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Tools and Technology

Noninvasive and Cost-Effective Trapping Method for Monitoring Sensitive Mammal Populations

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ABSTRACT Noninvasive sampling methods provide a means to monitor endangered, threatened, or sensitive species or populations while increasing the efficacy of personnel effort and time. We developed a monitoring protocol that utilizes single-capture hair snares and analysis of morphological features of hair for evaluating populations. During 2015, we used the West Virginia northern flying squirrel (*Glaucomys sabrinus fuscus*) in the Monongahela National Forest, West Virginia, USA, to test the feasibility of using this protocol to sample a sensitive mammal species found at low densities in challenging terrain and inclement weather conditions. Our hair snare was successful in collecting hair from 316 squirrels of 3 species with 99.4% single captures and only 1 permanent capture. Using morphological analysis, we differentiated among northern flying squirrels, southern flying squirrels (*G. volans*), and red squirrels (*Tamiasciurus hudsonicus*) using 8 morphological measurements and an orthogonal discriminant function analysis to successfully refine and confirm identification of the hair. We advocate the use of this relatively noninvasive and inexpensive protocol for studying other sensitive wildlife species. © 2017 The Wildlife Society.

KEY WORDS *Glaucomys sabrinus fuscus*, hair snare, Monongahela National Forest, morphometrics, population monitoring, single-capture, West Virginia.

Small population size and patchy distributions of many sensitive species makes monitoring difficult, time consuming, and expensive. Noninvasive sampling methods are valuable tools to monitor wildlife and minimally affect free-ranging animals while reducing the work effort of biologists (Gardner et al. 2010, Pauli et al. 2010, Frary et al. 2011). Many noninvasive methods (i.e., hair snares) successfully collect biological samples for use in research on occurrence and distribution (McDaniel et al. 2000, Kendall and McKelvey 2008), diet (Ben-David and Flaherty 2012), genetic structure (Foran et al. 1997, Dixon et al. 2006, Mondol et al. 2009), and physiological health of populations (Schwartz and Monfort 2008). Traditional live-capture monitoring methods are generally expensive in terms of time spent checking traps and maintaining equipment, which is amplified with low-density populations and in locations where access is constrained by challenging terrain or inclement weather conditions. Furthermore, traditional hair snares, such as wire and glue snares, are subject to multi-individual and multi-species captures (e.g., Zielinski et al. 2006). A single-capture hair snare requires less frequent personnel visits and ensures the sample is from 1 individual, thus providing appropriate samples for accurate genetic, physiological, and diet analyses (Belant 2003, Beier et al. 2005, Bremner-Harrison et al. 2006, Pauli et al. 2008, Ben-David and Flaherty 2012).

Identification of species from hair via genetic analysis and alternative designs that pair a remote camera with a hair snare for species identification are logistically (i.e., observer, effort) and economically costly (Hebert et al. 2003). Morphological measurements and analysis of collected hair provide an alternative approach in which samples are cost-effectively identified to species under a microscope (Moore et al. 1974, Teerink 2003, De Marinis and Asprea 2006). Identifying hair to species using morphological features has been used in previous studies to successfully distinguished several different groups of sympatric species such as domestic and wild ungulates (De Marinis and Asprea 2006), Tasmanian mammals (Taylor 1985), and felids (Harrison 2002).

Recently, the West Virginia northern flying squirrel (*Glaucomys sabrinus fuscus*) was delisted as threatened from the 1973 Endangered Species Act as amended, requiring that

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local management agencies provide continued population monitoring. Small population size and patchy distribution of the West Virginia northern flying squirrel necessitates a cost-effective monitoring method that accurately distinguishes northern flying squirrels from 2 common sympatric sciurid species, southern flying squirrels (*G. volans*) and red squirrels (*Tamiasciurus hudsonicus*; Weigl 1978).

Our objective was to develop a monitoring protocol for *G. s. fuscus* using a modified single-capture hair-snare design that allows for hair collection from a single individual and reduces the daily trap-checking effort required of traditional trapping methods. To evaluate the utility of collecting hair from a hair snare to monitor occurrence and distribution of mammals in a cost-effective way, we developed a method to identify sciurid species using morphological measurements of hair that are transferable to other mammalian species of concern.

