

Collective Movement and Habitat Use in a Declining Bat Species



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Tricolored Bat (*Perimyotis subflavus*)



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Purpose and Goals

Bats provide vital ecosystem services, particularly in agriculture, by consuming insects and saving farmers billions of dollars in pesticide costs (1,2). In the Ottawa Valley, they can also attract tourism to places like Bonnechere Caves. Unfortunately, white-nose syndrome (WNS) has drastically reduced the populations of several hibernating bat species in Ontario, prompting the Committee on the Status of Endangered Wildlife in Canada to list them as Endangered (3). As part of ongoing work contributing to the federal Species Recovery Plan (4), we discovered Canada's third known maternity colony of tricolored bats (*Perimyotis subflavus*) in 2023, the first found since the 1960s. This discovery provides a rare opportunity to characterize the maternity site's biophysical characteristics and investigate habitat use around the colony.

Maternity colonies are sites where females gather to rear their offspring and play an important role in juvenile recruitment, which drives population recovery (5). Little is known about maternity colony formation, and how bats select these areas or whether the females that gather there are related. Understanding relatedness within these maternity colonies may shed light on whether philopatry (i.e., females returning to their birthplace) or other habitat characteristics drive colony formation. Additionally, it is unclear how maternity colony size or relatedness may affect juvenile behavior and, by extension, survival.

Juvenile bats have been shown to acquire important information at social roosts in other species, which can influence offspring survival (6). Juvenile tricolored bats must quickly learn to forage effectively to achieve sufficient body condition to survive the winter hibernation, and must also locate a suitable hibernaculum (7,8). This can be particularly challenging for post-volant offspring because insect availability varies over time and they have limited knowledge of hibernaculum locations (7,9). Therefore, offspring in the first few months post-volant may rely heavily on information transferred from their mothers or other conspecifics (6–8).

The funds granted by Ontario Wildlife Foundation will support two primary objectives: (1) determining whether, and from whom, juvenile tricolored bats acquire information about critical sites, and (2) comparing the genetic structure of a maternity colony to the broader landscape-level genetic context. By integrating genetic relatedness with movement data from bats at a maternity colony, we will gain insight into information flow within the colony and better understand the spatial scale necessary to support social interactions. Disturbances that reduce colony size through structural changes or environmental degradation may disrupt information transfer, potentially harming juvenile survival and long-term recruitment. If juveniles primarily acquire information from their mothers, overall colony size may have less influence on recruitment dynamics. Consequently, conserving habitat at the landscape scale and facilitating movement among roosts may buffer populations against localized threats such as predation, habitat loss, severe weather, and disease transmission.

We will extend this work by examining how genetic composition is structured across the landscape by comparing the genetic makeup of the maternity colony to that of bats sampled at

multiple nearby swarming sites. Swarming sites are critical locations where individuals from across the landscape converge to assess hibernacula and mate. By integrating genetic samples from these sites, we can evaluate individuals' relatedness within the maternity colony relative to the broader population. By assessing genetic connectivity across the landscape, we can determine whether individuals from a single maternity colony utilize multiple swarming sites. Furthermore, if genetic diversity within a maternity colony is high, disturbance at a single site could disproportionately affect landscape-level genetic diversity.

Fieldwork

In summer 2023 and 2024, we captured 57 adults and newly volant pups at a maternity colony using a hoop trap and mist nets (Figure 1). We collected morphometric data, sex (by examining genitalia), and age (by assessing ossification levels in the finger joints). To monitor movement, we affixed a nanotag to 47 individuals weighing more than 6 grams (NTQB2; 0.29 g weight; Lotek Wireless Inc., Newmarket, Ontario). The tags emit signals every ~3 seconds and are compatible with the Motus wildlife tracking system.

