

## Sleep and vigilance linked to melanism in wild barn owls

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### Abstract

Understanding the function of variation in sleep requires studies in the natural ecological conditions in which sleep evolved. Sleep has an impact on individual performance and hence may integrate the costs and benefits of investing in processes that are sensitive to sleep, such as immunity or coping with stress. Because dark and pale melanic animals differentially regulate energy homeostasis, immunity and stress hormone levels, the amount and/or organization of sleep may covary with melanin-based colour. We show here that wild, cross-fostered nestling barn owls (*Tyto alba*) born from mothers displaying more black spots had shorter non-REM (rapid eye movement) sleep bouts, a shorter latency until the occurrence of REM sleep after a bout of wakefulness and more wakefulness bouts. In male nestlings, the same sleep traits also correlated with their own level of spotting. Because heavily spotted male nestlings and the offspring of heavily spotted biological mothers switched sleep–wakefulness states more frequently, we propose the hypothesis that they could be also behaviourally more vigilant. Accordingly, nestlings from mothers displaying many black spots looked more often towards the nest entrance where their parents bring food and towards their sibling against whom they compete. Owlets from heavily spotted mothers might invest more in vigilance, thereby possibly increasing associated costs due to sleep fragmentation. We conclude that different strategies of the regulation of brain activity have evolved and are correlated with melanin-based coloration.

### Introduction

Naturally occurring inter-individual variation in sleep provides a largely untapped opportunity to gain insight into the function(s) of sleep. Although the timing of sleep and its substates differ between and within individuals depending on age (Kurth *et al.*, 2010), sex (Dijk *et al.*, 1989) and the environment (Lesku *et al.*, 2008), the factors responsible for the observed variation are mostly unknown. At least some of the variation in sleep may reflect trade-offs between the costs and benefits of different sleep/wakefulness strategies (Donlea *et al.*, 2012). Sleep entails costs because the invested time cannot be spent in other activities such as feeding, mating or territory defence, and while sleeping, an

individual is vulnerable to predation (Rattenborg *et al.*, 1999; Lima *et al.*, 2005). Conversely, the benefits of sleep include memory consolidation (Diekelmann & Born, 2010), brain maintenance (Vyazovskiy *et al.*, 2008; Xie *et al.*, 2013), energy homeostasis (St-Onge, 2013) and immune system regulation (Opp, 2009). However, most prior work on the benefits of sleep has not taken advantage of inter-individual variation and has been performed in inbred domesticated animals living under artificial laboratory conditions (Grosmark *et al.*, 2012; Binder *et al.*, 2014). Although a few studies have examined sleep in wild populations, they were based on indirect behavioural measures of sleep such as whether eyes are closed (e.g. Christe *et al.*, 1996), which can be misleading in some species (Lesku *et al.*, 2011), and cannot be used to differentiate between rapid eye movement (REM) and non-REM sleep, sleep substates distinguished from each other and from wakefulness by changes in brain and muscle activity. Until recently, technological constraints precluded

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electrophysiological studies of animals sleeping in the wild. However, the recent development of small, light-weight devices that record sleep-related changes in brain and muscle activity (Vyssotski *et al.*, 2006, 2009) has opened the door to studies of animals sleeping in the natural environment in which sleep evolved (Rattenborg *et al.*, 2008), as well as the examination of potential real-world trade-offs between different sleep strategies (Lesku *et al.*, 2012).

Species in which naturally occurring inter-individual variation in a variety of behavioural and physiological traits has been well characterized in the wild may serve as powerful systems for gaining insight into sleep/wakefulness trade-offs and their functional implications. For instance, in a wide range of vertebrates, dark and pale melanin individuals differentially regulate immunity, sexual activity and energy homeostasis (West & Packer, 2002; Horth, 2003; Kittilsen *et al.*, 2012; Kim *et al.*, 2013), raising the hypothesis that the amount or architecture of sleep covaries with coloration. Accordingly, recent studies in the colour polymorphic barn owl (*Tyto alba*) showed that aspects of the diel rhythm are associated with pigmentation. Nestlings had low blood-circulating levels of corticosterone in the morning and high levels in late afternoon when their mother displayed a plumage typical for males (i.e. small black spots on the feather tips) or when their father displayed a plumage typical for females (i.e. large black spots). Nestlings showed a reversed pattern of corticosterone regulation when their mother was large-spotted or when their father was small-spotted (Roulin *et al.*, 2010a). This observation is consistent with the finding that the daily variation in adult body mass is correlated with melanin-based coloration, with females displaying larger black spots being heavier than those with smaller spots in the afternoon but not in the morning (Roulin, 2009). This raises the question of whether sleep-wakefulness architecture is also related to pigmentation.

To examine whether sleep is associated with melanin-based coloration, we performed a study in nestling barn owls, which vary in the degree of heritable pigmentation. Moreover, we recently demonstrated that a minimally invasive method can be used to measure electrophysiologically defined sleep in owls in the wild (Scriba *et al.*, 2013a). Because sleep affects investment in behavioural vigilance (Rattenborg *et al.*, 1999) for detecting predators (Beauchamp, 2003) and vigilance in the context of social interactions (Knight & Knight, 1986), we examined whether nestling vigilance during family interactions was related to pigmentation in another sample of birds than the ones used to record sleep. A previous study (Dreiss *et al.*, 2013) showed that nestlings are highly vigilant towards the nest entrance where their parents predictably deliver food probably because a rapid begging reaction towards the parents increases the likelihood of being fed before siblings (Roulin, 2001). This study also showed that nestlings

were looking towards their siblings when close to the entrance more often than expected by chance when positioned in the back of the nest box probably to follow the behaviour of competitors. As discussed by Dreiss *et al.* (2013), measuring the proportion of time a nestling is observing the nest entrance and/or its siblings is related to vigilance, a process that requires substantial energy (Illius & Fitzgibbon, 1994). Due to this investment, nestlings might adopt different vigilance trade-offs in conjunction with sleep-wakefulness pattern and colour-related life history strategies.

