Differential fitness effects of moonlight on plumage colour morphs in barn owls

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Abstract

The Moon cycle exposes nocturnal life to variation in environmental light. However, whether moonlight shapes the fitness of nocturnal species with distinct colour variants remains unknown. Combining long-term monitoring, high-resolution GPS tracking, and experiments on prey, we show that barn owls (*Tyto alba*) with distinct plumage colourations are differently affected by moonlight. The reddest owls are less successful hunting and providing food to their offspring during moonlit nights, which associates with lower body mass and survival of the youngest nestlings and with female mates starting to lay eggs at low moonlight levels. Although moonlight should make white owls more conspicuous to prey, hunting and fitness of the whitest owls are positively or un-affected by moonlight. We experimentally show that, under full-moon conditions, white plumages trigger longer freezing times in the prey, which should facilitate prey catchability. We propose that the barn owl's white plumage, a rare trait among nocturnal predators, exploits the known aversion of rodents to bright light, explaining why, counterintuitively, moonlight impacts less the whitest owls. Our study provides evidence for the long-suspected influence of the Moon

on the evolution of colouration in nocturnal species, highlighting the importance of colour in nocturnal ecosystems.

Introduction

Colouration largely determines how animals interact with their biotic and abiotic environment[1]. Perception of an individual's colouration by conspecifics, predators, or prey depends upon the reflective properties of an individual's colour, its background, the viewer's visual system, and the environmental light[2,3]. The latter component may shape the evolution of colouration, with heterogeneous light conditions favouring distinct colourations as shown, for instance, in African cichlid fish living at different depths[4,5] or in birds exploiting the canopy and the understorey of tropical forests, inhabiting less *vs.* more cloudy environments, or nesting in the open or in dark cavities[6–8]. However, most of the studies linking variation in environmental light and colouration come from diurnal species and the consequences that variation in nocturnal light has for the evolution of animal colouration are barely known[9].

The Moon has shadowed the evolution of life, which adapts its endogenous rhythms to the lunar cycle[10–14]. Moonlight alters the activity patterns of animals[15–18] as it alters an individual's capacity to visually detect food or to remain concealed[19–22]. By producing contrasting changes in light conditions[23], the Moon might also drive the evolution of colouration in nocturnal animals, but this hypothesis has received little attention despite being proposed more than a hundred years ago[24]. In addition to the difficulty of observing behaviour in nocturnal species[25], some authors suggested that our limited night vision has "clouded" our expectations of the importance of colour and light variation for nocturnal species[26]. This could explain why colouration of nocturnal species has often been considered as an adaptation for diurnal camouflage rather than for a nocturnal life[27].

In line with the accumulation of studies highlighting the importance of colour vision in nocturnal species[23,28,29], a few recent studies have indirectly addressed how nocturnal light variation relates to animal colouration. Cuttlefish, *Sepia apama,* actively adapt their colour patterns to their background not only during the day but also during the night[25,30]. Eagle owls, *Bubo bubo*, call more often during full-moon nights when their white throat patches, a potential visual signal[31], seem to be more conspicuous[32]. Colour polymorphism is more common in owls living in light-heterogeneous habitats formed by both forested and open landscapes[33]. Variation in nocturnal light levels might thus act as a selective agent on animal colouration. However, evidence supporting that moonlight variation affects the fitness of individuals according to their colouration is still lacking.

Combining data from a breeding population monitored over the last 20 years and high-resolution GPS tracking, we investigated how moonlight affects foraging, as well as breeding success and timing in barn owls (*Tyto alba*), whose ventral plumage genetically varies from white to dark red[34,35] (Fig. 1). To identify the underlying mechanism behind colour-specific performance in barn owls, we experimentally investigated the antipredator response of the barn owls' main prey, the common vole (*Microtus arvalis*), exposed to white and red owls under different moonlight conditions. The adaptive role of the red and white plumage of barn owls remains unknown, although previous studies discarded colour

variation as neutral[36,37] and suggest a role in predator-prey interactions[38,39]. Because light variation might affect the ability of prey to visually detect predators[10], we predict that moonlight influences owl hunting efficiency and, thereby, breeding success and timing. Rodent prey are likely to perceive different owl plumages as different shades of grey (*i.e.*, as differences in luminance), with the less reflective plumage of red owls appearing darker than a white plumage[40,41]. As other vertebrates with duplex retinae, rodent prey are likely to rely on the sensitive yet colour-blind rod vision[28]. Even if some rodent species may have up to two cone types[42] and may see colours, chromatic vision in addition to luminance should make red owls appear less conspicuous than white owls. Thus, we expect that barn owls should be more conspicuous during full-moon nights and exhibit a lower foraging success. This negative effect of moonlight should be stronger in white than in red owls because a white plumage is expected to reflect light more efficiently. We expect smaller differences during new-moon nights because dark noise limitation in dim light is likely to result in less contrasted differences between red and white owls[28].

Results

Effect of plumage colouration and moonlight on food provisioning and hunting success

Using infrared cameras, we first investigated whether parental colour and moonlight (measured as the visible percentage of the Moon; see Methods and Supplementary Fig. 1) affect food provisioning (the total number of prey that adults brought to their offspring each night). On average, food provisioning was 4.78 prey per night ± 1.22 standard error (s.e.) and was significantly associated with moonlight in interaction with parental colour (Poisson-GLMM: $z = -2.33$, $P = 0.02$; Supplementary Table 1; Fig. 2a). The food provisioning of the reddest parents decreased from new-moon (5.67 prey ± 1.21) to full-moon nights (3.27 ± 1.25; $z = -3.72$, $P < 0.001$). There was no significant relationship between food provisioning and moonlight within the whitest parents ($z = -0.81$, $P = 0.42$), who brought 4.94 ± 1.21 and 4.61 ± 1.22 prey during new- and full-moon nights, respectively.

Hunting success measured in males equipped with GPS trackers significantly depended on the interaction between plumage colouration, moonlight, and hunting effort (Supplementary Table 2). We observed no effect of moonlight and plumage coloration on hunting success when owls performed a below-the-mean hunting effort (< 26 hunting events per night, Binomial-GLMM: $z = 0.54$, $P = 0.588$). When the owls' effort was above the mean, hunting success in the reddest owls decreased from 0.48 ± 0.3 s.e. at new-moon nights to 0.42 ± 0.2 at full-moon nights (plumage colouration × moonlight: $z = -2.28$, $P = 0.023$, contrast within the reddest owls: $z = -2.34$, $P = 0.019$, Fig. 2b). No significant effect of moonlight was detected within the whitest owls ($z = 1.45$, $P = 0.147$, Fig. 2c). Owls do not adjust their hunting effort to moonlight or plumage colouration but moonlight affected at which time of the night the owls hunted (Supplementary Table 3, Supplementary Fig. 2).

Effect of plumage colouration and moonlight on prey anti-predator behaviour

To investigate why full- and new-moon light conditions have different effects on the parental food provisioning and hunting success of white and red owls, we experimentally investigated how common voles (the staple prey in our owl population[38]) detect and react (by either

freezing or fleeing[43,44]) to red and white naturally mounted barn owls under light conditions mimicking full- and new-moon nights (see Methods). Regardless of owl colouration, the voles responded to the owls with a probability of 0.49 ± 0.07 s.e. under the full-moon conditions and with a significantly smaller probability of 0.20 ± 0.05 under the new-moon conditions (binomial-GLMM: moonlight: $\chi^2 = 31.36$, d.f. = 1, $P < 0.0001$, owl morph: $\chi^2 = 0.03$, d.f. = 1, $P = 0.862$, interaction: $\chi^2 = 0.58$, d.f. = 1, $P = 0.447$, Supplementary Table 4, Fig. 3a).