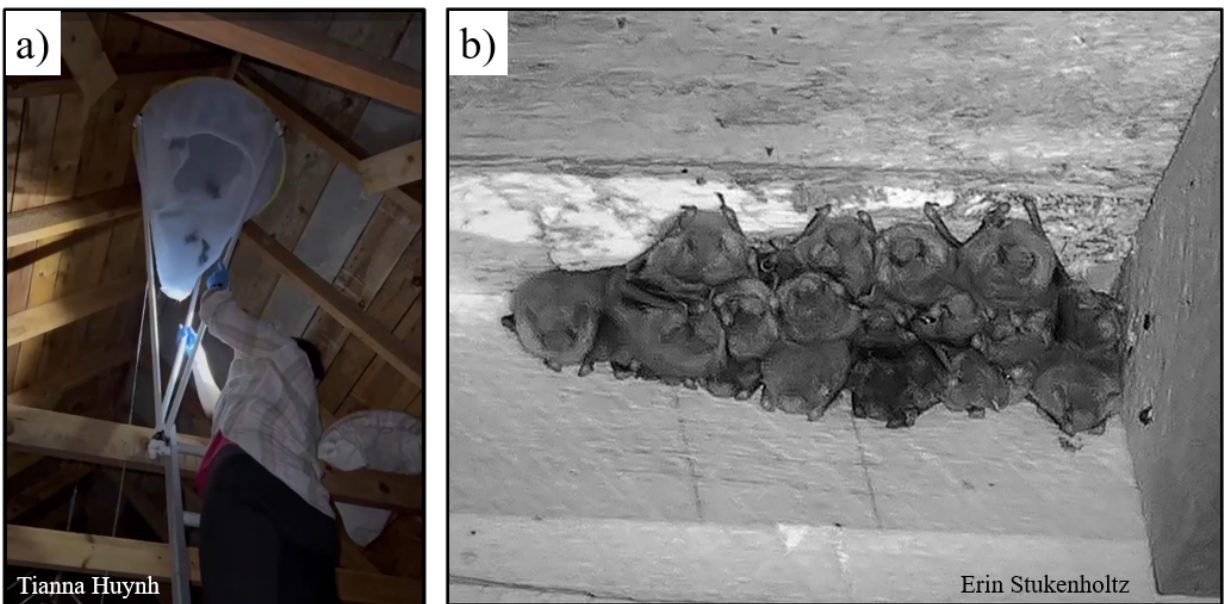


Figure 1. We captured tricolored bats using a hoop trap (a) and mist nets at a maternity colony (b) during the summers of 2023 and 2024. We attached transmitters to individuals to monitor movements across the landscape and better understand social information flow.

Social Movements

To assess dyadic movements and potentially information transfer, we deployed over 21 automated telemetry stations associated with the Motus Wildlife Tracking System across the landscape to monitor bat movements over two years (Figure 2). We used a variety of antenna types to monitor movements at both coarse and fine spatial scales: Yagi antennas (6- and 9-element) with an approximate detection range of 15 km, and omnidirectional antennas with a

detection radius of approximately 200 m, based on a calibration study conducted on the array. We deployed ten stations equipped with two to three Yagi antennas across the broader landscape, while two stations at known hibernacula had an additional omnidirectional antenna. Additionally, we deployed 11–14 stations around the maternity site, spaced about 300 m apart, to monitor finer-scale movements near the roost.

