Journal of Raptor Research

Advancing the science, conservation, and understanding of birds of prey

Journal of Raptor Research 59(3):jrr2446
doi: 10.3356/jrr2446

Polymorphism of Melanin-based Pigments in Barn Owls in California, USA

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ABSTRACT.—The barn owl group (Family Tytonidae, including *Tyto alba* and *T. furcata*) displays within-species variation in plumage, ranging from reddish to whitish and from heavily spotted to having no spots at all. One explanation for the persistence of polymorphism in raptors is the habitat matching choice hypothesis, which posits that different morphs exploit the different spatial or temporal environments to which they are best adapted. For example, in heterogeneous landscapes in Europe and the Middle East, reddish Western Barn Owls (*Tyto alba*) inhabit territories with proportionally more arable fields and consume proportionally more voles than mice, in contrast to their whiter counterparts (*Journal of Evolutionary Biology* 25:103–114; *Biological Journal of the Linnean Society* 106:447–454). We here sought to quantify polymorphism in female and male American Barn Owls (*Tyto furcata*) in Napa Valley, California, USA, and to test predictions about the relationships among landscape composition around nests, prey composition, and degree of reddishness in this population. We photographed adult owls and used MATLAB software to analyze plumage characteristics. We analyzed pellets to determine the proportion of mice (*Peromyscus*, *Reithrodontomys*, and *Mus*), voles (*Microtus*), and gophers (*Thomomys*) in the owls' diet. Analyses showed mixed support for the habitat matching choice hypothesis. As predicted, redder females occupied nest boxes with less forested area within their hunting area, but unlike the barn owls from Europe and the Middle East, they provisioned proportionately fewer voles than whiter females. The results provide further evidence for the existence of polymorphism in the barn owl group in heterogeneous landscapes, in this case within and surrounding vineyards.

KEY WORDS: *Color*; *diet*; *habitat*; *melanin*; *morph*; *plumage.*

POLIMORFISMO DE PIGMENTOS BASADOS EN MELANINA EN *TYTO FURCATA* EN CALIFORNIA, EE.UU.

RESUMEN.—El grupo de las lechuzas comunes (Family Tytonidae, que incluye a *Tyto alba* y *T. furcata*) presenta variación intraespecífica en el plumaje, que va desde rojizo a blanquecino y desde muy moteado hasta completamente sin manchas. Una explicación para la persistencia del polimorfismo en aves rapaces es la hipótesis de elección por coincidencia de hábitat, que plantea que los distintos morfos explotan los diferentes entornos espaciales o temporales a los que están mejor adaptados. Por ejemplo, en paisajes heterogéneos de Europa y Medio Oriente, *T. alba* habita territorios con proporcionalmente más campos de cultivo y consume proporcionalmente más topillos que ratones, en contraste con sus contrapartes más blancas (*Journal of Evolutionary Biology* 25:103–114; *Biological Journal of the Linnean Society* 106:447–454). En este estudio buscamos cuantificar el polimorfismo en hembras y machos de *T. furcata* en el Valle de Napa, California, EUA, y evaluar predicciones sobre la relación entre la composición del paisaje alrededor de los nidos, la composición de las presas y el grado de coloración

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rojiza en esta población. Fotografiamos lechuzas adultas y utilizamos el software MATLAB para analizar las características del plumaje. Analizamos egagrópilas para determinar la proporción de roedores pertenecientes a los géneros *Peromyscus, Reithrodontomys, Mus, Microtus* y *Thomomys* en la dieta de las lechuzas. Los análisis mostraron un apoyo mixto a la hipótesis de elección por coincidencia de hábitat. Como se predijo, las hembras más rojizas ocuparon cajas nido con menor área boscosa en las cercanías, pero a diferencia de las lechuzas de Europa y Medio Oriente, aportaron proporcionalmente menos *Microtus* spp. que las hembras más blancas. Los resultados aportan evidencia adicional de la existencia de polimorfismo en el grupo de las lechuzas comunes en paisajes heterogéneos, en este caso dentro y alrededor de viñedos.

[Traducción del equipo editorial]

INTRODUCTION

Polymorphism is the phenomenon in which individuals of a species of the same sex and age display more than one phenotype for the same trait within a population (Huxley 1955). Both natural and sexual selection have contributed to the evolution of polymorphism in spatially heterogeneous environments (Ravigné et al. 2004). Natural selection can favor the persistence of polymorphism by allowing conspecifics to avoid competition and to specialize or exploit different spatial or temporal environments and food resources most successfully acquired or maintained as a result of the phenotype expressed (Roulin 2004a). Individuals with different phenotypes can find and select the specific habitats to which they are best adapted (Ravigné et al. 2004, Dreiss et al. 2012). This is known as the “habitat matching choice hypothesis” (Edelaar et al. 2008), which is most likely to operate in circumstances where the cost of phenotype–habitat mismatch is high (Camacho and Hendry 2020), such as for polymorphic species in heterogeneous landscapes.

Sexual selection can also promote and sustain polymorphism when different phenotypes provide honest signals to potential mates about an individual’s intrinsic quality (Roulin 2004a). In species with strong patterns of mate selection, polymorphism in a population can be maintained if a phenotype is beneficial to one sex but detrimental to the other. For example, for raptors that pursue agile prey and have distinct sex roles while nesting, a small body size may be best for males to optimize hunting efficiency for provisioning prey to their mates and nestlings (Slagsvold and Sonerud 2007), but a large body size may be adaptive for females that compete for mates and produce and incubate eggs (Olsen and Olsen 1987). Consequently, small-bodied males produce comparatively maladapted daughters while large-bodied females produce maladapted sons, and this antagonistic selection likely helps maintain a range of body sizes and reverse sexual dimorphism in these species (Olsen and Cockburn 1991). Because color polymorphism is visually recognizable, species that express various color morphs are ideal for investigating the connections between morphs, physiology, or behavior (Robinson et al. 2024).

Color polymorphism occurs in only 3.5% of all bird species, including species in the orders Accipitriformes, Ciconiiformes, Galliformes, Cuculiformes, and especially Strigiformes, in which over 30% of species show polymorphism of some kind (Galeotti et al. 2003). Western Barn Owls (*Tyto alba*) and American Barn Owls (*Tyto furcata*), which we hereafter collectively refer to as “the barn owl group,” display polymorphism in melanin-based pigments, with their plumage ranging from reddish to whitish and from heavily spotted with black spots to having no spots at all, two traits characterized by the expression of pheomelanin and eumelanin, respectively (Roulin 1999). The production of these pigments is regulated by genetically inherited processes, not environmental conditions (Roulin et al. 1998, Roulin 2003, Roulin and Dijkstra 2003, Antoniazza et al. 2010).

