ORIGINAL ARTICLE

Melanin-based plumage coloration is associated with exposure in tawny owls under novel conditions

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Abstract

Intraspecific color variation is often associated with camouflage or protection, but melanin-based color variation is also linked to behavioral and physiological aspects including boldness. In the melanin-based plumage color polymorphic tawny owl (*Strix aluco*), the grey morph is known to be more cryptic than the brown morph. Using 19 captive tawny owls (11 grey and 8 brown), we tested if these two color morphs that differ in camouflage tend to differently use exposed perches in a familiar environment (home aviary) and a novel environment (experimental aviary), as well as whether their response to predation risk and mobbing cues differs. The two color morphs did not differ in their exposure under known conditions (in their home aviary), but brown tawny owls were more likely to use exposed perches than grey tawny owls after release in a novel environment.

Significance statement

Melanin-based coloration can be associated with several behavioral traits. However, it is still unknown how predators with genetically-based color polymorphism vary in their efficiency of active background choice and risk-prone behavior while facing predation risk or mobbing harassment. Using captive tawny owls, we investigated predators' behavior (attention towards the stimulus and use of space) while confronted to mobbing events and predation risk. We showed that tawny owls were using their space differently according to their color morph in a novel environment (experimental aviary) but not in a familiar environment (home aviary).

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Introduction

Camouflage is arguably among one of the most important functions of coloration since it is the potential for an individual to match the background of its environment. Animal camouflage is an example of natural selection (Kettlewell 1955) and is defined as a morphological adaptation including all kinds of concealment (Stevens and Merilaita 2011) consisting of strategies preventing detection. Camouflage includes different types of approaches to avoid being detected such as background matching, which occurs when certain patterns resemble the background, thus reducing the risk of detection (Stevens and Merilaita 2011). Both prey and predators may use a background matching strategy. Prey can benefit from background matching to escape predation. For example, tadpoles of *B. alvarengay*, when exposed to a threat, will move to

backgrounds similar to themselves in terms of lightness and color (Eterovick et al. 2010). Predators can also benefit from background matching in order to be invisible to their prey, for example, by matching the coloration of the substrate where the prey is foraging on, like in predatory crab spiders (Heiling et al. 2005).

Coloration is also very often genetically correlated with a suite of other traits including body size, life-history characters, morphology, physiology, and behavior (Roulin 2004; McKinnon and Pierotti 2010). In particular, melanin-based pigmentation, creating a variation in coloration from non-pigmented (pale) to black (eumelanin) or reddish brown (phaeomelanin) is associated with behavior and other traits via the melanocortin system (Ducrest et al. 2008). This association has been found in many organisms such as lions (*Panthera leo*), barn owls (*Tyto alba*), white-tailed deer (*Odocoileus virginianus*), and vipers (*Vipera aspis*) (Ducrest et al. 2008). Thus, individuals of different color may differ in their capacity to camouflage (depending on the background) but may also be genetically pre-disposed to differ behaviorally, e.g., in terms of exposure propensity.

For a predator, lower detectability not only allows higher hunting success but also allows avoiding harassment such as mobbing by potential prey. Mobbing is an antipredator behavior conducted by potential prey in order to make the predator leave the area (Curio 1978). Predators try to avoid mobbing (Flasskamp 1994). In the Australian powerful owls (*Ninox strenua*), persistent mobbing forces individuals to change roost sites and select roosting habitats where mobbing occurs less frequently (Pettifor 1990; Pavey and Smyth 1998). Being harassed and forced to move induces additional energy expenditure and may have negative effects on predators' hunting success as well as increasing their own predation risk by alerting top predators.

We here study the use of exposed vs. camouflaged perches, and behavioral response to the risk of predation and mobbing in captive tawny owls (*Strix aluco*), a nocturnal raptor. This species shows a melanin-based polymorphism in plumage color independent of age and sex that can be characterized into two different morphs: grey and brown (Brommer et al. 2005). This highly heritable plumage color polymorphism is based on different levels of pheomelanin deposition in the feathers, whereas the levels of eumelanin in the feathers are similar (Piault et al. 2009). The grey tawny owl morph is more cryptic than the brown morph for the human eye and also based on a computational avian vision model (Koskenpato et al. 2020). This difference in camouflage could affect the propensity of individuals of different morphs to use camouflage. However, apart from its effect on camouflage, melanin deposition is also positively associated with aggressive and bold behavior (Ducrest et al. 2008). In tawny owls, the exposure of an individual's roosting site determined the probability to be detected by

