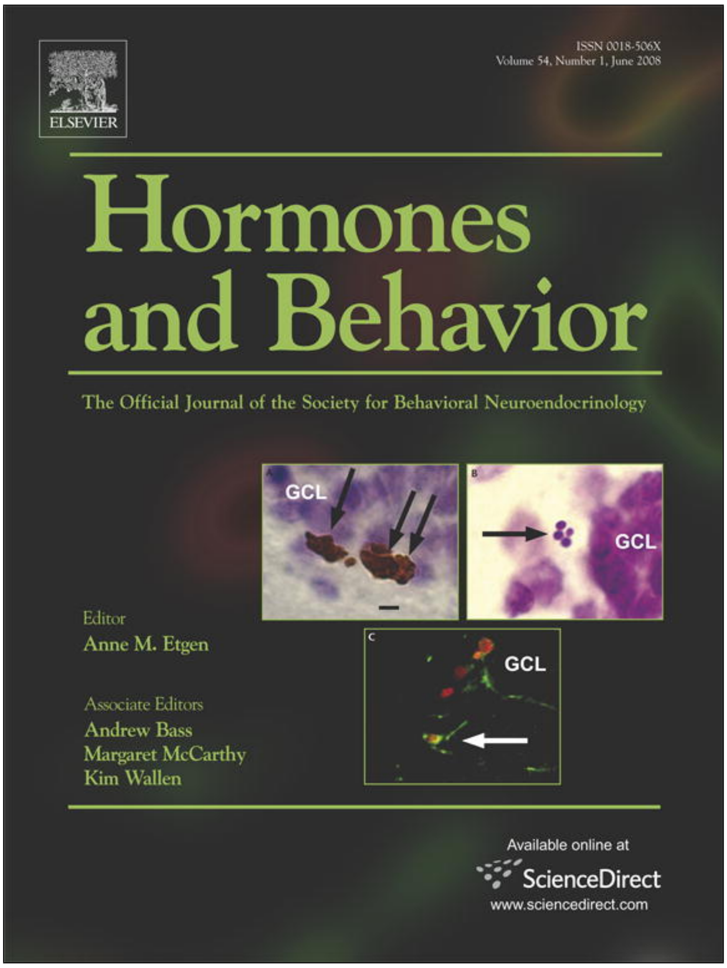

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Parental investment and its sensitivity to corticosterone is linked to melanin-based coloration in barn owls

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ABSTRACT

Behavioral and physiological responses to unpredictable changes in environmental conditions are, in part, mediated by glucocorticoids (corticosterone in birds). In polymorphic species, individuals of the same sex and age display different heritable melanin-based color morphs, associated with physiological and reproductive parameters and possibly alternative strategies to cope with variation in environmental conditions. We examined whether the role of corticosterone in resolving the trade-off between self-maintenance and reproductive activities covaries with the size of melanin-based spots displayed on the ventral body side of male barn owls. Administration of corticosterone to simulate physiological stress in males revealed pronounced changes in their food-provisioning rates to nestlings compared to control males. Corticosterone-treated males with small eumelanic spots reduced nestling provisioning rates as compared to controls, and also to a greater degree than did corticosterone-treated males with large spots. Large-spotted males generally exhibited lower parental provisioning and appear insensitive to exogenous corticosterone suggesting that the size of the black spots on the breast feathers predicts the ability to cope with stressful situations. The reduced provisioning rate of corticosterone-treated males caused a temporary reduction in nestling growth rates but, did not affect fledgling success. This suggests that moderately elevated corticosterone levels are not inhibitory to current reproduction but rather trigger behavioral responses to maximize lifetime reproductive success.

Introduction

The resolution of the trade-off between self-maintenance and reproductive effort is mediated by physiological mechanisms, such as the hypothalamic-pituitary-adrenal (HPA) axis. Environmental perturbations may activate the HPA axis and lead to the release of glucocorticoids, which help mobilize stored energy, redirect behavior to self-maintenance and enhanced restfulness at the expense of reproductive investment (reviewed in Sapolsky et al., 2000). While a short-term release of glucocorticoids is considered beneficial in allowing individuals to overcome stressful situations and reestablish homeostasis (Wingfield and Kitaysky, 2002; McEwen and Wingfield, 2003), chronically elevated levels can entail negative long-term effects (Sapolsky et al., 1986, 2000). Evidence for an adaptive role of corticosterone comes from experimental studies where a short-term elevation of corticosterone level increased survival (Meylan and Clobert, 2005; Cote et al., 2006).

The responsiveness of the HPA axis to environmental perturbation changes seasonally (Schwabl et al., 1980; Romero, 2002) and can vary between individuals (Cockrem and Silverin, 2002). Inter-individual variation in physiological, hormonal and behavioral traits can result not only from variation in condition, but may also be the outcome of selection having favored alternative heritable strategies to cope with unpredictable changes in key environmental factors. Accordingly, individual coping styles can have a genetic component and be associated with consistent differences in endocrine and behavioral responses to stressful situations. For example, aggressive pigs explore a novel object sooner than non-aggressive pigs and show an increase in cortisol levels, whereas in non-aggressive pigs cortisol levels remain low (Hessing et al., 1994). Great tits (*Parus major*) of two different selection lines for coping styles (slow vs. fast explorers) show different levels of corticosterone secretion when confronted with an aggressive resident male (Carere et al., 2003).

