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Signalling value of maternal and paternal melanism in the barn owl: implication for the resolution of the lek paradox

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Secondary sexual characters often signal qualities such as physiological processes associated with resistance to various sources of stress. When the expression of an ornament is not sex-limited, we can identify the costs and benefits of displaying a trait that is typical of its own sex or of the other sex. Indeed, the magnitude and sign of the covariation between physiology and the extent to which an ornament is expressed could differ between males and females if, for instance, the regulation of physiological processes is sensitive to sex hormones. Using data collected over 14 years in the nocturnal barn owl *Tyto alba*, we investigated how nestling body mass covaries with a heritable melanin-based sex-trait, females displaying on average larger black feather spots than males. Independently of nestling sex, year and time of the day large-spotted nestlings were heavier than small-spotted nestlings. In contrast, the magnitude and sign of the covariation between nestling body mass and the size of parental spots varied along the day in a way that depended on the year and parental gender. In poor years, offspring of smaller-spotted mothers were heavier throughout the resting period; in the morning, offspring sired by larger-spotted fathers were heavier than offspring of smaller-spotted fathers, while in the evening the opposite pattern was found. Thus, maternal and paternal coloration is differentially associated with behaviour or physiology, processes that are sensitive to time of the day and environmental factors. Interestingly, the covariation between offspring body mass and paternal coloration is more sensitive to these environmental factors than the covariation with maternal coloration. This indicates that the benefit of pairing with differently spotted males may depend on environmental conditions, which could help maintain genetic variation in the face of intense directional (sexual) selection.

ADDITIONAL KEYWORDS: colour polymorphism – female ornament – melanin – sexually antagonistic selection – sexual dimorphism.

INTRODUCTION

Secondary sexual traits often covary with life-history traits, physiology and behaviour with, for instance, individuals displaying an extravagant version of a sex trait being more aggressive (Senar & Camerino, 1998) or more immunocompetent (Folstad & Karter, 1992) than drab conspecifics. In species in which a secondary sexual character is not sex limited, and hence expressed in both sexes, the magnitude (or even the sign) of the covariation between the sex trait and other phenotypes may differ between males and females. From an ultimate point of view, secondary sexual characters may covary with other traits in a sex-specific manner because the fitness costs and benefits derived from expressing a trait to a different magnitude differs between the sexes (e.g. natural selection favours cryptic females and sexual selection attractive males) (Cox & Calsbeek, 2009). For example, in the barn swallow (*Hirundo rustica*) darker reddish males, but not females, are more likely to disperse (Saino *et al*., 2014). From a proximate point of view, the secondary sexual character

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may be associated with sex-specific physiological processes that have different consequences for males and females. For instance, in bank voles (*Myodes glareolus*), sons from lines of individuals artificially selected for high testosterone levels achieved a high reproductive success in contrast to daughters, who performed poorly. The opposite finding applied to individuals issued from lines of individuals artificially selected for low testosterone levels (Mills, Koskela & Mappes, 2012). Thus, females may be physiologically less successful when expressing secondary sexual characters at levels that are typical to males, and vice versa for males. When selection on some physiological traits is sex specific (e.g. Boratynski *et al*., 2010), these traits may therefore be differentially associated with secondary sexual characters in the two sexes.

Recent studies in the barn owl (*Tyto alba*) have shown that the size of black eumelanic spots displayed at the tip of ventral body feathers is positively selected in females and negatively in males (Roulin *et al*., 2010a; Roulin, Antoniazza & Burri, 2011). These selective processes may be exerted on spots themselves and/or on genetically correlated traits. Indeed, the size of maternal black spots covaries with several physiological traits expressed in their offspring (Roulin & Ducrest, 2011). Of particular interest is the finding that larger-spotted mothers produce offspring that better regulate glucocorticoids in response to stress than offspring born from smaller-spotted mothers (Almasi *et al*., 2012). Furthermore, daily variation in baseline corticosterone levels measured in nestlings was associated with maternal and paternal spot diameter but in opposite directions (Roulin, Almasi & Jenni, 2010b). Nestlings showed a typical daily variation in corticosterone levels for a nocturnal animal (i.e. lower levels in the morning and higher levels in the evening) when the mother displayed a male-like plumage (i.e. small black spots) and the father a female-like plumage (i.e. large black spots). When the parents displayed a plumage typical of their own sex (i.e. large spots in mothers and small spots in fathers), the pattern of variation was opposite to what expected, i.e. higher corticosterone levels in the morning and lower levels in the evening. As corticosterone is associated with metabolism (e.g. corticosterone levels increase when hunger increases, Sapolsky, Romero & Munck, 2000), these observations raise the hypothesis that offspring born from differently spotted parents show different genetically inherited patterns of daily variation in growth or digestion. These observations are interesting because they suggest that maternal and paternal spot sizes are differently related to offspring physiological processes potentially explaining, at least in part, why spot size is sexually antagonistically selected. We indeed found that smaller-spotted mothers and larger-spotted father produce sons and daughters with a high survival prospect, respectively (Roulin *et al*., 2010a). In order to get an insight into this hypothesis, we analysed nestling body mass during the resting phase (i.e. daylight hours) in relation to spot diameter measured in the nestlings themselves and in their biological parents using a large dataset collected during 14 years including 13595 measurements of 2193 nestlings. As we did for baseline corticosterone levels (Roulin *et al*., 2010b), we tested whether the covariation between nestling body mass and parental spot diameter changes in magnitude and/or sign from the morning to the evening and whether the sign of the change differs between mothers and fathers. Furthermore, we assessed whether these associations vary between years characterized by poor and high barn owl reproduction. Thus, for the intended purpose growth curves are not useful because they do not allow to test whether the covariation between body mass and plumage spottiness varies in sign and magnitude from the morning to the evening. Body mass measured in the morning should be more strongly correlated with parental feeding rates (Roulin, Ducrest & Dijkstra, 1999) and the ability to compete with siblings to monopolize parental food resources, whereas the decrease in body mass during the resting phase from the morning to the evening should be associated not only with the amount of food consumed the previous night but also with the efficiency of digestion (Roulin, 2009). Therefore, body mass measured in the morning, at noon and in the evening should reflect different processes. Our large dataset allows us to look at patterns otherwise hidden by variation in environmental conditions. For instance, in years with good breeding conditions nestlings may be heavier in the morning when their father displays large rather than small spots, whereas in the evening the opposite pattern may prevail. The opposite may be true for years with suboptimal breeding conditions. These associations may differ in magnitude and possibly in sign when comparing nestling body mass and maternal spot diameter.

Whatever the exact cause of the potential association between nestling body mass during the resting phase and parental pigmentation is, such a study can give insight in the exact signalling value of secondary sexual characters used in mate choice (Roulin, 1999; Roulin & Altwegg, 2007). This is important because identifying covariations between the degree of ornamentation and other phenotypes can be hidden if these covariations are sex specific and sensitive to other factors such as time of the day and environmental conditions. Furthermore, the covariation between nestling body mass and pigmentation can depend on whether we consider nestling, maternal or

paternal pigmentation. Indeed, at adulthood pigmentation is not equally selected in males and females (Roulin *et al*., 2010a), a phenomenon that might not yet prevail at an earlier stage in the ontogeny (Chippindale, Gibson & Rice, 2001).