We propose three scenarios regarding a potential link between pigmentation and sleep duration and/or architecture. First, darker individuals are selectively superior to paler conspecifics, and hence, they can reduce their sleep amount to benefit from having more time for other activities. This scenario therefore predicts that darker-pigmented individuals sleep less. Second, darker-pigmented individuals show superior performance with respect to many phenotypic traits, and to sustain all of these costly activities, they have to sleep more to profit from sleep benefits. This scenario predicts that darker-pigmented individuals sleep more. The third scenario posits that darker- and lighter-pigmented individuals have different life history strategies or exploit different habitats that require different amounts of sleep. In this case, we cannot propose *a priori* predictions regarding the sign of the association between pigmentation and sleep. Also, it might not be possible to distinguish the last scenario from the two others.

In this study, we first investigated whether different characteristics of sleep in barn owl nestlings are associated with the degree of melanin-based coloration measured in the nestlings themselves and in their parents. To examine whether such an association is most likely due to genetic factors rather than environmentally mediated, we performed a cross-fostering experiment to allocate nestling genotypes randomly among rearing environments. If sleep in nestlings raised by foster parents is correlated with coloration of their biological parents, we can suppose that this relationship is mediated by genetic factors. We then investigated whether the degree of nestling vigilance in the context of sibling competition is associated with the same melanin-based plumage traits.

## Materials and methods

### Assessment of plumage traits

Barn owls vary in both number and size of eumelanin black spots located on the tip of ventral feathers and in the degree of coloration from white to dark reddish, a pheomelanin-based trait. These three plumage traits are correlated (Roulin, 2004) and under strong genetic control with heritabilities between 0.80 and 0.90 (Roulin

& Dijkstra, 2003; Roulin, 2004; Roulin *et al.*, 2010b); their expression is not or only weakly sensitive to the environmental and physiological conditions (Roulin *et al.*, 1998; Roulin & Dijkstra, 2003). Numerous experimental studies showed that the size of black spots is associated with immunity, appetite, ability to withstand food depletion, resistance to oxidative stress and regulation of corticosterone (Roulin & Ducrest, 2011).

We counted black spots and measured their size using a calliper within a 60 × 40 mm frame placed on the breast, and a mean value was used in the statistical analyses. Reddish coloration of the breast was reliably compared with colour chips, ranging from −8 for white to −1 for reddish brown (Roulin, 1999). These measures are accurate and highly correlated with spectrophotometer estimates (Dreiss & Roulin, 2010). Female parents were distinguished from male parents by the presence of a brood patch, and the sex of nestlings was identified using molecular markers (Py *et al.*, 2006).

### General method of sleep recording

We obtained electroencephalograms (EEGs) of 66 barn owl nestlings (31 males and 35 females) from a free-living population located in Switzerland (46°49'N, 06°56'E). We recorded owlets at an age of 27–48 days (mean: 38.2 ± 0.55 days) when they were still in the nest box and therefore could be caught by hand. The nestlings stayed in broods containing two to eight individuals located in nest boxes (62 × 56 × 37 cm) fixed on the external wall of barns surrounded by farmland. At the time of recording, the (nocturnal) parents were naturally not resting anymore in their nests during the daylight hours because nestlings were old enough to thermoregulate and consume food by themselves without parental help. Parents were coming back to their nest at night to deliver small mammals.

Because our aim was to examine whether potential associations between sleep states and plumage traits of the nestlings and biological parents have a genetic component, as we did in the past for various other phenotypic traits (Roulin & Ducrest, 2011), we performed a cross-fostering experiment. We exchanged about half of the hatchlings between randomly chosen nests so that 41 (20 males and 21 females) of the 66 nestlings were raised by foster parents, while 25 nestlings (11 males and 14 females) were not cross-fostered and hence raised by their biological parents. Nests were matched in pairs with the criterion that nestlings of the two nests hatched on similar dates differing in 1–2 days at most. To determine the biological parents, molecular analyses from blood samples were conducted (for details see Henry *et al.*, 2013). As biological and foster parents did not resemble each other with respect to the plumage traits (Pearson's correlation,  $P$ -values > 0.31), we conclude that the cross-fostered nestlings were correctly allocated randomly among the rearing

environments. Therefore, any relationship between sleep measured in the cross-fostered nestlings and plumage traits of the biological parents is more likely due to genetic factors or early maternal effects but not confounded by the rearing environment. Experiments took place between May and October 2011.

### Sleep recording

The EEG was recorded using minimally invasive wire electrodes (stainless steel, diameter: 0.13 mm, 2 mm of the insulation exposed; Cooner Wire, Owensmouth, Chatsworth, CA, USA), which were inserted with a needle under the superficial layers of skin (~1 mm) after having locally anaesthetized the skin (Gingicain, Tetracain 754 mg 65 g<sup>-1</sup>). During the attachment of the recording device, nestlings stayed calm and only the eyes were covered to reduce stress. Briefly, two electrodes were placed over the posterior part of the visual hyperpallium (visual Wulst) of each brain hemisphere and referenced each to a posterior electrode placed over the caudal nidopallium of the same hemisphere. The ground electrode was centred between the other electrodes. The electrodes were held in place by a drop of superglue and a data logger (Neurologger 2, www.vyssotski.ch/neurologger2, Vyssotski *et al.*, 2009) containing a 3D-accelerometer (LIS302DLH; STMicroelectronics, Geneva, Switzerland) was connected with the electrodes. After gluing the data logger to the head, the nestlings were put back into their nest box. The logger was powered by two Renata zinc-air13 batteries, each 1.4 V, 310 mAh connected in series to apply voltage above 2.0 V necessary for the logger. The total weight was about 5 g (logger board weighs 0.92 g, accelerometer board 0.36 g, batteries 3 g plus tape for mechanical protection of the device). The EEG signals from each brain hemisphere and the acceleration of the movements were recorded at 200 Hz for up to 5 days (see Scriba *et al.*, 2013a for more information on the procedure). During this period, nestlings were left undisturbed together with siblings in the nest box in the field where they were naturally fed by the parents. We analysed the brain activity for the last complete 24-h period by taking care to start the analysis after a habituation period of a mean of 65 h 55 min (range: 21 h 15 min – 92 h 29 min) after we placed the electrodes. The length of the habituation period did not influence sleep parameters, indicating that nestlings habituated quickly to the electrodes and logger on the head (Scriba *et al.*, 2013b). Given that the standard method for recording brain activity in animals requires the surgical implantation of EEG electrodes onto the brain surface, our method is minimally invasive. Electrodes are inserted only in the first layer of skin, and the recording device weighed at most 2.1% of the bird's body weight. We video-recorded the behaviour of 17 owlets over the whole period of EEG recording to