The pheomelanic reddish to white gradient in Western Barn Owls is known to be influenced by natural selection as birds adapt to their environmental surroundings and hunting preferences, resulting in an effect on predator-prey dynamics and interactions with conspecifics (Roulin 2020). A pronounced geographic variation in coloration persists among female Western Barn Owls; there are more reddish owls in northeastern Europe, where the landscape consists of open habitats and where voles (*Microtus*) are more abundant (Roulin 2004b). On the other hand, more whitish owls are found across southern Europe, a landscape made up of predominantly wooded areas where wood mice (*Apodemus* spp.) are more common. The habitat matching choice has also been observed at a smaller scale in heterogeneous landscapes in both Switzerland (Dreiss et al. 2012) and Israel (Charter et al. 2012), where reddish owls inhabit territories with proportionally more arable fields and grasslands than their whiter counterparts. Color polymorphism can render advantages under different abiotic conditions as well. The different morphs are maintained in a population, likely due to selection for reddish owls that are best suited to prey on voles, while their whiter counterparts are more successful at capturing mice (Roulin 2004a, Antoniazza et al. 2014).

In contrast to pheomelanic plumage coloration, the number and size of spots displayed by an owl (both eumelanic traits) are believed to be influenced by sexual selection, acting as honest signals of an individual's intrinsic quality (Roulin 1999). In Switzerland, large-spotted female Western Barn Owls are more likely to survive their first year, while smaller-spotted males have greater chances of surviving their first year, though to a lesser degree (Roulin et al. 2010). Phenotypic variation of spot diameter overlaps across the sexes in Western Barn Owl populations (Roulin and Jensen 2015) and may well be maintained under sexually antagonistic selection due to the conflicting advantages the different plumages bring to the two sexes. Furthermore, there may be adaptive functions to having larger spots. During the nestling stage, food shortage is one of the main causes of mortality to offspring, resulting in positive selection for nestlings that can withstand periods of food shortage. One controlled experiment with nestlings assigned to two groups (starved or offered food ad libitum) found that nestlings with larger black spots had lower appetites and lost less body mass than nestlings with smaller black spots, suggesting that larger-spotted nestlings may be more resistant to periods of low prey availability (Dreiss et al. 2010). In another study, offspring of larger-spotted females had larger spots regardless of the sire's spot size, suggesting that the plumage-linked capacity to withstand prey shortages is inherited from the maternal side (Almasi et al. 2010).

The barn owl group hunts in a variety of landscapes, including open grassy areas and vegetated, wooded areas. They have successfully maintained a presence near human developments with agricultural practices, in some cases because farmers encourage birds in the barn owl group by installing human-made wooden nest boxes to encourage the owls to aid in pest control (Marti et al. 1979). Recent work in Napa Valley (Napa County, California, USA) that American Barn Owls are capable of reducing the abundance and activity of both gophers (Hansen and Johnson 2022) and smaller rodents like voles and mice (Larson 2024). Though not always an accurate representation of rodent abundance (Avenant 2005, Bernard et al. 2010), prey composition in pellets collected from birds in the barn owl group has been associated with local pest abundance. For example, pellets from Western Barn Owls breeding in nest boxes placed within orange groves in Spain contain mostly *Mus* and *Rattus* spp. (Martínez and López 1999). Rodriguez and Peris (2007) found *Mus spretus* were most abundant in arable lands in Spain, whereas more voles were found in grazed habitats. In California's Central Valley, American Barn Owls prey more on gophers when their breeding territory is near vineyards and other orchard crops (Kross et al. 2016). In California's Napa Valley, proportionately more voles and gophers were delivered to nest boxes with abundant grasslands nearby, whereas proportionally more mice were delivered to nest boxes with extensive oak woodland nearby (St. George and Johnson 2021). However, only a few studies have investigated the relationship between prey distribution, habitat, and the owl polymorphism within these agricultural ecosystems (Charter et al. 2012, Dreiss et al. 2012). It is important to understand how the different morphs of owls may use land cover types in agricultural ecosystems, both to evaluate the habitat matching choice hypothesis and to reveal how different morphs may affect the delivery of rodent pest control on farms.

We investigated polymorphism in a population of American Barn Owls in the managed wine-grape vineyard ecosystem of Napa Valley. Specifically, we aimed to quantify the degree of plumage variation and polymorphism in male and female American Barn Owls in this study system, focusing on the reddish-to-white breast color gradient and the number and size of black breast spots as described for Western Barn Owls in other systems. Additionally, we examined the hypothesis that polymorphism in females affects the habitats they use and the species of rodents they hunt by testing the following predictions. (1) Based on the habitat matching choice hypothesis and previous work on the Western Barn Owl, we predict that the degree of reddishness (in the reddish-to-white color morph gradient) is positively associated with the amount of open and grassy land cover types and negatively associated with forests near the owl's nest site. (2) The breeding sites selected by owls with different plumages is reflected in their prey composition, as evidence by contents of pellets retrieved at the nest. Specifically, the degree of reddishness is positively correlated with the proportion of prey associated with open and grassy habitats (voles and gophers) compared to prey associated with more wooded habitats (mice). (3) Assuming a female's spot size reflects her