mobbing birds (Sunde et al. 2003), but to date, no study has assessed whether there are morph-specific differences in detection probability. Here, we use 19 captive tawny owls of both color morphs to study whether tawny owl color morphs behave differently in terms of (1) roost choice in a familiar environment (home aviary), (2) roost choice in a novel environment (experimental aviary), and (3) their behavioral reaction to simulated mobbing and/or predation risk.

We expect that both morphs would try to camouflage when being exposed to predation risk and mobbing cues. On the other hand, when released in a novel environment, we expect the brown morph to be more prone to roost in the outer part of the experimental aviary due to the pheomelanin association with aggressive behavior (Ducrest et al. 2008; Da Silva et al. 2013a) and thus boldness. Similarly, we expect that in a familiar environment (i.e., home aviary), the brown tawny owl morph may chooses a more exposed perch. Lastly, we predicted that owls of both morphs would react to cues of predation risk and mobbing.

Material and methods

Experimental owls

The experiment was conducted in January 2021 on 19 adult captive tawny owls (11 grey (3 females and 8 males) and 8 brown morphs (3 females and 5 males)). The owls were taken to captivity from their nest boxes just before fledging at the age of 23–27 days in spring 2020. Each owl came from a different nest. The owls were kept in a purpose-built aviary with separate identical aviary compartments (3 m × 3 m × 6 m, hereafter "home aviary") at Lund University in Sweden (see below "roost site selection in home aviary" for the description of the aviary). At the beginning of the experiments, they were aged from 266 to 288 days.

Roost site selection in home aviary

To detect owls' space use in their familiar environment, when undisturbed by human presence, we observed owl roosting habits by recording pellets' position in the aviary. Owls regurgitate indigestible prey remains via the pellets (i.e., bones, teeth; Errington 1930; Glue 1970), usually while roosting. To identify the preferred perch for roosting, we collected owl pellets in the aviaries during summer 2020, from the 13th of June to the 13th of August when the owls were still in their growing phase. Each week, two observers collected pellets. For 5 min, we were looking for pellets in the inner and outer part of the aviary. All the home aviaries looked the same in terms of availability of perches in inner or outer area, but the disposition of perches differed from the

experimental aviary. The location of the pellets inside the aviary was recorded. We used the number of pellets under a perch as a measure of the time the owl spent on that perch.

The open field test

The experimental aviary (Fig. 1, SI Picture 1) was created as an open field test (Dingemanse et al. 2004; Brown and Braithwaite 2004; Carere et al. 2005; Dall and Griffith 2014; Toscano et al. 2016; Perals et al. 2017; Yuen et al. 2017). The aviary was composed of two parts, one covered by a roof and the other one without a roof. The outer, exposed area had fences of metal wire on both sides and above and was thus more exposed to both predators and mobbers as well as climate (SI Picture 2). The inner part of the experimental aviary was composed of two different camouflage backgrounds made with camouflage nets painted in grey and brown colors, respectively. There were three perches in the experimental aviary: one with a grey camouflage background, one with a brown camouflage background, and a perch in an exposed zone without background (Fig. 1, SI Picture 2). The door was located in the upper right corner of the scheme (Fig. 1) and was always covered by the camouflage background so that the owl could not perceive the
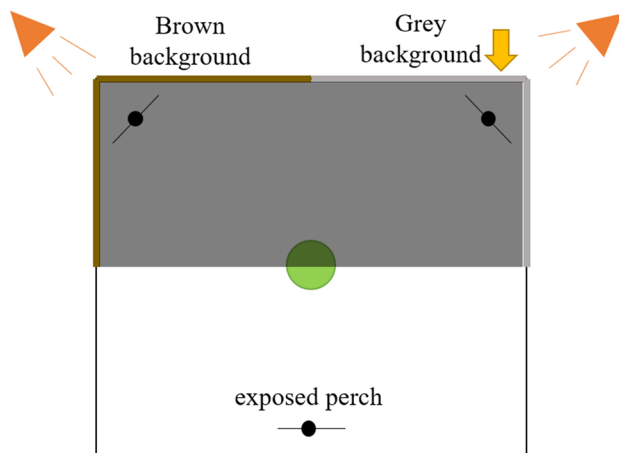
door when inside the experimental aviary. The backgrounds were switched after each trial so that in every trial each color background was on the opposite side than in the previous trial. The speakers were placed in the inner corridor, just behind the fence of the aviary, and at the same height as the perches inside. Two speakers were used, one behind each background perch. There was no speaker next to the exposed perch.

