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Genetic Confirmation of Predation of an Adult Female Eastern Spotted Skunk by a Barred Owl

Kendyl N. Hassler[1,3], Brin E. Kessinger[2], Caroline E. Harms[2], Lucas E. Price[2], Ethan P. Barton[3], Kevin J. Oxenrider[3], Rich E. Rogers[3], Kelly J. Pearce[4], Thomas L. Serfass[1,*], and Amy B. Welsh[2]

Abstract - Avian predation is a primary cause of mortality for *Spilogale putorius* (Eastern Spotted Skunk) and is generally attributed to *Bubo virginianus* (Great Horned Owl). We report the first confirmed avian predation of an Eastern Spotted Skunk by a *Strix varia* (Barred Owl). The radio-collar of an adult female Eastern Spotted Skunk was recovered alongside an owl pellet containing Eastern Spotted Skunk remains in eastern West Virginia. DNA sampled from the surface of the pellet was amplified, sequenced, and compared to known Barred Owl and Great Horned Owl sequences. Differences in 2 single nucleotide polymorphisms confirmed a Barred Owl produced the pellet. Our findings show Barred Owls are at least occasionally an avian predator of this species in eastern forest ecosystems.

Introduction

Spilogale putorius (L.) (Eastern Spotted Skunk) populations began declining across their range in the early 1940s (Gompper and Hackett 2005). Knowledge of the species' natural history prior to the decline is poorly understood and has led to many mutually non-exclusive hypotheses for the decline. These hypotheses include historical overharvest, synthetic pesticide use, large-scale habitat alteration, shifts in predator communities, disease, and/or natural population dynamics (Gompper 2017, Gompper and Hackett 2005, Gompper and Jachowski 2016). Several studies have been initiated to better understand the natural history of Eastern Spotted Skunks. However, research or observational information pertaining to cause-specific mortality for the species remains limited.

Just prior to the species' initial decline, mortalities of Eastern Spotted Skunks in an agricultural area in Iowa were reported to be primarily human-related (96% of 77 mortalities) through fur trapping, nuisance killings, predation by domestic pets, and road collisions (Crabb 1948). Today, most states within the species' historical range recognize the Eastern Spotted Skunk as a species of conservation concern, and several do not allow the species to be harvested (Eastern Spotted Skunk Cooperative Study Group 2020).

The only comprehensive analysis on cause-specific mortality of Eastern Spotted Skunks since the species' initial decline was conducted in the Ouachita Mountains

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of Arkansas, where the primary predator of Eastern Spotted Skunks was reported to be *Bubo virginianus* (J.F. Gmelin) (Great Horned Owl; Lesmeister et al. 2010). That study reported most Eastern Spotted Skunk mortalities were caused by predation (89% of 19 mortalities, 11% unknown cause of mortality), of which 71% were attributed to Great Horned Owls and 29% to various mammalian predators (Lesmeister et al. 2010).

There is little direct evidence attributing Eastern Spotted Skunk mortalities to a specific predator. Instead, most information on mortality is inferred from clues at the recovery site (e.g., perching branch or excrement from a predatory bird) and known predators in the area (Lesmeister et al. 2010). Avian predation is typically attributed to Great Horned Owls because of previous reports of Eastern Spotted Skunks in the Great Horned Owl's diet (Errington et al. 1940, Marti 1974), and the Great Horned Owl's use of a broad range of habitats across the United States (Johnsgard 1988). Although not previously shown to prey on Eastern Spotted Skunks, *Strix varia* Barton (Barred Owl) overlaps in range with the Eastern Spotted Skunk, uses a variety of habitats, including upland forested habitats (Johnsgard 1988), and has been recorded opportunistically taking prey similar in size to Eastern Spotted Skunks (Crabb 1944, Errington 1932, Livezey 2007).