examine whether birds were disturbed by the electrodes. When the nestlings were returned to the nest box after being instrumented for EEG recording, they did not show behavioural signs of distress (such as hissing and stereotyped movements), ate normally and did not try to remove the device from themselves or their siblings.

One 24-h period of the EEG was scored in 4-s epochs for wakefulness, non-REM and REM sleep (for details see Scriba *et al.*, 2013b) by one experienced investigator blind to nonsleep variables using SOMNOLOGICA software (Medcare, Embla, the Netherlands). Epochs containing more than one state were scored according to the predominant state. If right and left brain hemispheres showed different activities, the predominant state was scored. The total percentage of time spent in each state was calculated, and the number of bouts in each state was counted for each nestling. The durations of all individual bouts in each state were determined and averaged for each nestling and state. The latency between the end of a wakefulness bout of any length and beginning of REM sleep was computed, and the mean value was denoted 'REM sleep latency'.

### Vigilance in the context of family interactions

Between 1997 and 2005, we noted the position within the nest of two nestlings during the 15 min preceding the first parental feeding visit of the night. We used video cameras and infrared light to record the behaviour of the owlets in the nest box. Other nestlings but the two recorded ones were temporarily removed from the nests before recording. A nestling was considered vigilant towards the nest entrance or its sibling when the nest entrance or its sibling was in its field of view and hence visible for the nestling (i.e. in the 160 degrees in front of it, as eye movements in barn owls are highly limited; Steinbach & Money, 1973; Knudsen & Knudsen, 1989). We hence assessed the angle between the direction where an individual was looking and the nest entrance or sibling every 20 s (45 times in 15 min) using the vertical line that connected the bill and the centre of the head (midsagittal plane) and the line that connected the centre of the head and the entrance or sibling. We analysed the mean vigilance, estimated as the proportion of time (over the 45 observations) each nestling had the nest entrance or its sibling in its field of view. We also measured the proportion of time each nestling was closer to the nest entrance than its sibling during the observation session. For detailed methods, see Dreiss *et al.* (2013). Nestlings are probably able to see the nest box entrance and the shade of their siblings or parents during the dark period. However, owls move their head in the direction where they hear a noise, because in this species not only are the eyes frontally oriented, but also barn owls are highly specialized in hunting during the night and have an adaption, the

facial ruff, for this, to focus the sound to the ears (Ohayon *et al.*, 2006). Most of the communication is acoustic rather than visual in this nocturnal species.

Because we did not measure the amount of time watching the nest entrance and sibling in individuals for which sleep had been measured, we cannot relate these measures of vigilance to sleep architecture, but only to melanin-based plumage coloration. Only 22 of 147 nestlings had not been swapped between nests at hatching and hence raised by biological parents; therefore, sample size was not large enough to examine whether nestling vigilance behaviour is associated with plumage traits displayed by the biological or foster parents. In the statistical analyses, we therefore only considered the biological parents.

### Statistical procedure

Statistical analyses were conducted with the software JMP (version 9.0.0; SAS Institute Inc., Cary, NC, USA). The sleep data set was used before to describe the ontogenetic changes of sleep in barn owl nestlings (Scriba *et al.*, 2013b). To test whether sleep variables in this nocturnal species were associated with plumage traits separately for night and day, we averaged hourly values for daylight and dark period (based on sunrise and sunset times obtained from the Astronomical Almanac by H.M. Nautical Almanac Office in the U.K. and the United States Naval Observatory). Thus, for each individual and period (i.e. day and night), we calculated the proportion of time spent awake, in non-REM and REM sleep, the number of wakefulness, non-REM and REM sleep bouts as well as their mean durations. We also calculated the mean REM sleep latency. To reveal essential dependencies, we performed principal component analyses and extracted principal components (Table 1) with eigenvalues larger than 1.0 to be used in linear mixed models including the nest of origin as random variable. We implemented the nest of origin rather than nest of rearing because our aim is to compare nestling sleep with plumage traits of the biological rather than foster parents; it is noted, however, that if we use nest of rearing as random variable, we obtain similar results, and hence, our findings do not depend on which random variable is used. Eigenvectors indicate the loadings of each sleep variable on the first and second principal components (Table 1). Independent variables were the number and size of black spots measured in the nestlings and biological parents as well as the degree of reddish coloration, plus nestling sex and age. Because sample size was relatively low ( $n = 66$  nestlings), we performed separate analyses for maternal, paternal and offspring plumage traits. In the models we introduced nestling sex, nestling age and date when sleep was recorded to demonstrate that any relationship between sleep and plumage is not confounded by these factors. It is noted that mater-

**Table 1** Principal component analyses that summarize sleep–wakefulness data in a few indices in 66 barn owl nestlings. Sleep was measured during 24 h. First two principal components (PCs) of sleep–wakefulness states based on ten variables for which we calculated a mean value for each individual and each hour. Eigenvectors indicate the loadings of each sleep variable on the first and second principal components.