Assuming that inter-individual variation in coping styles is heritable, as the above examples suggest, selection may favor the evolution of phenotypic traits that advertise the ability to overcome stressful conditions. This situation may have evolved several times in different color-polymorphic species, whose individuals display alternative heritable morphs that are associated with difference in survival, behavior, physiological, and reproductive parameters (review in Roulin, 2004c). Each color morph can therefore reflect alternative strategies to cope with environmental conditions that fluctuate in space and time. An example for the coexistence of morphs is the tawny owl (*Strix aluco*) in which grey females produce offspring of

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higher quality while reddish-brown females breed more often (Roulin et al., 2003b). Most examples of color polymorphism come from species for which variation in coloration is due to differential deposition of melanin pigments raising the possibility that alternative melanin-based color patterns are associated with different physiological adaptations to cope with stressful environmental factors (Roulin et al., 2008). This is plausible because there is a functional link between the glucocorticoid response to environmental stressors and melanogenesis. Production of melanin pigments is, amongst other, regulated through the melanocortin 1-receptor, its agonists the melanocortins melanin-stimulating-hormones (MSH) and adrenocorticotropic hormone (ACTH) which are post-translational products of the pro-opiomelanocortin (POMC) gene (Slominski et al., 2004). Melanocortins not only regulate melanogenesis, but also other physiological functions including the stress response mainly through ACTH (Slominski et al., 2006).

In the present study, our aim is to investigate whether the role of corticosterone in resolving the trade-off between self-maintenance and reproductive activities covaries with the degree of melanin-based coloration. We chose the barn owl (*Tyto alba*) as study species because individuals from the same population vary in the degree of eumelanin-based coloration, from a complete absence of black spots to being heavily marked with spots and in the degree of phaeomelanin-based coloration from reddish-brown to white. This offers us the opportunity to examine whether an experimentally elevated level of corticosterone affects parental investment differently in alternatively colored individuals. This is plausible because in the barn owl, melanin-based coloration is associated with several behavioral, morphological, and physiological characteristics that have been linked to the ability to cope with stressful factors including heart size (Roulin et al., 2001a), feeding rate of nestlings (Roulin et al., 2001a), foraging method (Roulin, 2004d), recruitment into the population (Roulin and Altwegg, 2007), parasite resistance (Roulin et al., 2001b) and developmental homeostasis (Roulin et al., 2003a).

We tested whether the effect of experimentally elevated corticosterone levels in breeding males, the sex which provides most prey items to the offspring, was color-dependent. Our method of administering corticosterone allowed us to manipulate the mediator of the stress response (i.e. corticosterone), in the absence of stressful environmental factors. Because corticosterone stimulates foraging for self-maintenance (Silverin, 1986; Wingfield and Silverin, 1986; Lohmus et al., 2006) and reduces parental investment (Silverin, 1986; Silverin and Wingfield, 1998; Love et al., 2004), we predicted that corticosterone administration would reduce males' provisioning rates. Based on the hypothesis that the degree of eumelanism (but not of phaeomelanism) signals the ability to cope with stressful factors (Roulin et al., 2008), we also predicted that males displaying smaller black spots reduce provisioning rate following an experimental increase in circulating corticosterone more than males with larger black spots. Finally, we determined how the manipulation of corticosterone levels in breeding males affected the growth of their offspring.

Material and methods

Study organism

The barn owl is a medium-sized predator of small mammals (99% of the diet, Roulin, 2004a). The two to eleven eggs are laid between February and August, and eggs hatch asynchronously on average every second or third day creating a pronounced within-brood age hierarchy. Only females incubate the eggs and thus we can recognize them from males by the presence of a brood patch. Nestling growth rate peaks at 17 days post-hatch and nestlings are heaviest at 40 days post-hatch, from which point they gradually lose weight until fledging at ca. 56 days. Three-week old nestlings can thermoregulate and, hence, are no longer brooded by their mother whose daytime roost is at some distance from the nest. Extra-pair paternity is rare (Roulin et al., 2004). Variation in plumage coloration is already visible in nestlings. Although individuals of the two sexes can express any phenotype, males are, on average, less reddish-brown and display less and smaller black spots than females. All birds become slightly lighter in color when molting body plumage between the first and second year of life (Roulin, 1999a), but a bird that was darker than another individual in its first year is still darker in its second year (Roulin and Dijkstra, 2003). Plumage coloration and spottiness are genetically correlated within both sexes (spottier individuals are darker reddish-brown; Roulin, 2004b) and between the sexes (darker fathers and darker mothers produce darker sons and daughters, spottier fathers and spottier mothers produce spottier sons and daughters; Roulin et al., 2001a).