We report on factors associated with the mortality of an Eastern Spotted Skunk that was being monitored via radio-telemetry in eastern West Virginia. We were motivated to evaluate the Barred Owl as a potential predator of this Eastern Spotted Skunk after observing Barred Owls in the vicinity of the recovery site of the individual's radio-collar and an owl pellet. Herein, we provide the first evidence of predation on an Eastern Spotted Skunk by a Barred Owl and the genetic approach used to confirm the predator as a Barred Owl using DNA extracted from the owl pellet. To provide geographic context for the predation event, we also provide the home-range estimates of the predated individual.

Field-site Description

Our study took place on Nathaniel Mountain Wildlife Management Area (WMA; 4496 ha) in Hampshire and Hardy counties, WV (39°11'26.10"N 78°49'16.40"W; Fig. 1). Mountain ridges in the WMA are narrow and northeast–southwest ranging with dry, steep slopes. Steep side-slopes of secondary ridges drain to form several ephemeral streams and a perennial stream, Mill Run. Emergent sandstone and limestone outcrops are common along ridges, and talus is common in drainages. Elevations on the WMA vary from 300 m along Mill Run to 935 m on ridge tops. The WMA is primarily forested (95%) and dominated by mixed *Quercus* spp. (oak)–*Carya* spp. (hickory) and mixed oak–*Pinus* spp. (pine) forest stands. Ericaceous shrubs such as *Kalmia latifolia* L. (Mountain Laurel) and *Vaccinium* spp. (blueberry) are common understory species (West Virginia Division of Natural Resources, South Charleston, WV, unpubl. report).

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Methods, Results, and Discussion

On 15 March 2018, we fitted a live-trapped adult female Eastern Spotted Skunk (weight = 375 g) with a very high frequency (VHF) radio collar (Model M1740 [16 g]; Advanced Telemetry Systems, Inc., Isanti, MN) equipped with an 8-hr motion-sensitive mortality sensor as part of a study on den-site selection by Eastern Spotted Skunks (Hassler 2020). Protocols for live-trapping, radio-collaring, and radio-tracking followed guidelines of the American Society of Mammalogists (Sikes et al. 2016) and were approved by the Frostburg State University Institutional Animal Care and Use Committee (Approval #A2018-004) and the West Virginia Division of Natural Resources.

We located by homing (i.e., following VHF signals to the den/resting site) the day-time rest sites of the Eastern Spotted Skunk at least weekly for ~10 months after affixing the radio-collar, except for 7 weeks between 25 August and 18 October 2018 because of transmitter failure (we trapped and recollared the skunk [weight at recapture = 505 g] on 18 October 2018), and for 3 non-consecutive weeks in winter when heavy snowfall and ice prevented access to the study area. We calculated 50% and 95% kernel density estimate (KDE) home ranges from rest-site locations using the plug-in method in the 'ks' package in Program R version 3.6.1 (Duong 2020, R