	Principal components of sleep	
	First PC	Second PC
Eigenvalues	5.10	2.06
% Variance	51.0	20.6
<i>Eigenvectors of each sleep variable</i>		
% of time spent awake	−0.40	−0.11
% of time spent in non-REM sleep	0.40	0.16
% of time spent in REM sleep	0.38	−0.02
Mean wake bout duration	−0.27	0.09
Mean non-REM bout duration	0.12	0.63
Mean REM bout duration	0.17	0.10
Number of wake bouts	0.26	−0.41
Number of non-REM bouts	0.40	−0.25
Number of REM bouts	0.41	−0.07
Mean REM latency	0.11	0.56

nal and paternal numbers of spots (the only plumage trait to be associated with sleep variables) were not significantly correlated (1997:  $r = -0.05$ ,  $n = 12$ ,  $P = 0.88$ ; 2000:  $r = 0.17$ ,  $n = 20$ ,  $P = 0.47$ ; 2001:  $r = 0.13$ ,  $n = 9$ ,  $P = 0.75$ ; 2004:  $r = 0.17$ ,  $n = 18$ ,  $P = 0.49$ ; 2005:  $r = 0.17$ ,  $n = 15$ ,  $P = 0.07$ ; 2011:  $r = 0.11$ ,  $n = 19$ ,  $P = 0.65$ ), indicating random mating with respect to this plumage trait.

Nestling mean vigilance and relative position over the 15 min of observation were normalized with arcsine-root transformation. We used exactly the same vigilance data and performed similar linear mixed models as in Dreiss *et al.* (2013), except that we added plumage traits as covariates. We added in the model whether the sibling of the focal individual had a higher or lower rank in the within-brood age hierarchy. Video recording nested in both brood identity and year was set as random factor.

$P$ -values lower than 0.05 are considered significant in two-tailed analyses. In all models, we removed nonsignificant variables. It is noted, however, that all significant effects in the reduced models were already significant before removing nonsignificant variables from the full model.

### Ethical note

We analysed in another sample of owl nestlings the time it took for each nestling after placing the electrodes on the head to fall asleep when back in their nest box. This latency was very short ( $n = 27$  birds, mean  $\pm$  SD latency:  $172 \pm 125$  s), indicating that the

nestlings were not very stressed by the placement procedure and that they can get used to electrodes very quickly. Additionally, all nestlings from this study fledged, and the return rate for breeding in the following year was even higher in owls in which we recorded sleep (26.3%), than in those without sleep recording (19.0%). This research was approved by the Service Vétérinaire du Canton de Vaud (license 1508.5) and adhered to the National Institutes of Health standards regarding the care and use of animals in research.

## Results

### Sleep–wakefulness in relation to parental plumage traits

For each individual, we calculated a mean value for each of the two principal components (hereafter PC1 and PC2) based on the hourly values of sleep–wakefulness states (Table 1). In a linear mixed model, PC1, which mainly captured time spent in the three states, as well as the number of non-REM and REM sleep bouts (Table 1), was not associated with parental plumage traits (Table 2), but with nestling age as already shown in Scriba *et al.* (2013b).

The second principal component of sleep–wakefulness states that mainly described the duration of non-REM sleep bouts, the number of wakefulness bouts and the REM sleep latency (Table 1) was lower in nestlings born from mothers displaying more black spots (Fig. 1a) and in younger nestlings, but not linked with the degree of maternal reddish coloration and spot size as well as nestling sex ( $P$ -values  $> 0.41$ ) (Table 2). The association between PC2 and the maternal number of spots was not significantly different when comparing day to night, whereas PC2 differed between day and night (linear mixed model with nest of origin and nestling identity as two random variables, number of maternal spots:  $F_{1,25,95} = 9.27$ ,  $P = 0.0053$ ; nestling age:  $F_{1,59,59} = 9.81$ ,  $P = 0.0027$ ; day/night:  $F_{1,60,98} = 150.93$ ,  $P < 0.0001$ ; interaction number of maternal spots  $\times$  day/night:  $F_{1,60,88} = 0.76$ ,  $P = 0.39$ ). In a similar model where we replaced the variable ‘day/night’ by whether nestlings were raised by their biological or foster parents (i.e. ‘cross-fostering’), the variable maternal number of spots was significant alone ( $F_{1,25,51} = 7.27$ ,  $P = 0.012$ ) but not in interaction with the cross-fostering status ( $F_{1,42,39} = 0.09$ ,  $P = 0.77$ ). Finally, when considering only the cross-fostered nestlings, the association between PC2 and the number of spots of the biological mother was significant ( $F_{1,13,51} = 7.09$ ,  $P = 0.019$ ), but not of the foster mother ( $F_{1,15,95} = 0.09$ ,  $P = 0.77$ ).

Our results were not confounded by a number of variables. Indeed, the number of siblings in the nest box was associated neither with PC1 nor with PC2 (PC1  $\times$  brood size: Pearson’s correlation:  $r = 0.03$ ,

**Table 2** Sleep architecture of nestlings in relation to plumage traits of their biological parents in the barn owl. We performed separate linear mixed models on the first and second principal components for which the loading of each single sleep variable is given in Table 1. A first model is on maternal plumage traits and a second model on paternal plumage traits. 'Date' refers to the Julian date when sleep was measured. Nonsignificant variables are removed starting with the least significant ones, and final models are written in bold (these variables were already significant in the initial model). Percentage of the variance explained by the random variable 'nest of origin' is also given.