Our specific aims in the present study are the following: (i) we analysed the association between nestling body mass and the size of the melanic spots, when spot size is measured in the nestlings themselves and in the parents; (ii) we investigate whether these associations vary throughout the day and between years; and (iii) we examined whether interannual variations in the magnitude and sign of the covariations between nestling body mass and spot diameters is explained by environmental factors estimated by barn owl reproductive success. To this end, for each year we calculated the slopes of the regression of nestling body mass on parental spot diameters and correlated these values with indices of barn owl reproductive success (population size, laying date, brood size at fledging). A positive correlation would indicate that the offspring of larger-spotted parents are heavier when barn owl breeding conditions improve. This study has the potential to reveal that parental coloration is associated with offspring physiological processes in a different way when coloration is measured in the mother and father. This is important because it would indicate that under some specific environmental conditions a given value of an ornament is positively selected in females and negatively selected in males, and vice versa with other environmental conditions. Indeed, daily variation in nestling body mass may reflect trade-offs between several processes associated with digestion, food intake and more generally energy homeostasis. As a consequence, maternal and paternal spot diameter may be differentially selected through their covariation with nestling body condition.

MATERIAL AND METHODS

Study species

In the barn owl, the plumage varies from white to dark reddish-brown, a pheomelanin-based trait, and from immaculate to marked with black spots of varying size. Although members of the two sexes exhibit a trait in the same range of possible values, mean plumage trait values differ between males and females. Black spots are probably due to the deposition of eumelanin pigments, although we do not exclude the possibility that some pheomelanin is also stored. Feather concentration in porphyrin was not associated with plumage traits and we did not detect carotenoids in feathers (Roulin *et al*., 2008). Here, we considered only the size of eumelanic spots, since the hypothesis about a link between daily variation in body mass and the size of spots measured in the two parents holds for this trait only. Spot size is strongly heritable ($h^2 = 0.82$, Roulin *et al*., 2010a) and about 27% of the between-individual variation is explained by genes located on the Z sex chromosome and 44% by genes located on the autosomes (Larsen *et al*., 2014). The between nest variance in spot size is 0.72 and the within nest variances 1.04 indicating that the variance between the individuals within the same nest may be even bigger than the variance of the mean of the nests. Interestingly, the pronounced directional selection on female spot size leads to a change in the estimated autosomal breeding value but not in the sex-linked breeding value (Roulin *et al*., 2010a). An analysis of various fitness components showed that directional selection is exerted on survival in the first-year of life with large-spotted females having an advantage over small-spotted females (Roulin *et al*., 2010a). Mothers displaying smaller black spots (i.e. a typical male-like trait) produced sons with a high survival prospect, whereas fathers showing larger black spots (i.e. a typical female-like trait) sired daughters with particularly high survival prospects (Roulin *et al*., 2010a). This further emphasises the fact that the correlation between offspring phenotype and parental spot size can be of opposite sign when spots are measured in the mother and father. Black spots play a role in male mate choice. In an experiment in which we reduced the number of black eumelanic spots in breeding females, their male mate reduced feeding rate (Roulin, 1999). Furthermore, in the following year, females with experimentally reduced spottiness were less likely to breed again in the study area compared to control females (Roulin & Altwegg, 2007). Extra-pair paternity is rare in the barn owl (Henry *et al*., 2013).

Data collection

Between 1996 and 2009, we measured 2193 nestlings from 550 nests giving a total of 13 595 records of body mass (Table 1). In the present study, mean clutch size was 6 (range: 2 to 11). Nestling age was estimated after measuring wing length soon after hatching (Roulin, 2004) and sex was identified using molecular markers (1107 females and 1086 males; Py *et al*., 2006). Broods were repeatedly visited for other study purposes between 8 a.m. in the morning and midnight (mean ± SE: 2 p.m. ± 0.03) from 8 March until 29 October (mean ± SE: 28 June ± 0.3 days). At each nest visit, we always weighed and measured one wing of all nestlings of each brood; because eggs hatch asynchronously every 2.5 days, there is a pronounced within-brood age hierarchy. At the time of body mass measurement, nestlings were between 0 and 64 days

Table 1. Number of nests, individual nestlings and body mass measurements taken between 1996 and 2009 in the barn owl. * Numbers in parentheses are number of females (or males) producing a second clutch

	Number of nests of origin	Number of nestlings	Number of body mass measurements	Mean number (and range) of body mass measurements per nestling	Range in nestling age (in days)	Period when body mass measurements were taken	Number of different biological mothers*	Number of different biological fathers*
1996	61	213	2 112	9.9 (3,19)	0, 64	8 a.m.–12 p.m.	53 (8)	56 (5)
1997	32	115	501	4.4 (1,33)	0, 64	8 a.m.–11 p.m.	31 (1)	32 (0)
1998	43	183	1 821	10.0 (2,28)	0, 64	8 a.m.–12 p.m.	37 (6)	39 (4)
1999	34	133	1 065	8.0 (2,12)	0, 64	8 a.m.–12 p.m.	33 (1)	33 (1)
2000	11	22	159	7.2 (2,13)	0, 54	8 a.m.–12 p.m.	11 (0)	11 (0)
2001	42	187	1 494	8.0 (1,32)	0, 63	8 a.m.–12 p.m.	42 (0)	42 (0)
2002	63	260	1 324	5.1 (1,9)	0, 61	8 a.m.–12 p.m.	62 (1)	61 (2)
2003	40	169	692	4.1 (2,6)	0, 57	8 a.m.–10 p.m.	40 (0)	40 (0)
2004	37	148	1 075	7.3 (1,11)	0, 63	8 a.m.–12 p.m.	27 (10)	34 (3)
2005	39	171	788	4.6 (1,6)	0, 63	8 a.m.–11 p.m.	35 (3)	38 (1)
2006	19	65	637	9.8 (7,10)	1, 61	8 a.m.–8 p.m.	19 (0)	19 (0)
2007	56	267	961	3.6 (1,6)	0, 64	8 a.m.–10 p.m.	40 (16)	49 (7)
2008	58	208	633	3.0 (1,7)	1, 64	8 a.m.–11 p.m.	58 (0)	57 (1)
2009	15	52	333	6.4 (4,9)	0, 64	8 a.m.–11 p.m.	13 (2)	14 (1)
Total	550	2193	13 595					

of age (mean ± SE: 30.7 ± 0.14). Because our aim is to analyse the relationship between body mass and plumage traits at different times of the day, it is impossible to obtain growth curves for each individual nestlings at different time points of the day. This would have required the monitoring of each individual in the morning, noon and evening on many occasions, something that is impossible to perform in the field (the study area covers 1070 km^2). We thus did not compare individual growth curves but we examined whether nestlings born from differently spotted parents differ in body mass at different time points of the day. Therefore, our goal is rather to examine whether the covariation between offspring body mass and melanin-based coloration varies not only between years and sex of the parents, but also along the day.