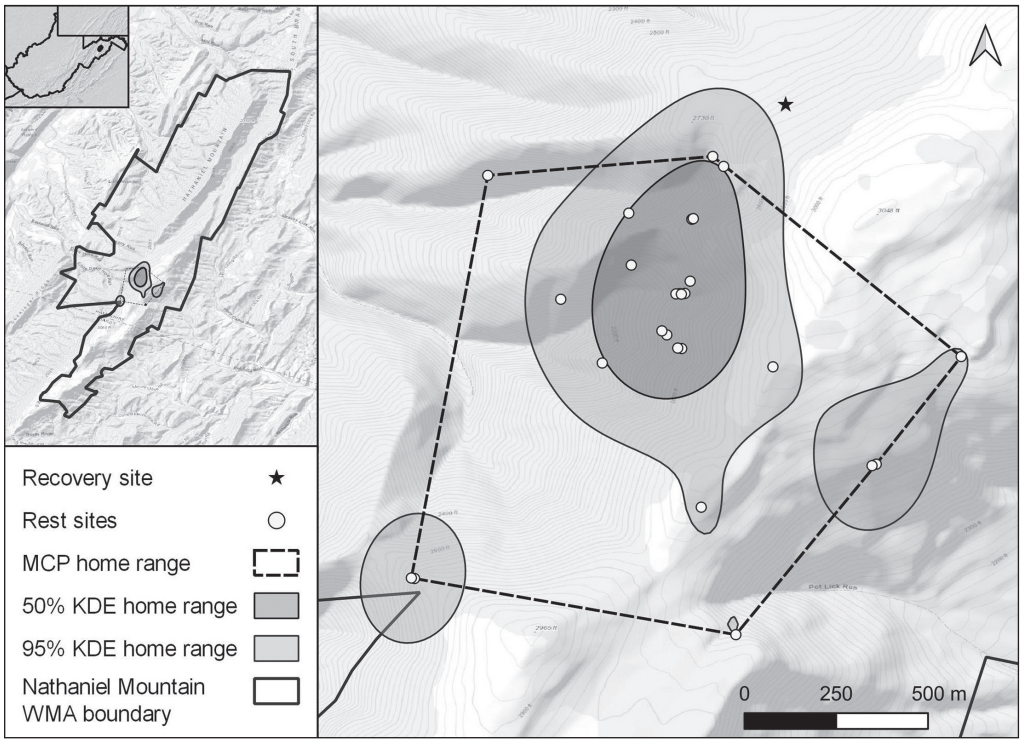

Figure 1. The 50% and 95% kernel density estimate (KDE) and 100% minimum convex polygon (MCP) home-range estimates and post-mortality recovery site of a female Eastern Spotted Skunk tracked by telemetry to day-time rest sites from March 2018 to February 2019 on Nathaniel Mountain Wildlife Management Area, WV.

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Core Team 2019, RStudio Team 2019) following guidelines by Walter and Fischer (2016). We also calculated the 100% minimum convex polygon (MCP) home range using the 'adehabitatHR' package to ensure compatibility to future studies (Calenge 2006). We mapped the Eastern Spotted Skunk's home range using QGIS version Bucureşti 3.12 (QGIS Development Team 2020).

We located 25 unique den sites from 39 relocations, from which we estimated the Eastern Spotted Skunk's 50% KDE, 95% KDE, and 100% MCP home range to be 27 ha, 102 ha, and 171 ha, respectively (Fig. 1). Although we were unable to monitor this individual continuously during all seasons, the 95% KDE home range was within the seasonal home-range estimates (54–135 ha) of female Eastern Spotted Skunks (Lesmeister et al. 2009).

On 24 January and 6 February 2019, we located the signal of the Eastern Spotted Skunk's radio-collar in a *Quercus montana* Willd. (Chestnut Oak) snag with a broken top (height = 4.5 m and diameter at breast height = 39 cm), during which time the radio-collar was not emitting a mortality signal. On 27 February, we located the individual in the same location emitting a mortality signal. We recovered the radio-collar and an owl pellet containing 2 Eastern Spotted Skunk mandibles and several cranial fragments (Fig. 2), from a shallow depression in the top of the snag. We referenced measurements, illustrations, and descriptions of Eastern Spotted Skunk skulls to confirm our identification (Van Gelder 1959). No cavities for denning were found upon further inspection of the snag, likely signifying the individual was deceased prior to 24 January, although the radio-collar was not emitting a mortality signal. We calculated distance from the recovery site to the nearest known rest site and to the nearest

