Original Article

Melanic color-dependent antipredator behavior strategies in barn owl nestlings

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The arms race between predators and prey has led to morphological and behavioral adaptations. Different antipredator strategies can coexist within a population if each strategy is the result of a trade-off with competing demands. Antipredator behavior can be associated with morphological traits, like color patterns, either because in the context of sexual selection, coloration signals the ability to avoid predators or because coloration is a naturally selected trait useful in avoiding predators. Because in the barn owl (*Tyto alba*), heritable eumelanic plumage coloration is associated with the glucocorticoid-dependent response to stress, we tested whether antipredator behavior is also related to this trait. Compared with small-spotted nestlings, individuals displaying larger black spots hissed more intensely in the presence of humans, feigned death longer, had a lower breathing rate under stress, and were more docile when handled. Cross-fostering experiments showed that the covariation between the spot size and the duration of feigning death was inherited from the biological mother, whereas covariation between spot size and docility was inherited from the biological father. Our results confirm that melanin-based coloration is associated with suites of behavioral traits, which are under both genetic and environmental influence. Coloration can thus evolve as a direct or indirect response to predation, but it can also be a signal of antipredator strategies to potential mates. *Key words:* animal personalities, melanin, natural selection, predation, sexual selection. *[Behav Ecol 23:473–480 (2012)]*

INTRODUCTION

An important factor in the evolution of morphology and behavior is the arms race between predators and their prey. While predators evolve more efficient foraging techniques, their prey is in turn selected for more refined adaptive strategies to escape them (Dawkins and Krebs 1979). These adaptations can involve specific color patterns (Stuart-Fox et al. 2004) or shapes (Hoso and Hori 2008) to enhance camouflage. They can also concern behavior including hissing calls in insects (Davis and Heslop 2004) and lizards (Labra et al. 2007), aimed at scaring predators, or feigning death in the presence of predators that do not eat or react to dead animals (Miyatake et al. 2004), or even feigning of injuries by parents to redirect the attention of predators toward themselves rather than toward their vulnerable offspring (Grimes 1936). Although a wide variety of antipredator strategies have evolved in many species, only in the last decade with the development of the field of behavioral syndromes or "animal personality" (Gosling 2001; Sih et al. 2004) has interest been devoted to possible individual differences in antipredator strategies (Bell 2005). A growing body of literature supports the proposition that individuals can differ in their response to predators (e.g., Cockrem 2007; Thaker et al. 2009; Jones and Godin 2010).

Different mechanisms could favor the coexistence of several antipredator strategies within a population. Polymorphism in the way individuals escape from predators can be maintained if the balance between the costs and the benefits of alternative strategies to avoid predation is the same (Roff 1996). For instance, morphs may exploit habitats where different predators are found, requiring alternative strategies to escape them, including specific colorations that allow genotypes to be cryptic in alternative habitats (Hoekstra et al. 2005), or they may adopt different antipredator behavior specifically directed to the size or type of predators (Seyfarth et al. 1980; Templeton et al. 2005). Another mechanism favoring strategy diversity is negative frequency-dependent selection, where predators are used to a prey having certain characteristics, leading to a disproportionate consumption of the most common type of prey, thereby favoring rare alternative antipredator strategies (Punzalan et al. 2005; Bond and Kamil 2006).

In several species, alternative antipredator strategies have been shown to be associated with morphological traits such as coloration, probably because viability selection mediated by predators can favor certain combinations of antipredator behavior and color patterns. In the pygmy grasshopper (*Tetrix subulata*), experimentally altered combinations of camouflage color patterns and behavior changed survival probability when confronted with a predator (Forsman and Appelqvist 1998). In the eastern red-backed salamander (*Plethodon cinereus*), a conspicuous red morph displays different antipredator behavior than a cryptic lead-colored morph (Venesky and Anthony 2007). In Hermann's tortoises (*Eurotestudo boettgeri*), darker eumelanic individuals are bolder in the presence of humans suggesting that the behavior toward predators is color specific (Mafli et al. 2011; see also Thomas 2002). Further proof for this proposition comes from a recent study

performed in the marsh harrier (*Circus aeruginosus*), which shows that 2 different color morphs of male harriers display different antipredator behavioral strategies. Gray individuals mobbed intruders less and recruited fewer helpers for mobbing than did brown males (Sternalski and Bretagnolle 2010). Finally, artificial selection for high and low stress responsiveness in the rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) showed that heavily spotted individuals display a lower physiological and behavioral stress response than lightly spotted individuals (Kittilsen et al. 2009). Thus, differently colored fish may respond differently to predation risk due to a link between the hypothalamic-pituitary-adrenal (HPA) stress response and the eumelanic coloration. Such a link could be mediated by the melanocortin system with products of the proopiomelanocortin (*POMC*) gene binding not only to the MC1R (melanocortin 1 receptor), resulting in melanin-based coloration, but also to 4 other receptors (MC2R–MC5R), which regulate other traits such as the HPA-axis, aggressiveness, physical activity, and immune function (Boswell and Takeuchi 2005; Ducrest et al. 2008). This can result in a covariance between these traits and melanin-based coloration (McKinnon and Pierotti 2010).

