

Does egg colouration reflect male condition in birds?

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Abstract How colouration provides information about individuals in birds has been a central issue in recent decades. Although much information has been derived, little is known about the adaptive significance of egg colouration in birds. A recent idea suggests that biliverdin- and porphyrin-pigmented eggs may act as a post-mating sexual signal for males to assess female quality. In birds, it is common for males to influence prelaying female condition by courtship feeding. Using Eurasian kestrels, a species that lays protoporphyrin-pigmented eggs, we descriptively assessed the influence of male feeding on egg pigmentation by considering female phenotype, condition, breeding parameters and male body condition. We found that older females and females with greyer tails (an index of individual quality) produce highly pigmented eggs. However, male body condition was the only variable that explained egg colouration when considered together with the female-related variables. Therefore, females that

mated with males in better condition laid highly pigmented eggs. With the same species, we also explored the cost of producing protoporphyrin-pigmented eggs using a food-supply experiment before the laying period. Food supplementation did not increase egg pigmentation, but hatching success and egg mass were positively related to egg colouration only in food supplied pairs. We suggest that egg colouration might be costly to produce and probably suggests egg quality. However, this cost cannot be explained by female quality, but by male condition instead. In general, our results do not support the theory that egg colouration is a post-mating sexual signal in species where males determine female condition at the time of laying.

Keywords Eurasian kestrel · Food supplementation · Egg colouration · Male condition · Female phenotype

Introduction

How colour-related traits are produced and expressed and the type of information they may transmit to conspecifics has intrigued ecologists since the time of Darwin (1871). Ornamentation in general, and ornamental colouration in particular, plays a pivotal role in sexual selection (Hill and McGraw 2006) and may suggest individual survival probability (Hamilton and Zuk 1982) by indicating the quality of the bearer. It is often assumed that production or maintenance of coloured ornaments imposes a cost for the bearer (Zahavi 1975). The trade-offs between ornament colouration and other costly functions such as immunity (Kurtz et al. 2007), mating success (Bitton et al. 2007; Loyau et al. 2007; Markert and Arnegard 2007; Olsson et al. 2005; Punzalan et al. 2008) or aggressiveness (Mougeot

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et al. 2005a, b) may confer honesty to ornament signalling (Andersson 1994). Therefore, how colouration provides information about individuals has become a central issue in evolutionary and behavioural ecology.

The meaning of egg pigmentation is an aspect of colouration that has intrigued ecologists in recent decades, but this has attracted less attention than other pigmented traits in birds (Faivre et al. 2003; Hill and McGraw 2006; Martínez-Padilla et al. 2007; Mougeot et al. 2007; Velando et al. 2005) and remains uncertain (Underwood and Sealy 2002). It has been suggested that egg colouration may be beneficial in decreasing predation by increasing crypticity (Solis and de Lope 1995), reducing brood parasitism (Soler and Møller 2007; Stokke et al. 2002) or increasing eggshell strength (Gosler et al. 2005). A recent hypothesis has suggested that eggshell colouration may function as a post-mating sexually selected signal of female quality to their mates (Moreno and Osorno 2003). Support for this hypothesis comes from correlative studies and experimental cross-fostering manipulation of clutches (Moreno et al. 2004, 2006; Sanz and García-Navas 2009; Soler et al. 2008) which have found positive relationships between egg colouration and male effort. However, other results suggest that the idea needs further consideration (Hargitai et al. 2008; Krist and Grim 2007; López-Rull et al. 2007; Reynolds et al. 2009). In several bird species, courtship feeding represents a substantial benefit which may offset the energetic or nutritional expenditure of egg production (Lifjeld and Slagsvold 1986); this has been experimentally supported (Helfenstein et al. 2003; Nisbet 1973; Salzer and Larkin 1990). However, species that use courtship feeding have been extensively ignored when examining the idea of egg colouration as a post-mating sexual signal. This behaviour effect is especially important in bird species in which males play a key role in providing food to females during the pre-laying and laying periods (Krebs 1970; Lundberg and Alatalo 1992; Newton 1979; Village 1990). Males feeding their mates during courtship is a wide phenomenon that is found in several bird species, from raptors (Newton 1979) to passerines (nearly 50% of North American passerines: Kendeigh 1952; Lack 1940). During this period, males provide food to females, and the quantity or quality of the food can be used by females in mate choice decisions (Mougeot et al. 2002). This suggests that it is common in birds for breeding allocation decisions made by females to be based on male quality. Consequently, body condition of laying females may depend on male feeding behaviour. If male quality affects female condition, it is likely that this can be mirrored in egg pigmentation. This would not support the idea of egg colouration as a post-mating sexual signal. However, the relationship between male quality and egg pigmentation remains almost unexplored (Sanz and García-Navas 2009).