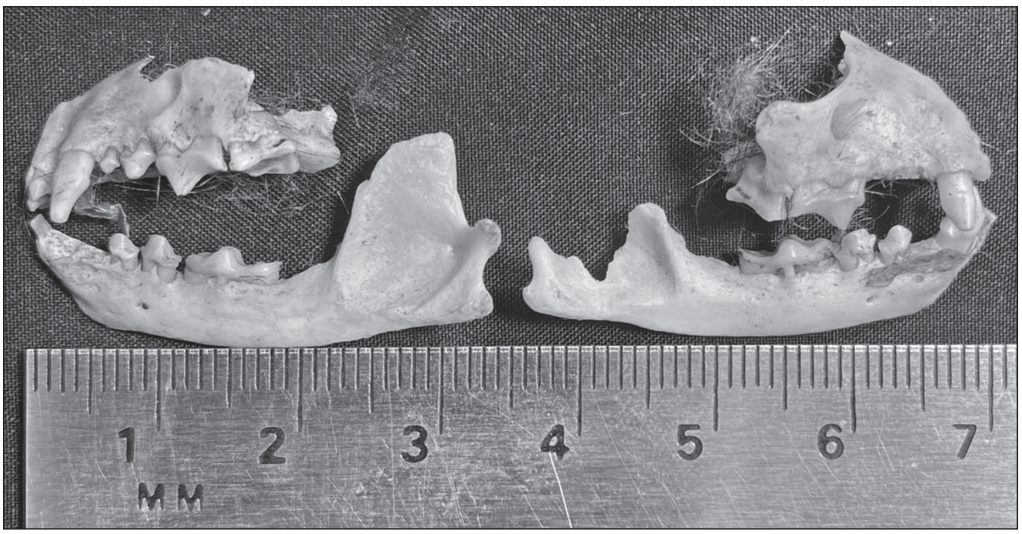

Figure 2. The mandibles and cranial fragments of an Eastern Spotted Skunk recovered from a Barred Owl pellet. We referenced measurements, illustrations, and descriptions of Eastern Spotted Skunk skulls to confirm our identification (Van Gelder 1959). The owl pellet was recovered alongside the radio-collar of a deceased female Eastern Spotted Skunk tracked by telemetry from March 2018 to February 2019 on Nathaniel Mountain Wildlife Management Area, WV.

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home-range boundary using the 'Measurement tool' with feature snapping enabled in QGIS version București 3.12 (QGIS Development Team 2020).

The recovery site of the radio-collar and the owl pellet were outside the boundaries of both MCP and 95% KDE home-range estimates calculated with prior point locations. Distances from the recovery site to the nearest known day-time rest site, which occurred along the MCP home-range boundary, and to the 95% KDE home-range boundary were 269 m and 62 m, respectively. Because of the proximity of the recovery site to the estimated home-range boundary, the Eastern Spotted Skunk may have been preyed upon within the home-range boundary and subsequently carried by the predator to the recovery site.

We observed Barred Owls in the vicinity of the recovery site on several occasions during this study, including on the day the owl pellet was recovered, prompting us to consider a Barred Owl as the potential predator. To determine which owl species produced the owl pellet, we extracted genomic DNA from the pellet following the protocol of Wasser et al. (2012). After dissecting the pellet to identify prey remains, we swabbed the exterior of the remains with 4 buccal swabs (Puritan® Cap-Shure® Sterile Cotton Tipped Applicators with Aerated Tip Protector; Puritan Medical Products, Guilford, ME) saturated with 1X PBS buffer, rotating the applicators as we swabbed the entire surface for mucosal cells (Ball et al. 2007). We then placed the applicators in 1.5-ml microcentrifuge tubes with 500 µl ATL lysis buffer (Qiagen Inc., Valencia, CA). We left the swabs overnight at room temperature and extracted DNA using a modified version of Qiagen's DNeasy Tissue DNA (Hilden, Germany) extraction protocol where 20 µl DTT was added and DNA was eluted in 200 µl AE buffer. In addition to sampling the owl pellet, we extracted DNA from known blood and feather samples from 5 owls being rehabilitated by the Avian Conservation Center of Appalachia in Morgantown, WV (all from West Virginia: 3 Barred Owls and 2 Great Horned Owls) to serve as a reference to compare with the unknown pellet sample. The known samples included feathers from 1 Great Horned Owl, blood from 1 Great Horned Owl, feathers from 1 Barred Owl, and blood from 2 Barred Owls. We extracted DNA from known Barred Owl and Great Horned Owl samples after the pellet sample to avoid contamination. We extracted these 5 known samples using Qiagen's DNeasy Blood and Tissue kit, following the nucleated blood protocol for the blood samples and adding 220 µl PBS to the digestion step. We modified the tissue extraction protocol for the feather digestion by including 20 µl DTT.