On all nestlings and their parents, A.R. measured spot size on the breast by placing a 60 × 40 mm frame and measured the size of 10 spots using a calliper. In 1086 male nestlings and 1107 female nestlings mean (± SD) spot size was 1.11 ± 0.42 and 1.42 ± 0.36 mm, respectively. In 333 different fathers and in 283 different mothers mean (± SD) spot size was 1.12 ± 0.48 and 1.59 ± 0.42 mm, respectively.

In 12 of the 14 years, we carried out cross-fostering experiments by swapping eggs or hatchlings between pairs of randomly chosen nests (no cross-fostering was carried out in 1997 and 2005). This design was useful to partition phenotypic variation into genetic and environmental components. These experiments have already been the topic of several publications where we explain the method in details (e.g. Roulin, 2006); 910 nestlings for which we measured body mass were raised in a different nest (so-called 'nest of rearing', $N = 556$ nests) from the one where they were born (so-called 'nest of origin', $N = 550$ nests), whereas 1283 nestlings were raised in the same nest as the one where they were born. The number of nests of rearing and origin differ because of nestling mortality and breeding failure.

In 10 out of 12 years, spot diameter of the foster and the biological mother was not correlated (Pearson's correlation, all P-values > 0.07, 1999: $t_{26} = -2.58$, $P = 0.02$, 2004: $t_{18} = 2.5$, $P = 0.02$) and in 8 out of 12 years spot diameter of the biological and the foster father was not correlated (all P-values > 0.1; 1996: $t_{30} = 4.1$, $P = 0.001$, 2002: $t_{51} = -2.4$, $P = 0.02$, 2003: $t_{34} = 4.6$, P = 0.001, 2009: $t_5 = -5.0$, $P = 0.004$).

Statistical procedure

Statistical analyses were done using the statistical software package R version 2.15.1 (R Development Core Team, 2012). For mixed-effect models, we used the function *lmer* of the package *lme4* (Bates, Maechler & Bolker, 2012) and to estimate the credible intervals, the function *sim* of the package *arm* (Gelman & Hill, 2007). To investigate whether variation in nestling body mass over the course of the day is associated with the size of black spots measured in the nestlings themselves or in their biological

parents, we performed a mixed-effects model analysis with log-transformed nestling body mass as the dependent variable. Independent variables were spot diameter of the nestling (diaN), biological mother (diaMo) and biological father (diaFa), nestling sex and year as two factors, and as covariates the date of nestling measurement (i.e. number of days since the first of January), current brood size (range: 1 and 9; mean ± SE: 4.4 ± 0.06), nestling rank in the within-brood age hierarchy (first-born nestlings have rank 1, second-born rank 2, and so on), age (in days), age^2, age^3, age^4, wing length (mm) and time of the day (hour). We implemented 'wing length' to control for body size (age was estimated with wing length measured around hatching). All numeric covariates are centred to their mean and divided by their standard deviation to account for the different units. We also included the following biologically relevant interactions: 'year × diaN', 'hour × diaN', 'year × hour', 'year × hour × diaN', 'year × diaMo', 'hour × diaMo', 'year × hour × diaMo', 'year × diaFa', 'hour × diaFa', 'year × hour × diaFa'; preliminary analyses showed that the relationships did not differ between male and female nestlings and for this reason we did not include interactions with nestling sex to simplify the statistical analyses. We fitted 'time of the day' as linear covariate only (i.e. no quadratic or cubic terms), since there was no recognizable non-linear relationship between body mass and time of the day (not shown). To correct for repeated measurements of body mass in the same individuals, we introduced nestling identity as random intercepts and an individual random slope for age (note that the model with a random slope for the second, third and fourth power of age did not converge even if we used orthogonal polynomials). We also incorporated the nests of origin and rearing as random intercepts to account for the genetic non-independence of siblings and of shared environment among nestlings reared in the same nest, respectively. We included the identity of the biological mother and father as two separate random intercepts to account for repeated measures of the same breeders between the years. In preliminary analyses, we included year as random intercept and parental and nestling spot diameter and hour as random slopes for each year but this model did not converge. For this reason, we included year as a fixed effect. With our model, we estimated 101 parameters, which gave us, given our dataset of 13 595 measurements, slightly more than 134 data points for each parameter estimate.

The significance of the random factors was tested with the method described by Faraway (2006). The distribution of the likelihood ratio for comparing an alternative model (containing a given random factor) with a null model (model without this random factor) is approximated using Monte Carlo simulation. We simulated 1000 times a set of response values from the null model and calculated the log likelihood ratio between the alternative and the null model for each set of simulated response values. From these 1000 likelihood ratios, an approximation of the distribution of the log likelihood ratio was obtained and used to obtain the *P*-value (Faraway, 2006).

The fixed effects were tested with the log likelihood ratio test (LR) using maximum likelihood estimation. In the final model, we only included significant interactions and used a stepwise backward procedure for model selection using the LR test. All main effects were kept in the final model. The main effects were tested against a model without any interaction, and two-way interactions were tested against a model without any three-way interactions. The linear effect of age was tested against in a model without the polynomials of age and each polynomials of age was tested in a model without the higher polynomials of age.

Because interactions of nestling and parental spot diameters with the factor 'year' and 'hour' were significant, we estimated the slopes of the regression of nestling mass on spot diameter in their biological parents (diaMo and diaFa) for each year and each hour (8 a.m. until 12 p.m.) from our final mixed-effect model. To obtain a 95 % credible interval (CrI), we simulated from the final model a random sample ($N = 1000$) from the joint posterior distribution of the model parameters using the function *sim* of the package *arm*, which uses a MCMC algorithm that samples from the posterior distribution of the parameters (assuming uninformative priors) (Gelman & Hill, 2007). From this sample, we used the 2.5% and 97.5% quantiles as lower and upper limit of the 95% CrI and a slope was significantly different from zero if the 95% CrI does not contain zero.

To examine whether the environment may account for inter-annual variation in the sign and magnitude of the covariation between nestling body mass and parental spot diameter, we estimated for each year the slopes of the regression of nestling body mass on spot diameter of the biological mother and father from the final mixed-effect model. This was done for morning (8 a.m.), midday (12 a.m.) and evening (8 p.m.). As surrogates of ecological parameters, we considered number of breeding pairs, mean annual laying date and mean number of fledglings at the first annual breeding attempt (Chausson *et al.*, 2014). Since the latter two variables were strongly correlated (Pearson's correlation: $r = -0.75$, $N = 14$ years, $P = 0.002$), we performed a principal components analysis. The first principal component (eigenvalue 1.75, variance explained 0.87) was negative for mean annual laying date (correlation coefficients between

the first principal component and the original variables: –0.93) and positive for mean annual number of fledglings (0.93). Thus, larger values characterized better years for barn owl reproduction. For each parent (father and mother separately), we performed one linear mixed-effects model with parameter estimates as dependent variable, year as random factor and time of day (morning, noon, evening), number of breeding pairs and the first principal component of laying date and number of fledglings plus their interactions with time of day (Table 3) as fixed effects. The fixed effects were tested as described above with the log likelihood ratio test (LR) and non-significant interactions were removed from the final model.