Barn owls have been studied intensively to understand the adaptive function and maintenance of variation in their plumage color. Both within and among populations, barn owls vary in the size of eumelanic black spots, a heritable sexually dimorphic trait with females displaying on average larger spots than males, and variation in spot diameter is due to both sex-linked and autosomal genes (Roulin et al. 2010). This trait is associated with many physiological and behavioral functions (Roulin and Ducrest 2011). Of particular interest is the finding of Almasi et al. (2010), who showed that the size of black spots of barn owls is associated with the (HPA) stress response as in the rainbow trout and Atlantic salmon (Kittilsen et al. 2009). In their study, barn owl nestlings born from a mother with larger spots mounted a lower corticosterone stress response and had lower levels of corticosterone after implanting corticosterone-releasing pellets. As shown experimentally in other species, the presence of a predator induces a rise in blood circulating corticosterone (Müller et al. 2009; see also Cockrem 2007), and hence, we predict spot diameter to be associated with personality and more specifically with individual differences in the reaction to predation risk.

In a free-living Swiss population of barn owls, we examined 3 antipredator behaviors in relation to melanin-based coloration. 1) When a predator is close to a brood, nestlings often start to hiss loudly, probably to scare the predator. In various animals, this behavior is also observed (Sibley 1955; Apel and Weise 1986; Labra et al. 2007), with the apparent goal to make the predator mistake the identity of the prey with another predator so that it is scared away (Dobkin 1979). We thus simulated a predator intrusion by opening nest-boxes and recorded the intensity of hissing calls. 2) Because nestling barn owls can become aggressive and agitated once captured by humans, probably in an attempt to escape, we recorded the degree of agitation when handled. 3) We carried out the tonic immobility test to measure the extent to which nestling owls feign death. Animals are put on their back, and the time taken to turn back and stand on the legs again is measured. Tonic immobility is commonly observed in a wide variety of taxa in response to external stimuli, and it is considered an adaptive defense mechanism against predators (Boissy 1995). Because the way individuals react in the presence of a predator is probably due to the ability to cope with stressful events, we also measured the breathing rate after capture, a measure of stress and fear (Carere and van Oers 2004; Fucikova et al. 2009). As larger spotted individuals show a lower glucocorticoid stress response, we predict larger spotted nestlings to

breath at a lower rate. To investigate whether the relationships between antipredator behavior and coloration are environmentally mediated or genetically inherited, we allocated nestlings randomly among environments by swapping hatchlings between randomly chosen pairs of nests.

MATERIALS AND METHODS

Study species

The worldwide-distributed barn owl is nocturnal and hunts mainly small mammals in the open landscape. In Switzerland (46°49′ N, 06°56′ E), where the study was performed, most barn owls breed in nest-boxes (1.0 × 0.6 × 0.5 m) and lay between 2 and 11 eggs (mean ± standard error [SE]: 6.1 ± 1.6) between the end of February and August (mean laying date: 29 April ± 24 days). Eggs are incubated for 32 days and hatch asynchronously on average every 2.5 days implying that in large broods, the first-hatched nestling can be up to 3 weeks older than its last-hatched sibling. During the first 2 weeks of life, the father forages, whereas the mother broods and distributes food to the nestlings. Once the offspring can eat without maternal help, the mother participates in foraging. From this time until fledging at around 55 days of age, the offspring are in their nest without their parents who sleep in barns located sometimes several kilometers away from the nest. At night, nestlings produce not only begging calls in the presence of their parents but also several hundreds of calls during their prolonged absence to pacifically resolve conflicts over the single indivisible food item brought at each parental feeding visit, a sib–sib communication process referred to as "sibling negotiation" (Roulin et al. 2000). These calls are noisy and hence may attract mammalian predators (Roulin 2001; McDonald et al. 2009), which may explain why barn owls evolved several antipredator strategies. This includes aggressive behavior toward predators using their claws and beak, extremely noisy hissing calls that may frighten predators (such as mustelids, cats, and humans; Roulin A., Dolivo V, personal observation), and feigning death by staying immobile.