Experimental manipulation of corticosterone level

The study was carried out in an area of 190 km^2 located at an altitude ranging from 430 to 520 m in western Switzerland (46°49'N, 06°56'E) in 2005. The area is dominated by agriculture and holds a barn owl population of 20–80 pairs breeding in 110 nest boxes put in barns. To experimentally investigate the effect of moderately elevated plasma corticosterone levels on provisioning rate, we captured 41 male barn owls at night when they were feeding their 24±3 days old offspring (mean±SE). Within 3 min of capturing a male, a blood sample was taken to determine the baseline plasma corticosterone level (Romero and Reed, 2005); we were able to blood samples 36 of 41 males within 3 min. Blood samples were taken by puncturing the brachial vein and collected into heparinized capillary tubes. Samples were centrifuged within 15 min of collection and the plasma immediately stored in liquid nitrogen in the field and at −20 °C once in the laboratory in the evening. We determined plasma corticosterone concentrations with an enzyme-immunoassay (Munro and Stabenfeldt, 1984; Munro and Lasley, 1988) after extraction of 5 μl plasma and 195 μl water in 4 ml dichlormethane. All samples were run in triplicates. The dilution of the corticosterone antibody (Chemicon; cross-reactivity: 11-dehydrocorticosterone 0.35%, progesterone 0.004%, 18-OH-DOC 0.01%, cortisol 0.12%, 18-OH-B 0.02% and aldosterone 0.06%) was 1:8000. HRP (1:400,000) linked to corticosterone served as enzyme label and ABTS as substrate. The concentration of corticosterone in plasma samples was calculated by using a standard curve run in duplicates on each plate. Plasma pools from chicken with a low and a high corticosterone concentration were included as internal controls on each plate. Intra-assay variation ranges from 5 to 13% and inter-assay variation from 12 to 21%, depending on the concentration of the internal controls.

After blood sampling, we implanted 21 males with a corticosterone pellet (hereafter cort-male) and 20 others with a placebo pellet (placebo-male). The pellets (diameter 5 mm) are made up of a biodegradable carrier-binder containing 15 mg corticosterone or, for placebo, only of the biodegradable carrier-binder (Innovative Research of America, Sarasota, Florida). We implanted the pellet under the skin of the flank above the knee through a small incision, which was closed with tissue adhesive (Histoacryl, BBraun, Germany). We could not determine the increase in plasma corticosterone levels in the experimental males, because repeated captures would have caused serious disturbance possibly increasing the risk of brood abandonment. However, the company specified that pellets releases corticosterone tonically over a seven-day period in rats. From a previous unpublished study in 2004 with nestling barn owls, which have a similar body mass as breeding males, implantation of the same implants increased circulating corticosterone levels by about 18 ng/ml (plasma baseline corticosterone levels before implantation±SE: 10 ng/ml±1.13, 2 days post-implantation: 28 ng/ml±2.81, 6 days post-implantation: 12 ng/ml±2.16). Following an acute stressor (i.e. handling), barn owls increase plasma corticosterone levels up to 60 ng/ml (B. Almasi, unpublished data) and therefore the effect of the corticosterone implants can be considered as within the physiological range. The Swiss committee for animal research approved the study (animal experiment permit from the 'service vétérinaire du canton de Vaud' no. 1736).

Assessment of plumage traits

In males, we scored breast phaeomelanin-based coloration by comparison with eight color chips from 1 for dark reddish-brown to 8 for white. From a previous study we know, that two measures of coloration taken on a same individual are highly correlated (female: $r_s=0.95$, $p<0.001$, $n=125$ individuals; male: $r_s=0.96$, $p<0.001$, $n=31$), and thus the method of assessing this plumage trait is repeatable (Roulin, 1999b). We also counted black spots located near the tip of feathers within a 60×40 mm area on the breast and measured their diameter to the nearest 0.1.mm. A mean spot diameter value was calculated and used for analysis. The repeatability of measuring number of spots in a recent study was 0.93 in breeding males (one-way ANOVA: $F_{29,36}=26.54$, $p<0.0001$) and 0.89 in breeding females ($F_{194,174}=15.96$, $p<0.0001$), and the repeatability of measuring spot diameter 0.92 in breeding males ($F_{29,36}=24.88$, $p<0.0001$) and 0.84 in breeding females ($F_{91,140}=11.45$, $p<0.0001$) (Roulin, 2004b). The age of twenty breeding males was known precisely as we banded them as nestlings ($n=20$). Unknown aged males were classified as 'yearling' if all primary and secondary feathers belonged to the same generation, and as 'adult' otherwise (Taylor, 1993). In our sample of pairs, male and female partners did not resemble each other with respect to plumage coloration (Pearson correlation: $df=39$, $r=0.05$, $p=0.76$) and spot diameter ($df=39$, $r=0.19$, $p=0.29$). Laying-date was not correlated with male and female plumage traits (p-values>0.09).