RESULTS

General pattern of variation in nestling body mass

Among the six random variables included in our statistical model (Table 2), the random intercept nestling identity and random slope nestling age significantly explained variation in nestling growth indicating that growth curves differ between individuals. Nestlings raised in the same nest had comparable body mass (term 'Nest of rearing'). In contrast, the term 'Nest of origin' was not significantly related to nestling body mass but the identity of the biological father and mother was.

Female nestlings were heavier than male nestlings (term 'Nestling sex' in Table 2), male body mass being on average 95% of females between 41 and 50 days of age (Fig. 1B). There were pronounced annual variation in body mass (term 'Year', Fig. 2A). Nestling body mass decreased from the morning until the evening when parents presumably started to bring back food at around 10 p.m. (term 'Hour', Fig. 2B). Nestling body mass decreased along the season (term 'Date') and with rank in the within-brood age hierarchy (term 'Rank raised') but increased with brood size (term 'Current brood size'). Nestlings with longer wings were heavier (term 'Nestling wing length') and as can be seen in Figure 1A body mass varies with age (terms 'Nestling age', 'Nestling age^2' and 'Nestlings age^3' and 'Nestling age^4'). Body mass reaches a plateau at about 38 days of age and remains stable during about 15 days followed by a small decrease in body mass, so-called body mass recession (Fig. 1A).

Relationship between nestling body mass and nestling pigmentation

Nestling body mass was significantly positively associated with their own spot diameter (term 'diaN' in Table 2) but not in interaction with year and hour. Larger-spotted nestlings were heavier than their conspecifics with smaller eumelanic spots and this relationship was similar throughout the 14 years and from the morning to the evening. The relationship between nestling body mass and nestling spot diameter remained significant if we exclude parental spot diameters from the model (LR = 10.9, df = 1, $P < 0.0001$, parameter estimate: 0.005 ± 0.002) and all other interactions with nestling spot diameter remained non-significant (P-values > 0.80). Note that wing length was not associated with nestling spot diameter (mixed-effect model with wing length as dependent variable and sex, age, age^2, age^3, and nestling spot diameter as fixed variables and nestling identity, brood identity and parent identity as random factors; LR test for spot diameter: 0.5, df = 1, $P = 0.5$).

Relationship between nestling body mass and parental pigmentation

We found significant interactions 'year × hour × spot diameter biological mother' and 'year × hour × spot diameter biological father' (Table 2). Nestlings were heavier if their mother displayed small rather than large black spots (significantly so in the morning in 1998, 2006, 2009 and in the evening in 1999 and 2007, Fig. 3A) and if their father displayed small rather than large black spots (significantly so in the morning in 1997 and 2007 and in the evening in 2008 and 2009, Fig. 3B). In the years 2008 and 2009, we found just the opposite associations in the morning (nestlings were heavier if their father displayed large rather than small black spots, Fig. 3B). The interactions 'year × hour × diaMo' and 'year × hour × diaFa' reported in Table 2 are still significant ($P < 0.001$) if considering only the nestlings raised in a foster nest. Figure 3C, D show similar patterns when we restricted the analyses to the cross-fostered nestlings. This suggests that the change in the covariation between offspring body mass and parental spot diameter along the day may have a genetic component (indicating genotype by environment interaction). Furthermore, if we run the final model with only cross-fostered nestlings and spot diameter of their foster parents, the interaction of 'year × hour × dia foster mother' is not significant ($P > 0.8$), but the interaction of 'year × hour × dia foster father' is significant ($P = 0.01$). However, if we look at the slopes of the regression of nestling body mass on diameter of the foster father, the interaction was significant only in 2009 probably because in this year spot diameters of biological and foster fathers were correlated (cross-fostered nestlings of small-spotted foster fathers were heavier in the morning than in the evening). This suggests that the associations between daily variation in nestling body mass and parental spot diameter are genetically rather than environmentally determined.

Table 2. Relationship between nestling body mass and nestling vs. parental melanin-based coloration in interaction with time of the day and year in the barn owl. Results of the mixed-effect model (LR test using Monte Carlo simulations (mcs) for random effects and LR test for fixed effects) with nestling body mass as dependent variable and spot diameter of nestlings and biological parents as covariates

Random effects					
Intercept	Slope	Variance	df	LR	P_{mcs}
Nestling identity		1.3	1	245	**0.001**
	Nestling age	2.5	2	1904	**0.001**
Nest of origin		0.00	1	0.1	0.7
Nest of rearing		0.68	1	67.9	**0.001**
Mother identity		0.40	1	13.5	**0.001**
Father identity		0.35	1	19.6	**0.001**

Fixed effects				
	Parameter estimates ± SE	df	LR	*P*
Nestling sex (female)	0.030 ± 0.002	1	139	**<0.001**
Year		13	37.7	**<0.001**
Hour	−0.022 ± 0.001	1	506	**<0.001**
Date	−0.011 ± 0.002	1	26.2	**<0.001**
Rank raised	−0.010 ± 0.001	1	60.9	**<0.001**
Current brood size	0.010 ± 0.001	1	23.4	**<0.001**
Nestling wing length	0.280 ± 0.009	1	745	**<0.001**
Nestling age	−0.069 ± 0.011	1	689	**<0.001**
Nestling age^2	−0.268 ± 0.003	1	28212	**<0.001**
Nestling age^3	0.176 ± 0.001	1	9679	**<0.001**
Nestling age^4	−0.044 ± 0.001	1	2321	**<0.001**
Nestling spot diameter (diaN)	0.006 ± 0.002	1	11.6	**<0.001**
Spot diameter biological mother (diaMo)	0.001 ± 0.006	1	3.4	0.07
Spot diameter biological father (diaFa)	0.003 ± 0.006	1	0.3	0.6
Hour × year		13	46.2	**<0.001**
Hour × diaN				NS
Hour × diaMo		1	0.2	0.9
Hour × diaFa		1	0.1	0.9
Year × diaN				NS
Year × diaMo		13	20.8	0.08
Year × diaFa		13	10.2	0.7
Year × hour × diaN				NS
Year × hour × diaMo		13	26.1	**0.02**
Year × hour × diaFa		13	44.3	**<0.001**

The dependent variable 'nestling body mass' was log-transformed to obtain normally distributed residuals. We removed non-significant interactions (NS) if they were not included in higher order interactions. Main effects were tested with a model without interactions; two-way interactions were tested with a model without three-way interactions. Significant *P*-values are written in bold. For random effects, we approximated the *P*-values (P_{mcs}) with a Monte Carlo Simulation. Numeric variables were centred and standardised for comparison of effect sizes. Current brood size gives the number of nestlings when a given individual had been weighed. We included the date when nestlings were measured to control for seasonal variation in nestling body mass. 'Rank raised' defines the position of the nestlings in the within-brood age hierarchy. The variance of the random effects is given as ×1000 and residual variance is 0.79. Parameter estimates are presented only for covariates and binary variables.