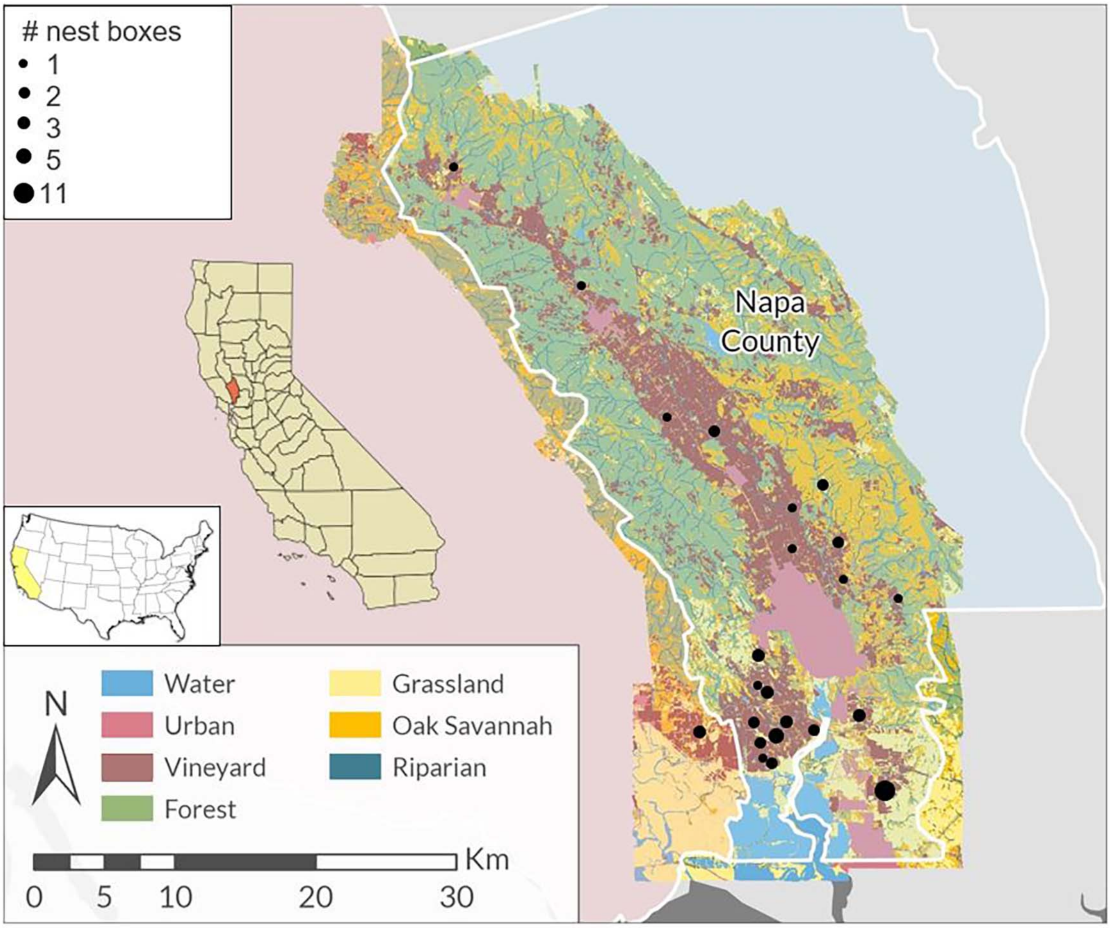

Figure 1. Map of Napa Valley, California depicting seven land cover types (from Coro 2021 and Huysman and Johnson 2021), with inset locator maps of Napa County and California. Locations of 22 vineyards (jittered to preserve anonymity) with number of American Barn Owl nest boxes sampled per vineyard in this study (total *n* = 54 boxes).

intrinsic quality and ability to care and provide for her nestlings, we predict that female spot size is positively associated with the number of nestlings fledged.

METHODS

Study Area. Napa Valley is 100 km north of San Francisco, California, and stretches for 50 km between the Vaca and Mayacamas Mountains. Napa Valley is characterized by a Mediterranean climate that renders the area ideal for viticulture. There are almost 500 vineyards spread across the region, yet the landscape remains relatively heterogeneous, and the vineyards are surrounded by foothills that consist of native mixed forests, oak savanna, and grasslands, and the valley floor contains some riparian areas and urban habitats mixed among farms (Napa County 2010, Wendt and Johnson 2017). The valley narrows and its margins become increasingly wooded from south to north (Fig. 1).

Our team monitors about 300 nest boxes annually, all located on vineyard properties, either within the vine rows themselves or along field margins and wooded edges. Nest box occupancy rate in this system varies from ~25–55%; it was 41% in this study (2021; M. Johnson unpubl. data). Nest box designs vary in this system, though most are wooden (40–61 cm tall, 45–61 cm wide, and 40–61 cm deep), mounted on wooden or metal poles (3–5 m), with entrance holes (10–15 cm diameter) facing various orientations. See Johnson et al. (2024) for detail and analysis of nest box attribute preferences. Our focus here was on habitat features around the nest boxes. The heterogeneous landscape ensured a wide variation in the proportion of land cover types near nest boxes (Fig. 1). For example, for the 54 occupied nest boxes used in this analysis (see below), the percentage of land cover that was grassland within the mean maximum distance from the nest box traveled by hunting American Barn Owls fitted with GPS tags in this system (2.81 km; Huysman and Johnson 2021) ranged from 3% to 77%, with a mean of 21%. The percentage that was wooded (mixed forest + riparian land cover types) ranged from <1% to 59%, with a mean of 12% (M. Johnson unpubl. data). The percentage of other land cover types within 2.81 km of the nest boxes was: vineyard (mean ± 1 SE: 44 ± 10%), oak savannah (14 ± 0.4%), riparian (4 ± 0.1%), urban (9 ± 0.4%), and water (4 ± 3%). The owls' prey within this system was primarily voles (*Microtus californicus*, 49.6%), gophers (*Thomomys bottae*, 22.0%), and mice (*Peromyscus* and *Mus*, 14.5%; St. George and Johnson 2021). Rodent surveys in the study region indicated that gophers and mice (>90% of mice were *Peromyscus sonoriensis*) were relatively common in the vineyard habitat, whereas vole populations were highly variable in space and time, being abundant in some vineyards or years and rare in others (Hansen and Johnson 2022, Larson 2024). St. George and Johnson (2021) established that proportionately more voles and gophers were delivered to nest boxes with abundant grasslands nearby, whereas proportionally more mice were delivered to nest boxes with extensive oak woodland nearby, prompting the predictions about color morph and prey tested in this study.

Field Methods. *Capture of adults.* We began monitoring nest boxes in early- to mid-February 2021. Following Wendt and Johnson (2017), we conducted periodic non-invasive occupancy checks by using a GoPro camera attached to an extendable painter's pole that was wirelessly connected to a smartphone. We captured females roosting diurnally in the nest boxes when nestlings were 10–25 d old. Using an orchard ladder, we gained access and removed the adult owl through a side or top door. When adults (male or female) were no longer reliably roosting with the nestlings, we used a box-mounted "patio-trap," which consisted of a roughly shoe-box-shaped device that was attached to the exterior of the nest box. An adult returning to the nest box at night to deliver prey must crawl through the device, triggering a door to shut behind it, trapping the owl in the nest box. An external light triggered by the shut door signaled when an owl entered the trap, upon which it was immediately captured by hand as previously described. We targeted the capture of adults in a variety of different habitats; therefore, though occupancy was concentrated in the southern part of the valley (Fig. 1), we spread our capture effort as much as feasible to obtain adults from occupied boxes farther north. After capture, each owl was placed in a cloth bag and lowered to the ground for processing. Individual owls received a United States Geological Survey (USGS) aluminum band and an Acraft (Edmonton, Alberta, Canada) anodized aluminum color band. For each owl, we quantified the melanin-based traits as described below. All methods were approved by Cal Poly Humboldt's Institutional Animal Care and Use Committee Protocol (IACUC No. 20/21 W.12).