	First principal component of sleep	Second principal component of sleep
Random variable 'nest of origin'	40.30%	25.82%
<i>Plumage traits of biological mother</i>		
Reddish pheomelanin-based colour	$F_{1,25.5} = 0.88, P = 0.36$	$F_{1,22.98} = 0.01, P = 0.92$
Number of black spots	$F_{1,22.6} = 0.005, P = 0.95$	<b><math>F_{1,25.05} = 10.06, P = 0.004</math></b>
Spot diameter	$F_{1,30.38} = 2.23, P = 0.15$	$F_{1,35.19} = 0.32, P = 0.57$
Nestling age	<b><math>F_{1,64} = 7.72, P = 0.007</math></b>	<b><math>F_{1,59.95} = 9.15, P = 0.004</math></b>
Nestling sex	$F_{1,56.86} = 0.01, P = 0.91$	$F_{1,59.91} = 0.65, P = 0.43$
Date	$F_{1,27.95} = 2.84, P = 0.10$	$F_{1,23.91} = 0.53, P = 0.47$
<i>Plumage traits of biological father</i>		
Reddish pheomelanin-based colour	$F_{1,16.96} = 0.17, P = 0.69$	$F_{1,17.81} = 0.79, P = 0.39$
Number of black spots	$F_{1,21.64} = 0.64, P = 0.43$	$F_{1,19.48} = 0.12, P = 0.74$
Spot diameter	$F_{1,17.57} = 0.008, P = 0.93$	$F_{1,17.62} = 0.09, P = 0.76$
Nestling age	<b><math>F_{1,64} = 7.72, P = 0.007</math></b>	<b><math>F_{1,63.97} = 11.27, P = 0.0013</math></b>
Nestling sex	$F_{1,48.12} = 0.002, P = 0.96$	$F_{1,50.54} = 0.18, P = 0.67$
Date	$F_{1,27.95} = 2.84, P = 0.10$	$F_{1,16.33} = 0.03, P = 0.87$

$n = 24$  broods,  $P = 0.88$ ; PC2  $\times$  brood size:  $r = -0.25$ ,  $n = 24$  broods,  $P = 0.23$ ). Additionally, sleep variables had been shown not to be associated with ambient temperature (Scriba *et al.*, 2013b). Nestling mass and wing length were not linked with maternal colour traits (linear mixed models with mother identity as random variable; nestling sex and nestling age as independent variables, all  $P$ -values  $> 0.24$ ).

To understand why maternal number of spots was related to the second principal component of sleep–wakefulness, we performed analyses with the variables showing the strongest loadings (non-REM sleep bout duration, number of non-REM sleep and wakefulness bouts and REM sleep latency; Table 1). By applying a linear mixed model for each of these four variables where we controlled for nestling age, we found that nestlings born from mothers with more black spots showed a shorter REM sleep latency ( $F_{1,27.33} = 10.34, P = 0.003$ ), shorter non-REM sleep bouts ( $F_{1,26.39} = 10.53, P = 0.003$ ) and more wakefulness bouts ( $F_{1,19.28} = 4.26, P = 0.05$ ). In contrast, the number of non-REM sleep bouts was not significantly associated with maternal number of spots ( $F_{1,20.39} = 1.27, P = 0.27$ ).

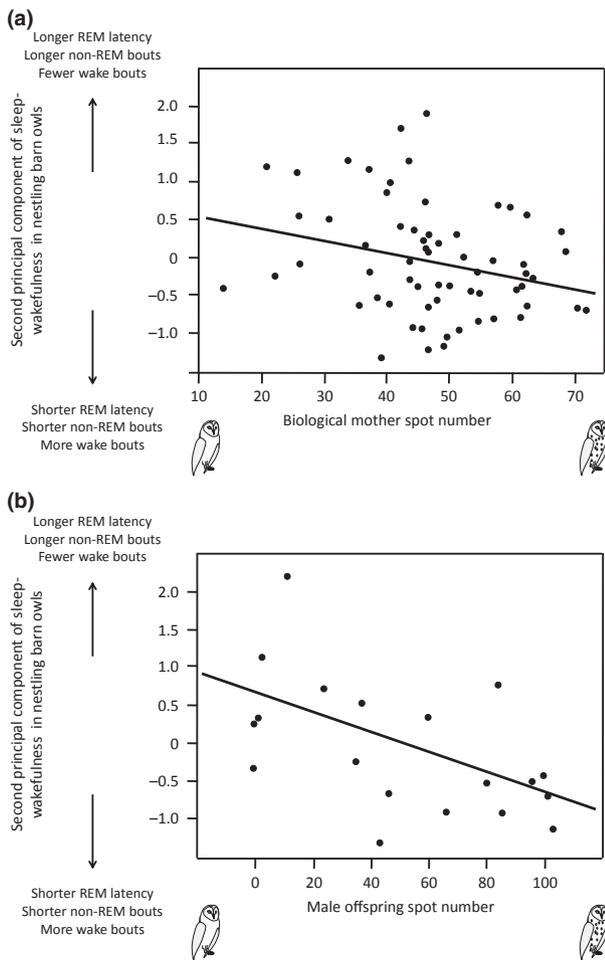
### Sleep–wakefulness in relation to nestling plumage traits

In our sample of birds, nestling spot number was positively associated with maternal and paternal spot number in sons (multiple regression on mean family values, mother:  $F_{1,10} = 7.91, P = 0.018$ ; father:  $F_{1,10} = 11.14, P = 0.008$ ) but not significantly in daughters (mother:  $F_{1,11} = 0.27, P = 0.61$ ; father:  $F_{1,10} = 1.83,$

$P = 0.20$ ), which is in line with a sex-linked component of spot number (A. Roulin & H. Jensen, unpublished). We thus tested whether the second principal component of sleep was associated with nestling spot number in each sex. In sons this component of sleep was positively related to their number of black spots (linear mixed model with nest of origin as random variable:  $F_{1,15.92} = 6.68, P = 0.02$ , Fig. 1b) but not in daughters ( $F_{1,30.39} = 0.61, P = 0.44$ ). By applying a linear mixed model for each of the four variables that are most related to PC2, sons displaying more black spots had shorter REM sleep latencies ( $F_{1,9.772} = 7.16, P = 0.024$ ), shorter non-REM sleep bouts ( $F_{1,11.42} = 5.34, P = 0.04$ ), more wake bouts ( $F_{1,22.47} = 7.16, P = 0.0056$ ) and more non-REM bouts ( $F_{1,24.81} = 7.25, P = 0.013$ ).