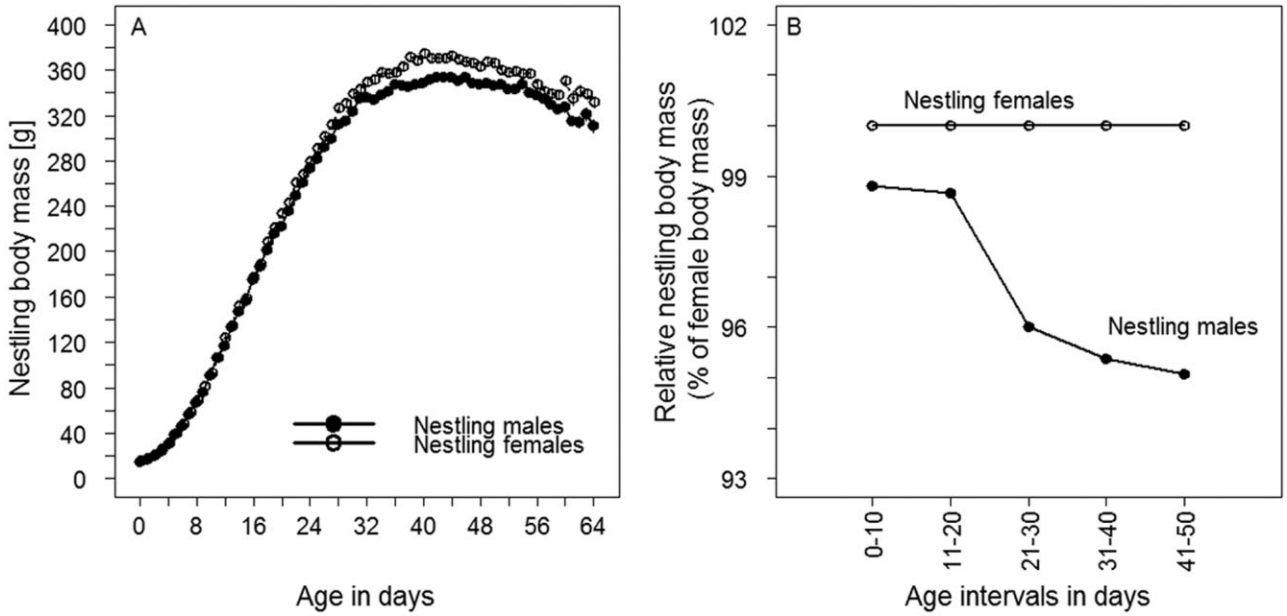

Figure 1. Nestling body mass in relation to nestling age and sex in barn owls. (A) Residual body mass corrected for hour of the day plotted as mean per age for male and female nestlings separately. (B) Age-related difference in body mass growth between male and female nestlings. Female nestlings were set as the reference level (e.g. between 41 and 50 days male body mass is 95% that of female body mass). This figure is based on the measurement of 1107 female and 1086 male nestlings totalling 13 595 measures.

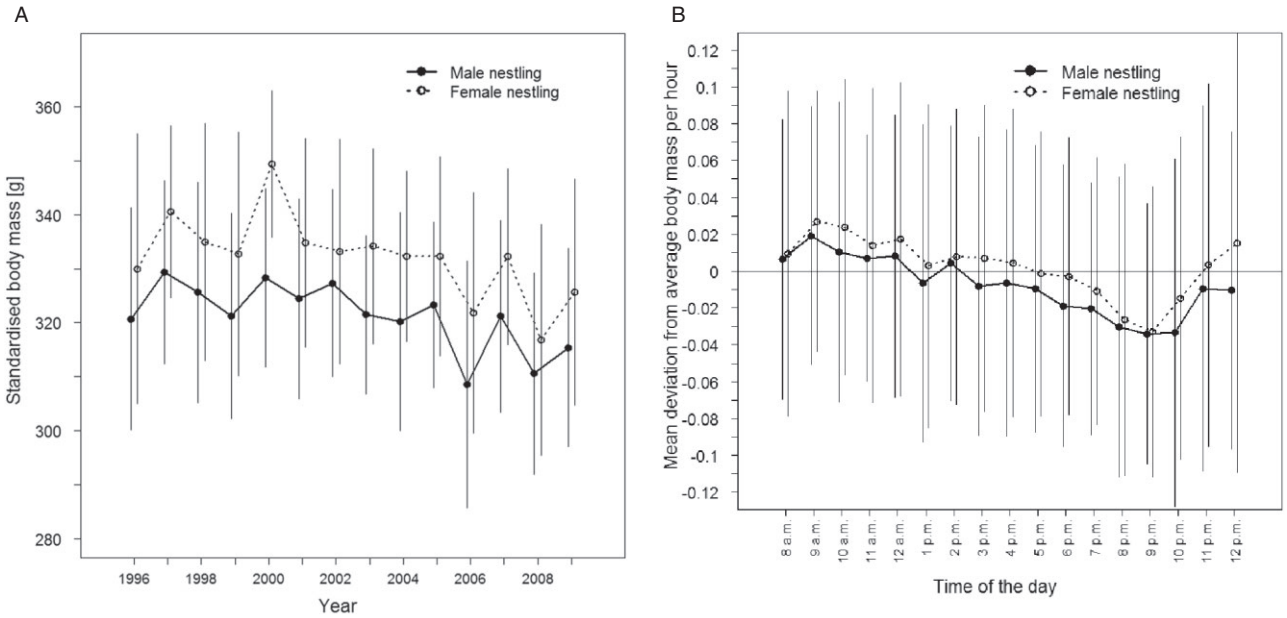

Figure 2. (A) Body mass standardised per individual for the first to the fourth polynomial of age plotted as mean (± SD) per year and (B) mean individual deviation of the average body mass plotted as mean (± SD) per hour. We included nestling identity as random intercept and age as random slope in the model for graph (A) and nestling identity nested in year as random intercept and age as random slope in the model for graph (B).