STUDY AREA

We deployed traps in the Monongahela National Forest (MNF) of West Virginia, USA, in 2 independent locations; Canaan Valley State Park (39.074°N, −79.471°W) and Blackwater State Park (39.112°N, −79.491°W). The forest habitat at both sites consisted of mixed hardwood–conifer stands composed of predominantly sugar maple (*Acer saccharum*), yellow birch (*Betula alleghaniensis*), American beech (*Fagus grandifolia*), red spruce (*Picea rubens*), and eastern hemlock (*Tsuga canadensis*; Stephenson and Clovis 1983). Elevation of study sites ranged from 270 to 1,400 m above mean sea level.

METHODS

Hair-snare Design

We modified Tomahawk live traps (No. 201; Tomahawk Live Traps, Hazelhurst, WI, USA) similar to Belant (2003) by disabling the door and attaching brushes to function as noninvasive, single-capture hair snares to collect sciurid hair in the MNF of West Virginia. We deployed hair snares at 2 sites in Canaan Valley and 2 sites in Blackwater State Park from November 2013 to November 2015. We selected sites based on presence of quality West Virginia northern flying squirrel habitat that contained red spruce and mixed hardwood–conifer forest and were >1 km from nest-box transect lines used in the current monitoring protocol (C. Johnson, U.S. Forest Service, personal communication). We set 10 traps at each location, which was the number of traps that one field technician could logistically pack into a location, set traps in a transect with 50-m spacing, and initially checked traps daily to test the assumption that individuals who entered the hair snare could easily exit the trap after the entry door closed. Once confident that animals could escape the traps, we checked traps every 72 hr. In circumstances in which it was not possible to check traps according to schedule, we closed the traps until regular checking could resume.

We disabled the locking mechanism using a combination of zip ties and twisted wire; thereby allowing a captured individual to push open the door from inside the trap. In contrast to traps modified for carnivore trapping (Belant 2003) that attached curry combs (min. mass of ~300 g) to the door, along the perimeter of the door we attached 3–4 small wire brushes (6.35-mm diam, 12 g each; The Mill-Rose Company, Mentor, OH, USA) to reduce the mass of the door to allow small mammals to exit the trap. The wire brushes collected hair samples from the dorsal region of the squirrel as it pushed through the closed door to exit the trap. In addition to steel brushes, we inserted a 1-cm-diameter wooden dowel wrapped with double-sided tape behind the treadle to increase the volume of hair collected (Suckling 1978, Sanecki and Green 2005, Schwingel and Norment 2010). Upon exiting, the door closed behind the escaping individual thereby preventing subsequent captures (Fig. 1). The supplies needed to modify each Tomahawk live trap cost US$2–3 and a new Tomahawk live trap No. 201 costs US $34–37.75.

We attached traps horizontally 1.5 m above ground on the bole of trees, following the protocol used in Carey et al. (1991). We baited traps with a mixture of molasses, oats, peanuts, and peanut butter, switching to black oil sunflower seeds during periods of high American black bear (*Ursus americanus*) activity. We wrapped bait with wax paper and used a paper clip to suspend it from the top of the trap to reduce the occurrence of bait theft by mice (*Peromyscus* spp.). We enclosed the traps with tarp to protect the bait and brushes from rain.

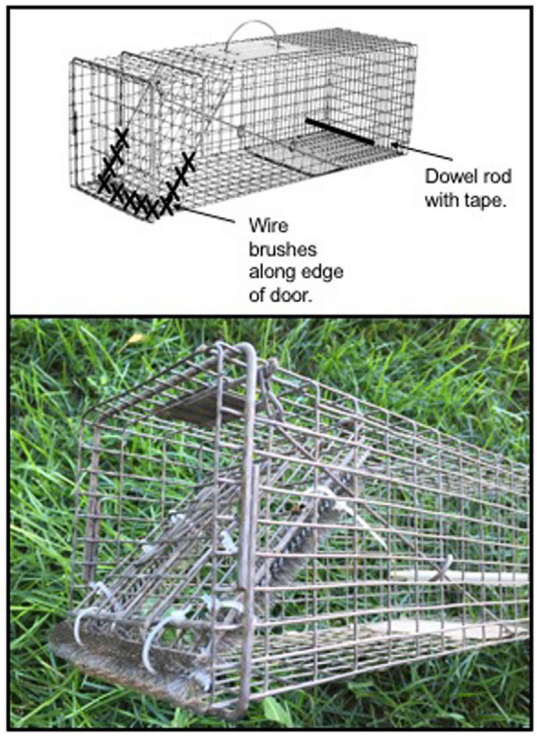

Figure 1. Modified Tomahawk trap design for West Virginia northern flying squirrels (*Glaucomys sabrinus fuscus*) from West Virginia, USA, in 2015, showing locations of wire brushes and wooden dowel rods. The modifications to the door allow trapped animals to escape while removing hair samples from the back of the squirrel as it pushed the door open to escape.