Moreno and Osorno (2003) partly based their idea on biliverdin- (blue-green) pigmented eggs, and it has been recently suggested that this pigmentation might be costly to produce (Soler et al. 2008). However, Moreno and Osorno's idea (2003) was also based on protoporphyrin- (brown-reddish) pigmented eggs. Protoporphyrins are added to the eggshell at the end of the oviduct during egg formation (Deeming 2002). An enhancement of reproductive effort reduced resistance to oxidative stress (Alonso-Alvarez et al. 2004), affecting ornament signalling (Doutrelant et al. 2008). This has led Moreno and Osorno (2003) to suggest that the degree of eggshell protoporphyrin pigmentation may be a potential signal of female quality, as only females in prime health condition could withstand the oxidative cost associated with the deposition of these pigments on the eggshell. However, the cost of producing protoporphyrin-pigmented eggs remains to be tested. Thus, it is possible that egg pigmentation might be costly to produce, but considering the potential additive effect of male quality on female condition, it is necessary to assess whether males or females pay this potential cost. If males do, this might have key implications for an accurate understanding of the hypothesis of egg pigmentation as post-mating sexual signal, and the premises should be reframed more accurately.

Here, we present a combination of correlative and experimental results in the Eurasian kestrel (*Falco tinnunculus*), a non-passerine and cavity-nesting bird species that lays protoporphyrin-pigmented eggs (Village 1990, whitish brown to dark reddish brown). In this study, the first aim was to explore the relationship between female quality and egg pigmentation at the correlative level. As a proxy of female quality, we considered the percentage of grey colouration in rump and tail, age and body condition in breeder females: these characteristics have been described as reliable fitness-related traits (Vergara et al. 2009). Second, we explored the relative influence of male quality on egg pigmentation. Female kestrels are fed by their mates starting at courtship until halfway through the nestling period (Village 1990), and male quality may influence reproductive decisions in females (Lack 1940; Salzer and Larkin 1990; Steele 1986). Third, we experimentally manipulated food conditions prior to laying in order to assess the potential cost of producing protoporphyrin-pigmented eggs as previously described (Martínez-Padilla 2006; Martínez-Padilla and Fargallo 2007). According to the aims described above and considering that males feed their mates during courtship, we expect (1) a positive relationship between female body condition or quality (assessed by their phenotypic traits) and eggshell pigmentation (Morales et al. 2006; Soler et al. 2008), but (2) a stronger relationship between male condition and eggshell pigmentation, and (3) that supplemented females will lay eggs with more pigments than control females.