General methods

A single person measured the diameter of black spots located at the tip of feathers of the ventral body side of adults and their offspring aged approximately 50 days. This was done on the breast, belly, flanks, and undersides of the wings to the nearest 0.1 mm using a caliper. A representative number of spots were measured on each body part, and then a mean value was calculated. The mean of both flanks (and both wings) was calculated, and finally, a mean value over the 4 body parts to be used in the statistical analyses. The assessment of this plumage trait is reliable (Roulin 1999, 2004). From 2008 to 2010, we carried out partial cross-fostering experiments by exchanging approximately half of the hatchlings between pairs of nests with the criterion that the matched broods had a similar hatching date. To recognize nestlings, we marked them with nontoxic color paint until we could ring them with a numbered aluminum ring. We collected a blood sample to determine nestling sex from blood cell DNA using sex-specific molecular markers.

Hissing behavior

In 2009, we studied hissing behavior in 15 broods containing 2–6 (3.9 ± 0.3) nestlings aged 40 ± 0.9 days. At the beginning of the night (mean time: 22h13 ± 12 min), a recorder (Marantz Professional Audio PMD-670) was placed at 10 m from the nest-boxes. The top of the nest-box was opened,

a microphone (Beyerdynamic M69N) installed inside of it, the nestlings touched and briefly illuminated with a flashlight to mimic the presence of a human predator, and the top of the nest-box was closed. We then retreated to 10 m away from the nest-box and did not make any noise while recording nestling behavior. This procedure lasted approximately 30 s. We recorded until 10 min after hissing had ended or in those cases where no hissing was induced, for 10 min. To obtain a reliable mean estimate of hissing behavior, we repeated this procedure 6.7 ± 0.6 times (range: 2–12) in a row and induced hissing in the nestlings in 95 of 103 cases. The number of times our presence induced hissing behavior was not associated with mean nestling spot diameter (Pearson's correlation: $r = 0.21$, $n =$ 15 nests, $P = 0.45$). The beginning of each hissing period was defined as the moment when the top of the nest-box was closed after having disturbed the brood. It ended when the last nestling stopped hissing and either siblings resumed negotiation calls (68 cases) or did not produce any hissing call in the next 10 min (35 cases).

In order to quantify the acoustic sound levels of hissing calls, a first calibration procedure of the acoustic device was done in the lab so that every recording was free of distortion and digitalized with a good quality and an adequate quantification. Because in situ acoustic calibration before each recording was not possible, we assumed all nest-boxes as closed boxes with the same volume and a microphone placed at equal distance to the nestlings during all experiments. Even though these assumptions could induce some minor changes of acoustic levels of the recordings, the analysis of the recordings validates the acoustic protocol permitting then to postprocess the whole acoustic data set. For each recording sequence selected with Adobe Audition 3.0 software, the energy contained in hissing calls was quantified using a script written in Matlab R2008b.

A mean value per brood was calculated over all the measurements. Because we measured hissing behavior of entire broods and not individual nestlings, we could not investigate whether this behavior was associated with spot diameter measured in the biological and foster parents (every brood contained nestlings of 2 origins since we performed a partial cross-fostering experiment) but only with the mean spot diameter measured in the nestlings themselves. Before calculating mean nestmates' spot diameter, we also had to remove variation explained by sex for the entire brood because females have on average larger spots than males (Roulin 1999). We did this by extracting residuals from a one-way analysis of variance with nestling spot diameter as dependent variable and nestling sex as a factor. Then, for each brood, we calculated a mean residual value. To analyze the relationship between residual nestling spot diameter and hissing behavior, we incorporated date and number of nestlings as covariates.

Because of the short time interval, the number of inductions within the same night cannot be considered as independent events. Ideally, to obtain reliable repeatability estimates of hissing behavior, we should have repeated the measurements on several independent visits. We therefore calculated repeatability over the consecutive measurements made on the same night to decide whether we can calculate a mean hissing value.