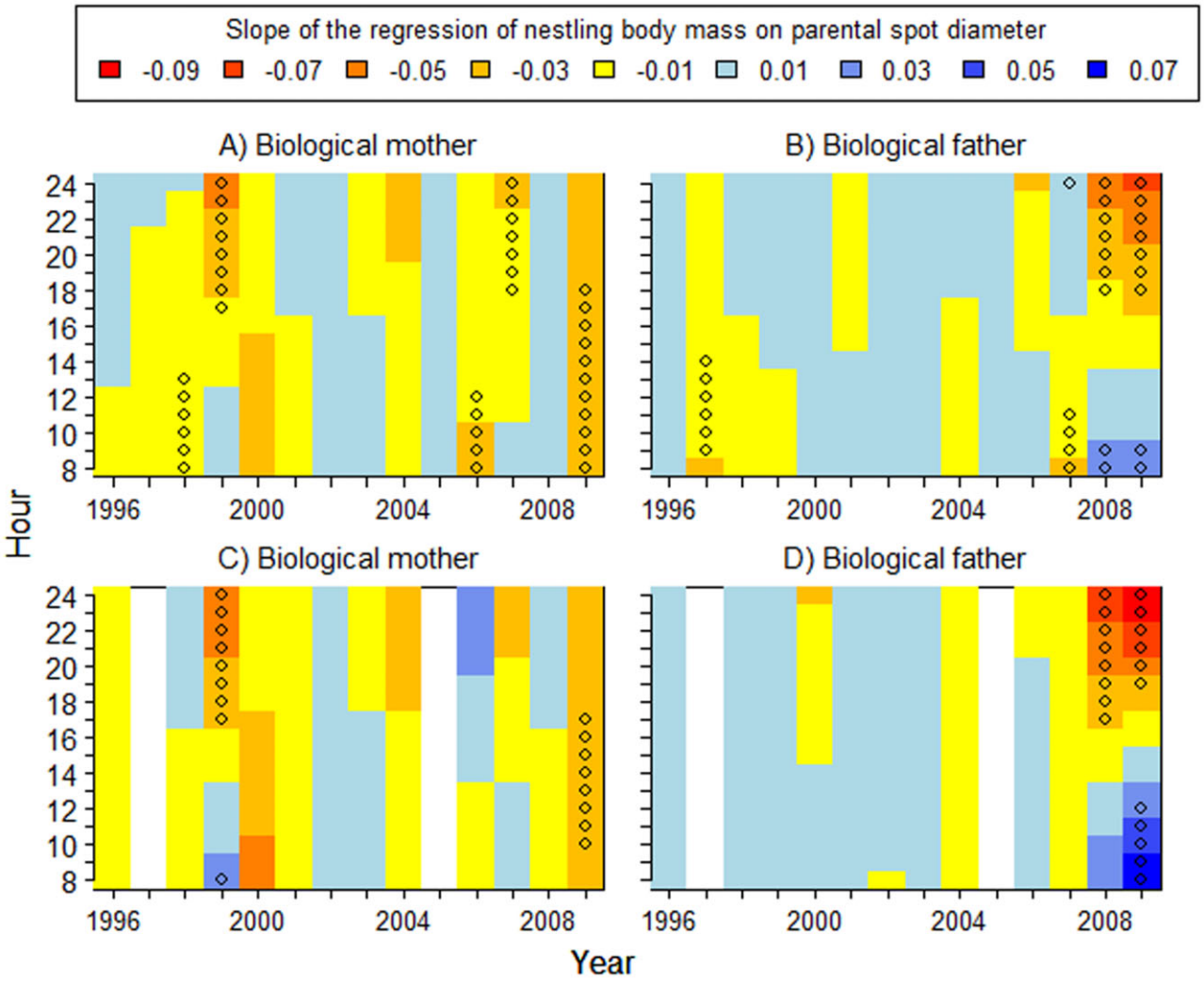

Figure 3. Slope for each hour and year of the regression of nestling body mass on spot diameter of the biological parents. (A) All nestlings (cross-fostered or not) and spot diameter of their biological mother, (B) all nestlings (cross-fostered or not) and spot diameter of their biological father, (C) only the cross-fostered nestlings and spot diameter of their biological mother, and (D) only the cross-fostered nestlings and spot diameter of the biological father. A positive slope indicates that nestling body mass and parental spot diameter is positively associated. Circles indicate when the slopes are significantly different from zero (95% CrI does not include zero).

The interactions 'year × hour × diaMo' and 'year × hour × diaFa' remained significant if we exclude nestling spot diameter from the model (LR = 31.1, df = 13, $P = 0.003$ and LR = 43.9, df = 13, $P < 0.001$, respectively).

Environmental determinism of the association between nestling body mass and parental pigmentation

As can be seen in Figure 3, the magnitude of the covariation between nestling body mass and parental pigmentation varied between years and time of the day. We found that the slopes of the regression of nestling body mass on mother spot diameter were significantly positively related to the number of breeding pairs independently of time of the day (Table 3). Indeed, mean slopes, over the values obtained for morning, noon and evening, were significantly associated with number of breeding pairs in females; Pearson's correlation in females: $r = 0.65$, $N = 14$ years, $P = 0.012$; in males: $r = 0.43$, $N = 14$ years, $P = 0.12$) (Fig. 4A). The interaction between time of the day and the first principal component of laying date and number of fledglings was significant for the slopes of the regression of nestling body mass on father spot diameter but not for mother spot diameter (Table 3). This significant interaction is explained by

Table 3. Covariation between reproductive parameters (number of breeding pairs, mean annual laying date and number of fledglings at the first annual breeding attempt) and parameter estimates of the covariation between nestling body mass and parental spot diameter

	Mother parameter estimate			Father parameter estimate		
	df	LR	*P*	df	LR	*P*
Time of the day	2	0.01	0.9	2	2.4	0.3
Number of breeding pairs	1	8.3	**0.004**	1	2.1	0.2
First principal component (PC1)	1	0.6	0.4	1	0.3	0.6
Time of the day × number of pairs			NS			NS
Time of the day × PC1			NS	2	19.5	**<0.001**

Results of two linear mixed models with year (1996 to 2009) as random variable and as three fixed effects 'time of day', 'number of breeding pairs' and 'the first principal component' of a principal components analysis with mean annual laying date (negative loading) and mean number of fledglings (positive loading) at first breeding attempts plus all two-way interactions with time of the day. For each year and parent (i.e. father and mother), we had three parameter estimates for morning, noon and evening (variable 'time of the day'). Main effects were tested with a model without interactions and we removed non-significant interactions (NS).
Mother (father) parameter estimates indicate the extent to which maternal (paternal) spot size covaries with offspring body mass.