Assessment of parental provisioning rate

The night after having implanted males, we started to record provisioning rates during four successive nights using infrared cameras installed outside or inside the nestboxes. The position of the cameras had no effect on male and female provisioning rates (Student's *t*-test: *p*-values$>$0.36). Provisioning frequency of males and females, banded on a different leg to recognize them on the video footage, was defined as the number of prey items brought to the nestbox from 10 p.m. until sunrise. Since prey species differ markedly in body mass, we estimated total prey mass delivered per night. To this end, we identified prey items on video footage either as voles (416 items), woodmice (156) or undetermined (450). Because it was difficult to identify common voles (*Microtus arvalis*) from water voles (*Arvicola terrestris*) from video footage, for each nest we identified prey remains found during daylight hours (in total we found 11±6 prey remains per nest) and calculated the proportion of voles that were common voles (72.7%) and water voles (27.3%). Based on a previous study on prey remains found in barn owl nests, common voles weigh on average 29.1 g, water voles 49.2 g and woodmice 33.5 g (Roulin, 2004c). To obtain an estimate of the amount of grams of prey brought by males and females each night, we used the following formula: *provisioning rate*$=n\times(v\times vm+m\times mm)$, where n is the number of prey items brought to the brood per night (provisioning frequency), v is the percentage of voles brought to the brood per night determined on video footage, vm is the mean vole mass calculated from the proportion of common voles and water voles of prey remains, m is the percentage of woodmice brought to the brood per night determined on video footage, and mm is the mean woodmouse mass. Due to technical failures and nestlings sitting in front of the cameras we obtained provisioning rates from 102 nights in the 41 experimental nests.

Assessment of nestling growth

To investigate the indirect effect of manipulating corticosterone level in breeding males on their offspring, we weighed all 212 nestlings to the nearest 0.1 g the day before males were captured, as well as all surviving nestlings at 6 and 27 days after implantation. For each nestling we calculated mean body-mass gain per day from day 0 to day 6 and from day 6 to day 27. The rank of each nestling in the within-brood age hierarchy was determined around hatching, a time when we clipped off the tip of one claw to recognize each individual until being ringed with an aluminium ring at ca. two weeks of age. There were no differences between the corticosterone and placebo treatments in mean hatching date, brood size on the first day of the experiment, mean nestling age, male and female plumage traits (Student's *t*-tests: all *p*-values$>$0.13), and male and female age (Pearson's Chi-squared test: *p*-values$>$0.58).

Statistical procedure

To test the hypothesis that individual variation in provisioning rate of cort- and placebo-male barn owls is linked to plumage traits, we performed a repeated mixed-effect model analysis. We included into the model male provisioning rate as the dependent variable, male identity as a random factor to control for pseudo-replication, the three categorical variables treatment (corticosterone vs. placebo), night (nights 1, 2, 3 and 4 post-implantation) and male age (yearling vs. adult), and the four covariates date, male body condition (male body mass divided by cubic wing length), phaeomelanin-based coloration and spot diameter. Since spot diameter and number of spots correlated significantly (Pearson correlation: $n=41$, $r=0.774$, $p<0.001$), we considered only spot diameter; we chose spot diameter instead of number of spots because this trait is more often associated with other phenotypic traits than number of spots (Roulin, 2004a). The best models were selected based on Aikaike's Information Criterion for small sample size (AICc) (Akaike, 1974; Burnham and Anderson, 2002), where the model with the lowest AICc-value is the most parsimonious. To avoid having an unreasonably large number of models, we first built models with the variables male age, night, number of nestlings, date and male treatment and their biologically relevant interactions ('night×treatment', 'male age×treatment', 'male body condition×treatment'). Models with a ΔAICc <2 compared to the model with the lowest AICc were selected to be expanded in the second step by including male plumage traits (color score and spot diameter). All models with a ΔAICc<2 were chosen for model averaging to estimate the predicted means and their SE. For model averaging we calculated Akaike model weights (ωi) (Anderson et al., 2000) which indicate the probability that a given model is the best among the whole set of candidate models. Weights of models sum up to 1 by definition. The model with the highest weight is considered as the best. Predicted parameters are multiplied by the weight of the particular model and summed over all selected models to give the weighted average of the predicted parameters (Burnham and Anderson, 2002). We calculated standard errors (SE) of the predicted values with the bootstrap method using 5000 iterations (Crawley, 2006). We also used model averaging to obtain model-averaged effect sizes for main effects without interactions and their SE.

To test for a potential effect of male treatment (cort- vs. placebo-implanted males) on offspring, we applied analogous repeated mixed-effect models with offspring body-mass gain as the dependent variable. We first included male treatment, growth period (0–6 days, 6–27 days after the start of the experiment), and nestling rank in the within-brood age hierarchy as categorical variables, and date and number of nestlings in the brood as covariates. In a second step, we expanded the best models with male and nestling phaeomelanin-based coloration and spot diameter. We included site and nestling identity nested in site as random factors to account for repeated measurements of the same nestling. The averaged predicted parameters were calculated as described above.

Results

Individual variation in baseline corticosterone

Baseline levels of circulating corticosterone before treatment did not differ between cort- (11.12 ng ml^{-1}±0.9) and placebo-males (14.4 ng ml^{-1}±1; Student's t-test: $t=-1.542$, df$=33$, $p=0.133$) and was not correlated with male phaeomelanin-based coloration (Pearson correlation: $n=36$, $r=0.136$, $p=0.465$) or spot diameter ($n=36$, $r=-0.396$, $p=0.695$).