We collected brushes and dowel rods from triggered traps for processing and replaced them with fresh brushes and dowel rods before rebaiting the traps. We extracted hair using tweezers, stored hair in coin envelopes or micro-capillary tubes with 28–200-mesh silica desiccant (Fischer Scientific, Pittsburgh, PA, USA), and recorded the date, trap location, and trap number associated with each sample. We stored the envelopes in a freezer at -18°C for up to 8 months and desiccant tubes at room temperature until laboratory identification and processing at Purdue University, Indiana, USA. After removing all visible hairs, we exposed brush bristles to an open flame to remove any residual hair and replaced the tape on the dowel rods.

Hair Identification

Three sciurid species—West Virginia northern flying squirrels, southern flying squirrels, and red squirrels—inhabit this region of the MNF (Healy and Brooks 1988, Stihler et al. 1995). We distinguished the hair of red squirrels from northern flying squirrel and southern flying squirrel hair using pelage color; red squirrel hair is 2-toned with red and black coloring, whereas northern flying squirrel and southern flying squirrel hair lacks red pigment (Moore et al. 1974). To determine and quantify the morphological differences between flying squirrel species, we used 10 known, independent West Virginia northern flying squirrel hair samples collected from the upper hind leg during nest box surveys in the MNF in 2013; we collected 10 dorsal hairs from 10 museum specimens of mature southern flying squirrels from the vertebrate collection at Purdue University. We photographed 5 hairs/individual under a compound microscope fixed with an Olympus DP70 digital camera system (Olympus America Inc., Melville, NY, USA), and analyzed the photos in ImageJ (http://imagej.net/Welcome; Rasband 1997) at 30× resolution. We measured 8 attributes at 5 randomly selected locations along each hair: A) width of the hair, B) height of the medulla from the center of the hair, C) height of the medulla at the edge of the medulla, D) the distance between 2 medulla at the center of the hair, E) distance between 2 medulla at the edge of the hair, F) distance from the medulla to the edge of the hair, G) ratio of measurements B and C (B:C), and H) ratio of measurements D and E (D:E; Fig. 2). We selected locations using a random number generator and counting down that number of dark medulla spots from the tip of the hair toward the root. We expected some differences in these 8 attributes between West Virginia northern flying squirrels and southern flying squirrels based on physical examination, which would allow for discrimination between the 2 species using these morphological characteristics.

We calculated the mean of the 5 locations/hair for each variable to obtain a mean for each hair. We then calculated the mean of the 5 hairs for the same individual to obtain an overall mean value for each measurement/individual. Therefore, we collected measurements for 5 samples/hair with 5 hairs/individual. We used an orthogonal discriminant function analysis to determine how well our 8 morphological measurements correctly classified each hair sample to species

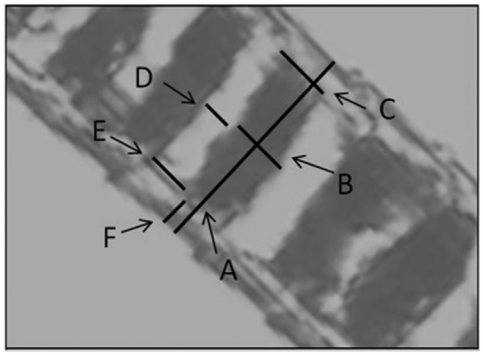

Figure 2. Morphological hair measurements A–F obtained from West Virginia northern flying squirrel (*Glaucomys sabrinus fuscus*) and southern flying squirrel (*G. volans*) for identification to species in West Virginia, USA, in 2015.

to determine which measurements statistically differed between the 2 species using Program R (R Development Core Team 2008). We set $\alpha = 0.05$ to indicate statistical significance. All methods were approved by Purdue University's Institutional Animal Care and Use Committee (PACUC Protocol #1310000959) and methods were developed using guidelines from the American Society of Mammalogists (Sikes et al. 2016).