Voles responded to the owls mainly by freezing (83% of the trials). The time they spent frozen was on average 9.5 s ± 1.3 s.e. and significantly depended on the owl morph in interaction with moonlight (LMM: $t_{41.78} = 2.02$, $P = 0.049$, Supplementary Table 4). Under full-moon conditions, voles froze for 5.15 s ± 1.6 longer when facing a white owl than a red owl ($t_{47.46} = 2.42$, $P = 0.039$) and voles froze for 9.6 s ± 2.0 longer when facing a white owl under full-moon compared to new-moon conditions ($t_{47.41} = 3.59$, $P = 0.003$, Fig. 3b). No significant differences were found between white and red owls under new-moon conditions ($t_{39.50} = -1.10$, $P = 0.368$) or in the response to red owls under full- and new-moon conditions ($t_{56.26} = 0.85$, $P = 0.424$).

To confirm that the increased freezing times observed were caused by the amount of light reflected from a white plumage during full-moon conditions, we experimentally tested the prediction that voles should spend less time frozen after facing a white owl of reduced plumage reflectance. To test this, we masked the plumage reflectance of one mounted white owl by applying duck preen wax (CDC) on the feathers (see Methods, Fig. 3c). Under full-moon conditions, decreased plumage reflectance resulted in significantly shorter freezing times in voles (13.6 s ± 1.2 s.e.) compared to the effect of an untreated white colouration (26.4 s ± 1.2, LMM, moonlight $\chi^2_1 = 5.56$, $P = 0.018$, CDC treatment: $\chi^2_2 = 21.69$, $P < 0.001$, interaction: $\chi^2_2 = 21.24$, $P < 0.001$, contrast under full-moon conditions: $t_{24.05} = 4.66$, $P < 0.001$; Fig. 3d). No differences were found under new-moon conditions ($t_{6.21} = 1.35$, $P = 0.223$).

Effect of moonlight and parental colouration on nestling mass and survival

We investigated the potential fitness consequences of the effect of moonlight and plumage colouration by testing whether offspring body mass and fledging success reflect the effects observed in food provisioning. The offspring body mass depended on the moonlight in interaction with the father's colour (LMM: $\chi^2_1 = 4.49$, $P = 0.034$; Supplementary Table 5). Consistent with the patterns of food provisioning, the offspring mass decreased from new-moon (224.1 g ± 2.2 s.e., estimated at the mean nestling age [30 days]) to full-moon nights (220.7 g ± 2.3) in nests raised by the reddest fathers ($\chi^2_1 = 4.49$, $P = 0.04$, Figure 4a-b). The body mass of owlets raised by the whitest males was not significantly different from new- to full-moon nights (228.0 g ± 1.5 and 228.8 g ± 1.5, respectively, $\chi^2_1 = 2.23$, $P = 0.13$). Thus, the major differences between colour morphs occurred during full-moon nights (6.6 g ± 1.0, $\chi^2_1 = 5.37$, $P = 0.021$) but not during new-moon nights (2.3 g ± 1.0, $\chi^2_1 = 0.36$, $P = 0.55$).

We observed no effect of the moonlight and father plumage colour on fledging success (binomial-GLMM: $\chi^2_1 = 0.04$, $P = 0.52$, Supplementary Table 6). However, due to a marked age hierarchy (rank) among barn owl siblings, with first-born (high rank) nestlings

exhibiting highest survival probability (above 75%; Supplementary Fig. 3a), we expect the youngest nestlings to be more affected by reduced food provisioning. When only low-ranking offspring (rank ≥ 7) were considered, fledging success depended on the moonlight in interaction with father colouration (quasibinomial GLMM: t_{62} = -2.58, P = 0.012, Supplementary Table 7). From new- to full-moon nights, fledging success increased in nestlings raised by the whitest parents (from 0.35 ± 0.2 s.e. to 0.95 ± 0.1, t_{62} = 3.03, P = 0.008), while it tended to decrease in nestlings raised by the reddest parents (from 0.61 ± 0.3 to 0.14 ± 0.1, t_{62} = -1.97, P = 0.071; Figure 4c-d). When accounting for cloud cover, which can mask moonlight effects (see Methods), the contrast remained significant for the whitest parents (t_{61} = 3.65, P = 0.001) and became significant for the reddest parents (t_{55} = -2.09, P = 0.040) (Supplementary Table 8).

Association between plumage colouration and moonlight and breeding

We observed a significant negative association between male colouration and the moonlight levels the night a male's mate laid the first egg (z = -2.87 P = 0.004, Supplementary Table 9a). Females that mate with the whitest males had a higher probability of laying the first egg of their clutch (0.58 ± 0.02 s.e.) during nights with at least 50% of the Moon's surface illuminated, whereas females mating with the reddest males had a higher probability of laying the first egg (0.62 ± 0.06) when less than 50% of the Moon's surface was illuminated (Fig. 5). Given the Moon cycle of *ca.* 29 days, we can expect similar negative associations between moonlight and plumage colouration at other moments of the barn owl's breeding cycle: more relevantly, when the first in-nest copulations are expected to occur (~27 days before laying the first egg[45], z = -2.27 P = 0.023), and when nestlings of rank 6 to 8 are expected to reach an age of 15 days old (between 59 and 63 days, *ca.* two moon cycles, after a female laid the first egg: z = -2.61 P = 0.009, Supplementary Table 9b,c).

Discussion

Our study shows that the Moon differently affects the hunting performance and the reproductive success and timing of barn owls with contrasting plumage colourations. This supports the long-standing, un-tested hypothesis that moonlight influences colouration of nocturnal animals[9,24,30], particularly by uncovering a link between fitness proxies, moonlight and colouration that was missing in previous studies[25,32]. Moreover, our study raises the possibility that the unique white colouration of barn owls might be favoured by moonlight, owing to the effect that the light being reflected from white plumages has on the prey's behaviour.

The reddest owls show diminished food provisioning and hunting success towards full-moon nights (Fig. 2a-c). Lower hunting success and food provisioning of the reddest owls during full-moon nights can be explained by the higher probability of voles to detect owls at full-moon conditions (Fig. 3a). The effect of the moonlight on hunting performance has a mirroring effect on reproductive success. In owlets raised by the reddest owls, body mass decreases from new- to full-moon nights, in line with the reddest parents bringing more prey during new-moon nights than during full-moon nights. Consequently, the survival prospects of nestlings raised by the reddest parents were lower when maximal nestling growth occurs

during full-moon nights (when owlets receive less food and weighed less). Survival impairment was only evident in the youngest chicks (age rank   7), likely because their smaller size makes them more vulnerable than their older siblings[46].

