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Cover Photograph: Artificial bat roosts at Warbler Ridge Conservation Area. A Hoary Bat was genetically identified from guano collected underneath the roost nearest to the camera. © Ricky Gieser.

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Hoary Not Sticking to the Story: Evidence of Hoary Bats (*Lasiurus cinereus*) Using Artificial Roosts in Illinois

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Abstract - Artificial roosts are a commonly used tool in bat conservation to supplement or replace natural habitat features. During routine surveys, we collected guano from directly underneath several artificial roost structures from 2 sites in Illinois. Genetic sequencing of the mitochondrial COI gene revealed several guano samples were from *Lasiurus cinereus* (Hoary Bats), in addition to expected species like *Eptesicus fuscus* (Big Brown Bat), *Myotis sodalis* (Indiana Bat), and *Nycticeius humeralis* (Evening Bat). Our repeated findings of Hoary Bat guano at artificial roosts were unexpected, because the species is believed to roost primarily in foliage. Detection of Hoary Bat DNA hints at the use of artificial roosts by this species in some fashion. Our results highlight the utility of genetic tools in uncovering cryptic behaviors and raise new questions about Hoary Bat behavioral ecology.

Anthropogenic change has resulted in numerous threats to biodiversity, with habitat loss among the most detrimental (Mantyka-Pringle et al. 2012, Sih et al. 2000, Swift and Hannon 2010). The loss of habitat has precipitated the development of novel conservation strategies to expand habitat opportunities and promote biodiversity. In some cases, this includes the installation of artificial habitat structures to promote survival, growth, reproduction, and abundance (Watchorn et al. 2022). Roosting structures for bats can vary widely by species and purpose (Kunz 1982). Generally, bats select roosts to protect themselves from environmental conditions. While some species may form large maternity colonies in summer, other species roost solitarily or in small groups (Klug et al. 2012, Mering and Chambers 2014). Males of many species tend to be found individually or in small groups during the summer (Fabianek et al. 2015). Bats can also rest between bouts of foraging in nocturnal roosts that are used for a shorter period than diurnal roosts (Ormsbee et al. 2007).

Artificial roosts have become a common strategy for conserving bats (Mering and Chambers 2014). The multi-chambered box, rocket box, and artificial bark are frequently used styles in North America (Mering and Chambers 2014). Success of artificial roosts depends on various factors including structure design, placement, and microclimate (Crawford and O'Keefe 2024). In Illinois, crevice-roosting bats, such as *Eptesicus fuscus* (Palisot de Beauvois) (Big Brown Bats), *Nycticeius humeralis* (Rafinesque) (Evening Bats), and bats in the genus *Myotis*, are common occupants of artificial roosts (Rueegger 2016), while foliage-roosting bats in the genus *Lasiurus* are not known to use artificial roosts.

Lasiurus cinereus (Palisot de Beauvois) (Hoary Bat) is a large-bodied, migratory, and solitary bat, with a native range extending across much of North America. These bats are not obligate foliage roosters, as they opportunistically roost in many microhabitats, including woodpecker holes (Cowan and Guiet 1965), squirrel nests (Neill 1952), the sides of buildings (Bowers et al. 1965), bridges (Hendricks et al. 2005), and even on a car door window

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(iNaturalist 2026). There are no published observations of Hoary Bats using artificial roosts, but Wilhelm (2012) detected Hoary Bat DNA from guano collected underneath 2 artificial bark structures in Arizona.

The Illinois Bat Conservation Program has been collecting information on roosts since 2016, including data on artificial roosts, natural roosts, and buildings used by bats. Recent efforts include collecting guano samples from underneath artificial roosts for genetic identification of the species that use these structures, while minimizing disturbance to bats. In this study, we present novel evidence from these surveys that suggests Hoary Bats occasionally use artificial roosts in eastern Illinois.

Field-site Description. We visited 2 sites with artificial bat roosts in this study, Embarras River Bottoms State Habitat Area (ERBSHA) and Warbler Ridge Conservation Area (WRCA), located about 100 km apart in east-central Illinois. Their habitats are a mix of restored forest and prairie, and ERBSHA is frequently subjected to flooding, as it is located at the confluence of the Embarras and Wabash Rivers. The artificial roosts are affixed to free-standing poles installed in prairies along forest edges, but are located a few meters from the tree line with no overhanging tree branches, thus allowing the structures to receive full sun for most of the day. At ERBSHA, the artificial roosts are all BrandenBark™, a simulated bark made from polyurethane (Copperhead Environmental Consulting 2026), and at WRCA, they are a mix of BrandenBark™ and rocket boxes.