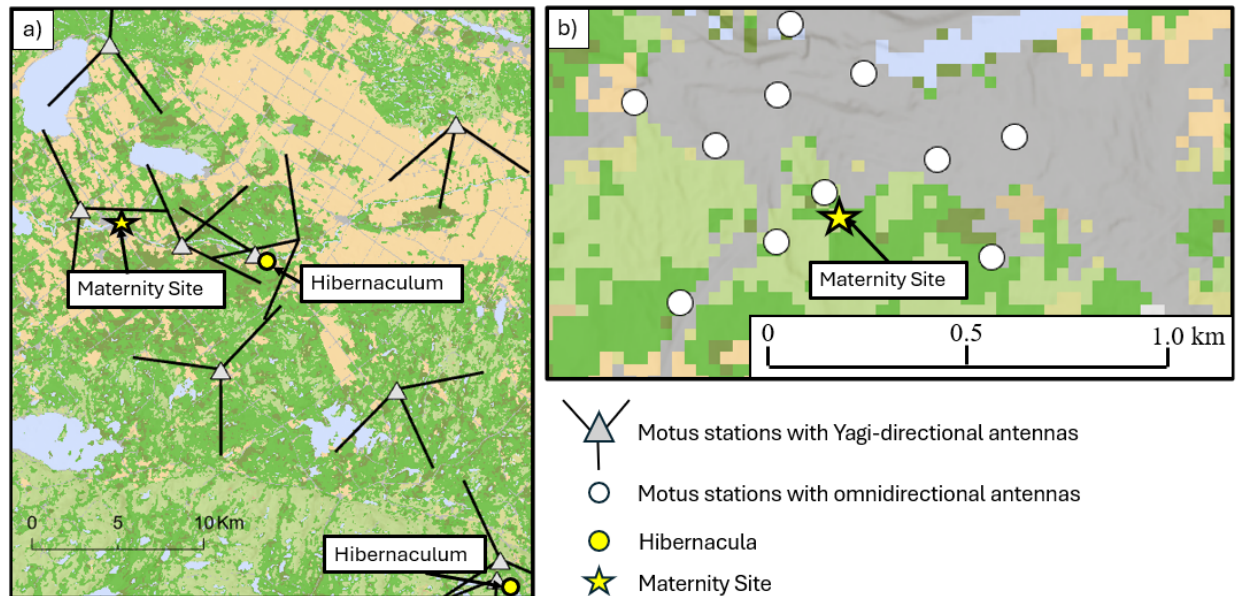


Figure 2. Motus stations were deployed across the landscape to monitor tricolored bat movements. (a) Stations designed to detect coarse-scale movements used 6- or 9-element Yagi-directional antennas, with detection ranges of approximately 15 km. We attached omnidirectional antennas to stations at two known hibernacula, with detection ranges of approximately 200 m. (b) To monitor fine-scale movements, we deployed stations equipped solely with omnidirectional antennas around the maternity colony.

Genetic Analysis

To examine relationships and the potential information flow across dyads at the maternity site, we collected 3-mm biopsy punches from bats' tail membranes during capture for DNA analysis. To assess genetic composition across the landscape, we collected tissue samples from 95 tricolored bats across six hibernacula over seven years. In spring 2026, we will extract DNA and sequence samples using double-digest restriction enzyme-associated DNA sequencing (ddRAD-seq).

ddRAD-seq is a reduced-representation genomic approach that samples loci across the genome rather than sequencing every base pair, making it more cost-effective than whole-genome sequencing while still providing high-resolution genetic information. This resolution will allow us to characterize mother–offspring relationships and detect finer degrees of

relatedness (e.g., cousins) that may be relevant to social dynamics within colonies and broader patterns of genetic structure. Additionally, this approach will facilitate genome-wide comparisons between the maternity colony and hibernacula, improving our understanding of connectivity between the maternity colony and the broader population.

Results

With financial assistance from OWF and in collaboration with Dr. Cullingham's lab at Carleton University, we extracted and sequenced DNA from 104 tricolored bats across five sites. We examined genetic relatedness across individuals using the Related package (10) and built pedigrees with Sequoia (11). We examined mean relatedness within sites and found kinship highest at the maternity site, followed by hibernaculum 1 (Figure 3). Genetic analysis from the other three hibernacula indicated very low relatedness across individuals. Mean genetic relatedness was low among individuals sampled across different sites, including among geographically proximate sites (Figure 4). These results may indicate that hibernacula, even ones near the maternity site, are used by individuals from multiple genetic lineages