Quantification of plumage characteristics. We quantified the red-to-white color gradient of each owl (Charter et al. 2012). Using a digital single-lens reflex camera (Canon Rebel T5; Melville, New York, USA) from a standard height on a tripod oriented directly over the owl, we photographed each owl's belly and upper breast. We manually customized the camera's white balance processing function at each nest box location by photographing a standard 18% neutral gray card before capturing the image of the bird. Each image was processed individually with a custom script for use in MATLAB version 2021a (the full script is available in Echávez 2023).

A standard 60 mm × 40 mm region of each owl's upper breast was selected to obtain the average red, green, and blue (RGB) values from the color-balanced image (Appendix). For analyses regarding coloration, we used the degree of reddishness measured by the ratio of red to green according to Vortman et al. (2011). To quantify the variation of eumelanin-based coloration expressed in the form of black spots at the tip of breast feathers, we measured the spots within the same 60 × 40 mm region. Auto- and manual-spot selection were both done in MATLAB; spots were counted and measured to the nearest ten-thousandth of a millimeter, using a 2-cm scale in the image for calibration. Spots were usually vertically oriented polygons or ellipses rather than true circles (Appendix), so we measured the maximum vertical dimension, which we hereafter refer to hereafter as "spot diameter" to be consistent with previous work (Charter et al. 2012). We extracted the number and average spot diameter for each owl to use in statistical analysis. Spots that were partially cut off by the edges of the region were excluded from analysis.

Pellet collections and analysis. We attempted to quantify the composition of the prey female owls captured to provision the young by examining the change in pellet composition over time. Males almost exclusively provide for the nestlings when they are recently hatched, and females start contributing to offspring provisioning once the oldest nestlings reach 21–25 d old (Roulin 2002). Prey delivery rates in this system peak when the young are 4–6 wk old (St. George and Johnson 2021). Therefore, we collected pellets once during the early nestling period when the oldest nestling was 2 wk old (i.e., when only the male was provisioning) and again after the onset of female contribution to provisioning the nestlings (when the oldest nestling was 5–6 wk old). The freshest pellets (intact, not too dry) were chosen for collection. Pellets from each collection were kept in paper bags labeled with the collection date and the box identifier. Pellets were dissected and prey identified to genus: vole (*Microtus*), gopher (*Thomomys*), mouse (*Peromyscus, Reithrodontomys*, and *Mus*), or other (rat [*Rattus*], shrew, bird, invertebrate). To quantify the minimum number of individual prey in each pellet, we counted the number of skulls or lower mandibles present, distinguishing left and right mandibles (Van Vuren et al. 1998).

Estimates of female prey provisions found in the pellets were calculated based on a weighted average approach that made use of observed changes in prey proportions as described below. We used the proportions of prey from pellets collected when nestlings were 2 wk old as a measure of prey captured when the male was the sole provisioner to the nest. Then we pooled the pellets collected at 5–6 wk to calculate the proportion of prey types when both the male and female were capturing prey for the nestlings. We used the difference in these two proportions to then estimate a female-contributed prey index for each nest box using three different scenarios to simulate various levels of the female-contributed prey composition: 50%, 75%, and 100% of the prey found in the dissected pellets collected in weeks 5–6 contributed by the female. We chose the values because 50% appears most biologically plausible (St. George and Johnson 2021), and because the higher values emphasized empirically observed pellet composition over a calculated composition from assumption. The results were used in linear regression models, in which we analyzed the relationships between the index of each main prey items (voles, gophers, and mice) as the response variable and the degree of reddishness of the female owls as the predictor.

Habitat sampling. We used a 4 × 4 m resolution GIS raster layer of land cover spanning all of Napa Valley adapted from Corro (2021) by Huysman and Johnson (2021) to measure land cover around each of the 53 nest boxes from which we captured and sampled a female. Specifically, we quantified the proportion of grassland and mixed forest within a 75-m radius of the nest box and a 2.81-km radius around the nest box. The former reflects the immediate flight environment of the nest box, and the latter radius reflects 95% of the area where owls hunt for prey within this study area (Huysman and Johnson 2021). We hereafter refer to these radii as the nest site scale (75 m) and the hunting radius scale (2.81 km). We classified the seven land cover types with ArcGIS Pro 2.8 from Esri and calculated the proportions with RStudio (packages *raster, rgeos, rgdal, dplyr, tidyr*, and *tidyverse*).

Statistical Analysis. We report the mean, SE, and range of the degree of reddishness and the number and diameter of breast spots for all measured female and male owls. Because of the geographic trend in land cover from south to north in Napa Valley, we examined correlations between these measurements and latitude for both sexes. We also tested for significant assortative or disassortative mating between pairs using a Pearson's correlation tests of reddishness, spot number, and spot diameter. We conducted all analyses with R (R core team 2024). For analyses of variance (ANOVAs) and simple linear correlations, we inferred significance by $P < 0.05$. For candidate generalized linear model sets, we used Akaike Information Criterion corrected for small sample size (AIC_c) to choose the best-supported models; models within $\Delta 2$ AIC_c were considered competitive, and significance was inferred by coefficients with 95% confidence intervals that did not overlap zero.

We used linear regression models to analyze the relationship between the plumage characteristic of degree of reddishness and the habitat surrounding the nest box. The habitats of interest were grasslands and forest, so the proportions of these two land cover types within 75 m (nest site) and 2.81 km (hunting radius) of each nest were used as the predictor variables (none were correlated with each other; all $r < 0.7$ and $P < 0.05$) and the degree of reddishness (red to green ratio, from RGB values) of each female was used as the response variable. To normalize the habitat variables, we performed a square root transformation. We built a small *a priori* candidate model set of models with various combinations of these nest site and hunting radius land cover variables and a null model.

We used generalized linear models with a Poisson distribution to model the effect of plumage and habitat

Table 1. Summary of the degree of reddishness (red to green ratio), number of spots, and spot diameter (mm) of breeding male and female American Barn Owls in Napa Valley, California, 2021.

Sex	Descriptive Statistic	Reddishness (Red to Green Ratio)	Number of Spots	Spot Diameter (mm)
Female ($n = 53$)	Mean ± SE	1.21 ± 0.01	38.5 ± 2.51	2.76 ± 0.01
	Range	1.04–1.34	15–110	1.78–4.01
Male ($n = 26$)	Mean ± SE	1.06 ± 0.01	33.0 ± 2.55	1.94 ± 0.07
	Range	1.01–1.15	1–52	1.39–3.04

on female reproductive success, measured as the number of nestlings in a box when the oldest reached ≥50 d of age (~80% of fledging age). During each visit to the nest box, nestling age was estimated based on feather growth patterns and the length of growing flight feathers. The visible feather characteristics were compared to a development chart provided by the Barn Owl Trust (Owlet Identification and Ageing, n.d.). The number of fledglings was used as the response variable, and we built a small *a priori* candidate model set with various combinations of female reddishness, spot diameter, and the amount of forest and grassland within 2.81 km as predictors. None of the plumage traits (reddishness, spot size, and number of spots) were significantly correlated with each other ($r < 0.4$, $P > 0.05$). In this system, the number of fledglings per box tends to show a unimodal distribution with date, peaking at the middle hatch dates with fewer fledglings from early and late nests (Carlino 2024), so we included a quadratic relationship with hatch day in all candidate models except the null model.