Contrarily, food provisioning, hunting success, and offspring body mass in the whitest owls is less or not affected by moonlight (Fig. 2, Fig. 4a-b). The whitest owls may actually perform better during full-moon nights as suggested by the survival of their youngest nestlings raised being positively related to moonlight (Fig. 4c-d). In our population, the diet of both white and red owls is dominated by common voles but white owls consume wood mice, *Apodemus spp.*, more frequently than red owls[38]. However, differences in the diet associated with colouration are unlikely to drive the observed effects because both *Apodemus* and *Microtus* show moon avoidance behaviours[47–49].

Contrary to our expectations, plumage colouration did not affect the probability that voles detect an owl (Fig. 3a). This suggests that white owls do not pay a higher cost of detectability than red owls (note that differences might still exist and have gone undetected in our study). However, there may be a benefit to being white under full-moon conditions, when a white plumage induces longer freezing times in rodents (Fig. 3b). Bright light is an aversive stimulus for rodents[50–53] and even small amounts of light (between 10^{-3} and 10^{-2} cm m^{-2} of luminance, *i.e.*, below the luminance of a full-moon night[23]) are aversive at least in rats[54]. In fact, light is often used in neurosciences to trigger freezing behaviour and study the mechanisms of fear[55]. Given this, it is possible to interpret longer freezing times in voles attacked by a white owl as the result of a greater aversion to the light reflected by a white plumage. This is further supported by the lower freezing times that voles showed after masking the plumage reflectance of a white owl (Fig. 3c-d). This experiment showed that the amount of light reflected from the plumage was the factor influencing voles' freezing times.

Inducing longer freezing times in the prey can be adaptive for barn owls because a barn owl's hunting success substantially increases when a prey stays immobile (up to 100% in laboratory conditions[43,44]). By exploiting sensory biases in the prey[56], white owls might enhance their hunting success during full-moon nights, explaining why moonlight had a smaller or no effect on food provisioning and hunting success of the whitest owls in comparison to the reddest owls. Whether the freezing response of prey species others than common voles are also affected by the white barn owl plumage still needs to be assessed. Light aversion has been observed in mice of the genus *Mus* and in rats (*Rattus norvegicus*), rendering likely that other prey species common in the barn owl's diet, such as mice of the genus *Apodemus* (~20% of the barn owl's diet[38]), are also aversive to light and might respond to white and red plumages differently. Evidence supporting that males make a larger hunting effort than females (this study) and are selected to have more immaculate plumages[57,58] is also suggestive that a white colouration might have evolved by enhancing male hunting capacity.

Given the effect of moonlight, we would expect red males to be rare in our population. However, a white colouration may incur costs that avoid white males to become more frequent. A white plumage might compromise camouflage during the daytime, particularly

against harassing competitors, such as carrion crows, *Corvus corone*. There may also be added benefits of displaying a redder plumage, particularly in harsh conditions when a higher melanin feather content may increase protection against feather abrasion, humidity, and/or cold temperatures[59]. Thus, the hunting-related benefits of a white plumage may trade off against survival to some extent. In this case, we expect a white plumage to be less frequent in owls exerting lower hunting efforts such as females and fledglings, which is in line with their, on average, redder plumage colouration[34,60,61]. We can then expect different selective agents inducing balancing selection on adult male colouration, which might maintain colour variation in this species, perhaps in combination with ontogenetic conflict within males and sexual antagonistic selection.

Small white patches are common among nocturnal species[9]. We predict that, as observed here, their ecological and evolutionary significance will be better understood when considering how their fitness effects change with varying moonlight levels. A question that remains open is whether selection exerted by moonlight is sufficiently strong to induce an evolutionary change in colouration. Here, we observed that moonlight acts on the total number of fledglings produced by males raising broods of at least seven owlets. This comprises 26.7% of the broods per year (Supplementary Fig. 3b), ranging from 8.3 to up to 48.5% depending on the year[62]. Thus, even though the fitness effect of the Moon is restricted to some individuals, it may affect a substantial part of the population and with particular strength in some years. Once they have fledged, the recruitment of nestlings in the local breeding population is not related to their rank in the brood (binomial GLMM: $\chi^2_1 = 0.37$, P = 0.54, mean recruitment: 15.73% $\pm$ 2.54), supporting that the differences in the breeding success of red and white males generated by moonlight may persist after nestlings fledge and have evolutionary consequences. Nevertheless, we cannot yet discard that the high juvenile mortality (the major fitness component in our population[62]) finally hinders any evolutionary response to moonlight. Thus, studies integrating the effects that the Moon has inside and outside the breeding season are still needed to understand better the evolutionary consequences of moonlight.

We observed that the Moon also influences breeding timing in barn owls of different plumage colouration (Fig. 5), suggesting that owls adjust their phenology to the Moon cycle. As observed in other colour polymorphic raptors[63], this effect is in line with owls having evolved mechanisms that minimize the negative impact of varying light conditions on their offspring. Thus, by laying their first egg during nights of lower moonlight levels, the period of maximal growth in the youngest nestlings of the reddest males will also occur during nights with low moonlight levels, which may help the reddest males to avoid the observed negative effects of high moonlight levels. Moonlight might also influence breeding timing for a different reason because males might indirectly drive oviposition through courtship feeding[64]. Thus, the Moon might also determine the onset of reproduction by affecting the number of prey that males of different colouration offer during courtship. In line with this hypothesis, we observed that first in-nest copulations are also more likely to occur with higher and lower moonlight levels in the whitest and the reddest males, respectively. However, *ca.* 60% of the first in-nest copulations occur without courtship feeding in barn owls[45], suggesting that the Moon might have a smaller influence at this time.

To conclude, our study shows that light variation associated with the Moon cycle exerts selection on the plumage colouration of a widespread nocturnal predator, the barn owl. Similar to the effect of varying diurnal light conditions[6,65], light variation during the night is also an important ecological factor to understand colouration of nocturnal species. Interestingly, our study provides supportive evidence that the white barn owls exploit sensory biases in the prey, which may enhance prey catchability and may help white owls to buffer the negative effects of moonlight. In line with the increasing evidence supporting the existence of accurate colour vision in numerous nocturnal species[26,40], our study contributes to reinforcing the idea that colour is important in nocturnal systems. This raises the concern that light pollution has the potential to interfere with the evolutionary and ecological dynamics associated with colouration of nocturnal species, which deserves the attention of future studies.

Methods

Study site and species

The study area comprises 1,070 km^2 between the lakes of Neuchatel and Leman in Western Switzerland. Since 1991, 360 nest-boxes installed in farms were regularly monitored for barn owl clutches. Eggs are laid every two to three days, and incubation starts with the first egg resulting in a marked age hierarchy (rank) among nestlings due to asynchronous hatching. The nests were revisited at least four times to capture the adults and record offspring development until fledging (*ca.* 55 days old). Owlets were weighed with a balance to the nearest 0.01 g, and their age was estimated based on wing length measured to the nearest 1 mm soon after hatching. An individual was considered to have successfully fledged if survived until the age of 55 days old. Fresh prey remnants (number and species) were recorded on every visit to the nests. Prey remnants are those prey items that were not consumed by the nestlings and/or the females before our visit and therefore do not directly reflect total parental food provisioning.

Moonlight and colour measurements

The plumage colouration of adults was scored on the breast, belly, flank, and the underside of the wings using an eight-colour chip ranging from -8 (white) to -1 (dark reddish), a method that highly correlates with objective spectrophotometric measurements of brown chroma (the ratio of long-wavelength reflectance, R600-700, over total reflectance, R300-700, see ref. [61] for further details). The average colour of all body parts was used for the statistical analyses. Barn owls also present a varying number of dark spots on their ventral plumage that are subject to sexual selection[66]. Including plumage spottiness on the models did not alter the results of the study (Supplementary Table 10).