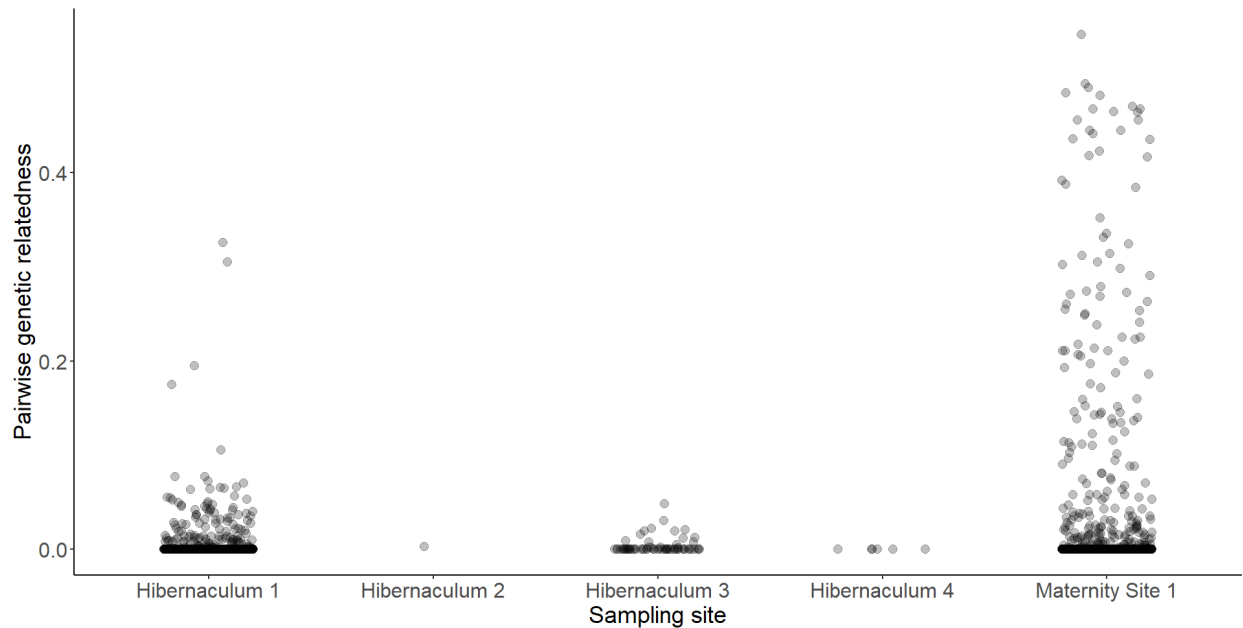


Figure 3. We examined mean pairwise genetic relatedness of tricolored bats (*Perimyotis subflavus*) among four hibernacula and one maternity site across seven years. The maternity site yielded the highest relatedness across individuals, followed by hibernaculum 1.