### Vigilance in the context of family interactions

As already shown in a previous study (Dreiss *et al.*, 2013), vigilance towards the nest entrance was significantly related to the rank in the within-brood age hierarchy of the siblings alone and in interaction with position in the nest, but also with maternal spot diameter alone and in interaction with position in the nest (Table 3). When a nestling was positioned further away from the nest entrance than its sibling, it was more vigilant towards the nest entrance when its mother displayed larger black spots (similar linear mixed model:  $F_{1,13} = 7.15, P = 0.019$ ; controlling for rank ( $F_{1,13} = 0.05, P = 0.83$ ) and position in the nest box ( $F_{1,13} = 3.25, P = 0.09$ ; Fig. 2a), a relationship that was not significant when nestlings were closer to the nest entrance than its sibling (maternal spot diameter:



**Fig. 1** Nestling sleep–wakefulness pattern in relation to number of eumelanin spots in the barn owl. (a) Relationship with maternal number of spots and (b) with mean sons’ number of spots (Pearson’s correlation:  $r = -0.61$ ,  $n = 18$ ,  $P = 0.008$ ). Predicted values are presented. For the loadings of the ten sleep variables, see Table 1.

$F_{1,13} = 2.69$ ,  $P = 0.13$ ; rank:  $F_{1,13} = 1.30$ ,  $P = 0.27$ ; and position:  $F_{1,13} = 0.97$ ,  $P = 0.34$ ). Maternal reddish coloration and number of spots were not significant (Table 3). In other linear mixed models, nestling and paternal plumage traits were not significantly related to vigilance, indicating that nestling vigilance towards nest entrance was only related to maternal spot diameter (Table 3).

Vigilance towards the sibling was significantly positively related to maternal number of spots (Table 3:  $F_{1,82} = 4.50$ ,  $P = 0.037$ , Fig. 2b); as shown in a previous study (Dreiss *et al.*, 2013), vigilance was also related to the rank in the within-brood age hierarchy and the position in the nest relative to its sibling (Table 3). Maternal reddish coloration and spot diameter were not significant, and replacing maternal plumage traits with

paternal (Table 3) or nestling plumage traits also did not yield significant results (results not shown).

## Discussion

### Relationship between sleep and melanin-based coloration

In line with the hypothesis that melanin-based coloration is associated with behavioural syndromes (Ducrest *et al.*, 2008), in cross-fostered nestling barn owls sleep architecture and behavioural vigilance in the context of family interactions were related to maternal eumelanin-based coloration. Although the amount of time spent in each state was not related to plumage traits, nestlings born from mothers displaying more black spots showed shorter REM sleep latencies (i.e. once in non-REM sleep, these individuals entered faster into REM sleep), shorter non-REM sleep bouts and more wakefulness bouts compared with owlets of weakly spotted mothers. Also, males with more black spots in the plumage showed the same characteristics of the sleep pattern. This indicates that those nestlings switch more frequently between sleep states.

We found links between nestling sleep architecture and plumage traits of the mother, but not those of the father. Plumage traits are highly heritable, and many phenotypes are correlated with maternal plumage (e.g. corticosterone levels, Almasi *et al.*, 2010; immunity, Roulin *et al.*, 2000; resistance to ectoparasites, Roulin *et al.*, 2001; anti-predatory response, Van den Brink *et al.*, 2012). Sleep measured in nestlings was also associated with spottiness measured in male nestlings, but not in female nestlings. We already found similar results with respect to fluctuating asymmetry (FA), where FA measured in nestlings was associated with plumage spottiness in nestling males but not in nestling females (Roulin *et al.*, 2003). From a mechanistic point of view, the reason why we detect these sex-specific relationships is still unclear but could be due to maternal effects (Groothuis & Schwabl, 2008; Dugovic *et al.*, 1999) or to genes having parent-of-origin effects (i.e. genes passed on by the mother have different effects than when passed on by the father, Lawson *et al.*, 2013), which can also depend on offspring sex (the parent-of-offspring effect may differ between sons and daughters, Hager *et al.*, 2008). From an ultimate point of view, the sex-specific association between sleep and plumage spottiness is in line with recent results showing that selection acting on this plumage trait is sex specific (Roulin *et al.*, 2010a,b, 2011; Steinsland *et al.*, 2014). Selection on spottiness is positive in females but negative in males, implying that physiological traits such as sleep could be associated with plumage spottiness in a sex-specific way as reported in the present study. Because sex-specific selection exerted on plumage spottiness appears to act on genetically correlated

**Table 3** Nestling vigilance towards nest entrance and sibling in relation to plumage traits of their biological parents in the barn owl. The video recording session nested in both brood identity and year was set as random factor. We performed separate linear mixed models on each vigilance components and for maternal and paternal plumage traits.