RESULTS

We captured, banded, measured, and photographed 53 female and 26 male American Barn Owls from 54 nest boxes. Females were significantly redder and had larger spots than males (Table 1; one-way ANOVAs; $F_{1,77} = 88.76$, $P < 0.01$; $F_{1,77} = 47.71$, $P < 0.01$, respectively). Female owls' reddishness was negatively associated with latitude in Napa Valley (Pearson correlation: $r = -0.31$, $P < 0.01$), though this result disappeared when the two most northerly boxes were removed ($r = -0.20$, $P = 0.16$), and males did not vary based on latitude ($r = -0.07$, $P = 0.74$). There was no evidence of significant assortative or disassortative mating based on reddishness (Pearson $r = 0.31$, df = 23, $P = 0.13$), spot number (Pearson $r = 0.17$, df = 23, $P = 0.41$), or spot diameter (Pearson $r = -0.28$, df = 23, $P = 0.17$).

Overall, the degree of reddishness in females was predicted by the amount of forest and to a lesser extent grassland land cover around each owl's nest site (Table 2). The top model included only forest land cover at the hunting radius scale, though the global model with grassland and forest land cover at both the nest site and hunting radius scales was competitive. In the global model, only the amount of forest within the hunting radius had a confidence interval that did not overlap zero (Fig. 2), and models including this variable collectively contained 0.74 of the model weight in the candidate set. More specifically, the degree of reddishness was significantly negatively correlated with forest within the birds' hunting radii (2.81 km; $\text{forest}_{\text{hunting radius}}$: $\beta = -0.15263 \pm -0.06491$ [SE], $P = 0.02$; Fig. 2). These results are consistent with the habitat matching choice hypothesis. Reddishness

Table 2. Candidate linear model set for the effect of the amount of grassland and forest land cover at nest site and hunting radius scales (75 m and 2.81 km radii, respectively) on the degree of plumage reddishness for 53 female American Barn Owls in Napa Valley, CA in 2021, including number of parameters (K), AIC_c, ΔAIC_c, and model weight (w).

Predictors of Female Plumage Reddishness	K	AIC_c	ΔAIC_c	w
$\text{forest}_{\text{hunting radius}}$	2	−125.5	0	0.45
$\text{grass}_{\text{nest site}} + \text{forest}_{\text{nest site}} + \text{grass}_{\text{hunting radius}} + \text{forest}_{\text{hunting radius}}$	5	−124.2	1.29	0.24
$\text{grass}_{\text{hunting radius}} + \text{forest}_{\text{hunting radius}}$	3	−123.3	2.24	0.15
Null	1	−122.3	3.20	0.09
$\text{grass}_{\text{hunting radius}}$	2	−121.8	3.70	0.07
$\text{grass}_{\text{nest site}} + \text{forest}_{\text{nest site}}$	3	−118.4	7.11	0.01

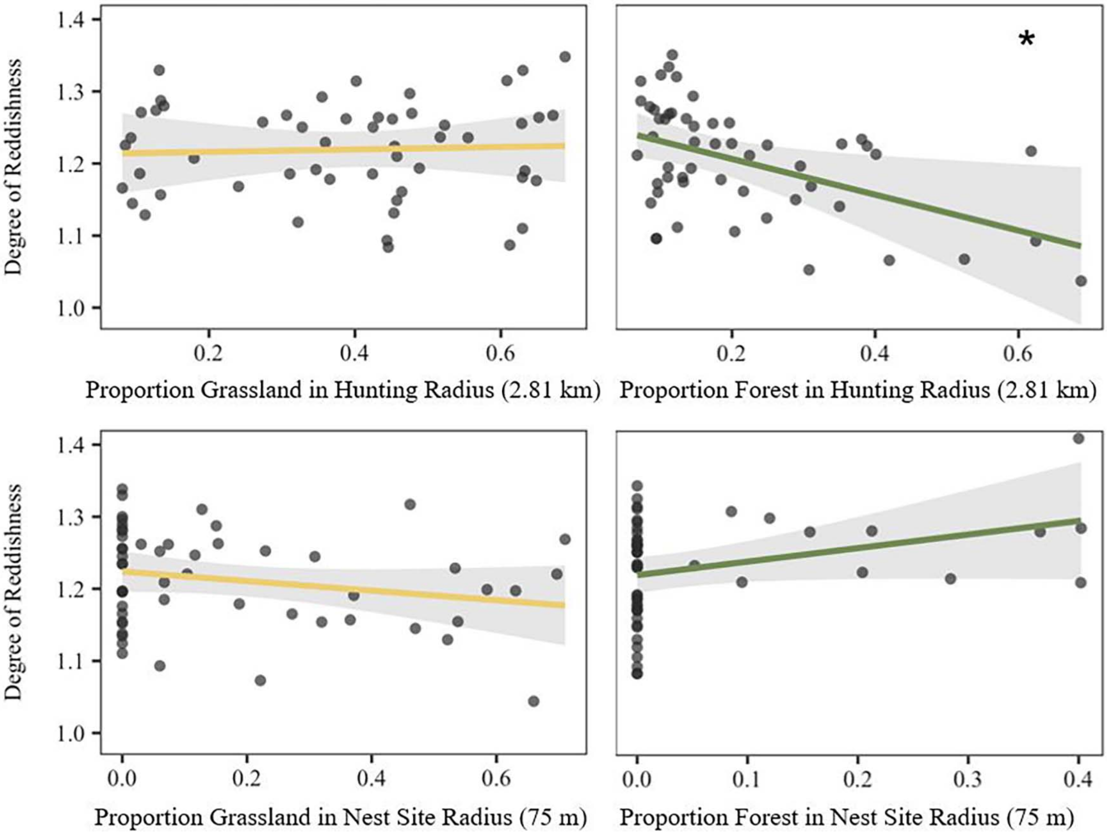

Figure 2. Partial response plots for relationship between female degree of reddishness and proportion of each land cover variables at nest site and hunting radius levels (square root transformed) in Napa Valley, California, in 2021. Each plot represents effect of the independent variable as others are held constant. Asterisk indicates confidence intervals that did not overlap zero.

was not significantly associated with either habitat at the nest site scale (Fig. 2).