Material and methods

Study species and breeding parameters

The study was carried out on a population of Eurasian kestrels in Campo Azávaro (40°40'N, 4°20'W, 1,300 m above sea level), central Spain, during the breeding seasons of 2002 and 2007. The study area is comprised of montane grasslands. The area is a flat and wide valley where the only trees present are located close to farms or in small, isolated patches (for more details, see Fargallo et al. 2001). The main use of the area is livestock rearing, and no agricultural use has been recorded at least since the end of the nineteenth century (Aitken 1940). Kestrels breed mainly in nest boxes provided since 1994 (Fargallo et al. 2001). In 2002, we monitored nest boxes every 2 days during egg laying and we marked eggs as they were laid in order to track laying date and sequence (Martínez-Padilla 2006; Martínez-Padilla and Fargallo 2007). Eggs were weighed to the nearest 0.25 g on the day of laying or the day after. We estimated incubation period as the number of days between the start of incubation and the day of hatching, following the method described in Blanco et al. (2003). To record hatching order, we started visiting nests every day from 25 days after the end of laying. When we detected the first evidence of hatching, we visited up to three times every day until every egg in the clutch had hatched. We estimated hatching asynchrony as the number of hours between the laying of each particular egg in relation to the first hatched egg (Viñuela 2000). We marked each chick with indelible ink on their egg-tooth (see details below) during the hatching process. Later, once all chicks had hatched, we marked them on the head every 5 days until they were ringed at 17–20 days. This allowed us to determine from which egg each chick had hatched. Nestlings were weighed with Pesola balances to the nearest 0.25 g on the day of hatching and at 24 days old. In 2007, we were only able to record laying date, egg mass, clutch size and brood size.

Adult male and female body condition

In 2007, we caught adult females on two occasions: during the incubation period (between a week after laying and a week before hatching) by netting and at the mid-nestling period by using nest-box traps (see below for further details). Males cannot be trapped during incubation because this may result in great disturbance to females and may interfere with breeding performance. Thus, males were trapped only at the mid-nestling period. In all cases and for both sexes, we recorded weight with Pesola balances (to the nearest gram) and wing and tarsus length with a ruler (to the nearest millimetre). For females, we also calculated the

change in weight between the first and the second capture. Breeding males and females were classified as yearlings or adults (>1 year old) according to plumage characteristics (Village 1990) or by ring codes if they were ringed in previous years.

Female traits

In 2007, we also estimated percentage of greyness of female tails and rumps as these are quality-dependent phenotypic traits in kestrels (Vergara et al. 2009). Using direct observation in the field, female tail and rump greyness were determined by the percentage of grey with respect to brown covering the whole rump and tail (0% = completely black-barred brown and 100% = completely black-barred grey). We estimated the repeatability of rump and tail colouration measurements in 17 dead individuals (Lessells and Boag 1987) by measuring each trait twice. Measurements within rumps ($r=0.99$, $F_{16,17}=236.81$, $p<0.001$) and within tail colouration were highly repeatable ($r=0.99$, $F_{16,17}=230.75$, $p<0.001$, $n=17$, see Vergara et al. 2009 for more details). Percentage of grey colouration estimated in the field reliably reflects the percentage of greyness measured from digital photographs, as previously reported (see for more details Fargallo et al. 2007a, b). Thus, we are confident that our measurements in the field reliably estimate percentage of grey colouration in rump and tail.

Food supplementation

In 2002, we performed a food supplementation experiment before egg formation (for more details, see Martínez-Padilla 2006; Martínez-Padilla and Fargallo 2007). Briefly, we supplemented food every 2 days with 120 g of farmed Japanese quail *Coturnix c. Japonica*, as previously reported for kestrels (Aparicio 1999). Feeding started at least 18 days before egg laying, thus ensuring that food supplementation started before egg formation (Meijer et al. 1989), thus covering the entire period of egg formation in this species (Meijer et al. 1989). Food supplementation prior to and during egg laying in Eurasian kestrels can increase clutch size (Aparicio 1994), rendering groups incomparable. Thus, we ceased food supplementation when the first egg was laid to avoid this possible effect. We confirmed that quails were eaten by finding their remains in or around food-supplemented nests. In order to explore the effect of the experiment on female body mass and condition, they were captured by netting at the nest-box between 1 week after the end of laying and 1 week before hatching. Therefore, the time elapsed from when we stopped food supplementation until female capture varied from 17 to 30 days (kestrels lay eggs every other day).