Effect of male corticosterone treatment on provisioning rate

Model selection revealed two best models to explain variation in the effect of male corticosterone treatment on male provision rate. The first model included night, male treatment, age, body condition, and spot diameter as well as the interactions 'male spot diameter×male treatment' and 'male spot diameter×male age'. The second best model in addition included the interaction 'male treatment×male body condition' (Table 1 A). Corticosterone treatment reduced male provisioning rate with cort-males feeding on average 68 g of prey mass per night less than placebo-males (mean prey mass per night±S.E. 281 g±16 versus 349 g±21). Males with smaller spots brought generally more food, but were more affected by the corticosterone treatment than males with larger spots (interaction male spot diameter by male treatment). Cort-males with small spots reduced their provisioning rate to the level of placebo-males displaying large spots (Fig.1 C and D). Age alone and in interaction with male spot diameter had a small positive effect, which was more pronounced in larger-spotted males, i.e. young larger-spotted males fed less than older males whereas the provisioning

Table 1

Model selection of repeated mixed-effect models to explain variation in male and female provisioning rates in relation to male corticosterone treatment (t), male spot diameter (md), male age (a), male body condition (c), female age (fa), number of nestlings (cl), night (n) and date (d)

	Model	Variables	LogL	K	AICc	ΔAICc	ωi
A. Males	**1**	**c+a+n+t+md+md*t+md*a**	**−623.64**	**12**	**1274.78**	**0.00**	**0.34**
	2	**c+a+n+t+md+c*t+md*t+md*a**	**−622.43**	**13**	**1274.99**	**0.21**	**0.31**
	3	c+a+n+t+md+md*a	−625.92	11	1276.78	2.00	0.13
	4	c+a+n+t+md+c*t+md*t	−624.93	12	1277.37	2.59	0.09
	5	c+a+n+t+md+c*t+md*a	−625.12	12	1277.75	2.97	0.08
B. Females	**1**	**d**	**−617.71**	**4**	**1243.84**	**0.00**	**0.39**
	2	**fa+d**	**−617.44**	**5**	**1245.50**	**1.66**	**0.17**
	3	fa+d+t	−616.91	6	1246.71	2.87	0.09
	4	cl	−619.38	4	1247.16	3.32	0.07
	5	fa+cl+t	−617.27	6	1247.42	3.58	0.06

Male identity was introduced as a random factor.

Log-Likelihood (LogL), number of estimated parameters (K), Akaike's information criterion (AICc), difference of AICc to the best model (ΔAICc) and Aikaike's weight (ωi) from the 5 best models are reported.

This model selection is based on the measurement of number of the feedings per night in 41 pairs over 102 nights. The best models (ΔAICc<2) are in bold.

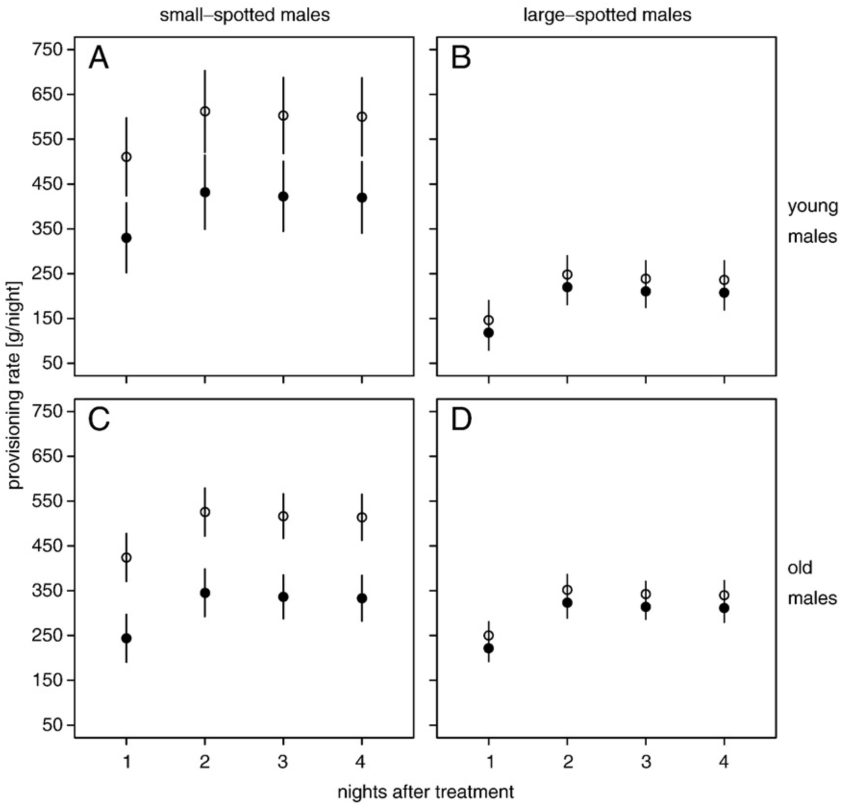

Fig. 1. Model-averaged predicted male provisioning rate in g night^{-1} (±SE) after treatment of (A) first year males with small spots, (B) first year males with large spots, (C) males older than 1 year with small spots and (D) males older than 1 year with large spots. The continous variable spot diameter ranged from 0 to 2.1 mm. For 'small-spotted males' spot diameter 0.1 mm was entered into the model and spot diameter 1.3 mm for 'large-spotted males'. Open symbols represent placebo-implanted males, closed symbols corticosterone-implanted males, respectively. These figures are based on the number of feedings per night in 41 pairs over 102 nights. There are significant differences in the provisioning rates of untreated small-spotted and large-spotted males. Cort-treated small-spotted males decreased their provisioning rates significantly compared to large-spotted males.

rate of young and old smaller-spotted males was the same. Body condition had a small positive effect on provisioning rate and the interaction of body condition and male treatment was only included in the second best model.