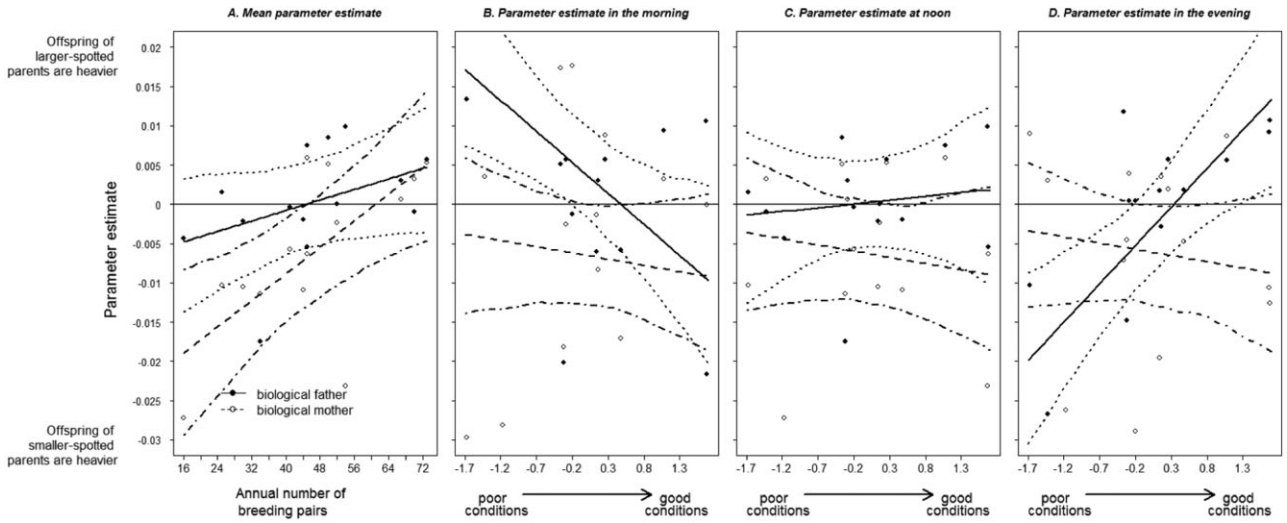

Figure 4. Relationship between reproductive parameters and parameter estimates of the covariation between nestling body mass and spot diameter of the biological parents in the barn owl. Reproductive parameters are 'annual number of breeding pairs' for panel (A) and the first principal component of a principal components analysis including mean annual number of fledglings (positive loading) and mean annual laying date (negative loading) at the first annual breeding attempts for panels (B), (C), and (D). Dashed lines represent fitted values (with 95% CrI) for biological mothers, while straight lines fitted values (with 95% CrI) for biological fathers. Open circles represent the parameter estimate of 1 year for biological mothers and closed circles for the biological fathers, respectively.

the fact that slopes were negatively associated with the first principal component in the morning ($r = -0.52$, $N = 14$ years, $P = 0.05$; in years when pairs bred later in the season and produced fewer fledglings large-spotted fathers sired heavier offspring in the morning, Fig. 4B) and strongly positively associated in the evening ($r = 0.73$, $N = 14$ years, $P = 0.003$; in years when pairs bred later in the season and produced fewer fledglings small-spotted fathers sired heavier offspring in the evening; Fig. 4D), but not significantly related at noon (Fig. 4C).

DISCUSSION

We investigated in the barn owl the covariation between a eumelanin-based plumage trait (size of

black feather spots) and nestling body mass throughout the resting phase (i.e. during the daylight hours) until the first feeding event at night. This approach was motivated by two previous studies. First, adult females displaying large black spots were heavier than small-spotted females in the afternoon but not in the morning (Roulin, 2009). Second, the covariation between baseline corticosterone levels measured in nestlings and parental spot diameter changed from the morning to the evening (Roulin *et al*., 2010b). The present study shows that indeed the covariation between nestling body mass and the size of black feather spots differs depending on whether spots are measured in the mother or father. Furthermore, the magnitude and sign of these covariations varied with time of the day and between years. Interestingly, in poor years offspring of larger-spotted fathers were heavier in the morning while in the evening the offspring of smaller-spotted fathers were heavier (Fig. 4). Pigmentation therefore may be a proxy for aspects of energetic processes that are sensitive to environmental factors (Husby, Hille & Visser, 2011) or digestion (Muller *et al*., 2013).

Relationship between nestling body mass and nestling pigmentation

Our study is based on a very large sample of nestling body mass measured on several occasions. We could thus run relatively complex statistical analyses where we simultaneously investigated whether nestling body mass covaries with the size of their own black spots and with those of their parents. The parameter estimate of nestling spot size (Table 2) shows that nestling spot size is positively correlated with nestling body mass independently of time of the day and year. Several non-mutually exclusive mechanisms could explain why larger-spotted nestlings are heavier. First, larger-spotted nestlings may better convert food into body mass. This aspect has not yet been examined but would deserve a detailed study since a review of the genetic literature showed that genes belonging to the melanocortin system regulate melanogenesis and energy homeostasis (Ducrest, Keller & Roulin, 2008). Second, larger-spotted nestling may better cope with stress and thereby be heavier. Indeed, we found that larger-spotted birds better cope with elevated corticosterone, a hormone released as a response to environmental stress situations (Almasi *et al*., 2012) and under the regulation of the melanocortin system. Third, large-spotted nestlings may be more competitive than small-spotted siblings, something that we are currently examining. Fourth, larger-spotted nestlings may have a higher food requirement during development and, assuming that they are more competitive, they obtain more food than smaller-spotted nestlings. However, a feeding experiment showed the opposite pattern with larger-spotted nestlings having a lower appetite than smaller-spotted nestlings (Dreiss *et al*., 2010). Finally, larger-spotted nestlings may better resist periods of poor feeding conditions. Accordingly, we found that larger-spotted nestlings lost less weight after experimental food deprivation (Dreiss *et al*., 2010). To conclude, the positive association between nestling body mass and nestling spot diameter possibly emerges because larger-spotted individuals possess alleles that allow them to gain weight faster or better withstand lack of food, or still because they may be more competitive than small-spotted individuals.

Relationship between nestling body mass and parental pigmentation

Nestling body mass did not always covary in the same way with maternal and paternal spottiness (Fig. 3). The proximate mechanism generating a positive or negative covariation between nestling body mass and mother spottiness can thus be independently regulated from the mechanism responsible for the covariation with father spottiness. Three mechanisms can account for this observation. First, nestling body mass could be a function of parental foraging behaviour, which is predicted by the plumage of their parents. This is however unlikely because the significant covariations with spot diameters measured in the biological parents were also detected when we restricted the analysis to nestlings that were not raised by their biological parents but by foster parents. Second, the results could be accounted for by sibling competition if the nestling ability to compete over parental food resources covaries with maternal and paternal spot size. This mechanism requires that the relationship between nestling competitiveness and maternal spottiness differs from the relationship with paternal spottiness. The third hypothesis relies on the idea that nestling growth is sensitive to a gene which regulates the expression of parental spot diameter and which is sensitive to environmental factors. The fact that the covariations between offspring body mass, maternal and paternal pigmentation were not similar across different years and periods within the day, the underlying gene(s) of these covariations may show parent-of-origin effects (i.e. epigenetic or genomic imprinting). Allele(s) inherited from the mother and father may have different effects on offspring body mass if their expression is differentially sensitive to environmental factors that fluctuate at several temporal scales (i.e. hour up to year). Parent-of-origin effects are plausible because the expression of a number of genes involved in development, including genes that pleiotropically regulate melanogenesis,

is not the same if inherited from the mother or father (Coan, Burton & Ferguson-Smith, 2005; Isles & Holland, 2005). Accordingly, the agouti-related protein *ASIP*, which can be imprinted, is involved in both melanin production and body mass regulation (Wolff *et al*., 1998; Morgan *et al*., 1999; Ducrest *et al*., 2008).