Pellet collection required two properly timed visits to each sampled nest box, and with our timing and personnel constraints we were able to collect 258 pellets from 29 of the 53 boxes with sampled females. All analyses involving the relationships of diet with land cover or plumage were restricted to owls and pellets from these 29 boxes. A total of 529 prey items were identified, including voles (n = 180), mice (n = 100), gophers (n = 175), rats (n = 16), shrews (n = 1), birds (n = 29), and invertebrates (n = 28). Three pellets were used from each collection visit per box, except for one of the nest boxes where only one pellet was acquired during the second collection period (i.e., when the oldest nestling was 5 to 6 weeks old; see Methods). There was variation across boxes (Supplemental Material Table S1), but the average proportion of gophers was greater than the average proportion of voles and mice for pellets collected during the first collection, when males were doing most or all the prey provisioning (vole: 0.29 ± 0.05 SE; mouse: 0.18 ± 0.05 SE; gopher: 0.42 ± 0.06 SE). For the pooled results from the 5–6-wk collections, when both male and female were provisioning, the average relative amounts were similar, with a slight shift toward more gophers and fewer mice (vole: 0.29 ± 0.04 SE; mouse: 0.14 ± 0.03 SE; gopher: 0.47 ± 0.04 SE).

Under the scenario in which the female contributes 75% of the prey found in pellets in weeks 5–6, reddishness was negatively associated with the amount of voles ($F_{1,27} = 10.92$, $P = 0.03$, $R^2 = 0.29$) and positively associated with the composition of gophers ($F_{1,27} = 4.79$, $P = 0.04$, $R^2 = 0.15$; Fig. 3); there was no significant relationship with mice

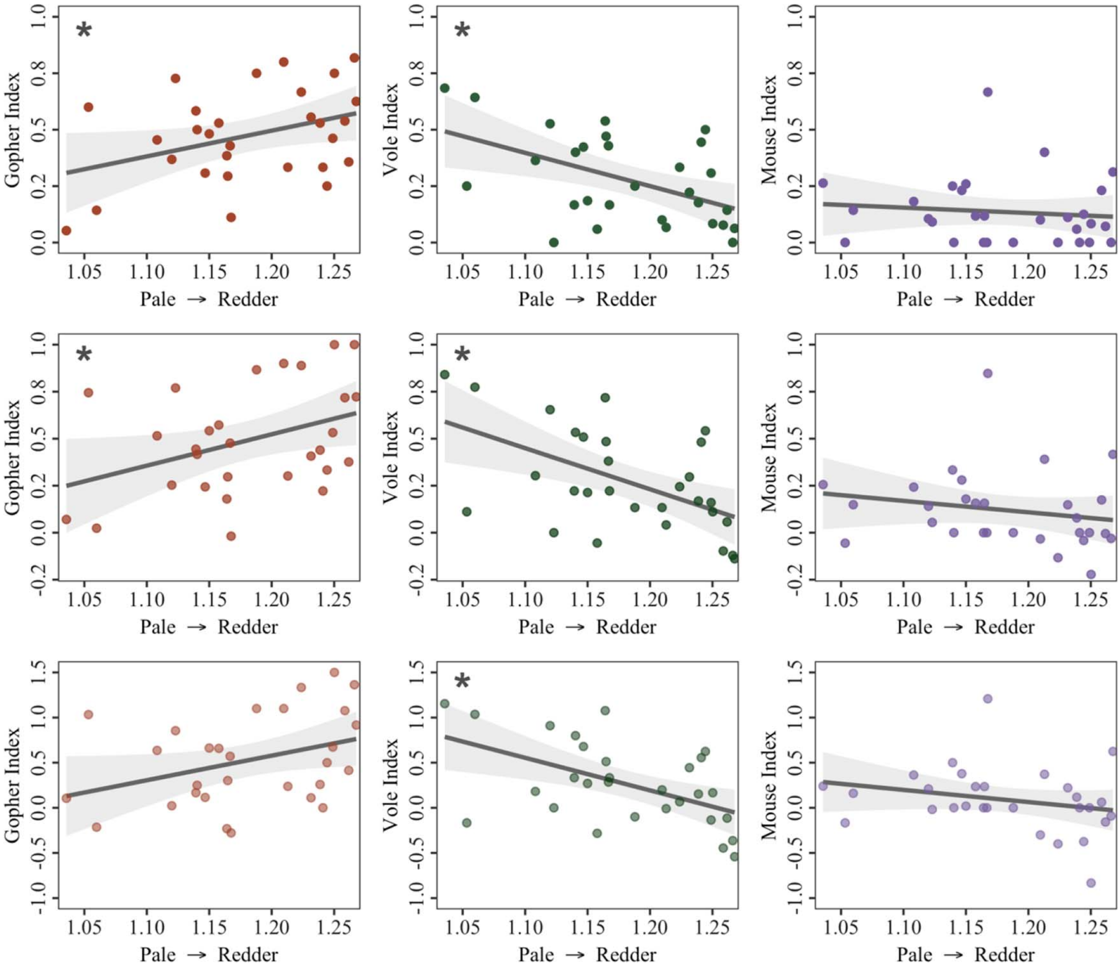

Figure 3. Relationship between female degree of reddishness and an estimated index of the proportion of prey species found in pellets and brought back by females ($n = 29$) in Napa Valley, CA in 2021. Top: Female prey contribution was estimated at 100% (top row)75% (middle row) and 50% (bottom row). Asterisks indicate significance ($P < 0.05$).

($F_{1,27} = 1.1$, $P = 0.3$, $R^2 = 0.04$). The relationships were very similar for the scenario in which the female contributes all the prey found in pellets, with reddishness again negatively associated with voles ($F_{1,27} = 11.39$, $P = 0.002$, $R^2 = 0.36$) and positively associated with gophers ($F_{1,27} = 5.08$, $P = 0.03$, $R^2 = 0.18$; Fig. 3). In the scenario where male and female contribute equally to the prey found in pellets, the relationships between plumage and prey amounts were similar, but only the negative association with voles was significant ($F_{1,27} = 10.28$, $P = 0.003$, $R^2 = 0.28$; Fig. 3).