For the open field tests, the owl was released into the aviary from the door (yellow arrow, Fig. 1). One trial was divided into two distinct parts. First, space use was measured during the first 4 min after the owl entered the aviary. The observer (CP), hidden from owl sight, recorded perch use and any switching between perches by the owl. The data were not recorded blind because the observer could see the color of the owl and backgrounds. The exposed perch was considered in the outer part of the aviary, and the two background perches were considered the inner part of the aviary.

Second, in the remaining 5 min and 40 s of the trial, a series of alternating cues of only playback or playback and visual cues combined were performed, each lasting 20 s. Each owl was tested 14 times in total, and the order in which each owl was tested during separate trials was randomized. During the first five trials, only playback cues (mobbing, predator, and control) were presented to the owl. The last nine trials were composed of a combination of playback and visual cues presented simultaneously (mobbing, predator and control) (see Table 1). The visual cue was pulled down at the same level as the owl's eyes when perched in the experimental aviary when the playback was on and pulled up against the ceiling when the playback was stopping. The owls could still see the visual cues up to the ceiling but flattened. All the playbacks (for the three treatment groups: mobbing, predator, and control) were made using sounds from the website xeno-canto (https://www.xeno-canto.org/). For mobbing, a mix of blue tits (*Cyanistes caeruleus*), great tits (*Parus major*), willow tits (*Poecile montanus*), and trushes (Turdus spp.) alarm calls from Europe were used, since these are among the most common passerines in Northern Europe, where the study was performed. The soundtracks chosen for the mix had for each species 3 or more individuals calling. We created only one final soundtrack to be played for all the trials. The playback part, which in total lasted 5 min and 40 s, included nine sessions of 20-s playbacks separated by

Fig. 1 Scheme of the experimental aviary, seen from above. The black lines with a dot represent perches, the orange devices represent speakers. The inner part of the aviary is represented by a darker color. The position of the door is represented by a yellow arrow. The green circle is representing where the visual cues were presented to the owls

Table 1 The different trials of the experiment and how many times they have been conducted with the different cues (–, no observation). Values presented in "total" columns are highlighted in bold font

	Mobbing		Predator		Control		**Total**	
							Right	**Left**
Side of the aviary where the brown background is	Right	Left	Right	Left	Right	Left		
Playback only	1 time	1 time	1 time	1 time	1 time	–	**3 times**	**2 times**
Visual cue + playback	2 times	2 times	2 times	2 times	–	1 time	**4 times**	**5 times**

20 s of silence in between each playback. The predator playback was made using northern goshawks (*Accipiter gentilis*) alarm playbacks, while the control playback was made using geese (*Anser anser*) alarm playbacks. Playback sessions lasted 20 s alternated with 20 s of silence as in the mobbing treatment. In total, one trial was composed of nine playback sessions.

The visual cues (mobbing passerines cue, predator cue, and control cue) were shapes hanging from the ceiling by a transparent string at a similar distance from each perch in the experimental aviary (Fig. 2, SI Picture 3). The mobbing passerine cue consisted of four origami birds colored as blue tits and great tits; the predator cue was a shape of a black bird of prey, and the control cue was two circle shapes embedded together, one all black and one blue and yellow like the mobbing birds.

We repeated the same treatment once for each owl. In the repeat, we switched background colors between the left and right side (i.e., one time grey background on the left side, one time brown background on the left side).