Moonlight was measured as the visible percentage of the Moon. Except for the analyses on hunting success, we used the Moon visible percentage when the Moon passes the meridian as a single moonlight value for each night. For those nights that the moonset occurred within one hour after the sunset or that the moonrise occurred within one hour before the sunrise (*i.e.*, the Moon was not visible during most of the night) were assigned a value of zero. The analysis of hunting success was based on observations at specific time points of the night

and hence, we obtained values of the visible percentage of the Moon at each specific time point. For the analyses on offspring body mass, we collected data on moonlight of the night previous to the capture of the nestlings. For the analyses on offspring survival, we collected data on the moonlight levels at the nestling age of maximal growth rate (15 days of age, mass gain 14.3 g day^{-1}, Supplementary Fig. 4), when nestlings are more sensitive to reduced food provisioning (nestlings that did not survive to fledging received less prey at age 15 than those who survived; $t_{36} = -2.86$, $P = 0.007$). For the analyses on the association between moonlight and breeding, we collected data on moonlight for the night the females laid the first egg (assessed based on the developmental stage of the clutch at the first time we visit the nest). We also collected data on moonlight for the nights when the first in-nest copulations are expected to occur (27 days before the first egg was laid[45]) and for the nights when nestlings of rank six to eight are expected to reach an age of 15 days old (given 14 days between the first and the seventh egg are laid, plus 31 days of incubation, and 15 days after hatching).

All the moonlight data were obtained for a locality within the study area (Yverdon-les-Bains; 46°46'44" N, 6°38'24" E) using a Javascript library (MeeusJs) developed by Fabio Soldati (www.github.com, last access on November 2018). The visible percentage of the Moon was square-root transformed for all the statistical analyses as this transformation improves the association with night illumination (Supplementary Fig. 1). Because nights with intense cloud cover are likely to introduce error in the effect of moonlight measured as the visible percentage of the Moon, we repeated the analyses including cloudiness as a predictor whenever possible. The models were re-ran considering cloudiness (percentage covered by clouds), which may affect light variation during the night. Including cloudiness in the models did not qualitatively change the results unless indicated otherwise in the Results section.

Parental food provisioning, GPS tracking, and hunting success

Food provisioning was measured using infrared cameras at a total of 131 nest boxes ($N = 1154$ observations of 201 different parents) in 5 years (1997, 2001, 2005, 2006, 2016). During the years 1997, 2001, 2005, and 2016, we used infrared video cameras (CCTV miniature cameras, Active Media Concept, France) connected to a recorder (Monacor International, Germany) and, in 2016, we equipped the nests with motion-sensitive camera traps (HC500 Hyperfire, RECONYX, USA). Food provisioning was recorded between 21.5 and 5.5 h for 3.4 nights ± 2.4 standard deviation (s.d.) per nest on average. From the videos and pictures, we counted the total number of prey items brought on each night by the male and female parents, which were previously captured and ringed on different legs to facilitate their identification in the videos and pictures (for further details see ref.[67]).

We monitored the foraging behaviour of 34 breeding male barn owls in 2016 and 45 in 2017 using GPS trackers. We used GiPSy-5 GPS tags (Technosmart, Italy), measuring 30 × 20 × 10 mm with battery and coupled with a 40-mm-long antenna. The tags weighed between 12 and 13 grams (less than 5% of an owl's body mass) and were attached as a backpack with a Teflon harness. Each tag collected location, time and speed over ground every ten seconds at night, from 30 minutes before dusk to 30 minutes after dawn, to ensure a complete

measurement of the activity period. In 2016 and 2017, breeding males were captured at their nest site when the oldest nestling was 19 to 34 days-old (mean = 25.4; SD = 2.8), equipped with GPS tags and released at the capture site. Approximately two weeks later, the owls were recaptured at the nest site to recover the GPS tags with the data. The trackers recorded the spatial location of each owl for an average of 8.1 nights ± 2.6 s.d. per owl. Prior to any analysis, GPS data were pre-processed and filtered for aberrant positions based on either speed or location.

An Expectation-Maximization binary Clustering (EMbC) algorithm[68] was applied to classify barn owl movement data into different behaviours. EMbC uses an unsupervised approach (*i.e.*, based on no previous classification of the data) to cluster location data based on speed and turning angle between locations. We were interested in describing three main behaviours: perching, commuting and hunting. Perching was defined as a stationary behaviour, characterized by null or low speed and a wide range of turning angles, due to the GPS error. Commuting was defined as a rapid straight flight, characterized by high speeds and low turning angles, often displayed after a prey capture between the hunting grounds and the nest box. Lastly, hunting was characterized by a slow and sinuous flight, with low to medium speed and medium to high turning angles. For validation, the EMbC behavioural classification was compared to a visual classification performed on a random subsample of the whole dataset (20 individuals). The correspondence between EMbC and the visual classification was high: 92.7% on average (perching = 94.5% ± 2.3 s.e.; commuting = 91.1% ± 3.8 s.e.; hunting = 92.6% ± 4.9 s.e.). Therefore, we considered EMbC's classification as reliable.

After detecting each event when the owls were likely to be hunting (N = 13,558), we classified a hunting event as successful if the owl flew back to its nest immediately afterwards (*i.e.*, it commuted) or as unsuccessful, if the owl resumed hunting. Although this indirect measure of hunting success might include as successful those cases when males visit the nest without a prey and might leave out cases when an owl hunts close to and from a perching site, we found a good correspondence between the mean prey delivery rate of males per night observed using infrared cameras (7.08 preys ± 3.52 s.d.) with that estimated using the GPS tracks (8.94 preys ± 5.31 s.d., exact Poisson test P = 0.45). Additionally, the observed mean hunting success (~0.41 ± 0.1 s.e.) is also within the range observed in a previous study measuring owl success in catching prey in captivity (0.42 ± 0.2 s.e.)[44]. Thus, we consider that our indirect measurement of success is a good proxy for real hunting success.

Behavioural experiments

We used common voles, *Microtus arvalis* to investigate how prey react to barn owls of different plumage colouration and under different light conditions. Common voles are the staple prey in our owl population (~55% of the diet[38]), and the analysis of fresh vole remains found in the nests suggests that the number of voles that owls capture depends on the interaction between moonlight and the owls' colouration in the same way as described above for food provisioning (z = 2.11, P = 0.035, fewer voles as prey remains the day after a full-moon night than after a new-moon night in the reddest owls: z = -1.98, P = 0.048, no effect

of Moon cycle on the number of voles as prey remains within the whitest owls: z = 1.46, P = 0.143, Supplementary Table 11 and Supplementary Fig. 5).

The voles were captured within the first 2 weeks of February 2015 ($N = 24$) and on the first 2 weeks of March 2016 ($N = 23$) using Longworth live traps in the surroundings of the University of Lausanne, Switzerland and housed individually in plastic terraria ($42.5 \times 26.6 \times 18.5$ cm) at the animal facilities of the University of Lausanne, Switzerland. The room temperature and humidity were kept constant at 22 ± 1ºC and 50%, respectively. Food (rodent food pellets, seeds, and apple pieces) and water were provided *ad libitum*. The terraria were equipped with a hiding place, and hay and sawdust served as the substrate. We left the voles to acclimate to the laboratory conditions for ten days and recorded their behaviour to the different owl colour morphs and light conditions during days 11 and 12. On day 13, they were released at the exact location where they were captured.