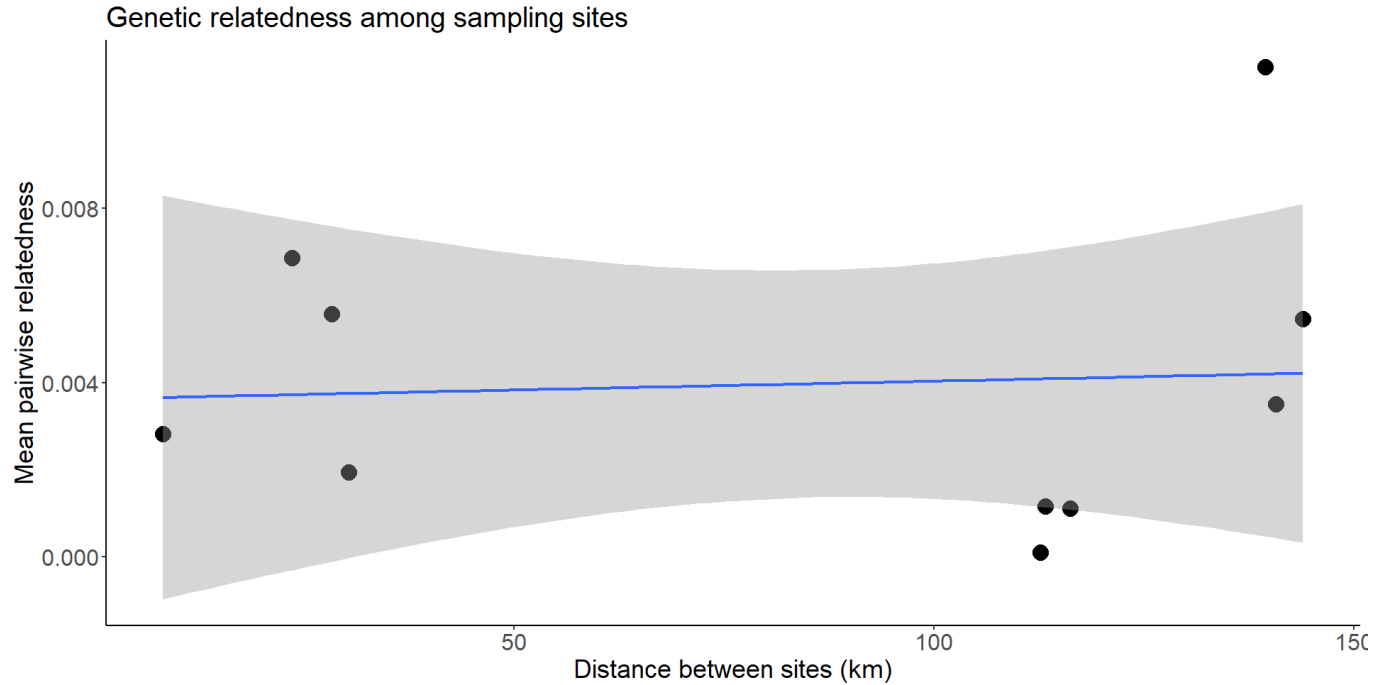


Figure 4. We examined mean pairwise genetic relatedness of tricolored bats (*Perimyotis subflavus*) across four hibernacula and one maternity site from 2017 to 2024. Genetic similarity was low across sites, and distance did not indicate genetic similarity.

Sequoia identified 10 pedigrees involving 25 individuals at the maternity site, including inferred first-degree relationships (e.g., parent–offspring or full-sibling) and second-degree relationships (e.g., half-sibling). We examined dyadic relatedness and the duration of association between individuals, beginning at the roost and tracking their movements across the landscape until they separated. Greater genetic similarity between dyads was associated with shorter durations of association (Figure 5). Although we did not tag or collect DNA from every individual in the maternity colony, our results indicate that individuals also associate with bats with low genetic relatedness, suggesting that social associations are not restricted to closely related individuals.

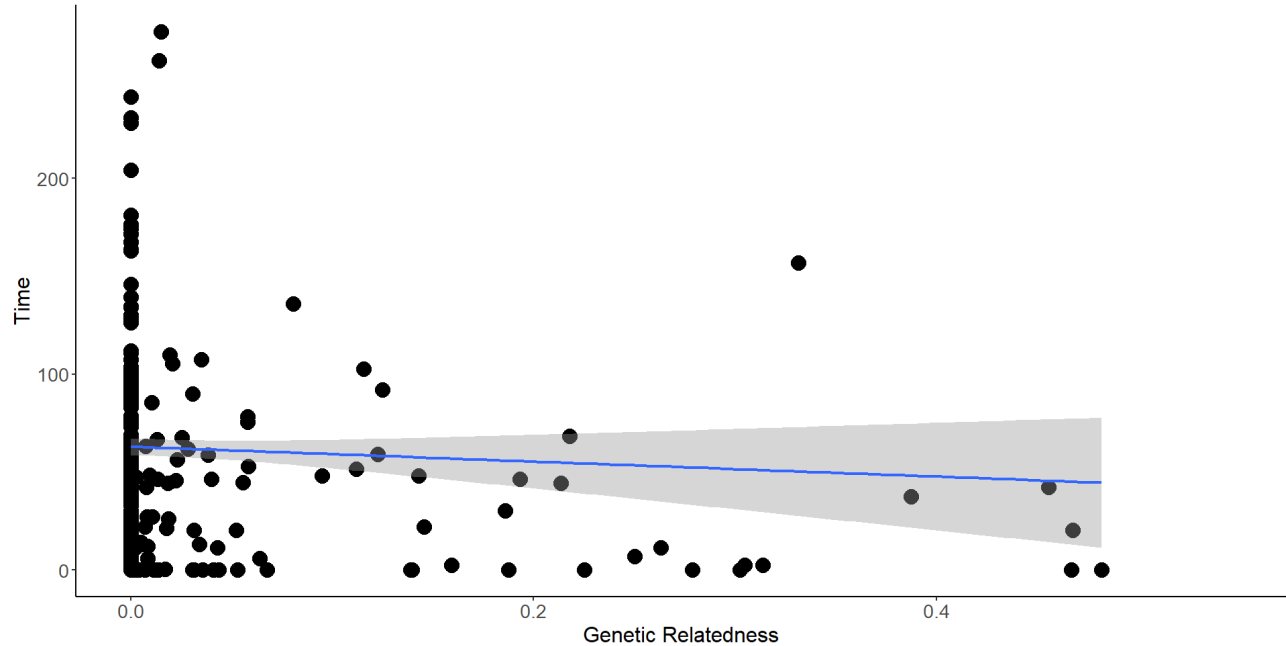


Figure 5. Using genetic similarity among tricolored bats (*Perimyotis subflavus*) sampled at a maternity site, we examined the duration of association between dyads from departure from the roost through movement across the landscape until they separated. We tracked movements using the Motus Wildlife Tracking System. While DNA and movements were not captured for every individual, dyads with low genetic relatedness were often associated for longer durations, potentially suggesting that social associations were not restricted to closely related individuals.