Tonic immobility

In 2009, we carried out the tonic immobility test in 37 cross-fostered nestlings from 13 origins and in 32 noncross-fostered nestlings from 10 origins. Each individual was tested between 1 and 3 times (2.0 ± 0.1) on different days at a mean age of 27.4 ± 1.5 days. Tonic immobility was recorded at the beginning

of the night (mean time: $22h24 \pm 5$ min) a few days before or after we assessed hissing behavior but never on the same day. Using the methods described by Jones and Faure (1981) and Jones (1986), a single person put each individual on its back on a flat illuminated surface and restrained it for 10 s with a hand on its breast. The hand was then removed, and the time until the nestling moved to turn and stand again was measured. The same person stayed nearby within sight of the nestling until the end of the test. In this test, individuals differ not only in the duration but also in the ease with which the immobile state can be induced. In addition, often the duration of tonic immobility is negatively correlated with the number of attempts needed to induce it (Hennig 1978, Mills and Faure 1991). Following the most common way to measure this tendency (Jones 1986), we obtained a measure of the motivation to not stay on their back by repeating the tonic immobility test up to 3 times. If an individual stayed on its back more than 15 s on the first occasion, we did not repeat the test; if it stayed less than 15 s, we tested it again; and if at the second trial it again stayed less than 15 s, we carried out a third and final trial. Thus, each individual was tested between 1 and 3 times. The mean number of times an individual was tested thus indicated the tendency or repeatability of an individual to stand up quickly after having been put on its back. Over the 1 to 3 trials, we considered the longest duration this individual stayed on its back as another measure of tonic immobility. If an individual stayed longer than 120 s, we stopped the test and hence 120 s was the maximum duration. The tests were done without prior knowledge of plumage traits because at that time color traits were not yet developed, and a different person measured spot diameter without being aware of the results of the tonic immobility test.

Breathing rate

The number of times an individual breathes in 1 min, as measured by counting the number of breast movements is an indication of response to handling stress (Carere and van Oers 2004; Fucikova et al. 2009). On the same day when the tonic immobility test was performed, we assessed breathing rate in 48 nestlings (29 cross-fostered and 19 noncross-fostered) immediately after being taken out of their nest-box. Because we recorded breathing rate in more than one nestling per visit, for each individual, we recorded the time between the moment when we opened the nest-box for the first time and when we started to count breast movements; however, this measure was not associated with breathing rate $(r = 0.08$, $n = 48$, $P = 0.61)$. Ambient temperature was recorded but was also not significantly correlated with breathing rate $(r = 0.23$, $n = 48$, $P = 0.10)$. The test was performed on 1–3 different days (2.7 ± 0.2) to allow calculation of repeatability. Unfortunately, we could not calculate heritability for breathing rate because we only started to measure this trait in 2009 in nestlings (but not in adults), and hence, we do not have yet enough data.

Docility

Between 2008 and 2010, a single person handled 448 nestlings and assessed aggressiveness toward the handler and the degree of agitation around the age of fledging (mean age: 48 ± 0.28 days). Score 0 was assigned to nestlings that did not express any aggressive behavior, score 1 when they tried to bite once or a few times, score 2 when they frequently scratched, attacked, or pinched with the beak, and score 3 when they were extremely aggressive by grabbing with their bill and claws and when trying to catch the bird to be handled they were on their

back with claws raised. Assessments of agitation were given according to a similar index (0–3), the minimal score (0) being assigned to nestlings that did not move or hiss during manipulation, whereas the maximal score (3) corresponds to nestlings that were struggling, flapping their wings, and/or hissing all the time. The values for aggression and agitation were summed to get an index of docility. The higher the value, the less docile we considered the individual to be. We only started to record docility in 2008, and although we measured this trait in both nestlings and adults, currently we cannot yet calculate heritability, as we need to compare nestling docility with parental docility measured at the same life history stage. Nestlings are much less docile than adults, and in adults, docility depends on several variables such as reproductive stage (van den Brink V, Roulin A, unpublished data). Thus, although it would be very interesting to have heritability estimates, the data are currently not yet available to do so.

Covariation of the measured behavioral traits

If the different behavioral traits are correlated with each other, this could indicate that differences between individuals are part of different antipredator strategies. To investigate possible covariation between the measured behavioral traits, we calculated mean values per individual for each behavioral trait (breathing rate, tonic immobility duration, and number of attempts and docility score). Not all traits were normally distributed, and because transformations did not improve normality, we used nonparametric Spearman's rank correlations.

Statistical methods

Statistical analyses were performed using the software programs JMP 7.1 and SAS v9.1 (SAS Institute Inc.). To analyze tonic immobility test in relation to spot diameter, we considered only the 37 cross-fostered nestlings and calculated the mean of the 1–3 recorded values. In a mixed model analysis of covariance (ANCOVA), we entered the nest of rearing as a random variable, nestling sex as a factor, and nestling age and body mass as well as spot diameter of the biological and foster mothers and fathers as 6 covariates, and the interaction between sex and spot diameter was also included. A similar procedure was applied to breathing rate. In these tests, we were able to enter sex as a factor in the model because contrary to the hissing experiment here, we obtained a behavioral value for each individual nestling.