Methods. Individuals from the Illinois Bat Conservation Program and the Illinois Department of Natural Resources collected fecal samples from underneath 13 individual free-standing artificial bat roosts at ERBSHA and at WRCA between 2022 and 2023. We collected the samples by picking up a single piece of guano from directly underneath the artificial roost with sterile forceps and placing the pellet into a collection tube filled with silica beads to preserve the DNA present (Corthals et al. 2015). At ERBSHA, we collected guano from a permanent device made of polyvinylchloride pipe and window screen, while at WRCA, we collected samples directly from the ground. We collected 5 guano samples from ERBSHA on 16 August 2022 and 12 samples on 13 July 2023. All ERBSHA samples were from the same 5 BrandenBark™ roosts. At WRCA we collected 8 samples from 8 roosts, of which 4 were BrandenBark™ and 4 were rocket boxes, on 19 September 2023. During the second collection event at ERBSHA, if an abundance of guano was present underneath a roost or if the guano appeared to be degraded, we collected more than 1 sample per roost for redundancy. For some sites, we also counted bats that emerged from the roosts, starting 30 min before sunset and ending 1 h after sunset. In 2024, we used a borescope (Depstech, WF010-3.5M, Shenzhen Deepsea Innovation Technology Company, Shenzhen, China) to try to identify which species were diurnally occupying the structures.

We used established genetic tools to analyze fecal samples at the Collaborative Conservation Genomics Laboratory of the Illinois Natural History Survey. We extracted total genomic DNA from individual guano pellets using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), with minor modifications, including increasing the dose of proteinase K to 40 µL and lysing for 24 h, adding an overnight refrigeration step, and warming the AE buffer to 70°C prior to elution in 20 µL of AE buffer. We used polymerase chain reaction (PCR) to amplify a region of cytochrome oxidase I (COI), using the Species from Feces SFF_145f and SFF_351r COI primers (Walker et al. 2016). We ran PCR twice to produce clear gel electrophoresis bands.

The first PCR was composed of 25-µL reactions, including 5 µL nuclease free water, 12.5 µL GoTaq Colorless Master Mix (Promega Corp., Madison, WI), 1 µL SFF_145f, 1 µL SFF_351r, 0.5 µL BSA, and 5 µL template DNA. Thermal cycling included an initial

denaturation step of 95°C for 2 min, followed by 35 cycles of 95°C for 45 sec, 51°C for 30 sec, and 72°C for 30 sec. A final extension step of 72°C for 5 min concluded the cycling. The PCR was run a second time with each 25-µL reaction composed of 7.5 µL nuclease free water, 12.5 µL GoTaq Colorless Master Mix, 1 µL SFF_145f, 1 µL SFF_351r, and 2 µL template DNA produced during the first PCR amplification. Thermal cycling was as described for the first PCR amplification again. We used ExoSAP-IT Express PCR Product Cleanup Reagent (Thermo Fisher, Waltham, MA) to clean and purify the second PCR product samples prior to sequencing.

We sent the samples to the Roy J. Carver Biotechnology Center at the University of Illinois Urbana-Champaign for Sanger Sequencing. We trimmed reads with Geneious Prime 2022.0.2 (https://www.geneious.com; Kearse et al. 2012) and assessed species identification with NCBI's Nucleotide Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990). Reads were targeted to the COI region and were compared against the GenBank database (ncbi.nlm.nih.gov/genbank). We based identification on pairwise identity, query coverage, E-value, and bit score. We considered matches with ≥98% pairwise identity and >95% query coverage as strong species-level matches. In cases for which identity values fell slightly below this threshold, consistent top hits across all high-scoring pairs, that showed the same species among the top 100 results, were used to support identification. We confirmed identifications only when top hits consistently aligned with a single species across multiple accessions.

Results. The most common species identified from guano samples was the Big Brown Bat, occurring at multiple roosts at both field sites (Table 1). *Myotis sodalis* Miller and Allen (Indiana Bat), an endangered species, was detected once at both sites. Guano from Evening Bats was collected only at WRCA. Hoary Bats were identified 5 times from 4 separate roosts across all 3 collection events, with Roost 16-01 at ERBSHA having a detection in both years (Table 1). All detections of Hoary Bats were made from feces collected at BrandenBark™ structures, Evening Bats from only rocket boxes, and Big Brown Bats and Indiana Bats from both BrandenBark™ and rocket boxes.

Efforts to gain additional information included the use of a borescope and emergence counts. After collecting guano at ERBSHA on 13 July 2023, the field team conducted an emergence count at Roost 16-01, where Hoary Bats were detected in 2022 and 2023. No bats emerged from this roost. In 2024 at WRCA, borescoping occurred on 17 May and 19 August at 2 roosts accessible with the equipment, including North Roost 1 (where a Hoary Bat was detected in 2023) and North Roost 2, both of which are Brandenbark™. Borescope videos showed only Big Brown Bats present in these roosts.