Sessions were not conducted when weather (i.e., heavy snow, heavy rain, heavy wind) or when sounds in the environment might have interfered with presentation of visual and auditory cues. The behavior of the tawny owls (i.e., roosting site, number of switches between the perches, time spent on each perch) were both recorded with a camera and observed directly behind a fence outside of the experimental aviary where the owl could not see the observer. We defined a reaction to the cue to occur when the owl oriented its head towards the speakers and/or the visual cue. The playback and visual cue were simultaneously presented to the owls nine times in one trial. Each time, the observer recorded if the owl oriented its head towards the speaker and/or the visual cue, or not. We established a variable explaining the reaction of the owls to the cues: the "probability to react" out of the 9 stimuli takes into consideration how many times the owl responded to the stimuli during one trial (ie 0 or 1 reaction level, 9 repetition per trial). The probability to react was then plotted as a percentage. Roosting site was recorded during the whole experiment. The experimental aviary can be perceived as an open field test, with a closed area and an open area (Brown and Braithwaite 2004; Dingemanse et al. 2004; Carere et al. 2005; Dall and Griffith 2014; Toscano et al. 2016; Perals et al. 2017; Yuen et al. 2017). At the end of each trial, the observer approached the owl from the outer corridor next to the exposed perch to record its reaction (change perches: yes or no, choice of background perch if changing perches).

Statistical analyses

For all the models, we used generalized linear mixed-effects models (GLMM), using the function "glmer"

implemented in package "lme4" (Bates et al. 2015) in R (R Development team 2019). Individual ID was consistently included as a random factor and we considered morph (scored as a binary variable: G = grey, B = brown) as a fixed factor. For all the models involving behavior in the experimental aviary, we included the fixed factors: the number of previous trials to control for the habituation of the aviary, sex, time of the day (morning or afternoon), and disturbance (binomial variable, i.e., wind, people talking nearby, truck passing by the nearest road) because they could affect the behavior of the owls. First, the efficiency of active background choice was analyzed as a binomial GLMM such as the use of the matching background corresponding to the owl color morph (1) versus the non-matching background or the exposed perch (0). The exposed perch data was combined in this analysis with the non-matching background choices since a lot of owls were choosing this perch (119 cases out of 266), making the analyses stronger when keeping those observations. The same data were used to study the binomial use of space in the experimental aviary, such as the use of the outer part of the experimental aviary (exposed perch, 1) versus the inner part of the experimental aviary (either grey or brown background, 0). The reaction level of the owls to mobbing cue, predator cue, and control cue was analyzed as a binomial GLMM. The type of playback (mobbing, predation, or control) was considered a fixed factor, to test if the owls would react differently. We also controlled for the presence of a visual cue (binomial variable) by including it as a fixed factor. Change of perch was not included since owls usually did not change roosting site after playback started, see results.
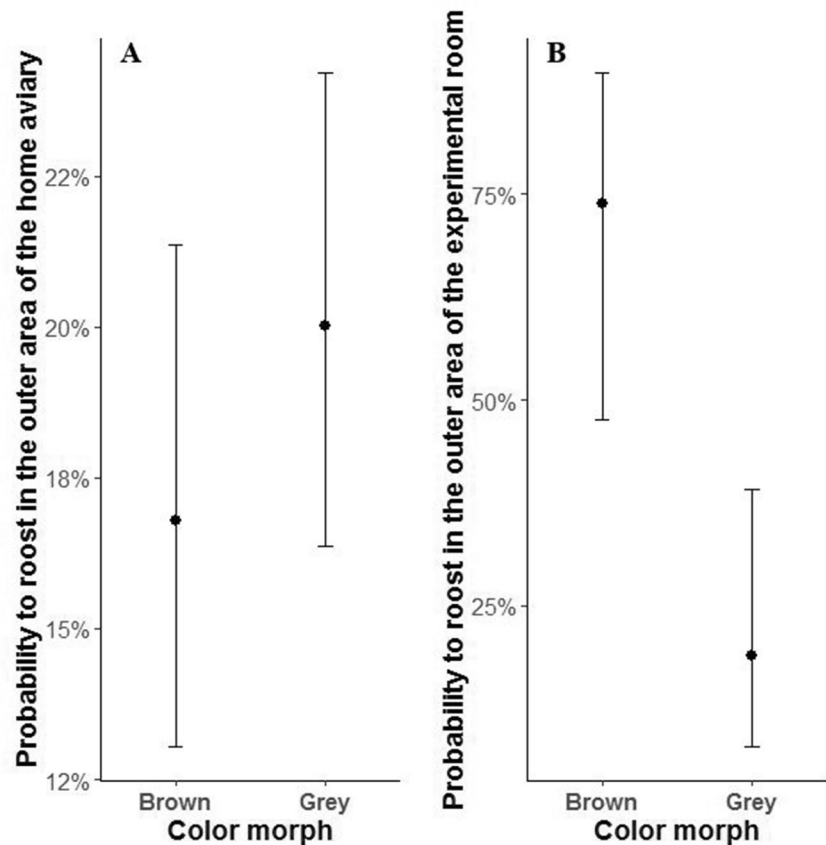
The proportion of pellets found in the outer part of the aviary (open, no roof) compared to the inner part (hidden, with roof) was modelled as a binomial GLMM. The proportion of pellets found in the outer part of the aviary (i.e., under the outer perch) compared to the inner part of the aviary was calculated using the function cbind (Bates et al. 2015) in R. To control for the weather effect, we included the number of rainy days during the week before the collection as a fixed factor. We also corrected for the impact of sex and the collection week as fixed factors since pellets were collected during owls' growth.