The number of offspring that reached fledgling age per female ranged from zero to seven, with an average of 3.7 ± 0.3 (SE). The top model predicting the number of fledglings included only the quadratic effect of hatch date, with reproductive success peaking just before Julian day 100 and steadily decreasing thereafter. Confidence intervals (95%) for this effect did not overlap zero, and models containing this effect had a cumulative model weight of >0.99 (Table 3). A competitive model also included a negative effect of reddishness, though this confidence interval overlapped zero. There was very little evidence that female spot diameter was associated with reproductive success; models containing this variable contained only 0.26 of the model weight in the candidate set. There was

Table 3. Candidate generalized linear model set (Poisson distribution) for the effect of plumage reddishness (red to green ratio; R/G), spot diameter, and a quadratic effect of hatch date on the reproductive success (number of fledglings) for 53 female American Barn Owls in Napa Valley, California, in 2021, including number of parameters (*K*), AIC_c, $\Delta\mathrm{AIC}_c$, and model weight (*w*).

Predictors of Female Reproductive Success	*K*	AIC_c	$\Delta\mathrm{AIC}_c$	*w*
poly(hatch day, 2)	3	217.5	0	0.39
R/G + poly(hatch day, 2)	4	218.5	0.97	0.24
spot diameter + poly(hatch day, 2)	4	219.9	2.33	0.12
spot diameter + R/G + poly(hatch day, 2)	5	220.8	3.30	0.08
R/G*grass + poly(hatch day, 2)	6	221.4	3.84	0.06
R/G*forest + poly(hatch day, 2)	6	221.6	4.07	0.05
spot diameter*R/G + poly(hatch day, 2)	6	223.1	5.54	0.02
spot diameter + R/G*grassland + poly(hatch day, 2)	7	223.9	6.35	0.02
spot diameter + R/G*forest + poly(hatch day, 2)	7	224.0	6.45	0.02
Null	1	238.3	20.72	<0.01

also little evidence for reddishness to interact with habitat in an effect on reproductive success.

DISCUSSION

We present the first study to explore the relationships between American Barn Owl plumage, diet, and habitat selection in North America. American Barn Owls in Napa Valley show some evidence for the habitat matching choice hypothesis based on the habitat-associated plumage variation observed in female American Barn Owls. The variation in pheomelanin-based coloration can be observed at various spatial scales in the barn owl group across the world (Roulin et al. 2009), and our work documents variation at a local scale in Napa Valley. Specifically, we found a significant negative relationship between the female's degree of reddishness and the proportion of forests within the owls' hunting radii (2.81 km), suggesting that reddish female American Barn Owls are found in habitats with a lower proportion of forests within their home ranges than their whiter counterparts, as predicted. Additionally, the correlation between degree of reddishness and latitude in Napa Valley, although weak, indicates that females tend to be redder in the southern end of the valley, where the landscapes around vineyards consist mainly of grasslands. There were no significant associations with reddishness and habitat at a nest site scale (75 m), suggesting the habitat matching choice hypothesis operates at larger scales.

Local adaptation is an alternative explanation for correlations between plumage and habitat, but the spatial and temporal scales of our results better align with the habitat matching choice hypothesis. Under the local adaptation idea, the preponderance of a particular morph in a specific environment could accumulate over time due to differential reproduction and survival of morphs depending on their local habitat, rather than resulting from individual habitat choice. However, the landscape in Napa Valley is heterogeneous, and the spatial extent over which we observed variation in plumage and habitat was small, so nest boxes as close as 4 km apart often had markedly different proportions of habitats within their 2.81-km hunting radii. These distances are far less than the typical natal dispersal distance of juvenile American Barn Owls (15–57 km; Marti 1999, Roulin 2020). Therefore, the associations between plumage and habitat we observed are unlikely to have accumulated from dispersing juveniles sharing plumage characteristics inherited from nearby well-matched parents, as would be predicted by the local adaptation hypothesis. In addition, we do not suspect the match we observed between plumage and habitat accumulated over time via survival of older birds with morphs matched to their environment, as would be predicted by the local adaptation hypothesis. For example, some (13%) of the females in this study were in their first or second breeding season, and they also showed similar associations between plumage and land cover variables as did the older adults (signs of 3 of 4 coefficients were same as for older owls), suggesting these younger birds matched their local habitats after dispersing to their first (or second) breeding sites.

Our finding that redder females nested in areas with proportionally less forest reflects the same patterns observed for Western Barn Owls at a local scale in Israel (Charter et al. 2012) and Europe (Dreiss et al. 2012). The observed associations between reddishness and open grassy habitats in Europe and Israel for Western Barn Owls, and in

California for American Barn Owls is fascinating, but their evolutionary underpinning remains unclear: why the owls show these preferences for habitat or the prey located within them, and whether these patterns reflect any adaptive significance is uncertain and awaits further study. However, recent work shows that darker individual Western Barn Owls provide more prey to nestlings during darker nights, suggesting that light conditions in mixed habitat environments may play a role in raptors' hunting behavior (Tate et al. 2016, Passarotto et al. 2018, San-Jose et al. 2019). It is possible that owls of different morphs employ hunting strategies that may provide advantages during different stages of the breeding season. However, because it is hypothesized that Western and American Barn Owls started to diverge in the Late Miocene (~6 million yr ago; Uva et al. 2018), the adaptive significance of these morphs may not necessarily share common evolutionary histories.

Our results suggest some relationships between habitat use, plumage, and diet composition of female American Barn Owls in Napa Valley. However, in contrast to predictions stemming from patterns observed in Western Barn Owls in Europe and Israel, the whiter-breasted females in Napa Valley provided proportionately more voles to their offspring in areas with proportionately more forest within their hunting area. Interestingly, our results also indicated significant opposing relationships between degree of reddishness and proportion of voles versus gophers even though both rodents tend to occupy open grassy habitats (Rodríguez and Peris 2007, Charter et al. 2009, St. George and Johnson 2021). The proportion of mice had no significant relationship with plumage coloration. Thus, the complex relationships between habitat, rodent prey, and owl plumage need additional study to be resolved. For example, there are several factors that were not included in these analyses, most notably, the seasonal fluctuations in rodent populations and the management practices of the different vineyards.

One of the biggest sources of uncertainty in our analyses stemmed from the fact that we could not confirm which parent contributed the prey found within each pellet. Hence, our calculations of changing proportions of pellet composition from when only the male provisioned to when both sexes provisioned provided only a rough index of female-contributed prey. Nonetheless, the analyses of the varying levels of contribution from the mother (50, 75, or 100%) yielded similar conclusions, suggesting relative insensitivity to at least this portion of our analysis. It remains unclear how the owls select for the prey across the landscape, so deploying GPS transmitters on breeding adults may provide further evidence to evaluate predictions of the adaptive habitat choice hypothesis. Future studies should incorporate the use of cameras at the entrance to the nest box to confirm the prey brought back by the female and the male, ideally coupled with GPS trackers with frequent detections to reveal the habitat from which prey were captured (Wood et al. 2021). Among Western Barn Owls, there is no evidence that mates pass off prey to the other to deliver to the nest (Roulin 2020), though whether this is also the case for American Barn Owls is uncertain, and if present would complicate the use of cameras to assign parental prey delivery.