Model selection showed that females did not compensate for the decrease in provisioning rate of cort-males (Table 1 B). The two best models revealed that female provisioning rate increased slightly with date (model-averaged effect size±SE: 1.982 gd^{-1}±0.847) and decreased with female age (effect size±SE: −32 g±44).

Effect of male corticosterone treatment on nestling growth and survival

Overall, male provisioning rates correlated significantly with body-mass gain of the entire brood during the time of the male stress challenge (growth period 0 to 6, Pearson's correlation: r=0.501, n=41, p=0.002). The reduced food-provisioning rate by cort-males during the first 6 days resulted in a reduced body-mass growth rate (2.98 gd^{-1}±0.76 per nestling raised by cort-males versus 7.16 gd^{-1}±0.70 per nestling raised by placebo-males; Table 2). From day 6 to day 27 growth rate was lower than during the first 6 days and there was no difference in body-mass gain between nestlings raised by cort- and placebo-males.

Post-hoc, we examined whether male plumage traits were associated with the ability of offspring to cope with the food restriction caused by a lower provisioning rate of cort-males. Model selection yielded three best models with similar AICc and Aikaike's weights. All models included male treatment, nestling rank in the within-brood age hierarchy, and growth period as explanatory variables. The two best models also included male phaeomelanin-based coloration and the interaction of male phaeomelanin-based coloration with treatment (Table 2). The reduction in body-mass gain during the first 6 days post treatment in nestlings raised by cort-males was more pronounced when the father was reddish-brown (Fig. 2A) than when he was whitish (Fig. 2B). Nestling phaeomelanin-based coloration and spot diameter as well as father spot diameter were not

Table 2
Model selection of repeated mixed-effect models to explain variation in body-mass gain in relation to father corticosterone treatment (t), father phaeomelanin-based coloration (mc), father spot diameter (md), rank (r), date (d) and growth period (v)

	Model	Variables	LogL	K	AICc	Δ AICc	ωi
Body-mass gain	**1**	**d+r+t+v+v*t+mc+mc*t+mc*v**	**−1202.24**	**17**	**2440.12**	**0.00**	**0.35**
	2	**r+t+v+v*t+mc+mc*t+mc*v**	**−1203.97**	**16**	**2441.39**	**1.28**	**0.18**
	3	**d+r+t+v+v*t**	**−1206.40**	**14**	**2441.90**	**1.79**	**0.14**
	4	d+r+t+v+v*t+mc+mc*t	−1204.84	16	2443.13	3.02	0.08
	5	d+r+t+v+v*t+md	−1206.00	15	2443.27	3.15	0.07

Site and nestling identity nested in site were introduced as random factors. Log-Likelihood (LogL), number of estimated parameters (K), Akaike's information criterion (AICc), difference of AICc to the best model (ΔAICc) and Aikaike's weight (ωi) from the 5 best models are reported.
This model selection is based on the 392 measures of body-mass gain in 212 nestlings. The best models (ΔAICc<2) are in bold.

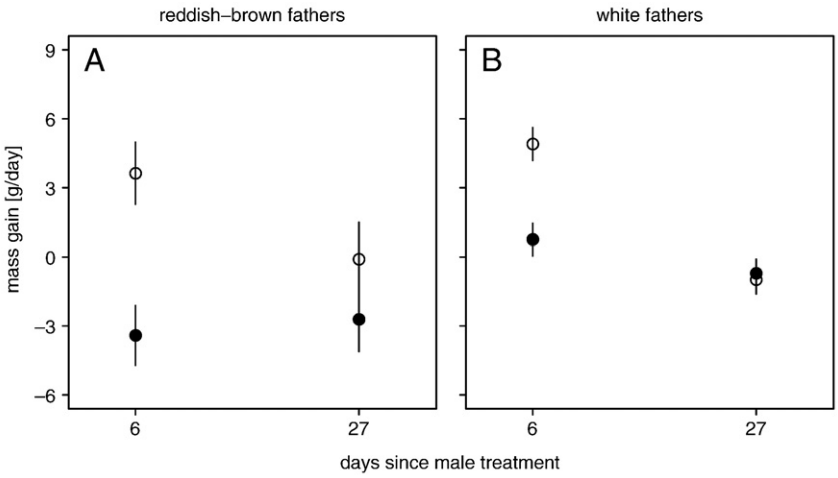

Fig. 2. Model-averaged predicted body-mass gain of nestlings in g day^{-1} (±SE) over the first 6 days after father treatment and from day 6 to day 27 after father treatment. The continous variable father phaeomelanin-based color which ranged from 1 (dark reddish brown) to 8 (white) is set in (A) at 3.6 (reddish-brown) and (B) at 7 (white). Open symbols represent nestlings of placebo-implanted fathers, closed symbols nestlings of corticosterone-implanted fathers, respectively. These figures are based on the 392 measures of body-mass gain in 212 nestlings. From day 0 to day 6 of the experiment body-mass gain of nestlings of cort-treated reddish-brown fathers was lower than body-mass gain of nestlings of cort-treated white fathers, whereas body-mass gain of nestlings from different morphs of untreated fathers did not differ. There was no difference between the groups from day 6 to day 27.