On the night of day 11, the voles were moved to a dark room enclosed by black cloth (Supplementary Fig. 6a) and placed individually in a larger terrarium ($80 \times 35 \times 40$ cm) with new substrate mixed with a handful of the substrate from the rodent terrarium to minimize stress. The room was divided into three lines (2.80 m large, 2 m high and 1 m wide) by black cloths, and two terraria were placed at the end of each line (the sides of the terraria were covered with black paper to avoid the voles seeing each other). To measure the vole response to owl colour morphs, we used two white (plumage colour score of -8) and two red (plumage colour score of -2 and -3.25) owls that were taxidermized in a flying posture (Supplementary Fig. 6c). The owls were suspended 1.60 m above the ground with a transparent nylon string and remained hidden under a black cloth at the end of each line at the opposite end to the voles' terraria. Twenty minutes after the voles were placed in the large terraria, we opened the cloth hiding the owl and let the owl slide through a 2-m long zip-line that went down to the opposite end of the line where the voles' terraria were placed (Supplementary Fig. 6b). The 2-m length of the zip-line was chosen given that the anti-predatory response of the only rodent species previously tested (the spiny mouse, *Acomys cahirinus*) against an attacking barn owl occurred mainly within a range of 0 to 2 m between the owl and the rodents[44]. The owls were moved backwards along the zip-line and released again two more times (spaced by five minutes) simulating the multiple attacks that owls often perform on their prey[43,44].

For each vole, the same procedure was repeated after one hour on the same night but with an owl of a different colour. On the following night, the procedure was repeated with a different light condition (*i.e.*, at the end of the experiment, each vole was exposed to owls of both colourations and under both light conditions). To mimic full-moon light conditions, we placed two halogen lights (470 lumen each) attached together in one side of the room and at a distance of 3 m (Supplementary Fig. 6b). Halogen lights have been successfully used in previous studies to trigger moon-dependent behaviours[14], given their spectral similarity to the moonlight[69,70]. The light source was separated from the rodents by several black cloths so that the light in the first line measured with a standard luxometer was 0.25 lux, equivalent to full-moon light conditions at temperate latitudes[71]. To mimic new-moon conditions, the halogen lights were turned off, resulting in values below the detection level of the luxometer (< 0.001 lux). The order with which the treatment (*i.e.*, the colour of the taxidermized owl)

and the light conditions were randomized for each vole but controlling that there were the same number of trials for each combination of treatment order and light conditions.

In 2016, we included an additional manipulation to test if the effect observed on the voles' freezing times was dependent on the amount of light that is reflected from the owls' white plumage. We exposed the rodents to a white owl, also taxidermized in a flying posture and whose plumage colouration was treated with duck preen wax ("cul de canard"; CDC, Petitjean Fishing Equipment, SA, Switzerland) in addition to the red and white owls used in 2015. CDC was gently applied with a brush (6 drops per cm^2 of plumage) and significantly decreased plumage reflectance within the UV and visible wavelength ranges (see Figure 3c).

During the trials, vole behaviour was recorded with two infrared video cameras, one located within the terraria and another located in a position above the terraria. From the video footage, we determined whether the voles responded to the owls (by either freezing or fleeing) or not (i.e., the voles' behaviour remained unaltered after owl presentation). The amount of time that each vole spent frozen (time between the voles froze and resumed their activity) was measured from the videos to the nearest second. Because we were interested in investigating the response of the voles in relation to owl colouration, all the observations where the voles' orientation made them unable to see the owls were excluded (49 % of the trials). The final mean number of observations per vole was 6.04 ± 2.23 s.d. and was not significantly related to the owl colouration, the light conditions, or the repeated exposure to the owls (all $t_{23} < 0.97$, $P > 0.34$).

Statistical analyses

All statistical analyses were conducted with R 3.0.2 (R Core Team, Vienna, Austria). Linear mixed (LM) and generalized linear mixed (GLM) models were fitted with the functions *lmer* and *glmer*, respectively, implemented in the package 'lme4'[72]. GLM models on food provisioning as a Poisson response variable included the random effect of Parent ID to account for repeated measurements on the same parents over several nights, Brood ID to account for male and female parents provisioning the same brood, and Year ID to account for inter-annual variability in prey abundance. Fixed factors included moonlight, plumage colouration of the parent and their interaction. The fixed effects of sex, brood size, and their interaction, and laying date (including quadratic effects to account for within-year variation in prey abundance) were also included. GLM models on hunting success as a binomial response variable included the random effects of Male ID to account for repeated measurements taken on the same male over several nights, and of Night ID (nested in Male ID) to account for repeated measurements on the same male within the same night. Year was included as a fixed factor given that the study only covers two years of GPS tracking. Fixed factors included moonlight at the beginning of a hunting event, male plumage colouration, hunting effort (the number of times a male was observed hunting over a given night) and all two-way and the three-way interaction among these terms. Hunting effort was included in the models given that it may affect hunting success owing to owl's fatigue when hunting many times over the same night or because effort can reflect how suitable the conditions for hunting were over a given night (*e.g.*, low efforts may reflect poor climatologic conditions). We considered hunting effort in interaction with plumage colouration (and moonlight)

because plumage colouration may associate with differences in stamina[73] and with different tail and wing morphologies that may affect how red and white owls hunt under different conditions[74]. We also included the linear, quadratic, and cubic effects of the time of each hunting observation, after visually detecting that hunting success strongly decays during dawn and dusk. As for the models on food provisioning, the fixed effects of brood size and the linear and quadratic effects of laying date were also included.

The statistical models on vole behaviour (GLMM on vole probability to detect an owl as a binary response and LMM on freezing time) included the random effects of vole ID to account for repeated measurements on voles (each vole faced the same treatment 3 consecutive times), session (each vole was tested in the two moonlight conditions and with the two owl morphs), lane to account for potential variation within the experimental room, and block (each year, voles were captured and tested in the experimental setting in groups of 7-8 voles). Fixed effects included owl morph (red *vs* white), moonlight condition (full- *vs* new-moon), and their interaction. The fixed effect of year (two-levels factor) and its interaction with the other terms was included to account for the fact that the experiment was repeated in two separate years by different observers. Repetition was included to account for differences in the response between the first, second, and third time the same owl was presented to the rodents. For the analysis of time spent frozen, mean times of the three repetitions were taken.

Linear mixed models on nestling body mass (log-transformed) were conducted on a total of 18,735 records of offspring body mass collected for 3,878 nestlings born over the last 20 years in 814 different broods. The models included the random effects of owlet ID to account for repeated measurements of body mass on the same nestlings, including the random slopes for nestling age, age^2, age^3. The IDs of the brood where nestlings were born and raised were included to account for the shared environment and origin of nestlings. The IDs of the foster parents were included to account for repeated breeding of the same parents across several years. Fixed effects included moonlight, father plumage colouration and their interaction as well as factors known to affect nestling body mass: age (up to the 4th power[75]), hour (up to the 3rd power), laying date (linear and quadratic), brood size, and rank within brood hierarchy. We only considered the colouration of the father given the larger male hunting effort. Males alone feed both their offspring and female partners until the first-born owlet is 2 to 3 weeks old[46]. From this time onward, females leave to produce another clutch[76] or stay but hunt significantly less than males (the average number of prey per night for females was 3.79 ± 0.67 [mean ± s.e.] *vs.* 7.08 ± 1.21 for males; $z = 9.38$, $P < 0.001$, Supplementary Table 1).