We analyzed nestling docility with a general linear mixed model. Separate models were built to assess the association between docility and spot diameter of cross-fostered nestlings or of the biological and foster parents. We analyzed cross-fostered nestlings separately so that we could assess the influence of color traits of the biological and foster parents on the behavior. Other factors in the models were nestling sex, nestling age, brood size, spot diameter of the nestlings or of the parents, and two-way interactions. For the model including nestling spot diameter, we added nestling nested in nest of rearing as a random factor to account for multiple measurements of the same individual. In addition to this, for the models with parental spot diameter, year and identity of the parent were added as random factors to account for multiple nestlings and multiple breeding seasons of the same individual. All statistical analyses are two-tailed and P values smaller than 0.05 considered significant. Nonsignificant variables were removed one after the other starting with the least significant interactions. Means are quoted ±SE.
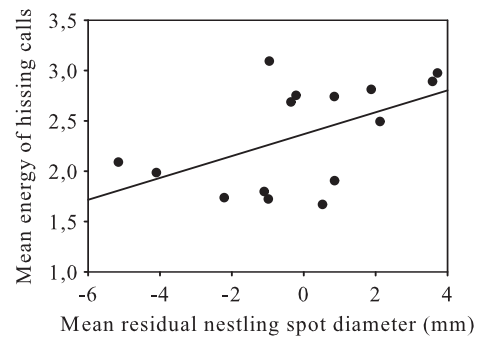
Figure 1
Energy contained in hissing calls (log + 1 transformed) in relation to residual nestling spot diameter in nestling barn owls. Mean values per nest were calculated so that each nest appears only once in the figure. We extracted residual nestling spot diameter to remove variation explained by sex. Regression line is drawn for illustrative purpose. Least squares values from the statistical model presented in the results are presented.

RESULTS

Hissing behavior

The total amount of energy contained in hissing calls was measured several times in a row on the same night in each site and was found to be significantly repeatable ($r = 0.50 \pm 0.04$; $F_{14,76} = 6.43$, $P < 0.0001$). The mean amount of energy per brood contained in hissing calls increased with mean residual nestling spot diameter (stepwise linear regression analysis on mean brood values: $F_{1,11} = 5.72$, $P = 0.036$; Figure 1) after controlling for brood size ($F_{1,11} = 10.04$, $P = 0.009$; more energy was invested in hissing by larger broods) and date ($F_{1,11} = 10.40$, $P = 0.008$; owls hissed more at the end than beginning of the season); brood sex ratio and nestling age were not significant ($P > 0.60$) and so we removed these 2 variables from the model. Note that in a preliminary model, the interaction between brood size and mean residual nestling spot diameter was not significant ($F_{1,10} = 0.19$, $P = 0.67$) indicating that the higher hissing response to stress by large- than small-spotted nestlings was probably not mediated by social interactions associated with brood size. This result is not confounded by habituation because the positive relationship between the amount of energy contained in hissing calls and mean residual nestling spot diameter was also detected when considering only hissing calls produced the very first time we disturbed nestlings ($r = 0.59$, $n = 13$ nests, $P = 0.032$).

Tonic immobility

When put on their back, nestlings took on average 62.8 ± 4.1 s to turn over and stand again on their feet. The mean speed with which nestlings turned back on their feet was significantly repeatable between days ($r = 0.19 \pm 0.07$; $F_{68,101} = 1.58$, $P = 0.018$). In a mixed model ANCOVA with the nest of origin entered as a random variable, nestlings stood up on their feet quicker when their biological mother displayed smaller than large black spots ($F_{1,6.9} = 13.24$, $P = 0.0085$; Figure 2A); spot diameter of the biological father and of the 2 foster parents were not significant (P values > 0.21). When replacing maternal spot diameter by nestling spot diameter, the relationship was no longer significant ($F_{1,30.43} = 0.43$, $P = 0.51$). The interaction terms, nestling sex and age were never significant and hence removed from the final models.