Egg colouration

In 2002 and 2007, high-resolution pictures ($2,272 \times 1,704$ pixels) of the eggs were taken at a standard distance (50 cm) using the flash of a digital camera (Nikon Coolpix 950ZS). All photos were taken by JM-P between 7 and 10 days after females finished laying. The same grey reference chip was placed beside the egg for each picture within years but not between years. Hence, we were unable to compare egg colouration variation between years. Kestrel egg pigmentation is extremely variable: eggs may be homogeneously pigmented or they may show spots that vary in size, shape, number, density and colour intensity. Also, background colouration may range from almost white (unpigmented) to dark reddish brown. This complex patterning is difficult to measure; therefore, as a conservative measure, we measured the average eggshell lightness–darkness as an estimate of the overall degree of pigmentation. JM-P analysed digital images using Adobe Photoshop 9.0.2, fitting the largest ellipsoid area within each egg to obtain its average red (R), green (G) and blue (B) components. The same was done for the grey reference of each photograph. With this methodology, we were able to follow the recommendations suggested by Stevens et al. (2007). We performed a principal component analysis (PCA) with the R, G and B of each egg and for each reference. This resulted in three components: the first one explained 95.95% (eigenvalue = 2.85) of the variance, the second 4.0% (eigenvalue = 0.13) and the third 0.05% (eigenvalue = 0.01). Similar results were obtained for the reference (first component: eigenvalue = 2.84, 94.9% of the variance; second component: eigenvalue = 0.14, 4.8% of the variance; third component: eigenvalue = 0.007, 0.3% of the variance). We considered the first component an “egg colouration index” for the eggs and the first component for the reference as a correcting factor. In the RGB colour system, a combination of high values of these three components (R, G and B) of the first axis corresponds to dull/light colours (less pigmented–dark–brownish eggs), so we transformed it to a positive value by multiplying by (–1) for a more intuitive interpretation of the results. We did the same for the reference value for consistency. Therefore, in every figure shown, higher indexes of egg colouration are associated with highly pigmented eggs.

In order to validate our method, we assessed the repeatability of egg colour measurements in two ways following Lessells and Boag (1987): (1) within the same picture and (2) between two different pictures of the same egg. From a subsample of 28 randomly selected eggs, we obtained an egg colouration index that was highly and significantly repeatable within pictures (repeatability = 0.99, $F_{27,55}=5,844.90$; $p<0.001$). Similarly, the egg colouration index between pictures was highly repeatable

(repeatability = 0.98, $F_{14,29}=111.09$, $p<0.001$). Thus, we are confident that our method reliably reflects egg colouration in kestrels.

Statistical procedures

General linear mixed models (GLMM) were performed with SAS software for all analyses. The response variable (egg colouration index) was fitted to a normal distribution and an identity link function. Since egg colouration from eggs in the same nest probably had more similar colouration than those from different nests due to the influence of sharing common parents and rearing environments, they were not considered independent samples. Thus, we considered nest as a random variable: this allowed us to treat colouration of each egg as the sample unit. Statistical tests associated with random terms denote significant nest variation in the variables examined (in all models, the random term was significant, $Z>3.33$, $p<0.001$). We performed three models to explore the relationships between egg colouration and breeding parameters. At a correlative level and considering only control broods in 2002, the first model explored the relationship between egg colouration and laying order, hatching order, hatching probability and brood reduction as fixed effects while nestling mass, egg mass, laying date, incubation period, brood size, clutch size and hatching/fledging success were considered as covariates. Laying and hatching order were encoded as first, middle or last laid egg, to allow a comparison of differences in egg mass within the laying sequence between different clutch sizes. Hatching probability of a particular egg was recorded as 0 (unhatched) and 1 (hatched), and brood reduction was noted when at least one chick died in the brood after hatching. Hatching and fledging successes were obtained by dividing the number of eggs hatched by clutch size and the number of nestlings successfully reared by initial brood size, respectively. We performed a second model with the data collected in 2007, with egg colouration as a dependent variable, brood reduction as a fixed effect and nestling mass, egg mass, laying date, brood size, clutch size and hatching/fledging success as covariates. As explanatory variables, we included body mass, size and age for both sexes and greyness of female rump and tail for females only. We considered body condition as body mass corrected by wing length in the models. In the third model, we explored the relationship between egg colouration and the experimental manipulation with GLMM models. Egg colouration index was the response variable and laying order, hatching order, egg mass, laying date, incubation period, brood size, clutch size and hatching success were explanatory terms. Treatment was a fixed effect and the interactions with all variables were included in the model. Mean and SD are given.