RESULTS

Single-capture Hair Snare

We collected 316 hair samples at the 2 study sites: 159 at Yellow Birch Trail and 157 at Canaan Loop Road. We identified 42 West Virginia northern flying squirrel and 40 southern flying squirrel samples, and 149 red squirrel samples. We had 1 trap-related mortality of a red squirrel. Of the confirmed squirrel samples, 85 samples (37%) had too few hairs present (i.e., <2) in the sample to confidently identify to species. Of the 42 West Virginia northern flying squirrel samples collected, 28% had >0.250 μg, which provides sufficient sample for stable isotope analysis, and all 42 samples had >10 hairs that would allow for DNA analysis based on protocols associated with the QIAGEN DNEASY® Blood and Tissue Kit (Qiagen Inc., Valencia, CA, USA) commonly used in wildlife genetics studies using hair (McKelvey et al. 2006, Henry and Russello 2011). Two southern flying squirrel samples (0.6%) had >1 species of hair present with the second species of hair unidentifiable but they were not hairs from 1 of the 3 sciurid species sampled in this study.

Hair Identification

Our results indicated that measurement F was smaller in West Virginia northern flying squirrels than southern flying squirrels ($F_{1,18} = 22.82$, $P < 0.01$; Tables 1 and 2). No other variables differed between West Virginia northern flying squirrels and southern flying squirrels (Table 2). Using all 8 measurements, the discriminant function analysis classified 90% of the measurements as the correct species. The 2 measurements with the highest canonical coefficients were variable F (0.60), which ranged from 0.81 to 1.30 μm in

Table 1. Mean (μm) and standard deviation values as well as the range of measurements (μm) for hair measurements A–F and 2 ratios for West Virginia northern flying squirrels (*Glaucomys sabrinus fuscus*) and southern flying squirrels (*G. volans*) from West Virginia, USA, in 2015.

	G. s. fuscus				*G. volans*			
Measurement[a]	$\bar{x}$	**SD**	**Min.**	**Max.**	$\bar{x}$	**SD**	**Min.**	**Max.**
A	6.86	1.37	5.40	10.0	7.42	0.84	5.16	9.10
B	2.39	0.30	1.99	2.83	2.38	0.35	1.78	3.03
C	1.72	0.20	1.45	2.05	1.69	0.19	1.34	1.88
D	1.43	0.36	0.99	2.15	1.31	0.23	0.99	1.62
E	1.88	0.42	1.41	2.58	1.92	0.31	1.44	2.36
F	1.04	0.28	0.81	1.30	1.43	0.17	1.13	1.80
Ratio 1[b]	1.35	0.23	0.83	1.85	1.49	0.07	1.32	1.80
Ratio 2[c]	0.84	0.18	0.71	1.44	0.71	0.18	0.59	0.80

[a] A, Width of the hair; B, Height of the medulla from the center of the hair; C, Height of the medulla at the edge of the medulla; D, Distance between 2 medulla at the center of the hair; E, Distance between 2 medulla at the edge of the hair; F, Distance from the medulla to the edge of the hair.
[b] Ratio of measurements B and C (B:C).
[c] Ratio of measurements D and E (D:E).

West Virginia northern flying squirrels and 1.13 to 1.80 μm in southern flying squirrels, and variable G (0.17) ranging from 0.83 to 1.85 μm in West Virginia northern flying squirrels and 1.32 to 1.80 μm in southern flying squirrels (Table 2).

DISCUSSION

The modified low-cost hair-snare design successfully collected hair samples for monitoring of a sensitive species found at low densities in challenging terrain. This method was successful in collecting hair samples in sufficient quantity for genetic and stable isotope analysis. Stable isotope analysis requires very small samples (1–2 μg; Ben-David and Flaherty 2012), and our small hair samples provided sufficient hair for quantifying $\delta^{13}C$ and $\delta^{15}N$ in a concurrent diet study. Using known samples of West Virginia northern flying squirrel and southern flying squirrel hair, we identified morphological features of sciurid hair that allowed for accurate identification of 2 closely related species with morphologically similar hair. The ability to identify hair without genetic analysis provides species identity results in less time and increases the cost-effective advantage of using hair snares to monitor wildlife. However, the hair collected using this trap design would allow for DNA analysis, which would provide data on population attributes including population abundance, density estimates, and sex ratios (Foran et al. 1997, Waits and Paetkau 2005) as well as dispersal, paternity, and kinship (DeYoung and Honeycutt 2005).