The mean number of times we tested nestlings, that is, the other measure of the nestling's motivation to turn over and stand again on their feet, was 1.70 ± 0.08. As we carried out

(A)

(B)
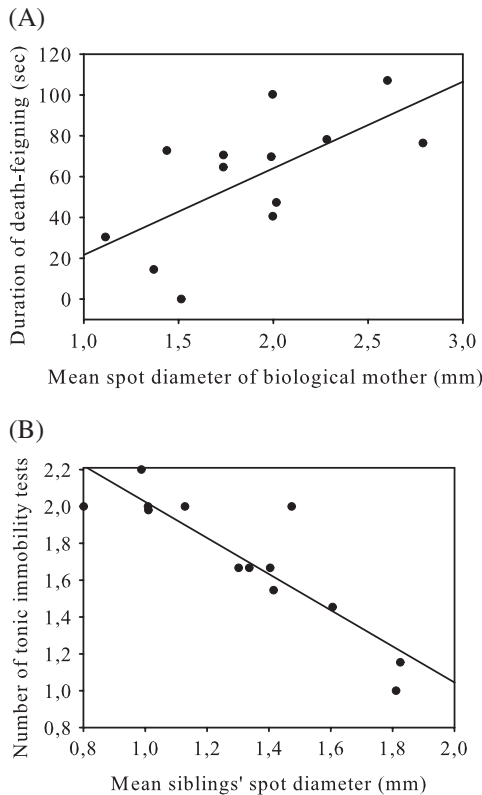
Figure 2
Tonic immobility in nestling barn owls raised by foster parents in relation to the size of black plumage spots. (A) Mean amount of time siblings took to feign death (i.e., amount of time between the moment when a nestling was put on its back and turned to stand back on its legs) in relation to the size of black spots of their biological mother. Pearson's correlation is $r = 0.64$, $n = 13$ nests, $P = 0.018$. (B) Mean number of attempts needed for nestlings to stay longer than 15 s on their back before turning back and standing again on their legs in relation to the size of black spots measured in the nestling themselves. Pearson's correlation is $r = -0.88$, $n = 13$ nests, $P = 0.0002$. In the 2 panels mean sibling values were calculated so that each origin appears only once. Regression lines are drawn for illustrative purpose.

the tonic immobility tests 2–3 times on different days, we could assess whether this second measure of tonic immobility was repeatable. This was the case ($r = 0.15 \pm 0.07$; $F_{68,101} = 1.44$, $P = 0.047$). In contrast to the previous measure of tonic immobility, this second measure was not significantly associated with spot diameter of the biological and foster parents (mixed model ANCOVA with nest of rearing as random factor: P values > 0.25; the interaction of nestling spot diameter and sex, nestling sex and age were also not significant, P values > 0.15) but with spot size measured in the nestlings themselves (another mixed model ANCOVA: $F_{1,8.3} = 13.78$, $P = 0.0056$). When all nestlings (cross-fostered and noncross-fostered) were considered simultaneously, qualitatively similar results were obtained ($F_{1,56.4} = 6.27$, $P = 0.015$). Nestlings displaying larger black spots were tested less often than individuals with smaller spots (Figure 2B) indicating that individuals with larger black spots were more prone to stay longer on their back. Again, nestling sex and age were never significant and hence removed from the final models.

Breathing rate

Breathing rate was repeatable within nestlings in consecutive visits ($r = 0.32 \pm 0.06$; $F_{47,93} = 2.305$, $P = 0.0027$). When con-
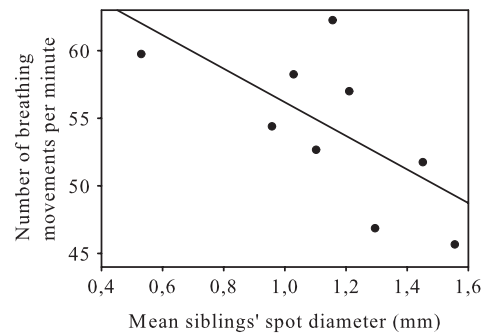
Figure 3
Number of breathing movements per minute recorded as result of handling stress in relation to siblings' spot diameter in the barn owl. We calculated mean values so that each nest only appears once. Pearson's correlation is $r = -0.68$, $n = 9$ nests, $P = 0.04$. Regression line is drawn for illustrative purpose.

sidering only the 29 cross-fostered nestlings for which we measured breathing rate, this variable was not associated with spot diameter measured in the biological and foster parents (mixed model ANCOVA, P values > 0.10). In a similar model using spot diameter measured in the nestlings themselves instead of in parents, individuals displaying larger black spots made fewer breathing movements per minute (Figure 3; mixed model ANCOVA: $F_{1,14.51} = 5.90$, $P = 0.029$; nestling sex and age were not significant). When measured in all nestlings, including noncross-fostered ones this was also significant ($F_{1,40.1} = 4.56$, $P = 0.04$).

