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ABSTRACT: Use of noninvasive DNA-based tissue sampling (e.g., hair, scats) for individual identification in wildlife studies has increased markedly in recent years. Although field techniques for collecting hair samples have been developed for several species, we are unaware of their use with free-ranging ungulates. From December 2004 to August 2005 we evaluated the efficacy of barbed wire for snaring hair samples suitable for genetic analyses from white-tailed deer (*Odocoileus virginianus*) on trails and at baited sites. During initial trials on a semi-captive deer herd in northern Ohio, deer demonstrated avoidance of barbed wire positioned on game trails through four weeks but entered baited sites with barbed wire in <3 days. Field trials on free-ranging deer in Michigan using two snare configurations at baited sites checked at one-or-two-week intervals also were successful in obtaining hair samples suitable for extracting DNA. Number of hair samples appeared to increase with deer activity. Number of hair samples and amount of hair in individual samples were greater during winter and spring than during summer. Adequate genetic material was present in 98% (n = 53) of samples collected during winter. Obtaining hair samples noninvasively from white-tailed deer has numerous applications including determining natal origin, population monitoring, and density estimates. We recommend use of baited sites encircled with a single strand of 15.5 gauge, four-point, barbed wire 80 cm above ground attached to ≥3 trees. In treeless areas, metal or wood posts could be substituted. Hair snare height and configuration could be adapted for other ungulate species.

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INTRODUCTION

Overabundance of white-tailed deer (*Odocoileus virginianus*) populations has become one of the most difficult issues facing wildlife managers (Warren 1997). At high densities, deer browsing and herbivory can adversely affect plant community composition and structure (Waller and Alverson 1997, Frankland and Nelson 2003, Pedersen and Wallis 2004). Cascading ecological effects include indirect influences on avian composition and insect abundance (Miller et al. 1992, deCalesta 1994, Ostfeld et al. 1996). Additionally, conflicts between humans and deer may include agricultural loss, zoonoses, property damage to landscaping, and collisions with vehicles (Conover et al. 1995, Conover 1997).

Similarly, deer overabundance is a pervasive management issue in National Park units in the eastern United States (e.g., Shafer-Nolan 1997); with deer-vehicle collisions and impacts on native plants the most frequently reported issues (Frost et al. 1997, Porter 1997). For example, six of nine national park units within the western Great Lakes region contain overabundant white-tailed deer populations that have or are presently adversely affecting native vegetation (e.g., Robinson 1980, Balgooyen and Waller 1995, EDAW 2003). Development of long-term monitoring and associated research is considered necessary to resolve these issues and ensure effective deer management (Waller and Alverson 1997).

Numerous techniques are available to monitor white-tailed deer abundance including aerial surveys, spotlighting, forward-looking infrared, and pellet counts (e.g., Beringer et al. 1998, Belant and Seamans 2000). More recently, genetic markers (e.g., microsatellite DNA) have been identified for numerous wildlife species (e.g., Foran et al. 1997). For example, a microsatellite DNA panel has been developed for white-tailed deer and validated for several populations (Anderson et al. 2002, DeYoung et al. 2003). Individual assignment testing for assessing natal origin can be used to determine dispersal and population history (Beaumont and Bruford 1999 [in DeYoung et al. 2003]), in addition to monitoring abundance and population estimates that include estimates of precision (Foran et al. 1997). An important advantage of using hair for DNA analysis is that it can be obtained from free-ranging animals without capture (e.g., Belant 2003, Belant et al. 2005).

Although hair snares have been developed for several wildlife species (Raphael 1994, Foran et al. 1997, Woods et al. 1999, McDaniel et al. 2000, Belant 2003), we are unaware of any techniques used to noninvasively collect hair from free-ranging white-tailed deer. Development of a hair snare could provide a cost-effective and accurate means to monitor deer abundance or estimate their population size in areas where deer are not harvested or where other techniques are impractical (e.g., large roadless forested areas). Our goal was to develop a noninvasive method for monitoring abundance and determining genetic relatedness of white-tailed deer. Specifically, we sought to determine the effectiveness of barbed wire to remove hair that is suitable for determining genotype from free-ranging white-tailed deer.