Although not statistically significant, there was a tendency of larger-spotted mothers producing heavier offspring (Table 2), a tendency that however disappeared when we removed nestling spot diameter from the model. In contrast, the relationship between offspring body mass and paternal spottiness was far from significant. These results concur with most other studies performed in the barn owl (e.g. Roulin & Ducrest, 2011) showing that maternal rather than paternal spottiness is associated with offspring phenotype. Further, offspring sired by large- or by small-spotted fathers have a similar mean body mass (variable 'spot diameter biological father, diaFa' in Table 2), but the daily regulation of nestling body mass is very different between individuals sired by differently spotted fathers, a phenomenon that varies between years (interaction 'year × hour × diaFa' in Table 2, see also Figs 3 and 4). This suggests that genes associated with father spottiness usually do not predict overall offspring body mass but are rather related to some physiological processes that vary across the day and among years. Accordingly, in years with a poor breeding success (i.e. owls breeding later in the season and producing fewer fledglings) larger-spotted fathers sired offspring that were heavier in the morning than those of smaller-spotted fathers, while in the evening the opposite pattern prevailed with offspring of smaller-spotted fathers being heavier (Fig. 4B–D). To sum up, the differences between maternal and paternal plumage variation with nestling mass suggests that if selection is positive for heavier young, then selection should be more consistent for female pigmentation among years, but selection should be more episodic and could be stronger for males depending on annual conditions (Table 2; Fig. 3). Although researchers have measured offspring body mass in relation to secondary sexual characters displayed by their parents (e.g. Kim *et al*., 2013), we are not aware of any study that performed similar analyses as the ones we report here. This would be of interest because such analyses can reveal trade-offs that might be otherwise difficult to detect. Temporal variation in the covariation between nestling body mass and parental melanin-based coloration indicates that the sensitivity of nestling body mass to temporarily varying environmental factors differs between genotypes identified by parental coloration.

Several research protocols can be proposed as follow-up studies to understand why body mass regulation is so closely associated with pigmentation. The ultimate goal is to identify the genes, metabolites and proteins that generate this association. However, if our aim is to use a candidate gene approach, we need to identify which physiological process is most likely to be involved in the covariation between body mass and pigmentation. A first study we can think of is to investigate aspects of digestion, i.e. the amount of time a meal is retained (i.e. time span between food absorption, defecation and pellet rejection) and the digestive efficiency to convert food to owl mass. Because these processes could be time-dependent they should be studied in relation to time of the day or night when food is consumed. Another aspect that should be tackled is the activity level of nestlings in relation to pigmentation. Because melanin-based coloration is associated with behaviour (Van den Brink *et al*., 2012), we could examine whether covariation between body mass and coloration is due to activity, *i.e.*, to catabolism rather than anabolism. Finally, because the covariation between body mass and coloration varies along the day, specific studies on colour-specific daily activity should be performed, something which is also underway.

Maintenance of genetic variation in melanin-based coloration

In poor years (i.e. when few owls breed) smaller-spotted mothers produced heavier offspring than larger-spotted mothers (Fig. 4A), a relationship that was independent of time of the day (Table 3). This suggests that offspring that inherit genes from smaller-spotted mothers may have a selective advantage in years when few pairs breed. Because the breeding population size is lower after a harsh winter and in years when pre-breeding food conditions are poor (Altwegg *et al*., 2006; Chausson *et al*., 2014), we conclude that small-spotted mothers may have a selective advantage over large-spotted mothers under those conditions, at least in terms of offspring growth. Furthermore, when breeding conditions were poor (i.e. in years when pairs bred later and produced fewer fledglings) offspring sired by smaller-spotted fathers were heavier at the end of the resting period (i.e. in the evening) after having digested the last meals many hours ago. This is again consistent with the proposition that smaller-spotted individuals may have a selective advantage, in terms of nestling body mass, when breeding conditions are poor. These findings mirror studies performed in other organisms showing that the intensity of selection exerted on traits such as melanin-based coloration varies between years. For instance, in the tawny owl (*Strix aluco*) reddish individuals have a poorer survival in years with deeper snow depth (Karell *et al*., 2011).

These year-specific selective effects may be explained by the strong links between pheomelanin-based coloration and physiology in this species (e.g. Piault *et al*., 2009). Similar findings apply to pied flycatchers (*Ficedula hypoleuca*) in Finland (Sirkiä, Virolainen & Laaksonen, 2010; Sirkiä *et al*., 2013) suggesting that temporally fluctuating selection on the degree of melanin-based coloration is accounted by the colour-specific physiological responses to environmental factors.

This discussion emphasises the importance of measuring fitness components in relation to sex-traits over a large range of ecological conditions to evaluate sex-specific selection. Indeed, genes may have a parent-of-origin effect (i.e. different effect if passed on by the mother or the father), which can also depend on offspring sex (the parent-of-origin effect may differ between sons and daughters, Hager *et al*., 2008). Our study is such an example showing that the association between body condition and secondary sexual characters can vary with time of the day.

IMPLICATION FOR THE RESOLUTION OF THE LEK PARADOX

A major unresolved issue of evolutionary biology is how genetic variation in sexually selected ornaments can be evolutionary stable despite intense directional selection (Pomiankowski & Møller, 1995). Our study demonstrates that phenotypic differences in offspring (here body mass) are more affected by environmental condition in regards to male than female phenotypes (Fig. 4). It appears that alleles inherited from the father act on nestling phenotype in a more plastic manner than alleles inherited from the mother. Therefore, the genetic benefits of mating with large- or small-spotted males will depend on prevailing environmental conditions. Such genotype by environment interaction can help maintain genetic variation because different genotypes maximize their fitness in different environments (Kawecki & Ebert, 2004), a phenomenon that may select for context-dependent mate choice (Roulin & Bize, 2007). Interestingly, paternal spottiness shows significant positive and negative relationships with offspring mass depending on year, while females only show positive or neutral relationships. This suggests that selection on male plumage spottiness has been shaped by selection for variability while selection for female spottiness has been more direct and directional. Accordingly, female spottiness is under stronger directional selection than male spottiness (Roulin *et al*., 2010a) and more often covaries with offspring phenotypes (Roulin & Ducrest, 2011). This is consistent with the hypothesis of Day & Bonduriansky (2004) proposing that intralocus sex conflict can be resolved through the evolution of genomic imprinting. Indeed, if selection on an ornament is sex-specific different alleles may have different fitness effects in males and females. These differential effects may vary along environmental gradients, and hence to facilitate each sex to reach its phenotypic optimum, selection may favour the evolution of genomic imprinting so that the phenotypic effect of an allele differs if inherited from the mother or father. As a consequence, offspring phenotype will be differently related to the degree of maternal and paternal ornamentation if the underlying genes undergo imprinting. Imprinting of paternally inherited genes may be a way for paternal spottiness to evolve under balancing selection through their effect on offspring phenotype, which strongly depend on environmental conditions, and for maternal spottiness to evolve under directional selection relatively independently of environmental conditions. Researchers interested in sexual selection usually consider, for simplification, that ornamented males always have a selective advantage over drab male conspecifics. Considering that the genetic benefit of displaying an extravagant or modest version of an ornament differs between environments would probably shed new light on how genetic variation can persist. The study of melanin-based colour traits appears to be fruitful in this context.

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