Earlier work points toward a strong genetic inheritance of eumelanic traits in Western Barn Owls (Antoniazza et al. 2010) that may signal physiological benefits. The trait of spot size in females, in particular, has been associated with nestling growth, increased feather quality, resistance to stress, and increased parental care (Almasi et al. 2008, 2010, Dreiss et al. 2010). If this pattern extends to American Barn Owls, we expect the number of fledglings in our study system to be greater for female owls with larger spot diameters. Although the analysis in this study failed to confirm a relationship between reproductive rate and spot size, we recommend further exploration, perhaps including offspring spot size and differences in male versus female nestlings. If owls select habitats that they are best suited for, we expect the reproductive success of redder females to be higher in areas with more grassland, but lower in areas of forest; i.e., we hypothesized an interaction between the effect of reddishness and habitat on reproductive success. However, we found little evidence for any effect, additive or interactive, with plumage and habitat on reproductive success. This contradicts previous work with Western Barn Owls in Europe and Israel showing that white and redder females had similar levels of reproductive success when they bred in their respective preferred habitats (Charter et al. 2012, Dreiss et al. 2012). Earlier work on Western Barn Owls also shows that degree of reddishness correlates with sociality, dispersal, and phenology. These behaviors can be expressed via huddling in nestlings, in which redder owls generally tend to be more social than whitish owls. Redder Western Barn Owls also disperse farther than their whiter counterparts (van den Brink et al. 2012, Roulin 2013), and on average, reddish females in Switzerland reproduce later in the season (Roulin and Altwegg 2007). This can become important as owls respond to the seasonal fluctuations in rodent prey populations, where in some cases the number of offspring can serve as an indicator of those populations (Horváth et al. 2020). Whether these patterns also occur in American Barn Owls remains unresolved.

Our results also confirm that the timing of breeding in American Barn Owls contributes to the successful fledging of offspring, with early and late nests resulting in fewer offspring than those with intermediate hatch dates. As extreme weather events increase in the coming years, it will be particularly important to account for abiotic factors that may induce early fledging and potentially endanger offspring during the post-fledging period, and how these patterns may vary among morphs (Romano et al. 2019). In addition to increasing the sample size, future work should aim to quantify and incorporate the eumelanic characteristics of nestlings into analyses.

Conclusions. This is the first study to examine the relationship between plumage, habitat, diet, and reproductive success of American Barn Owls in North America. We found evidence consistent with the habitat matching choice hypothesis that birds in the barn owl group, particularly females, choose particular habitats correlated to their plumage. Across this landscape, as in Europe and Israel, redder female barn owls nested in areas with proportionately less forest, while their whiter counterparts used more forested areas. Our study also suggested plumage-biased foraging by females. Though we expected whiter females to provide more mice than voles and gophers, our results suggested that they provided a greater proportion of voles to offspring. Thus, there appears to be a difference in foraging between morphs in our study system that does not align with what has been shown for Western Barn Owls in Europe and the Middle East. Whether this reflects a difference in prey availability due to habitat or a behavioral effect of certain morphs targeting particular prey is unknown. This may have relevance for the delivery of pest control on farms because certain rodent species are more problematic in some crops than in others, and our results suggested owls with different plumages capture, to some degree, different prey. Evidently, polymorphism has persisted in this population, but it remains somewhat unclear how the various morphs perform in this heterogeneous, yet predominantly agricultural environment.

SUPPLEMENTAL MATERIAL (available online). Table S1. Estimated indices of main prey (n = 529) found in dissected pellets that were contributed by Napa Valley female American Barn Owls (n = 29) in 2021 with three different scenarios that correspond to proportion of prey brought back to nest by female.

ACKNOWLEDGMENTS

This research would not have been possible without the collaboration of more than 60 vineyards in Napa Valley that welcome both barn owls and biologists on their land. Special thanks to I. Jeremaz of Grgich Hills, J. Johnson of Tres Sabores, and K. Bellair of Honig. Analyses would not have been completed without the generous contribution of P. Csernica. We thank A. Arreguin, M. Bach, K. Berger, D. Blackstone, J. Burroughs, K. Crouch, N. Derrik, A. Gebbing, K. Gerstenberg, J. Gerstenberg, P. Kazemi, K. Larson, V. Lawton, H. Lee, M. Lehman, L. Leuty, E. Luttrell, N. Morales, J. Ormiston, E. Palomares, V. Ramirez, A. Przecha, A. Rashe, C. Silva, C. Somerville, P. Stiles, A. Vargas, S. Vaughan, and W. Vickers for assisting with pellet dissections. Comments on an earlier draft of this report were provided by B. Clucas and J. Black. Funding was provided by the US Department of Agriculture's Hispanic Serving Institution program, and the California State University's Agricultural Research Institute.

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Received 14 June 2024; accepted 14 April 2025
Associate Editor: Christopher W. Briggs

APPENDIX

BARN OWL FRAME USED FOR ANALYSES

We photographed 53 female and 26 male adult American Barn Owls (five unknown sex adults were omitted from all analyses). Photographs were taken after all morphometrics were recorded and before aging under an ultraviolet (UV) light. When adults were captured during daylight, the owls were placed under a shaded area to avoid overexposed images. If capture took place after sundown, we turned off all nearby light sources (vehicle headlights or headlamps). The owl was placed on the ground, its legs bound by a red cloth and its face hooded with a knitted covering. An identification card with a premeasured scale bar 2 cm long was placed next to the owl around the same height as the breast. When capturing the photo, a digital single-lens reflex camera was mounted on a tripod and placed directly over the owl's ventral aspect (Fig. A1).

After importing the photo into MATLAB, we selected the length of the 2 cm scale bar to tell the program how many pixels made up the specified length. The 60 × 40 mm area used to measure the plumage characteristics was selected by choosing a center point along the upper ventral aspect as represented by the red dot and rectangle shown on Fig. A1. Once the program cropped the image around the center point (Fig. A2a), spots were selected and excluded from RGB measurement (Fig. A2b), followed by measuring the maximum vertical dimension of each spot (referred to as "diameter" to be consistent with Charter et al. 2012).

Figure A1. Raw photograph of male Barn Owl's ventral aspect. The rectangle corresponds to the 60 × 40 mm area used to measure red, green, and blue (RGB) values; count the number of spots; and measure the maximum vertical dimension of each spot (referred to as "diameter").

Figure A2. (a) Cropped 60 × 40 mm area of male Barn Owl's ventral aspect. (b) Spots (white polygons) selected for counting and measuring.