	Vigilance towards the nest entrance	Vigilance towards the sibling
<i>Plumage traits of biological mother</i>		
Reddish pheomelanin-based colour	$F_{1,79} = 0.01, P = 0.98$	$F_{1,75} = 1.95, P = 0.17$
Number of black spots	$F_{1,79} = 1.92, P = 0.17$	<b><math>F_{1,82} = 4.50, P = 0.037</math></b>
Spot diameter	<b><math>F_{1,81} = 16.31, P = 0.0001</math></b>	$F_{1,75} = 0.16, P = 0.69$
Nestling rank	<b><math>F_{1,81} = 5.42, P = 0.022</math></b>	<b><math>F_{1,82} = 6.00, P = 0.016</math></b>
Nestling sex	$F_{1,74} = 0.36, P = 0.55$	$F_{1,75} = 0.01, P = 0.98$
Nestling position in the nest box (position)	<b><math>F_{1,81} = 16.27, P = 0.0001</math></b>	<b><math>F_{1,82} = 4.50, P = 0.037</math></b>
Position × reddish pheomelanin-based colour	$F_{1,72} = 0.02, P = 0.90$	$F_{1,72} = 1.07, P = 0.31$
Position × number of black spots	$F_{1,72} = 2.72, P = 0.10$	$F_{1,72} = 1.46, P = 0.23$
Position × spot diameter	<b><math>F_{1,81} = 16.20, P = 0.0001</math></b>	$F_{1,72} = 0.32, P = 0.57$
Position × nestling rank	<b><math>F_{1,81} = 8.60, P = 0.004</math></b>	$F_{1,82} = 3.10, P = 0.081$
<i>Plumage traits of biological father</i>		
Reddish pheomelanin-based colour	$F_{1,69} = 1.16, P = 0.29$	$F_{1,69} = 0.37, P = 0.54$
Number of black spots	$F_{1,69} = 2.05, P = 0.16$	$F_{1,69} = 1.11, P = 0.30$
Spot diameter	$F_{1,69} = 2.22, P = 0.14$	$F_{1,69} = 0.30, P = 0.59$
Nestling rank	<b><math>F_{1,83} = 5.04, P = 0.028</math></b>	<b><math>F_{1,83} = 7.54, P = 0.007</math></b>
Nestling sex	$F_{1,69} = 0.02, P = 0.88$	$F_{1,69} = 0.19, P = 0.66$
Nestling position (position)	$F_{1,83} = 0.40, P = 0.53$	<b><math>F_{1,83} = 58.46, P &lt; 0.001</math></b>
Position × nestling rank	<b><math>F_{1,83} = 7.74, P = 0.006</math></b>	<b><math>F_{1,83} = 4.48, P = 0.037</math></b>

'Nestling position in the nest box' refers to the proportion of time each nestling was closer to the nest entrance than its sibling, and nestling rank whether the focal individual is older (i.e. senior) or younger (i.e. junior) than its sibling. Significant values are written in bold.

traits (Steinsland *et al.*, 2014), our study suggests that sleep may participate in sexually antagonistic selection, with heavily spotted males being counter-selected and heavily spotted females being positively selected. From an evolutionary ecological perspective, the adaptive function of sleep is still unclear due to the dearth of studies; therefore, we discuss a number of possible reasons why sleep architecture and vigilance were associated with melanin-based coloration.

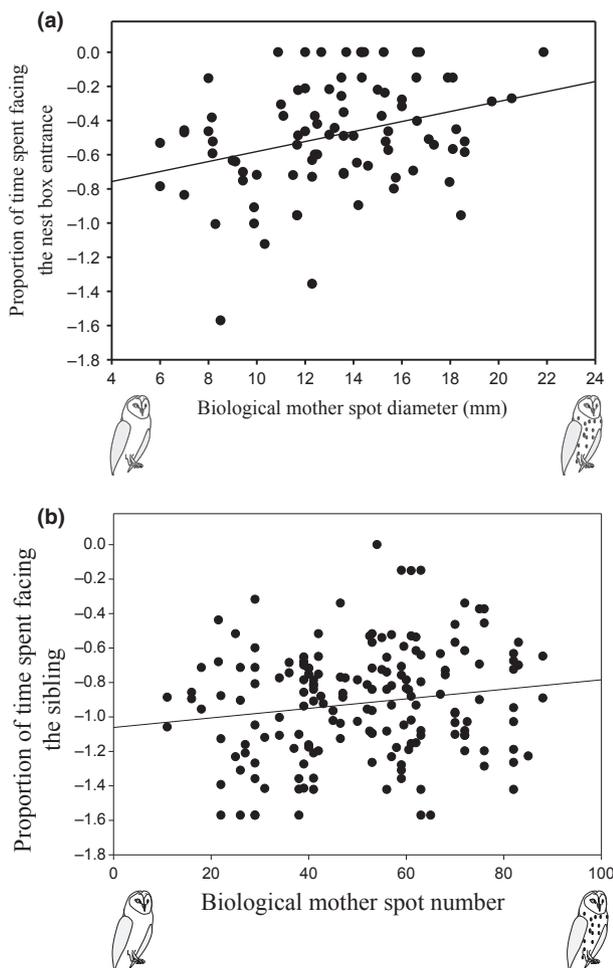
### Sleep-wakefulness states and vigilance in the context of sibling competition

The duration of non-REM sleep bouts, number of wakefulness bouts and REM sleep latency were different during day and night, indicating that nestlings change their behaviour at night. The more frequent changes between wakefulness and sleep states during the dark period may allow nestlings to detect faster the return of parents bringing food items. Accordingly, in several bird species, including barn owls (Roulin, 2001), nestlings that start to beg quicker at parents' return are more likely to be fed first than their less attentive nest mates (Stamps *et al.*, 1989; Smith & Montgomerie, 1991; Leonard & Horn, 1996). We found that nestlings were more often watching the nest box entrance and their sibling when their mother was heavily rather than weakly spotted. This raises the interesting possibility that nestlings of heavily spotted mothers are more vigilant towards the arrival of parents

with a food item or that the cost/benefit ratio of being vigilant is particularly low in those individuals. This may indicate that, at least at the beginning of the night, the intensity of sibling competition may be particularly pronounced in nests of heavily spotted mothers.