GLM models on nestling survival as a binary response variable included the same random and fixed terms as for models on nestling body mass except for owlet ID (no replication in survival within individuals), age and hour. These models were conducted on data from 4,504 nestlings from 944 broods monitored in the last 20 years while the analyses restricted to nestlings of rank 7 was conducted on 217 barn owl nestlings from 150 broods. The GLM initial full model on the survival of nestling of rank 7 show evidences of underdispersion and we used a penalized likelihood approach (function *glmmPQL*, package 'MASS')[77].

To test for an association between male colouration and moonlight levels on the date females laid their first egg (N = 1,293 clutches raised by 631 different males between 1994 and 2017), we created a binomial variable considering whether moonlight was 50% or < 50% on a given date. We then fit a GLM model with moonlight as a binary response and considering the random effects of Year and Male ID (to account for repeated observations on the same males), and the fixed effect of male colouration. The same approach was used but considering moonlight levels on the date when the first in-nest copulations are expected to occur and on the date when a females' nestlings of rank six or more are expected to reach the age of 15 days old.

For all models, Cook's D values were computed from the models to assess the influence of the observations on model performance and collinearity was assessed by calculating the variance inflation factor for each of the quantitative parameters in the models[77]. All full models were simplified by backward elimination of non-significant terms (P > 0.1), which provided qualitatively similar results as when using an information-based (AIC) approach[78] (Supplementary Table 12). For posterior contrasts on the interactions between colour and moonlight, we performed multiple 'simple slopes tests'[77] using the minimum and maximum values of colour and moonlight as conditional values. The P values from the contrast tests were adjusted to account for multiple testing using the Benjamini-Hochberg approach. Significance was set at 0.05 (two-tailed).

Ethics

The monitoring of barn owls was performed under the legal authorization of the 'Service vétérinaire du canton de Vaud', Switzerland. Barn owls were equipped with GPS under the authorization of the 'Service vétérinaire du canton de Vaud', Switzerland (Authorization VD2844.a and VD3213). The voles were captured under the permit 2154 of the Canton de Vaud, Switzerland, and the behavioural experiments were authorized by the 'Service vétérinaire du canton de Vaud', Switzerland (Authorization VD2934).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Colour variation in barn owls.
Barn owls exhibit continuous variation in plumage colouration from immaculate white to
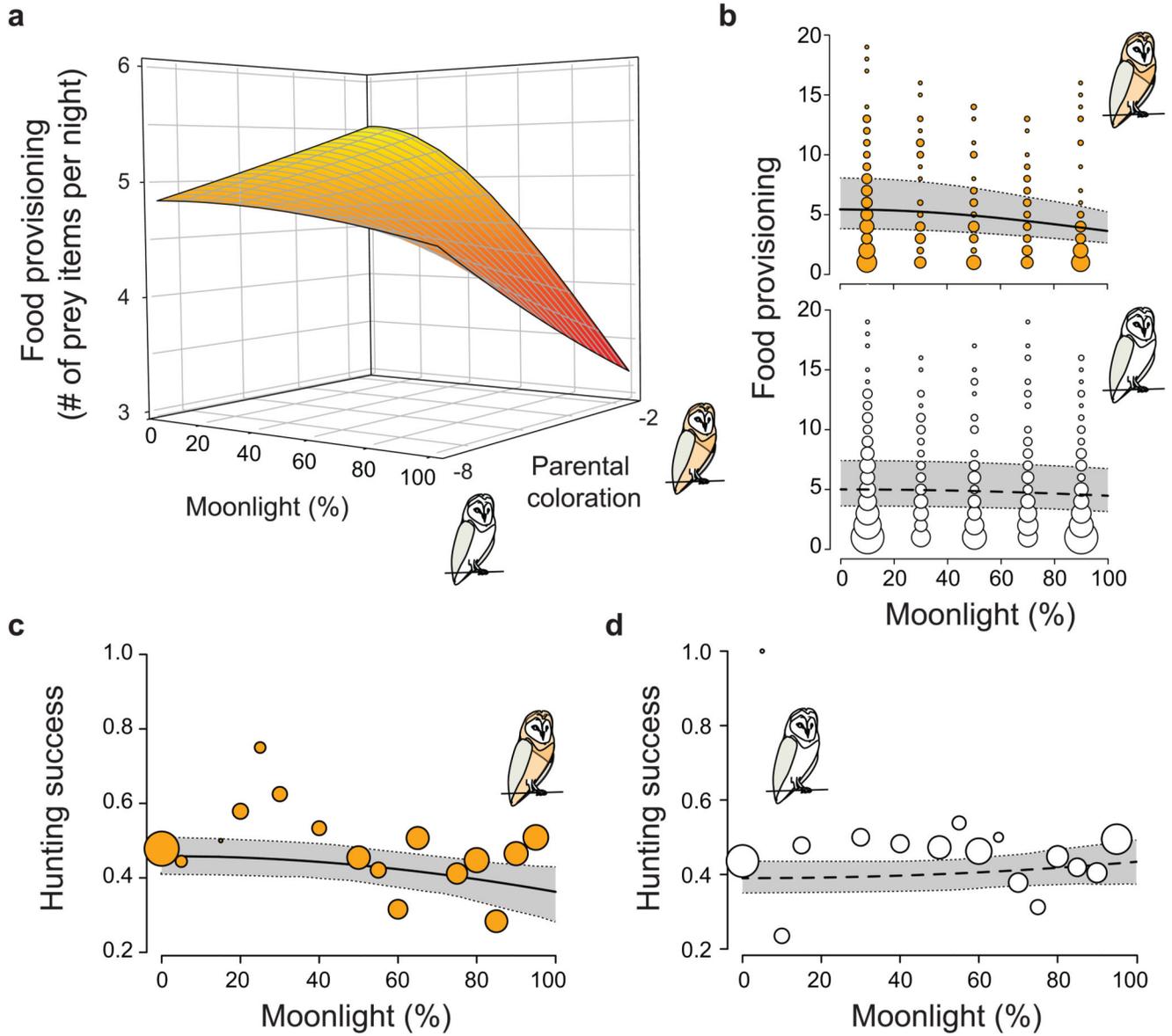dark reddish.

Figure 2. Parental food provisioning depends on moonlight and parental plumage colouration in the barn owl.