All DNA samples produced high-quality COI sequences that were suggestive of their respective bat species, including Hoary Bat, aside from 1 sample that yielded no result due to poor-quality DNA that could not be matched to a species identification. All BLAST results showed high identity scores, with the top matches for each sample consistently aligning to its identified species across all high-scoring pairs. Two sequences exhibited 100% pairwise identity and 100% query coverage (Table 2), confirming species-level identification with high confidence. Two additional sequences showed slightly lower pairwise identity (96.4%, 96.6%), but top matches were consistent across accessions, supporting reliable species assignment. One poorer quality sequence returned lower identity and coverage values (83.3% identity, 57.3% coverage) for its top BLAST hit, but as the other top sequences that produced significant alignment matches were all also for Hoary Bat DNA we are confident in this assignment. Low percent identity is likely due to degraded guano-based DNA, rather than the possibility of a different species being a more appropriate match.

Discussion. Our detection of Hoary Bat guano at artificial roosts suggests 1 of the following possible scenarios. First, Hoary Bats randomly and independently, in both time and space, defecated mid-flight over these locations. Second, Hoary Bats were utilizing foraging habitat near the artificial roosts and defecated non-randomly in space, but without interacting with the structure, perhaps drawn to the presence of other bat species. Third, 1 or more Hoary Bats rested on the exterior of the roost, or finally 1 or more Hoary Bats used, at least transiently, the interior of the artificial structure as a roost. Although bats are known to defecate while flying and roosting (Voight et al. 2010), we believe that scenario 1 (totally random occurrence) is unlikely, as we have recorded 5 separate occurrences of Hoary Bat guano collected at artificial roosts. We cannot rule out scenario 2, as the artificial structures may co-occur in locations with ideal foraging conditions. We only collected guano directly beneath the artificial roosts, and it remains possible that Hoary Bat guano could have been present in the wider surrounding environment. However, we posit that the most likely scenario is that Hoary Bats are, in some way, using these structures (landing, resting on the outside, etc.), if not fully roosting in them.

All samples that resulted in a Hoary Bat identification were collected from underneath BrandenBark™, artificial roosts that are created to mimic a tree with exfoliating bark. As the Hoary Bat is primarily a tree/foliage-dwelling species, these structures are more similar to its natural habitat than other artificial roost styles, like the rocket box or the traditional multi-chambered bat box. The only other known detections of Hoary Bats from artificial roosts are also from bark-style roosts (Wilhelm 2012). Hoary Bats are solitary and are known to switch

Table 1. Genetic species identifications from bat guano collected in Illinois for each artificial bat roost sampled at Embarras River Bottoms State Habitat Area in 2022 and 2023 and from Warbler Ridge Conservation Area in 2023. Values are reported as 1 species identification per roost. Samples reported as "no result" did not yield high-quality DNA and were unable to be matched to a species identification.

Bat species	Embarras 2022	Embarras 2023	Warbler Ridge 2023	Total
Big Brown Bat	1	4	3	8
Indiana Bat	1	-	1	2
Evening Bat	-	-	2	2
Hoary Bat	3	1	1	5
No result	-	-	1	1
Roosts sampled	5*	5*	8	13

*The same 5 roosts at Embarras River Bottoms were surveyed both years and the duplicate surveys are not counted towards the total number of roosts sampled.

roosts often, so it is reasonable to posit that a Hoary Bat would occupy an empty structure made from artificial bark for a period and then move on (Veilleux et al. 2009). Due to the low roost fidelity of Hoary Bats, it will be difficult to confirm how they may be using these structures. Future work should be conducted to collect more information on the roosting behavior of Hoary Bats to understand how they are using these structures, perhaps by continuing to examine roost interiors, by collecting guano samples for genetic identification, by using trail cameras, and/or by using acoustic equipment paired with emergence counts.

Our results highlight the value of guano collection and genetic surveillance for providing useful information about elusive or rare bat species. This method reduces the potential harm to individual bats and allows researchers to identify bats well after individuals have left the area, as long as the guano is not degraded by the elements. Previously, Hoary Bats have not been considered a species that would use artificial roosts, but with significant losses in populations due to habitat loss and wind-energy mortality (Frick et al. 2017, Frick et al. 2020), this species may benefit from additional conservation efforts. Artificial roosts may not target a large number of Hoary Bats, but they are another tool in the bat conservationist's toolkit.

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Table 2. Hoary Bat species identifications from guano collected under BrandenBark™ roosts in Illinois from 2022 and 2023. Species identities were informed by the proportion of pairwise identity reported from BLAST.

Site name	Roost name	Year	% Query coverage	Pairwise identity	E value	% Identical sites	GenBank accession number
Embarras	13	2022	100%	100%	2.4E-59	100%	MF990080
Embarras	14	2022	100%	96.4%	9.88E-66	94.6%	MF990080
Embarras	16-01	2022	100%	100%	8.28E-59	100%	MF990080
Embarras	16-01	2023	57.3%	83.3%	4.19E-52	76.7%	MF990080
Warbler Ridge	1 North	2023	96.1%	96.6%	1.03E-94	94.1%	MF990080

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