Results

Egg colouration and breeding success

In 2002 and from control broods, we analysed egg colouration from 86 eggs and 16 different clutches. We did not find any relationship between egg colouration and female mass, size (wing or tarsus length) or condition (GLM, all $p > 0.167$, in all cases grey reference as covariate, all $p < 0.047$). From all reproductive variables considered, we found that the degree of pigmentation decreased only with laying order (GLMM, $F_{2,56}=3.79$, $p=0.032$). Specifically, last laid eggs were duller than first (GLMM, $F_{1,22}=4.04$, $p=0.045$) and middle laid eggs (GLMM, $F_{1,43}=6.04$, $p=0.018$). No differences were found between first and middle laid eggs (GLMM, $F_{1,45}=0.08$, $p=0.788$). We did not find any relationship between the reproductive parameters considered and egg colouration (GLMM, all $p > 0.120$).

Egg colouration, adult body condition and female traits

In 2007, we were able to compare colouration from 175 eggs and 36 nests to parental condition. In females, we did not find any relationship between body condition (body mass corrected by wing length) and egg colouration, neither during incubation (GLMM, $F_{1,93}=0.13$, $p=0.718$) nor during early chick rearing (GLMM, $F_{1,93}=0.01$, $p=0.933$). Similarly, we found that egg colouration was not related to female weight (GLMM, both $p > 0.700$) or the change in female weight between captures (GLMM, $F_{1,95}=0.11$, $p=0.743$). Older females laid eggs with more pigment than younger females (GLMM, $F_{1,95}=7.16$, $p=0.008$). When considering female age, percentage of grey on the rump and grey on the tail, only the latter variable was related positively to egg colouration (GLMM, $F_{1,95}=5.04$, $p=0.026$; rest of variables, all $p > 0.342$). However, when body condition of the male was added to the model, the only variable related to egg colouration was male condition (GLMM, $F_{1,75}=4.57$, $p=0.036$, remaining variables, GLMM, all $p > 0.462$). Females paired with males in better condition laid darker eggs (Fig. 1).

Food-supplementation experiment

The average length of supplementary feeding before the onset of laying was 34.45 ± 5.07 days. The food-supplement experiment did not modify any of the breeding parameters considered (see Martínez-Padilla 2006; Martínez-Padilla and Fargallo 2007). Female parent mass, wing, tarsus length and condition did not differ between treatments (see Martínez-Padilla 2006; Martínez-Padilla and Fargallo 2007). In addition, food supplementation did not change the

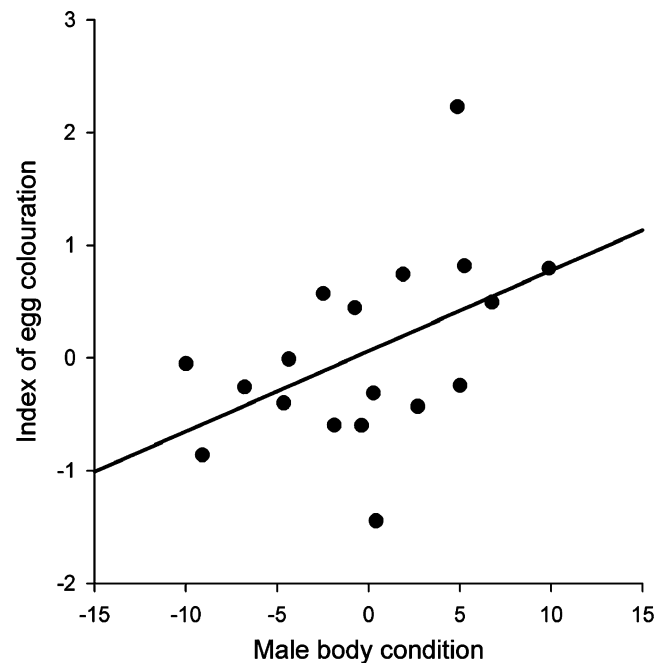


Fig. 1 Linear regression between average egg colouration in a clutch and body condition of male Eurasian kestrels