### Sleep-wakefulness states and vigilance in the context of predator-prey interactions

The frequent changes between wakefulness and sleep in nestlings may have another function unrelated to nocturnal feeding visits or family interactions. It has been proposed that shorter sleep cycles (alternating bouts of non-REM and REM sleep) lead to an increase in short awakenings, thereby reducing the risk of predation (Snyder, 1966; Lesku *et al.*, 2009). In rats, REM sleep latency was longer after a predatory encounter, possibly to reduce the risk of predation in case the predator returns (Lesku *et al.*, 2008), as high arousal thresholds may make REM sleep a particularly dangerous state (Dillon & Webb, 1965). This observation suggests that barn owls having short REM sleep latencies may feel safe, and this may be particularly the case in nestlings born from heavily spotted mothers and in heavily spotted males. In previous studies, we found that maternal spot size (a trait that is strongly correlated to spot number; Roulin, 2004) is also associated with anti-predator strategies (Van den Brink *et al.*, 2012). Individuals born from large-spotted mothers feign death in front of a predator for longer periods of time, suggesting that they adopt a more passive anti-predator strategy.



**Fig. 2** Vigilance in relation to eumelanin-based coloration in nestling barn owls. Inter-individual variation in the level of vigilance towards the nest entrance (a) and the sibling (b), estimated as the proportion of time a nestling had the nest box entrance (a) and its sibling (b) in its visual field, according to maternal diameter of black eumelanin feather spots (a) and maternal number of spots (b). Each data point represents a mean value for each nestling.

This may be so perhaps because they are more rapidly aware of the presence of a predator (given that they switch more often between wakefulness and sleep states) and may be quicker to adopt the correct anti-predator behaviour. In contrast, individuals of small-spotted mothers may adopt a more active anti-predator strategy, which is in line with the observation that they may detect a predator relatively late because they switch less often between sleep and wakefulness states.

### Sleep-wakefulness states and vigilance in the context of stress regulation

Another possibility is that our observation is associated with the way individuals deal with stressful situations.

In previous studies, we showed that the way nestlings regulate blood-circulating corticosterone is related to maternal eumelanin-based coloration (Almasi *et al.*, 2010). Therefore, the different sleep architectures observed in nestlings born from heavily and weakly spotted mothers may be an indirect consequence of glucocorticoid regulation. Unfortunately, data are missing in animals, as well as in humans, on the relationship between natural variation in glucocorticoid levels and sleep. When artificially administering glucocorticoids, the effect on sleep depends on the doses: high doses of cortisol were shown to decrease the time spent in non-REM sleep in rats, whereas low doses did not show any effect (Bradbury *et al.*, 1998; Vazquez-Palacios & Velazquez-Moctezuma, 2000). Consequently, it is unclear whether the relationship between melanism and REM sleep latency is related to stress levels.

### Costs and benefits of alternative sleep strategies

The above discussion assumed that switching states more frequently is beneficial. However, the opposite might also be true. Vigilance might be decreased shortly after awakening due to sleep inertia, which is characterized by impairment in cognitive and motor performance (Matchock, 2010). Also, in studies on humans more fragmented sleep was shown to have an adverse effect on daytime functioning via increasing daytime sleepiness even when total time spent in the sleep states did not differ (Downey & Bonnet, 1987; Stepanski *et al.*, 1987; Stepanski, 2002). Therefore, nestlings from mothers displaying more black spots and heavily spotted males might suffer from increased sleepiness, which could explain the shorter REM sleep latency, as experimental sleep fragmentation can lead to a decrease in non-REM and REM sleep latency (Stepanski, 2002). This further suggests that nestlings from differently spotted mothers adopt different sleep strategies that entail both costs and benefits.

### Alternative hypotheses to explain our results

Our main results show that sleep architecture is associated with melanin-based coloration in a sex-specific way. We discussed our results with adaptive arguments, but one could argue that these results are artefacts with different individuals being differentially sensitive to the electrodes. Unfortunately, given that sleep and its substates cannot be reliably quantified without measuring brain activity, we cannot measure the potential negative effect of placing the electrodes. Even though the method we used to record sleep is far less invasive than other methods commonly used in animal sleep research, we cannot discard the possibility that some owls modified their sleep architecture as an outcome of the electrodes. However, we do not

believe this possibility is likely for the following reasons. First, we placed the electrodes a couple of days before actually measuring sleep so that owls had time to get used to them, thereby reducing the possibility of negative impact of these electrodes on sleep. Second, we show that owlets fall asleep on average only 2 min after having placed electrodes, demonstrating that they can get used to them very quickly. Third, we did not observe owlets trying to remove actively their own electrodes or electrodes of their siblings. Finally, the results on vigilance behaviour were obtained without placing electrodes, and they are in line with the data on sleep. This suggests that our results are consistent, and we are confident that our study is biologically relevant and we hope that it will stimulate researchers to consider sleep as an important life history component in any biological system.

## Conclusion

Barn owl nestlings from heavily spotted mothers do better on a variety of phenotypic traits than those of lightly spotted mothers (Roulin & Ducrest, 2011). Here we further show that they differ in their sleep architecture and are more vigilant compared with individuals from mothers with smaller black spots. These individuals might be selectively superior to paler conspecifics, and hence, they can invest more in vigilance than siblings from paler-coloured mothers. Another possibility might be that nestlings from darker-pigmented mothers show superior performance with respect to many phenotypic traits, and to sustain all these costly activities, they have to invest more in vigilance. This investment might be at the costs of a fragmented sleep pattern. A third scenario posits that individuals from darker- and lighter-pigmented mothers have different life history strategies or exploit different habitats that require different sleep patterns. The costs and benefits of these potential strategies need to be resolved in future studies focusing on the fitness consequences of differences in sleep–wakefulness pattern early in life. We are aware that our work raises a number of unresolved issues, but we hope that it will stimulate research on sleep from an evolutionary ecological point, a topic that is still in its infancy.

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