included in the best models. Note that food-provisioning rate did not differ between males of different color (Table 1 A), although it clearly varied with spot size (see above).

Only four out of 212 nestlings died (1.9%) during the first four days after having implanted a corticosterone-releasing pellet in males precluding any analysis of an immediate effect of male corticosterone treatment on nestling survival. Between the start of the experiment and fledging a total of 30 nestlings died out of 212 (14.2%), but these mortality events were not associated with male treatment (Pearson's Chi-squared test: $\chi^2=0.232$, df = 1, $p=0.630$). Thus, cort- and placebo-males produced a similar number of fledglings (4.6 fledglings ± 0.2 of cort-males versus 4.9 fledglings ± 0.3 of placebo-males; Student's t-tests: $t=-0.658$, df = 39, p-values = 0.514).

Discussion

In this study, we demonstrated that the administration of corticosterone to breeding males decreased their provisioning rates, and that their mates did not compensate for it. The depression in provisioning rates in the first night after treatment in all males may be due to the implantation procedure or more likely due to the attachment of radio-tags, which were used for another study. The effect of exogenous corticosterone on provisioning rates was more pronounced in small-spotted males, with large-spotted males showing not only a minimal response to corticosterone but an overall lower level of provisioning. The reduced provisioning rate of corticosterone-treated males entailed a temporarily reduced growth rate in their nestlings, that was consistent with the estimated life-span of the implants, but no reduction in breeding success. Nestlings may cope differently with a reduced food supply depending on their fathers' phaeomelanin-based coloration.

Effect of corticosterone on provisioning rate

An effect of corticosterone on reproductive effort has been previously reported. As in barn owls, a moderate administration of corticosterone in male and female pied flycatchers (*Ficedula hyploeuca*) reduced feeding frequency and a higher dose provoked the abandonment of the brood (Silverin, 1986). In contrast, in black-legged kittiwakes (*Rissa tridactyla*) corticosterone-treated parents (either male or female) spent more time away from the brood without decreasing feeding rate (Kitaysky et al., 2001). Thus, corticosterone administration may differentially affect behaviors across species and, therefore, one might expect different species to react differently to environmental perturbations. We suggest that an increase in corticosterone level alters the most plastic behaviors of parental investment. In the kittiwake with only two chicks in need of guarding in an open nest, a modulation of time-budget seems to be used as a buffer against environmental variability (Angelier et al., 2007a), rather than a reduction of the feeding frequency. In contrast, the barn owl and the pied flycatcher have a variable number of nestlings (mostly 5–8) in a protected cavity in no need of guarding and parents are continuously in search for food. At least in barn owls, environmental variability is mainly charged to the brood and hardly buffered by the parents, because the parents are already working at their sustainable maximum (e.g. Roulin et al., 1999). Thus, when corticosterone is changing the trade-off between reproductive effort and self-maintenance in favor of the latter, kittiwakes reduce guarding, exposing the chicks to a higher risk of predation, while barn owls and pied flycatchers reduce feeding frequency, thus exposing the chicks to a higher risk of starvation. We do not know whether male barn owls increased foraging for their own need after corticosterone administration, but findings in the pied flycatcher (less body-mass loss during the feeding period in corticosterone-treated birds; Silverin, 1986), the black-legged kittiwake (increased time spent away from the brood; Kitaysky et al., 2001) and the wandering albatross (*Diomedea exulans*) (corticosterone levels correlate positively with daily distance traveled during a foraging trip; Angelier et al., 2007b) suggest an increase in foraging activity of these birds and a higher investment in self-maintenance at the expense of parental investment. This conclusion is consistent with the observation that prolactin levels, a hormone that regulates nest bond and parental behavior (Cherel et al., 1994; Buntin, 1996; Criscuolo et al., 2005; Chastel et al., 2005; Criscuolo et al., 2006), decrease when corticosterone levels increase in birds (Cherel et al., 1994; Criscuolo et al., 2005; Chastel et al., 2005; Criscuolo et al., 2006).

Under natural conditions, food shortage (possibly due to bad weather) and the presence of predators are the most common unpredictable environmental factors leading to a raise in corticosterone. When environmental conditions deteriorate, the costs to raise a brood successfully increases and, consequently, parents should invest more in their own condition and survival and, thus, in future reproduction. When corticosterone levels increase and reach a certain threshold the brood will most probably be abandoned (Silverin, 1986). If corticosterone levels are only moderately elevated (and below this certain threshold), elevated corticosterone levels may not be inhibitory to current reproduction, but rather trigger behavioral responses to maximize lifetime reproductive success of the parents.