relationship between egg colouration and female mass, size (wing or tarsus length) or condition between groups (see Martínez-Padilla 2006; Martínez-Padilla and Fargallo 2007). Hatching and fledging success were not significantly different between groups (see Martínez-Padilla 2006; Martínez-Padilla and Fargallo 2007). Treatment did not affect each egg's hatching time (in relation to the first hatched egg), egg size or hatching asynchrony (see Martínez-Padilla 2006; Martínez-Padilla and Fargallo 2007).

Food supplementation did not influence egg colouration (GLMM, $F_{1,139}=1.04$, $p=0.310$) and only became statistically significant when the interactions were introduced to the models (Table 1). However, food supplementation modified the relationship between egg size and egg colouration (Table 1). Egg size was positively related to the egg colouration index in food-supplemented pairs (GLMM, $F_{1,60}=10.14$, $p=0.002$), but not in control pairs (GLMM, $F_{1,63}=0.77$, $p=0.384$, Fig. 2). Also, the relationship between egg colouration and hatching success was different depending on treatment (Table 1). In food-supplemented nests, eggs were darker when they were from broods with higher hatching success (GLMM, $F_{1,56}=35.99$, $p < 0.001$). There was no relationship between hatch success and egg pigmentation in control nests (GLMM, $F_{1,67}=0.03$, $p=0.870$).

Table 1 GLMM with normal error and identity link function on egg colouration

Response Term	Rejected terms	Explanatory terms	<i>F</i>	Df.	<i>p</i>	Estimates ± SE
Egg colour index		Reference colour index	19.18	1,124	<0.001	0.438±0.006
		Treatment	15.48	1,124	<0.001	−6.369±3.246
		Hatching success	19.66	1,124	<0.001	−1.659±0.527
		Egg mass	2.04	1,124	0.155	−0.319±0.133
		Hatching success*treatment	13.80	1,124	0.003	−1.659±0.527
		Egg mass*treatment	9.62	1,124	0.002	0.318±0.154
	Clutch size		1.31	1,118	0.254	0.065±0.018
	Clutch size *treatment		2.11	1,118	0.149	−0.263±0.335
	Brood size		1.26	1,82	0.263	−0.276±0.122
	Brood size *treatment		0.37	1,82	0.544	0.166±0.187
	Incubation period		1.63	1,91	0.205	−0.028±0.100
	Incubation period*treatment		0.06	1,91	0.814	−0.140±0.123
	Hatching order		0.28	1,88	0.598	−0.035±0.248
	Hatching order*treatment		0.01	1,88	0.921	0.089±0.324
	Laying date		1.40	1,116	0.239	0.069±0.018
	Laying date*treatment		1.16	1,116	0.196	−0.035±0.023
	Laying order		2.93	2,122	0.089	−0.197±0.312
	Laying order*treatment		0.67	2,120	0.512	0.273±0.383

The model retained the variance of nests introduced into the model as a random term (see “Material and methods” for more details). Parameter estimates and SE for the levels of fixed factor were calculated by considering a reference value of zero for last-order level

Discussion

At a correlative level, a strong relationship between egg colouration and breeding parameters is not apparent as egg colouration decreased only with laying order. We did not

