- Vergara P, Fargallo JA, Martínez-Padilla J (2010) Reaching independence: food supply, parent quality, and offspring phenotypic characters in kestrels. *Behav Ecol* 21:507–512
- Vergara P, Fargallo JA (2011) Multiple coloured ornaments in male common kestrels: different mechanisms to convey quality. *Naturwissenschaften* 98:289–298
- Village A (1990) *The kestrel*. Poyser, London
- Wiehn J, Korpimäki E (1997) Food limitation on brood size: experimental evidence in the Eurasian kestrel. *Ecology* 78:2043–2050
- Zampiga E, Gaibani G, Csermely D (2008) Sexual dimorphic colours and female choice in the common kestrel. *It J Zool* 75:155–159