Results

Roost site selection in home aviary

We estimated the weekly roost site selection in the home aviaries of the owls as the number of pellets found under each of them. The proportion of pellets found under the outer area compared to inner area did not differ between the two morphs nor between males and females (Fig. 2A, Table 2).

Fig. 2 A Least squares means with 95% confidence intervals of the probability to roost in the outer area of the home aviary, according to color morph (brown or grey). **B** Least squares means with 95% confidence intervals of the probability to roost in the outer area of the experimental aviary, according to color morph (brown or grey)

The proportion of pellets found under the outer perch was lower when there were more rainy days during the week preceding the pellet collection (Fig. 2A, Table 2).

The open field test

Roost site selection in experimental aviary

When released in the experimental aviary, and thus in a novel environment, all the owls from all trials flew to the outer metal net fence before settling on a perch to roost. The probability that they chose to roost in the outer part of the experimental aviary depended strongly on the color morph of the tawny owl. Brown tawny owls had a higher probability to use the outer area of the experimental aviary than the grey tawny owls (Fig. 2B, Table 3). The owl usually chose its roosting perch within the 4 min of habituation time and did not change roosting site after the playback started (except in 19 cases out of 262).

Table 2 Generalized linear mixed model analyzing the proportion of pellets found under the outer perch compared to the inner perches ($n=19$)

	Variables	Estimate $\pm$ SE	Df	Chisq	*P*
Proportion of pellets found under the outer perch compared to inner perches	Morph (grey)	0.21 ± 0.20	1	1.18	0.28
	Week	-0.16 ± 0.03	*1*	*34.34*	*< 0.0001*
	Number of rainy days during the week before collection	-0.1 ± 0.05	1	0.26	0.07
	Sex (male)	-0.1 ± 0.2	1	3.21	0.61

The model includes fixed terms concerning individual traits such as the morph (grey compared to brown) and sex (male compared to female). The model includes environmental information such as the number of rainy days during the week before collection and the number of the collection week (from 1 to 8). The ID of the individual is considered a random factor. Significant fixed terms are indicated in bold font

Table 3 Generalized linear mixed model analyzing the probability to roost in the outer area of the experimental aviary ($n = 19$)

	Variables	Estimate $\pm$ SE	Df	Chisq	P
Probability to roost in the outer area of the experimental aviary	**Morph (grey)**	**-2.49 ± 0.76**	**1**	**10.63**	**< 0.01**
	Disturbance (yes)	-0.36 ± 0.36	1	1.01	0.31
	Time of the day (morning)	0.16 ± 0.33	1	0.23	0.63
	Number of previous trials	0.03 ± 0.04	1	0.63	0.43
	Sex (male)	0.3 ± 0.83	1	0.13	0.72

The model includes fixed terms concerning individual traits such as the morph (grey compared to brown) and sex (male compared to female). It also included environmental information such as the time of the day (morning compared to afternoon), the number of previous trials (from 0 to 13), and disturbance (yes compared to no). The ID of the individual is considered a random factor. Significant fixed terms are indicated in bold font