We had a relatively high percentage (37%) of samples that did not include enough hairs for confirmed identification. This failure rate is not uncommon for hair snares; a hair snare developed for use with San Joaquin kit foxes (*Vulpes macrotis mutica*) failed to collect hair in 33% of the instances when the snare door was tripped (Bremner-Harrison et al. 2006). We accepted our failure rate rather than increasing the mass of the door because of the low density and low trapping success of this sensitive and recently delisted species (Ford et al. 2004, Menzel et al. 2006, Weigl 2007). An increase in door mass would have increased the likelihood of hair capture as squirrels pushed out of the door; however, this may have increased the likelihood of trap mortality from squirrels unable to escape. The human effort required to maintain live-trapping grids for this species for demographic analysis, stable isotope studies of diet, or collect samples for DNA analysis would be time- and cost-prohibitive and indeed, nearly all of the current monitoring efforts for this species and the endangered Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*) use nest boxes for this reason (Stihler et al. 1995, Weigl et al. 1999, Ford et al. 2015), whereas Boulerice and Van Fleet (2016) and Diggins et al. (2016) recommend other noninvasive monitoring methods for this species. Similar methods using hair collection have been used to document presence–absence for other rare species of wildlife species including wolverines (*Gulo gulo*; Magoun et al. 2011), Eurasian lynx (*Lynx lynx*; Schmidt and Kowalczyk 2006), and rare tropical carnivores (Castro-Arellano et al. 2008) because of the challenges related to studying species that occur at low density or in challenging terrain.

Table 2. Difference between means of West Virginia northern flying squirrel (*Glaucomys sabrinus fuscus*) and southern flying squirrel (*G. volans*) morphological hair measurements and canonical loading values from West Virginia, USA, in 2015. The larger the canonical loading value, the greater the variable contributed to the discriminant function.

Variable[a]	**Canonical loading**	**Wilks' λ**	***F***	***P***
A[a]	0.12	0.95	0.92	0.35
B	−0.01	1.00	0.01	0.94
C	−0.50	0.99	0.16	0.69
D	−0.11	0.96	0.71	0.41
E	−0.03	1.00	0.05	0.82
F[f]	0.60	0.44	22.82	0.00
Ratio 1[b,*]	0.17	0.91	1.78	0.19
Ratio 2[c]	−0.13	0.85	3.08	0.10

[a] A, Width of the hair; B, Height of the medulla from the center of the hair; C, Height of the medulla at the edge of the medulla; D, Distance between 2 medulla at the center of the hair; E, Distance between 2 medulla at the edge of the hair; F, Distance from the medulla to the edge of the hair.
[b] Ratio of measurements B and C (B:C).
[c] Ratio of measurements D and E (D:E).
*Measurements with the highest canonical coefficients for both *G. sabrinus* and *G. volans*.

Additionally, the hair snare was successful in collecting hair from a single individual while preventing subsequent captures. Species-specific modifications to this trap design allow this to be used on a variety of species. In our study, our modified trap design reduced the mass of the door compared with the design proposed by Belant (2003) using curry combs allowing small mammals to easily push the trap door open and escape. However, we strongly advocate daily trap checks until observers are confident captured animals are able to escape. In our study, one red squirrel was not able to exit the trap because it chewed the zip tie and triggered the locking mechanism. The inclusion of wire around the locking mechanism in place of, or in addition to, zip ties prevented any subsequent permanent captures. If hair samples are collected for genetic analysis, we also suggest short intervals between trap visits (e.g., 1–3 days) for maintenance of high-quality hair samples (Taberlet et al. 1999). We also noted that some hairs collected on the tape-wrapped dowel lacked the hair follicle, which would prevent DNA analysis from those hairs.

Our method provided a reliable, cost-effective means to collect hair using a readily available trap with few additional and simple modifications. Wire brushes, zip ties, dowels, and double-sided tape only add approximately US$2–3 to the cost of the trap. Tomahawk traps are available in various sizes for the capture of a wide variety of wildlife species, allowing for great flexibility in application. Based on our results, we advocate the combined use of our noninvasive, single-capture hair snare and the development of hair identification techniques to survey or monitor target species, especially those that are difficult to capture or require prohibitively expensive trapping effort (Zielinski and Kucera 1995, Henry and Russello 2011). This method of species identification using hair morphology, measurements, and multivariate analysis has been applied successfully to other wildlife studies (Oli 1993, Harrison 2002, Pocock and Jennings 2006, Jones et al. 2014). We recommend including quantitative measurements of hair morphology in future studies using hair identification and suggest this would increase likelihood of successful hair identification (Lobert et al. 2001) at minimal additional cost when DNA analysis is not feasible.

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