(**A**) Relationship between the total number of prey items brought by male and female parents and its plumage colouration in interaction with moonlight. The predicted surface from a Poisson-GLM model is presented. (**B**) Detailed effects of moonlight on food provisioning. Shown are the observed values of food provisioning (pooled every 20 units of moonlight for clarity, with dot size proportional to the number of observations: smallest dots = 1 observation, largest dots = 77), regression lines (continuous and dashed lines reflect significant and non-significant associations, respectively) and 95% interval of confidence for barn owls above the 3rd quantile (reddest owls) and 1st quantile (whitest owls) of colour variation. (**C-D**) Hunting success within the reddest and whitest owls, respectively. Shown are the observed values of hunting success (pooled every 5 units of moonlight for clarity,

with dot size proportional to the number of observations: smallest dots = 1 observation, largest dots = 694), regression lines (continuous and dashed lines reflect significant and non-significant associations, respectively) and 95% interval of confidence for barn owls above the 3rd quantile (reddest owls) and 1st quantile (whitest owls) of colour variation.
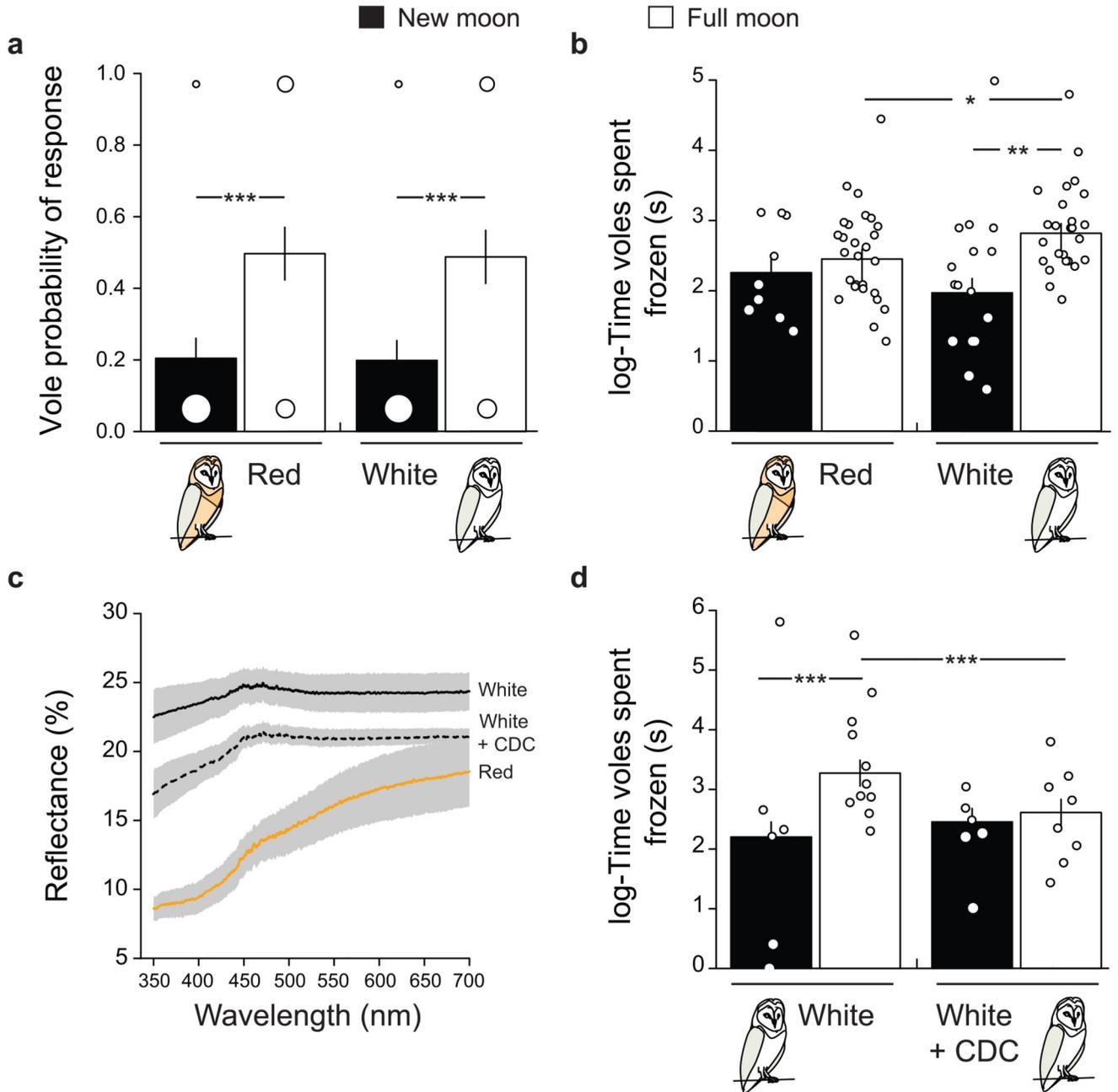
Figure 3. Probability to response and time spent frozen of common voles as a function of barn owl plumage colouration and moonlight conditions.

(**A**) Probability (± s.e.m.) that common voles detected (either freeze or flee) stuffed owls of white and red colour under light conditions mimicking full- and new-moon nights. The size of the white dots represents the number of observed responses of the voles (smallest dots = 21 observation, largest dots = 85). (**B**) Time (± s.e.m.) voles spent frozen (immobile) after observing white and red owls under light conditions mimicking full- and new-moon nights. (**C**) Mean reflectance spectra (± s.e.m.) of the plumage of a white owl, a white owl treated with CDC wax and a red owl (**D**) Time (± s.e.m.) voles spent frozen (immobile) after

observing a normally coloured white or a white owl treated with CDC under light conditions mimicking full- and new-moon nights. Significance of contrasts are indicated by *** P 0.001, ** P 0.01, * P 0.05.
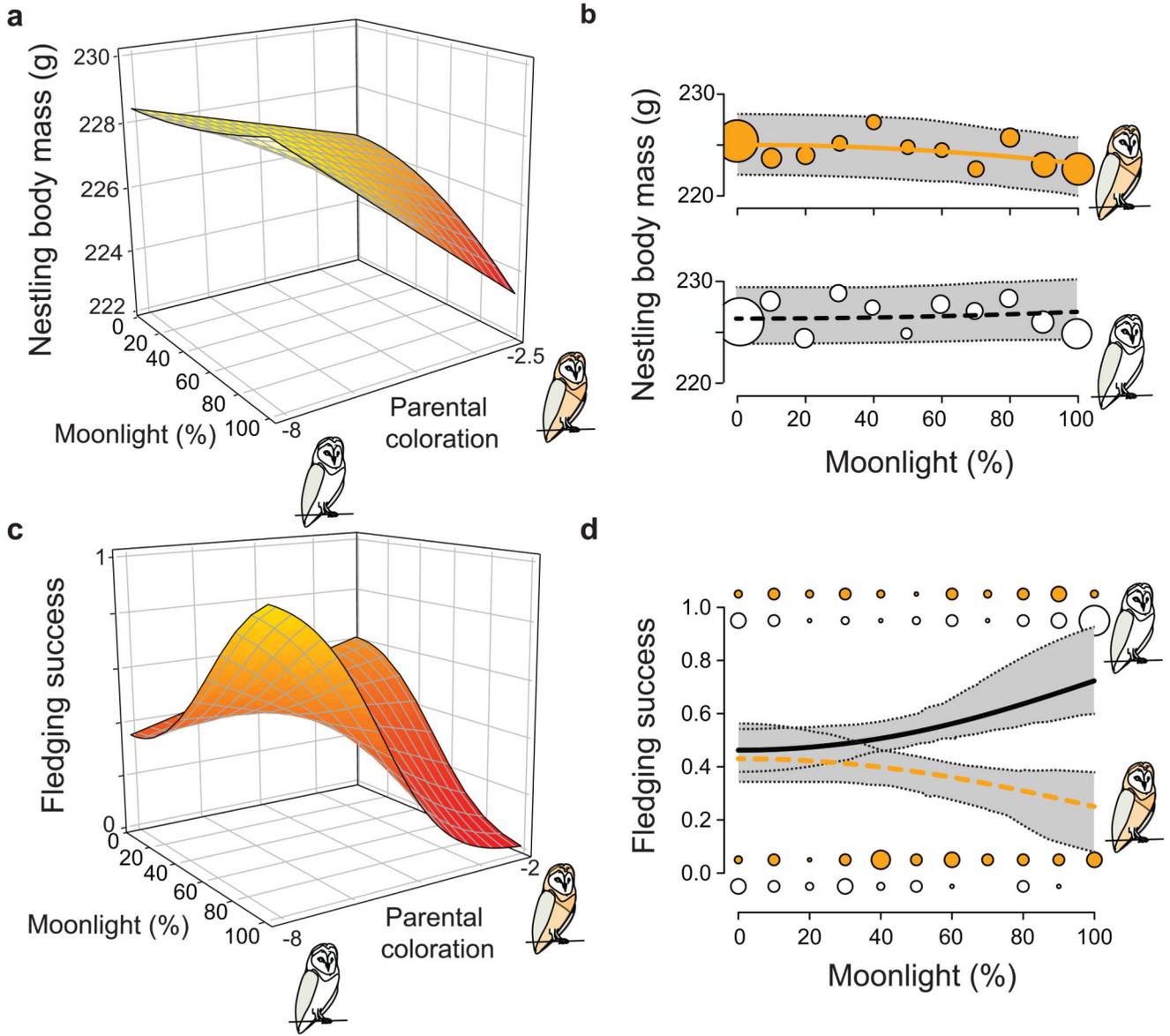
Figure 4. Offspring body mass and survival depend on moonlight and parental plumage colouration in the barn owl.