Efficiency of active background choice

Grey tawny owls were more likely to select the perch with a matching background compared to brown tawny owls (binomial GLM: estimate $\pm$ SE $= 1.57 \pm 0.47$, df $= 1$, Chisq $= 11.26$, $P < 0.001$). However, this difference in background matching choice is largely explained by brown tawny owls using more the exposed perch (without a camouflage background) compared to grey tawny owls (Tables 3 and 4). Nevertheless, while grey tawny owls were using the three perches approximately randomly in equal proportions, the brown morph used the perch with the matching brown background the least frequent (< 10%; Table 4). At the end of each trial, the observer approached the experimental aviary from the direction of the exposed perch and was visible to the owl. Neither morph, however, showed any tendency to background match when moving from the outer to an inner perch (background matching percentages; grey $= 48.5\%$, brown $= 38.9\%$).

Reaction to mobbing or predation risk

The tawny owls reacted clearly to both predators and mobbers, and the reactions were stronger when a visual cue was also present (Fig. 3, Table 5). The reaction level did not differ between color morphs (Fig. 3, Table 5). Reaction levels decreased over successive trials, and were stronger when there was a disturbance, but did not vary along the time of day (Table 5).

Table 4 Percentages of both owl color morphs (brown or grey) on each perch available in the experimental aviary (perch with brown background, perch with a grey background and exposed perch without background) ($n = 19$)

	Brown	Grey
Brown background	9.6%	40.1%
Grey background	21.9%	32.9%
Exposed perch	68.5%	27.0%
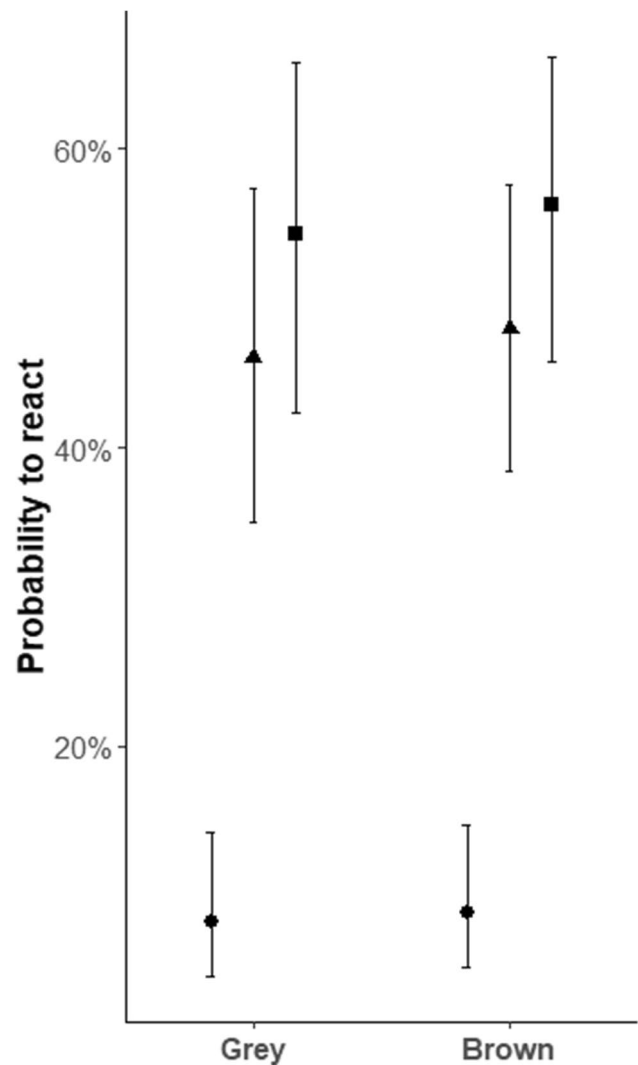
Fig. 3 Least squares means with 95% confidence intervals of the probability to react of the tawny owl, according to their color morph and the type of cue presented (mobbing, predation, or control) during experiment 1. Control is represented in dots, mobbing is represented in triangles, and predation is represented in squares. The number of trials was 14

Table 5 Generalized linear mixed model analyzing the reaction level of tawny owls ($n = 19$)