Melanin-based plumage coloration and coping with increased corticosterone levels

Our study is among the few that focused on individual variation to a physiological stress response (Carere et al., 2001; Pfeffer et al., 2002; Carere et al., 2003; Wada et al., in press). We showed that there is pronounced difference in parental investment between different phenotypes and further that there are pronounced differences in how birds with different phenotypes cope with physiological stress (in our study simulated by administering corticosterone). The untreated smaller-spotted males invested more into reproduction, but when corticosterone levels increased, they were more stress-sensitive, than the larger-spotted males with a lower parental investment and a lower susceptibility to stress.

Baseline circulating corticosterone levels were not related to male melanin-based traits and the associated parental investment while a short-term increase in corticosterone produced differential behavioral responses in differently colored individuals. We do not know whether baseline corticosterone levels mediate parental investment in different morphs, but a behavioral response might not only be due to circulating levels of corticosterone but also depend on receptor type and receptor density, which can be different between the morphs.

Our study showed that individual differences in coping with stress are signaled with a phenotypic trait, here plumage eumelanin-based coloration. The less stress-sensitive males displayed larger black spots than the more stress-sensitive males. We obtained similar results in barn owl nestlings in that more eumelanic nestlings and nestlings with more eumelanic mothers showed lower plasma corticosterone levels after corticosterone administration (B. Almasi, in preparation), suggesting a stronger negative feedback mechanism or clearance rate of these individuals. The same mechanism might explain the better stress-resistance of darker eumelanic males. In a similar line, nestlings raised by foster parents developed more symmetrical feathers of the left and right wings when their biological mother displayed larger black spots (Roulin et al., 2003a). Finally, under stressful rearing conditions nestling Alpine swifts (*Apus melba*) grew more rapidly only if their biological father was darker eumelanic (Bize et al., 2006). These various studies suggest that the degree of eumelanin-based coloration is associated with the ability to cope with stressful situations.

That less eumelanic birds are more stress-sensitive agrees with the hypothesis of a functional link between melanogenesis and melanocortins, including ACTH (Racca et al., 2005; A.L. Ducrest, L. Keller, A. Roulin, submitted). Such a functional link may be most operational during feather growth (i.e. in chicks and during molt in adults), but may reflect a more general individual variation in physiology. In the males of this study, molt of remiges regularly started during the period of chick feeding.

The link between stress-sensitivity and plumage coloration may contribute to the maintenance of genetic variation in eumelanin-based coloration in this population. In favorable environmental conditions more stress-sensitive, less eumelanic individuals may have a higher reproductive output or chicks of better quality through their higher parental investment, while in sub-optimal environmental conditions less stress-sensitive darker eumelanic individuals may attain a higher fitness, because they may abandon their brood less easily and because they may be genetically predisposed to resist stressful environmental situations. In this study, phaeomelanin-based coloration seems not to be involved in signaling the ability of an individual to cope with stress, a finding that is consistent with a recent study (Roulin et al., 2008).

Effect of increased corticosterone levels in fathers on nestling growth

Administration of corticosterone to free-living male barn owls decreased provisioning rates to their brood by about 70 g per night (i.e. the mass of 2.5 voles per night), which was not compensated for by the female. Not surprisingly, this food restriction resulted in a reduced body-mass gain in the nestlings compared with control broods.

The provisioning rate of fathers did not vary with the fathers' phaeomelanin-based coloration. However, when the fathers were stressed, fathers displaying more reddish-brown coloration produced offspring, which gained less body mass than offspring of whiter fathers. (Table 2). This is the result of a correlative post-hoc analysis and lacks experimental rigor (e.g. controlling food supply to the nestlings and cross-fostering nestlings to allocate genotypes randomly among rearing environments), but it points to two phenomena that lead to interesting further questions.

First, not eumelanin-based coloration, the trait, which correlated with stress-sensitivity of the fathers, but phaeomelanin-based coloration correlates with body-mass gain of the nestlings under food restriction. Thus, different plumage characters may signal different aspects of stress-sensitivity. Furthermore, the heritability of stress-sensitivity may be linked in a complex way with plumage characteristics and possibly other characteristics of the bird. This calls for a comprehensive recording of plumage characters in further studies.

Second, because the provisioning rate of both placebo- and cort-males did not vary with phaeomelanin-based coloration, variation in food supply cannot explain the variation in body-mass gain of chicks with cort-fathers. Different body-mass gain, when food supply is similar, may result from differences in energy-allocation between nestlings of more or less phaeomelanic fathers. For instance, under food restriction chicks of phaeomelanic fathers may allocate more energy into tissue maturation or immune function while chicks of white fathers try to maintain body-mass gain as best as they can. Reducing metabolic rate may be another means of maximizing growth.

These two observations call for further studies to elucidate (1) how stress coping styles are signaled in various plumage traits and (2) whether fathers pass on genes resulting in different strategies to cope with food restriction in chicks, which, however, are not detected in the chicks' coloration (there is no direct effect of nestling phaeomelanin-based coloration on their body-mass gain).

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