Contributions

This project has substantially contributed to the scientific training and professional development of both graduate and undergraduate students (Figure 6). During fieldwork, eight students gained experience monitoring bat movements using handheld receivers and Motus technology, including training in data collection, animal care, and handling endangered species. Many of these students are now applying these skills in their own research projects.

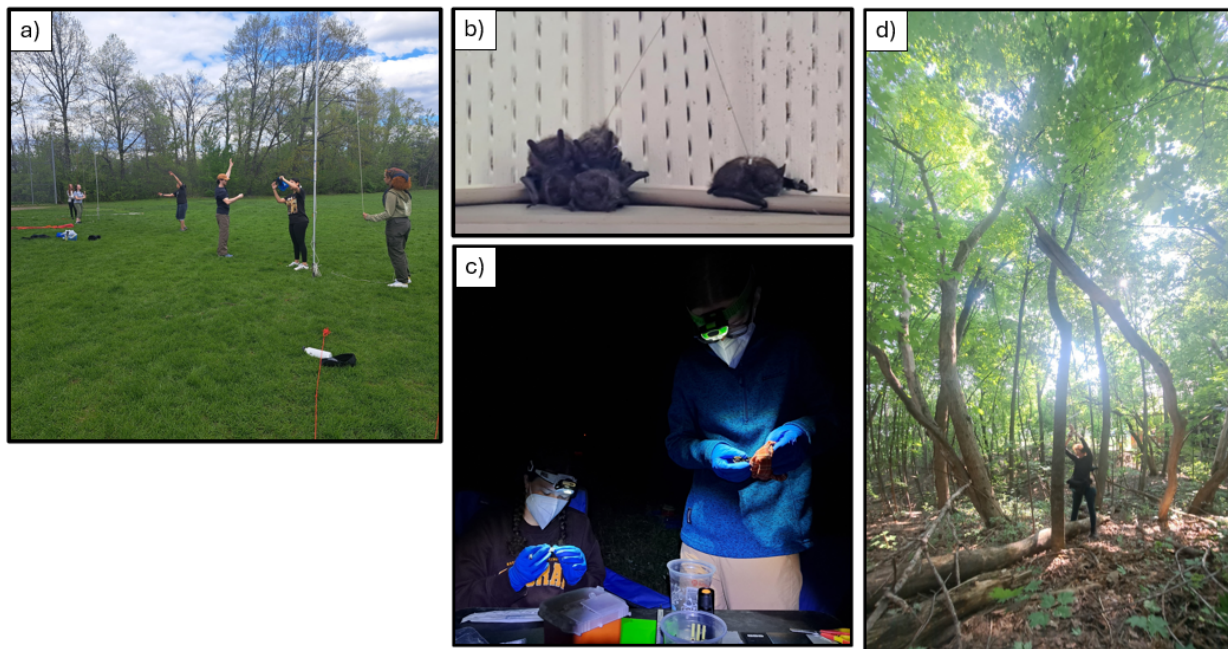


Figure 6. Several undergraduate and graduate students have learned a variety of skills, such as (a) a variety of trapping methods, (b) attaching transmitters to bats, (c) data collection and handling of endangered species, (d) tracking movements with handheld receivers, and using the Motus Wildlife Tracking System. These skills have helped students grow in science and in their career endeavors, with many conducting conservation-based research and collecting tracking information on a variety of species. All photos were taken by Erin Stukenholtz.