	Variables	Estimate $\pm$ SE	Df	Chisq	P
Reaction level to the cues	Morph (grey)	0.07 $\pm$ 0.28	1	0.06	0.80
	Type of cue (predator)	**2.72 $\pm$ 0.25**	**2**	**121.35**	**< 0.001**
	Type of cue (mobbing)	**2.39 $\pm$ 0.25**	**2**	**121.35**	**< 0.001**
	Number of previous trials	**−0.10 $\pm$ 0.03**	**1**	**13.11**	**< 0.001**
	Disturbance (yes)	**0.34 $\pm$ 0.12**	**1**	**7.50**	**< 0.01**
	Visual cue (yes)	**1.13 $\pm$ 0.21**	**1**	**27.87**	**< 0.001**
	Time of the day (morning)	0.18 $\pm$ 0.12	1	2.47	0.12

The model includes fixed terms concerning individual traits such as the morph (grey compared to brown) as well as experimental pieces of information such as the presence of a visual cue (yes compared to no) and the type of cue presented (predator compared to control and mobbing compared to control). It also included environmental information such as the time of the day (morning compared to afternoon), the number of previous trials (from 0 to 13), and disturbance (yes compared to no). The ID of the individual is considered a random factor. Significant fixed terms are indicated in bold font

Discussion

To summarize, neither morph showed evidence of background matching after release in an experimental aviary, and brown tawny owls often perched in the outer, exposed area. In their home aviaries, both morphs perched mainly in the non-exposed part of the aviary. The two morphs responded similarly to simulated mobbing and predation risk in the experimental aviary.

In general, wild animals can improve their camouflage abilities through active background choice (Stevens et al. 2017). Nevertheless, our findings here underline that under novel conditions both morphs rapidly chose to perch independently on the camouflage background and did not move during cues presentation. Camouflage is yet important for nocturnal predators like tawny owls, as it would allow them to rest during the day without being detected by mobbers or predators. Koskenpato et al. (2020) showed that human observers detect brown tawny owls with a higher probability and faster than grey tawny owls. However, the tawny owl brown morph does not behaviorally compensate for its poorer camouflage by using camouflaged perches.

We find that the two tawny owl morphs do not differ in space use when they reside in known conditions, in their home aviaries, while they do so in novel environments. Taken together, our work thus suggests that space use difference between the two morphs only occurs in a novel, presumably stressful environment. Indeed, our findings suggest brown tawny owls chose to roost on the exposed part of the experimental aviary. In nature, exposure can be a disadvantage since it leads to an increase in encounters with mobbers or predators, with diurnal predators being responsible of 73% of owls' mortality (Sunde et al. 2003). In the wild, being more prone to expose could also be correlated to being bolder in general and thus be an advantage to protect territories, explore, and disperse more. However, there is no difference between the two morphs on dispersal patterns, neither at natal dispersal (Passarotto et al. 2022) nor in breeding dispersal (authors unpublished). Moreover, Galeotti and Cesaris (1996) did not find that tawny owl color morphs would differ in their habitat choice which is unexpected with our results showing that both morphs are using different types of habitat in a novel environment. It has also been shown that the two color morphs display different behaviors such as aggression (Da Silva et al. 2013) and hunting strategies (Karell et al. 2021). In the latter, they showed that brown tawny owls are more generalists than grey tawny owls in years with low mammalian prey abundance. This diet difference could be a beneficial strategy to cope with unpredictable environmental changes such as low prey abundance. Using open habitats could present an ecological advantage when hunting, and it already has been shown that polymorphic species are more prone to live in open/closed habitats than intermediate habitats (Passarotto et al. 2018). Hence, in this system, the color morphs differ in several aspects of behavior, and our current finding shows that exposure differs between morphs. Such morph-specific exposure can be associated with the distribution of morphs and intraspecific competition for resources, which all are expected according to the theory of the evolution and maintenance of color polymorphism (reviewed in Roulin 2004).