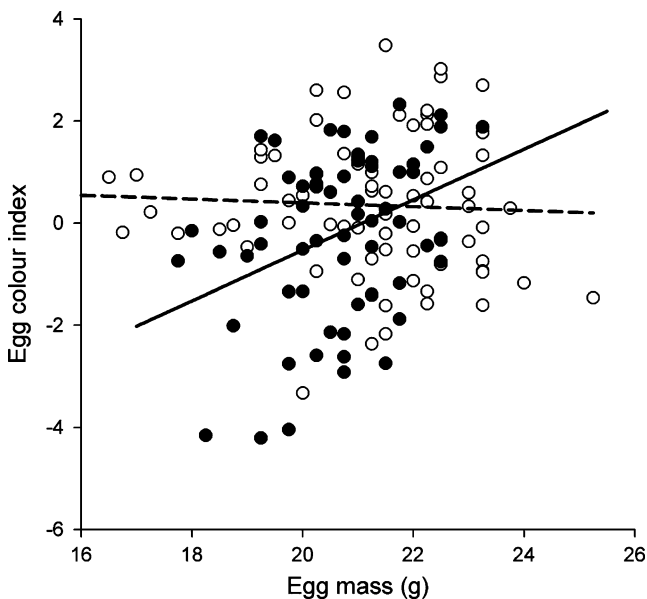


Fig. 2 Linear regression between egg mass and egg colour index for control (empty circles and dashed line) and food supplemented pairs (full circles and continuous line) in Eurasian kestrels

find any effect of female mass or body condition on egg colouration, but some indicators of female quality, such as age and plumage colouration, were positively related to egg colouration. Therefore, protoporphyrin-based egg colouration may be informative about female quality. However, male body condition better explained egg colouration than any measures of female quality (age, phenotype or body condition). The weak association between female phenotypic traits and egg colouration does not rule out that egg pigmentation might be a result of female physiological condition rather than suggesting female quality (Cassey et al. 2008). The results from the experiment show that egg colouration did not increase with extra food before laying, but suggest instead that larger eggs and eggs from clutches with higher hatching success were more pigmented only in food-supplied pairs.

There was no association between female condition and egg colouration in our correlative results, but we did see a relationship between egg colouration and female phenotype. One possible explanation for the lack of relationship between female body condition and egg colouration is that we captured females about 1 week after the end of laying, and this delay may not reflect their body condition during egg laying. However, this is unlikely, as female condition and egg colouration can be positively related in incubating females (Morales et al. 2006). We also found a significant relationship between egg colouration and embryo develop-

ment (expressed as hatching success) in food-supplied but not in control pairs. In contrast, egg colouration was positively related to the amount of grey colouration on the tail. It has been suggested that this melanin-dependent trait is an index of female quality (Vergara et al. 2009); thus, egg colouration may be informative about female quality. However, care should be taken at interpreting that egg colouration might be a proxy of female quality because it correlates with a secondary phenotypic trait (Reynolds et al. 2009). Reynolds et al. (2009) suggested that plumage quality may be a more reliable signal of female quality than egg pigmentation because it can be assessed outside the low-ambient-light conditions found inside a nest cavity or nest-box.

Male body condition was the best predictor of egg colouration even after considering female quality (understood as body condition or different phenotypic traits, see above). We assessed male body condition at the halfway point of the nestling period, but we are confident that this is a reliable index of male breeding condition. Male condition shows no, or very slight, variation from courtship to the nestling period (Village 1990). Male kestrels feed their mates from courtship to halfway through the nestling period (Village 1990), and female condition at the time of laying is strongly related to male quality (Village 1990). This may explain why egg colouration is related to male but not female body condition. As has been observed in insects, courtship or nuptial feeding in oviparous organisms may significantly enhance female fitness because of the nutrients derived from the male's gift (Thornhill and Alcock 1983; Vahed 1998). This potential benefit has also been described in birds (Lifjeld and Slagsvold 1986; Royama 1966; Tasker and Mills 1981). In fact, numerous studies and experiments have suggested that courtship feeding in birds represents a substantial compensation for the energy expended in egg production (Helfenstein et al. 2003; Lifjeld and Slagsvold 1986; Nisbet 1973; Salzer and Larkin 1990). In addition, in many bird species, such as passerines (Lundberg and Alatalo 1992) or raptors (Newton 1979), the male obtains the breeding territory according to his quality. He is often also the main food provider to the female and chicks (Palokangas et al. 1994; Village 1990). Therefore, it is clear that males can influence the conditions (resource abundance) in which females start laying. They likely influence egg colouration as well (Sanz and García-Navas 2009).