We initially anticipated that an undergraduate student would complete the DNA sequencing. However, the small tissue samples and project timeline made it difficult to complete the sequencing within the available timeframe. A graduate student in Dr. Cullingham's lab subsequently provided valuable assistance with the genetic analyses. During summer 2026, he focused on optimizing laboratory protocols to maximize DNA yield from the available tissue samples and subsequently generated a matrix of pairwise genetic relatedness among all sampled individuals. This project provided valuable hands-on training in molecular techniques and new analytical methods for generating relatedness matrices and developing pedigrees. The graduate student will also continue to support the application of these genetic results to examine the relationship between genetic relatedness and tricolored bat movements. These analyses will contribute to a manuscript we plan to submit to a peer-reviewed journal.

This project has also contributed to public education and outreach. We engaged with the local community and informed landowners hosting Motus stations about endangered bats, the Motus Wildlife Tracking System, and ways they can support bat conservation.

Our findings provide an initial indication of the relationship between genetic relatedness, social association, and landscape genetics in tricolored bats. As we continue to analyze our

genetic and movement data, we look forward to further exploring these relationships and providing updates on our findings. Future research will investigate whether genetically related individuals reunite at foraging sites after separating and whether age influences patterns of social association.

References

1. Maslo, B., Mau, R. L., Kerwin, K., McDonough, R., McHale, E., & Foster, J. T. (2022). Bats provide a critical ecosystem service by consuming a large diversity of agricultural pest insects. *Agriculture, Ecosystems & Environment*, 324, 107722. <https://doi.org/10.1016/j.agee.2021.107722>
2. Boyles, J. G., Cryan, P. M., McCracken, G. F., & Kunz, T. H. (2011). Economic Importance of Bats in Agriculture. *Science*, 332(6025), 41–42. <https://doi.org/10.1126/science.1201366>
3. COSEWIC. (2015). COSEWIC Assessment and Status Report on the Little Brown Myotis (*Myotis lucifugus*), Northern Myotis (*Myotis septentrionalis*), and Tri-colored Bat (*Perimyotis subflavus*) in Canada. https://www.registrelep-sararegistry.gc.ca/virtual_sara/files/cosewic/sr_Little%20Brown%20Myotis%26Northern%20Myotis%26Tri-colored%20Bat_2013_e.pdf
4. Environment Canada. 2015. Recovery Strategy for Little Brown Myotis (*Myotis lucifugus*), Northern Myotis (*Myotis septentrionalis*), and Tri-colored Bat (*Perimyotis subflavus*) in Canada [Proposed]. Species at Risk Act Recovery Strategy Series. Environment Canada, Ottawa. ix + 110 pp
5. O'Shea, T. J., Ellison, L. E., Neubaum, D. J., Neubaum, M. A., Reynolds, C. A., & Bowen, R. A. (2010). Recruitment in a Colorado population of big brown bats: Breeding probabilities, litter size, and first-year survival. *Journal of Mammalogy*, 91(2), 418–428.
6. Wilkinson, G. S. (1992). Information transfer at evening bat colonies. *Animal Behaviour*, 44, 501–518. [https://doi.org/10.1016/0003-3472\(92\)90059-I](https://doi.org/10.1016/0003-3472(92)90059-I)
7. Stumpf, M., Meier, F., Grosche, L., Halczok, T. K., Schaik, J. V., & Kerth, G. (2017). How Do Young Bats Find Suitable Swarming and Hibernation Sites? Assessing the Plausibility of the Maternal Guidance Hypothesis Using Genetic Maternity Assignment for two European Bat Species. *Acta Chiropterologica*, 19(2), 319–327. <https://doi.org/10.3161/15081109ACC2017.19.2.008>
8. Goldshtein, A., Harten, L., & Yovel, Y. (2022). Mother bats facilitate pup navigation learning. *Current Biology*, 32(2), 350-360.e4. <https://doi.org/10.1016/j.cub.2021.11.010>
9. Gager, Y. (2019). Information transfer about food as a reason for sociality in bats. *Mammal Review*, 49(2), 113–120. <https://doi.org/10.1111/mam.12146>

10. Pew, J., Muir, P. H., Wang, J., & Frasier, T. R. (2015). related: an R package for analysing pairwise relatedness from codominant molecular markers. *Molecular ecology resources*, 15(3), 557-561.
11. Huisman, J. (2017). Pedigree reconstruction from SNP data: parentage assignment, sibship clustering and beyond. *Molecular ecology resources*, 17(5), 1009-1024.