Our findings are based on tawny owls that were taken into captivity from their nest and hence have little experience of mobbing harassment or predators. Before the experiment, the tawny owls' experience of predation risk and mobbing was restricted to raptors flying over and small birds coming into their home aviaries. In general, naïve raptors react significantly less than experienced ones to mobbing sounds (Consla and Mumme 2012). Raptors may therefore have to learn where to perch to escape mobbing. This camouflage learning also occurs in nature because tawny owls choose less exposed places to roost after encountering goshawks, *Accipiter gentilis* (Sunde et al. 2003). Nevertheless, we find that tawny owls react

to predation risk and mobbing, in our setup simulating predation risk and mobbing. The behavioral responses also did not differ between the color morphs. From that perspective, our findings indicate that young brown tawny owls may have higher costs of predation and mobbing due to a lack of camouflage. Mortality in young tawny owls leaving the nest is very high (Sunde 2005), with 36% of young dying within 55 days after leaving the nest, predation from mammalian and avian predators being the most important cause of death (Overskaug et al. 1999), and a poor ability to camouflage when naïve may therefore have consequences for morph-specific juvenile survival. Nevertheless, little is known about morph-specific post-fledging survival. Brown offspring is bigger at fledging (which could give a benefit in terms of survival), but recruitment in the following years is not dependent on morph (Morosinotto et al. 2020). Of particular interest is therefore to what extent the two tawny owl color morphs are differentially detectable by wild birds (mobbers or predators) and how they actively react to those stressors in the wild.

Any methodological biases are important to consider when using open field tests (Brown and Braithwaite 2004; Dingemanse et al. 2004; Carere et al. 2005; Dall and Griffith 2014; Toscano et al. 2016; Perals et al. 2017; Yuen et al. 2017). For instance, in our design, the position of the speakers could have an impact on owls' choice of perch. However, in our experiment, all owls were settled after approximatively 2 min of habituation in the experimental aviary, which was way before the audial treatment was started. We observed only few cases (19 out of 262) when the owl changed roosting perch after the playback started meaning that the position of the speakers did not impact their choice. It also seems unlikely that the position of the releasing door could have influenced owls' roosting decision. This is because the door was always covered by a camouflage net during the experiment so that the owl could not see the door once being in the experimental aviary. At the end of each trial, the observer approached the experimental aviary from the direction of the exposed perch and was visible to the owl. The owls that perched at the outer perch always fled to an inner perch. In that sense, while both inner and outer parts could be potentially stressful for the owls, the outer one should have been perceived as even more stressful since the owl could actually see the observer approaching. Moreover, in cases when the owls were perching in the outer exposed part of the home aviaries (similar in its structure to the exposed area in experimental aviary), the owls performed antipredator behavior (elongated cryptic pose) when facing birds of prey flying above the aviaries (authors' personal observations). We therefore interpret that brown owls are likely bolder, and not shier, than grey owls when choosing to perch in the exposed part of the aviary.

Our experimental aviary is designed as an open field test, with an exposed area and a closed area. We find that brown tawny owls perch more in the exposed area of the aviary. Boldness can be defined as the willingness of individuals to engage in risky behavior (Ward et al. 2004). Boldness is often tested using behavioral tests such as the novel object experiment or an open field test (Brown and Braithwaite 2004; Dingemanse et al. 2004; Carere et al. 2005; Dall and Griffith 2014; Toscano et al. 2016; Perals et al. 2017; Yuen et al. 2017). In the open field test, risk-taking is measured by the amount of time an individual spends out in the open (i.e., away from the protected inner area with walls of the open field). Choosing to roost in the exposed area of the aviary strongly suggests choosing the riskier choice of perch. A review of over 40 animal species concluded that melanism is in general associated with aggressive and bold behavior (Ducrest et al. 2008). In tawny owls, the association between pigmentation and boldness in the wild is partly supported by breeding adult females guarding the nest site when offspring is big. Darker colored (brown) tawny owl females are indeed more likely to be present at the nest when a human intruder approaches compared to lighter colored (grey) females in the late nestling period (Da Silva et al. 2013), although Brommer et al. (2014) did not find such an association while studying nest defense during incubation. Future studies on color polymorphic differences in stress response and coping strategies would however be needed to understand the mechanisms behind the behaviors observed. Based on our present results, we would predict that the two color morphs have similar baseline levels of corticosterone, as suggested by overall similar behavior in a familiar environment, but may differ in their induced levels of corticosterone when facing novelty or stress.

Declarations

Ethical approval The ethical permits for the aviary work have been approved by the Swedish Board for animal experiments (permit number 5.8.18–06007/2019). Everyone working with the owls has passed the required ethical course in laboratory animal science and Swedish legislation, ethics, and animal at Lund University. All applicable international, national, and/or institutional guidelines for the use of animals were followed.

Conflict of interests The authors declare no competing interests.

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