We used a primer set designed to amplify a ~90-base pair segment of the 16S mitochondrial region for all bird species. We designed this primer set by aligning bird and mammal mitochondrial DNA using BioEdit (version 7.2.6; Hall 1999) and manually identifying DNA regions that were conserved among bird species, but different from mammal species. Primers were designed to anneal to avian conserved regions. Components of polymerase chain reactions (PCR) consisted of 2X QIAGEN multiplex PCR master mix, 0.6 10-µM forward and reverse primer (16S-AVES1F: 5'-GGT AGC GCA ATC AAT TGT CCC AT-3'; 16S-AVES1R: 5'-CCA CAG GGT CTT CTC GTC TTA TG-3'), and up to 30 ng DNA for a final volume of

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24 µl. We amplified the 16S region with an initial denaturation stage of 95 °C for 3 min followed by 35 cycles of 94 °C for 30 sec, 57 °C for 1.5 min, and 72 °C for 1 min, then a final extension of 72 °C for 15 min. We performed PCR on DNA extracted from the pellet swabs, known samples, and PCR negative controls. The pellet swab that successfully amplified was run 2 additional times. We cleaned the resulting PCR products using the GeneJET PCR Purification Kit (Thermo Fisher Scientific, Waltham, MA). We sequenced the amplified mitochondrial DNA using the reverse primer with the ABI BigDye V3.1 (Applied Biosystems, Foster City, CA) sequencing chemistry and cleaned it with Sephadex G-100 DNA Grade to remove unincorporated dye terminator. We conducted capillary electrophoresis on the ABI 3130XL Genetic Analyzer. We aligned the obtained sequences using BioEdit (version 7.2.6; Hall 1999). We also searched the pellet sample sequence against the National Center for Biotechnology Information (NCBI) nucleotide database (Altschul et al. 1990).

PCR amplification and sequencing were only successful for 1 of the 4 pellet swab samples from which DNA was extracted. We obtained 4.2 ng/µl of DNA from this swab sample and <1.5 ng/µl of DNA for the other 3 samples. No sequences were obtained from the negative controls, indicating no contamination. We identified 2 single nucleotide polymorphisms (SNPs) that appeared to be diagnostic between Barred Owls and Great Horned Owls within the amplified region of DNA from known genetic samples (Table 1). The pellet sample sequence matched the

Table 1. Sequence alignment of the 16S mitochondrial region for the unknown bird DNA from the owl pellet swab, 3 Barred Owls (BDOW), 2 Great Horned Owls (GHOW), and 3 sequences from GenBank identified by their accession numbers. Bases in bold indicate differences from the unknown sequence and the underlined bases are the 2 diagnostic single nucleotide polymorphisms. Superscripted letters indicate origin of reference samples: [A]West Virginia, [B]Ohio, [C]Georgia, and [D]unknown location.

Sample	Sequence
Owl pellet swab	ATCAATTTCACTGATTATCTGTAAGAGACAGTTAAGGCCTCGTTTAG CCATTCATACAGGTCCCGATTTATGGGACAATTGATTGCGCTACCA
BDOW 1[A]	ATCAATTTCACTGATTATCTGTAAGAGACAGTTAAGGCCTCGTTTAG CCATTCATACAGGTCCCGATTTATGGGACAATTGATTGCGCTACCA
BDOW 2[A]	ATCAATTTCACTGATTATCTGTANGAGACAGTTAAGGCCTCGTTTAG CCATTCATACANGTCCCGATTTATGGGACAATTGATTGCGCTACC -
BDOW 3[A]	ATCAATTTCACTGATTATCTGTAAGAGACAGTTAAGGCCTCGTTTAG CCATTCATACAGGTCCCGATTTATGGGACAATTGATTGCGCTACC -
GHOW 1[A]	- - - - - TTTCACTGATTATCTG**C**A**G**GAGACAGTTAAG**A**CCTCGTTTAG CCATTCATACA**A**GTC**T**CGATTTATGGGACAATTGATTGCGCTACCA
GHOW 2[A]	ATCAATTTCACTGATTATCTGTAAGAGACAGTTAAG**A**CCTCGTTT**G**G CC**G**TTCATACAGGTC**T**CGATTTATGGGACAATTGATTGNGCTACC-
MF431745.1 BDOW[B]	ATCAATTTCACTGATTATCTGTAAGAGACAGTTAAGGCCTCGTTTAG CCATTCATACAGGTCCCGATTTATG**A**GACAATTGATTGCGCTACC-
KM042913.1 BDOW[C]	ATCAATTTCACTGATTATCTGTAAGAGACAGTTAAGGCCTCGTTTAG CCATTCATACAGGTCCCGATTTATGAGACAATTGATTGCGCTACC-
AF173593.1 GHOW[D]	ATCAATTTCACTGATTATCTGTAAGAGACAGTTAAG**A**CCTCGTTT**G**G CC**G**TTCATACAGGTC**T**CGATTTATGGGACAATTGATTGCGCTACC-