We cannot clarify the mechanism behind this pattern. We suggest two potential explanations. First, it is possible that male courtship feeding can be a signal of quality to females (Vergara et al. 2007), leading them to allocate resources differently on or in their eggs according to their mate's quality. Second, it has been shown that protoporphyrin pigment in eggs can act as a surrogate of structural shell components, such as calcium, when these are scarce. Particularly, in the presence of dichloro-diphenyl-trichloroethane

(DDT), birds respond to the reduction in eggshell thickness by increasing protoporphyrin deposition (Jagannath et al. 2008). This suggests that pigment deposition has a structural function (Gosler et al. 2005). However, birds in our study area during the breeding period are free of carbamate or organophosphorus insecticides (Vergara et al. 2008), so the effect of chemical pesticides is probably low in our population. Furthermore, according to this function of pigment deposition, we would expect that females mated with poor-quality males would produce eggs with more pigments to compensate for males providing fewer resources for structural shell development. We found the opposite trend. We cannot rule out, however, the possibility that females mated with high-quality males (males with good body condition) may be able to afford a high allocation of both calcium and porphyrins on their eggshells (Jagannath et al. 2008). Regardless of the mechanism, it appears that egg colouration may be a side effect of male quality in kestrels. Only with cross-fostering experiments will it be possible to correctly evaluate the role of males in egg pigmentation. If male condition determines egg colouration, it would be inappropriate to consider this trait a post-mating sexual signal for males. It is likely that, even in kestrels, egg colouration may reflect female quality, but this relationship may be weaker in species in which female condition before laying depends on male quality. When exploring the meaning of egg colouration under Moreno and Osorno's hypothesis (2003), it is essential to either consider male condition or to use cross-fostering experiments to change male body condition.

Nonetheless, we found that the relationship between egg mass and egg colouration was different between control and food-supplemented groups: egg colouration and egg size were positively associated in food-supplemented females only. However, more subtle effects may emerge when other factors, such as the sex of the embryos, are taken into account (Martínez-Padilla and Fargallo 2007). This may also be the case for egg colouration. Producing larger and darker eggs may only be affordable for those females that received supplemental food before laying. Also, considering that the experiment did not alter egg mass (further explanations given in Martínez-Padilla 2006; Martínez-Padilla and Fargallo 2007), our results suggest a potential cost for producing brown-pigmented eggs. Thus, we suggest that (1) only females mated with high quality males may afford laying bigger and darker eggs and (2) females may allocate differentially on their eggs according to male condition. Interestingly, it has been shown in kestrels that food supplementation also changed the relationship between egg mass and T-cell-mediated immunity of offspring (Martínez-Padilla 2006) in a similar way that egg colouration and size covaried in this study: egg mass was negatively related to cell-mediated immunity in control broods, but positively in food-supplemented

broods. Only females supplied with extra food might increase resource allocation in larger eggs, resulting in increased cellular immunocompetence at the time of fledging (Martínez-Padilla 2006). We agree that female ornamentation is also being sexually selected, as Moreno and Osorno (2003) suggest. However, in light of our results, we think that it is unlikely that egg colouration is under similar selection pressures.

Considering our results together with the previous positive association between egg mass and nestling cellular immunity (Martínez-Padilla 2006), the potential cost of producing eggs with more pigmentation may rely on males rather than on females. Therefore, our results do not support the idea of considering egg colouration as a postmating-sexual signal, at least in species where males determine female condition during the laying period. Therefore, we suggest that egg colouration might be not adaptive, but a side effect of male quality. These results constitute a new framework for Moreno and Osorno's (2003) hypothesis and egg colouration in general that deserves future work in order to elucidate the meaning and consequences of egg colouration in birds.

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