A. Relationship between moonlight and father plumage colour for offspring body mass (predicted surface from a LMM). **B.** Detailed effects of moonlight on nestling body mass. Shown are the observed mean values of nestling body mass (pooled every 5 units of moonlight for clarity, with dot size proportional to the number of observations: smallest dots = 224 observations, largest dots = 993), regression lines (continuous and dashed lines reflect significant and non-significant associations, respectively) and 95% interval of confidence for barn owls above the 3rd quantile (reddest owls) and 1st quantile (whitest owls) of colour variation. **C.** Relationship between moonlight (at nestling age of 15 days) and father plumage colour for fledging success of low-rank owlets (rank 7). **D.** Relationship between fledging success of low-rank owlets and moonlight (at nestling age of 15 days) raised by the

whitest fathers (plumage colour 1st quantile) and the reddest fathers (plumage colour 3rd quantile). Lines indicate the regression lines (continuous and dashed lines reflect significant and non-significant associations, respectively) and 95% interval of confidence, and dots indicate the observed fledging success (pooled every 5 units of moonlight for clarity, with dot size proportional to the number of observations: smallest dots = 1 observation, largest dots = 8).
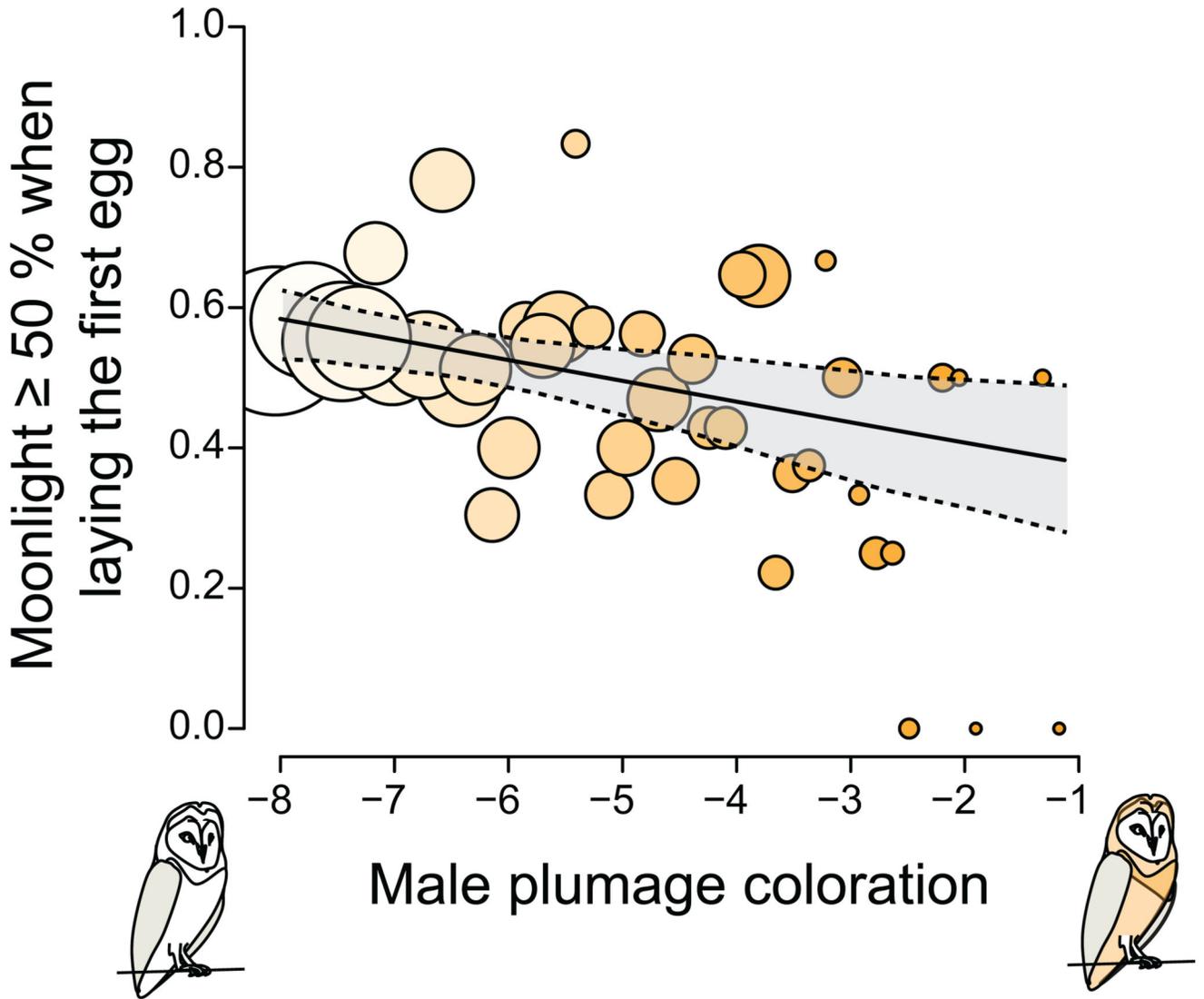
Figure 5. Plumage colouration in association with moonlight levels the night females laid the first egg.
Probability that a female laid the first egg of a clutch during nights with 50 % or more of the Moon surface illuminated in relation to male plumage colouration. Shown are the observed proportions of cases (pooled every colour unit to the first decimal, smallest dots = 1, largest dots = 181), the regression line and the 95% interval of confidence.