Docility

In 284 nestlings, we recorded docility on different days (3.9 ± 0.09 times) which proved repeatable ($r = 0.27 \pm 0.066$ $F_{1,494} = 1.992$, $P < 0.0001$). After correcting for nestling age ($F_{1,333} = 6.9$, $P = 0.014$; with age individuals became less docile), nestlings were less docile when their biological father displayed small than large black spots ($F_{1,88} = 5.64$, $P = 0.02$) (Figure 4). Nestling sex and brood size had no effect on docility (for all traits $P > 0.30$). For the nestlings themselves, only a positive association with age was found ($F_{1,445} = 4.64$, $P = 0.032$). When all nestlings (cross-fostered and noncross-fostered) were in the model, the results remain qualitatively the same ($F_{1,1307} = 34.8$, $P < 0.0001$). In the models investigating the effects of mean spot diameter of nestlings and mean spot diameters of biological mother and foster parents, no relations with color traits were found (all P values > 0.41).

Covariation between behavioral antipredator traits

Most individually tested traits were correlated with each other. Breathing rate was negatively correlated with tonic immobility duration (Spearman's correlation, $r_s = -0.48$, $n = 70$ individuals, $P = 0.0004$) and positively with tonic immobility number of attempts ($r_s = 0.34$, $n = 70$, $P = 0.018$). The tonic immobility duration was negatively correlated with the number of attempts needed to induce tonic immobility ($r_s = -0.67$, $n = 70$, $P < 0.0001$). Finally, less docile nestlings breathed at a higher rate ($r_s = 0.35$, $n = 70$, $P = 0.014$), tended to stay less long on their back ($r_s = -0.23$, $n = 70$, $P = 0.058$), and hissed at a lower level than docile individuals ($r_s = -0.63$, $n = 16$ nests, $P = 0.009$).

DISCUSSION

In the barn owl, several antipredator strategies were associated with the size of black eumelanic spots: larger spotted nestlings invested more energy in hissing behavior and feigned death
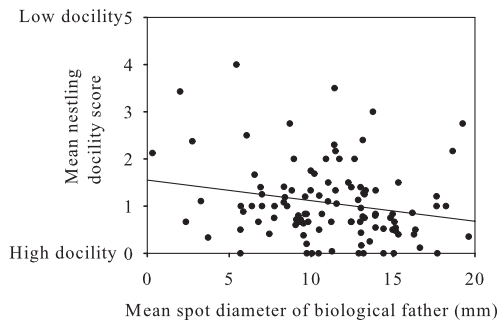
Figure 4
Mean docility score of nestling barn owls raised by foster parents in relation to the spot diameter of the biological father. A higher score means the individuals are less docile (see explanation in MATERIALS AND METHODS). We calculated mean sibling values so that a single value per foster parent pair is presented in the figure.

more easily and for longer than smaller spotted conspecifics, which were more agitated when handled and stressed as evidenced by breathing rate. Using cross-fostering experiments, we could test how 2 of these color-specific behaviors were inherited from one generation to the next. Interestingly, the covariance between spot size and tonic immobility was inherited from the biological mother, whereas the covariance between spot size and docility was passed on through the biological father. This indicates the presence of genetic or maternal/paternal effects.

Boldness–shyness

When confronted with a predator, small-spotted barn owl nestlings appeared to be particularly bold by being aggressive and agitated while handled, whereas large-spotted owls are rather shy by staying calm when handled, feigning death, and hissing loudly. Shy behavior seems to have as a goal the avoidance of a direct confrontation or even a fight. Given that docile individuals were hissing more intensely than less docile nestlings, we conclude that the hissing behavior reflects a shy and calm behavior. It might confuse or scare away the predator without the direct risk of a physical confrontation. In other species where a physical confrontation would pose a serious risk of injury or death, it is also used for instance by burrowing owls (*Athene cunicularia*) mimicking rattle snake calls to deter ground squirrels (Rowe et al. 1986) or skunks (Medill et al. 2011), who can advertise their danger by hissing. As shown in our study, hissing was more pronounced in large than small broods probably because the likelihood that there is an individual with a fearful personality that continues to hiss during long periods of time is higher in large than small broods. Thus, the higher hissing response to a predator of large- compared with small-spotted owlets could be socially mediated. However, we did not find support for this hypothesis given the absence of statistical interaction between brood size and spot diameter. Another possibility that remains to be tested is whether the intense hissing behavior of larger spotted individuals is mediated by social dominance interactions and aggressiveness within the brood. To sum up, more data are required to investigate whether the association between spot diameter and hissing is genetically inherited or socially driven.