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known Barred Owl samples for these 2 SNPs. NCBI BLAST results also identified the pellet sample as a significant 98.86% identity match to 2 Barred Owl samples (Accession nos. MF431745.1 [from Ohio], KM042913.1 [from Georgia]) and a 95.45% identity match to a Great Horned Owl sample (Accession AF173593.1 [unknown origin]). The NCBI Barred Owl sequences also matched the pellet sample at the 2 potentially diagnostic SNPs, whereas the NCBI Great Horned Owl sequence did not, which provides further evidence that these SNPs can be used to differentiate the 2 species (Table 1). Therefore, we conclude that a Barred Owl predated on the Eastern Spotted Skunk.

We provide evidence that our primer set can be used to differentiate Great Horned Owl and Barred Owl genetic samples in the region where samples were collected. However, reference samples in this study were from a limited number of individuals (n = 8) and a limited geographic range (West Virginia, Ohio, Georgia, and 1 unknown origin), therefore we cannot conclude that these SNPs would be diagnostic over the entire range of either owl species. Hence, these potentially diagnostic SNPs should be confirmed using samples from different geographic regions.

Our report provides evidence that Barred Owls are capable of successfully preying upon female Eastern Spotted Skunks. We do not know the physical condition and health of the Eastern Spotted Skunk when the predation event occurred. However, she appeared to be in good physical condition when live-captured in October 2018 to replace the radio-collar. Her weight increased from 375 g at the initial capture in March 2018 to 505 g in October 2018. The final known weight was typical of reported weights of female Eastern Spotted Skunks (482–539 g; Crabb 1944). We are unsure if Barred Owls will attempt to prey upon male Eastern Spotted Skunks, which are typically larger than females and weigh 652–737 g on average (Crabb 1944).

The predation of this Eastern Spotted Skunk occurred in late winter when overhead deciduous leaf cover was reduced and risk of predation may be increased. Eastern Spotted Skunks are reported to have a higher risk of mortality in the fall, likely because of changes in overhead leaf cover, and when travelling through suboptimal habitat (Gompper 2017, Lesmeister et al. 2010). For other small carnivores, like *Mustela erminea* L. (Short-tailed Weasel) and *M. frenata* Lichtenstein (Long-tailed Weasel), avian predation is most common in the winter (Linnell et al. 2017).

Our findings are limited to 1 observation. Therefore, we do not know with what regularity Barred Owls may attempt to prey upon Eastern Spotted Skunks. In the Pacific Northwest, remains of *Spilogale gracilis* Merriam (Western Spotted Skunk) and *Mephitis mephitis* (Schreber) (Striped Skunk) were not detected among nearly 5000 prey remains from over 1300 Barred Owl stomachs examined (Baumbusch et al. 2020). However, Barred Owls in their study frequently smelled of skunk odor, suggesting these birds may have previously attempted to prey on skunks (Baumbusch et al. 2020).

Previous reports of avian-caused mortality of Eastern Spotted Skunks have been attributed to Great Horned Owls (Errington et al. 1940, Lesmeister et al. 2010). Our

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findings show that Barred Owls may at least occasionally play a role as an avian predator of this species in eastern forest ecosystems.

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