Even though the tonic immobility test is widely regarded as a measure of fear in poultry (Jones 1986), differences in duration can also be regarded as different strategies (Erhard et al. 1999). Tonic immobility might increase survival chances after an attack, as demonstrated in ducks (Sargeant and Eberhardt 1975) and quails (Thompson et al. 1981). It can even be inter-

preted as a calculating, selfish strategy, where an individual tries to divert attention away from itself to redirect it toward its conspecifics, which are still moving (Miyatake et al. 2009). The negative association we found between tonic immobility and breathing rate supports the idea that in barn owls, tonic immobility is a calculating strategy because a higher breathing rate, which is an indicator of stress, goes together with shorter tonic immobility duration and more attempts needed to induce it.

As we have seen, in the barn owl, docility is inherited to offspring from the biological father. Because we know males are also selected to have fewer and smaller spots than females (Roulin et al. 2010, 2011), selection on boldness may indirectly affect the evolution of spot diameter and vice versa. In other words, antipredator behavior may indirectly influence the evolution of sexual dimorphism in the degree of eumelanin-based coloration. Thus, in males small and few spots may honestly signal the ability to survive in environments where predators are frequent given that boldness increases reproductive success early in life possibly at the cost of reduced lifespan (Smith and Blumstein 2008; Reale et al. 2009). We would thus predict that males displaying smaller black spots produce offspring with low survival prospects. Interestingly, this is exactly what we found in a recent study showing that smaller spotted males produce daughters with a particularly low survival (Figure 1c in Roulin et al. 2010). A similar pattern is found in a population of the common buzzard (*Buteo buteo*), where light males are more aggressive than dark males, whereas in females this pattern is reversed (Boerner and Krüger 2009). This might reflect the different demands made on the 2 sexes in antipredator behavior.

Consistency of behavior

Repeatabilities reported in this study range from 0.15 to 0.5, which indicates that individuals are consistent in their behavior toward potential predators. The repeatability of tonic immobility duration and number of attempts is clearly lower than found in other studies on chickens (rank scores within individuals; Jones 1988) or beetles ($r = 0.94$, Nakayama et al. 2010), but in a review on pigs, cattle, and poultry, repeatability was found to be very variable (Forkman et al. 2007). A meta-analysis of animal personality studies by Bell et al. (2009) shows that it is not uncommon to find low but significant repeatability values and our reported values are all still well within the range of repeatabilities reported there. In field studies, many variables are beyond our control that can impact individual behavior, thus, it remains complex to study consistency of behavior. This is highlighted by a recent study on rufous-collared sparrows (*Zonotrichia capensis*) where geographic variation in repeatability of the same traits in different populations of the same species was found (Van Dongen et al. 2010).

Melanin-based coloration and stress

Associations between melanin pigmentation and aggressiveness (Ducrest et al. 2008), courtship behavior (Yeh et al. 2006), glucocorticoid-dependent stress responses (Kittilsen et al. 2009), and antipredator strategies (Venesky and Anthony 2007) have already been observed in several species. A high response to stress could be a way to explain the agitation and aggressiveness against handlers observed among small-spotted barn owls because a previous study has shown that barn owls with larger spots mount a lower corticosterone response to capture-induced stress than those with smaller spots (Almasi et al. 2010). In rainbow trout, individuals with more spots also showed a lower response to stress, both in hormone levels and locomotor response (Kittilsen et al.

2009). There is evidence suggesting that individuals behaving bold when faced with a predator respond more strongly to handling stress (i.e., breathe faster) and explore their environment more intensely (Fucikova et al. 2009). The link between stress response and exploratory behavior proposes an interesting hypothesis that dispersing behavior is related to spot diameter, which could explain why in our barn owl population immigrant females (who have dispersed) have smaller spots than resident females (Roulin and Altwegg 2007). This proposition is currently studied further in our barn owl population.

Selection pressure on stress response

Prolonged exposure to predators, as experienced in areas with high predator pressure, might facilitate habituation, resulting in a decreased response to this stress source, to avoid the costs associated with chronic stress (McEwen and Wingfield 2003). A reduced response to repeated stressors has been demonstrated in a number of taxa including fish (Brown et al. 2005, but see Bell et al. 2010) and rats (Caldji et al. 2000). In our study, we found a reduced response to stressors in the large-spotted individuals, suggesting that predation pressure might have selected for this trait to coevolve with melanin-based coloration. It would therefore be interesting to test whether predation is a major selective force that could account for the observed pronounced worldwide variation in spot size in the barn owl (Roulin et al. 2009; Roulin and Salamin 2010).

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