MATERIALS AND METHODS

Study Area

We conducted initial trials at the National Aeronautic and Space Administration's Plum Brook Station (PBS), Erie County, Ohio (41° 22' N, 82° 41' W). The 22-km² facility is enclosed by a 2.4 m high chain-link fence with barbed-wire outriggers. Deer ingress and egress occurs through several gaps between the fence and ground. Vegetation within PBS consisted of canopy dogwood shrubs (*Cornus* spp.), grasslands, open woodlands, and mixed hardwood forests (Rose and Harder 1985). Estimated deer density during winter 2004-2005 was 54/km² (J. Cepek, U.S. Department of Agriculture, personal communication).

Field trials also were conducted at Sleeping Bear Dunes National Lakeshore (SBDNL), Pictured Rocks National Lakeshore (PRNL), and Grand Island National Recreation Area (GINRA). Sleeping Bear Dunes National Lakeshore comprises 242 km² and is located in the northwestern Lower Peninsula of Michigan (44° 77' N,

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86° 05' W). North Manitou Island (NMI; 60.7 km²) and South Manitou Island (SMI; 20.2 km²) are part of SBDNL and are each located about 11 km from the mainland. Dominant overstory vegetation types on the mainland portion of SBDNL are coastal forests (including red oak [*Quercus rubra*] and jack pine [*Pinus banksiana*] and mixed northern hardwood forests (including sugar maple [*Acer saccharum*] and American beech [*Fagus grandifolia*]) (Hazlett 1991). Overstory vegetation on NMI and SMI is predominantly American beech-sugar maple forest followed by mixed hardwood and conifer forests. Deer from captive stock were released on NMI during 1926 (McCullough and Case 1982). Deer apparently were not native to SMI and are believed to have emigrated from NMI located about 5.0 km northeast of SMI. Estimated deer density on the mainland portion of SBDNL during October 2004 was seven to 10 individuals/km² (T. Minzey, Michigan Department of Natural Resources [MDNR], personal communication). Estimated deer densities on NMI and SMI were about three and <1/km², respectively (S. Yancho, SBDNL, personal communication).

Pictured Rocks National Lakeshore (280 km²) is in the northcentral Upper Peninsula of Michigan (46°33' N, 86°20' W) along Lake Superior. About 59% of PRNL is dominated by northern hardwood forests containing predominantly sugar maple and American beech. Ten percent of PRNL contains upland conifer stands including red pine (*Pinus resinosa*), white pine (*P. strobes*), and jack pine. Estimated deer density in PRNL during October 2004 was about three individuals/km² (T. Minzey, MDNR, personal communication).

Grand Island National Recreational Area (GINRA) is a 54.6 km² island administered by the U.S. Forest Service and located in Lake Superior about 1.0 km offshore from the western portion of PRNL. Dominant vegetation types include northern hardwood and mixed hardwood and conifer forests similar to PRNL (M. Cole, U.S. Forest Service, unpublished data). Deer density on GINRA is unknown but was estimated to be comparable to PRNL (T. Minzey, MDNR, personal communication).

Hair Snares

During December 2004 we established eight 6.1 x 6.1-m feeding sites (Seamans et al. 2002) each ≥0.9 km from the nearest site. These sites were part of a long-term study to investigate techniques to abate deer damage (Belant et al. 1998*a*,*b*, Seamans et al. 2002). Feeding sites were selected based on deer activity and to maximize distance between individual sites. At each site a plastic snow fence 1.5 m high was erected on three sides with a 1.2 m long livestock feed trough centered within the fenced area and 1.0 m from the rear fence. Whole-kernel corn was placed in troughs as bait. An active infrared monitoring device (Trailmaster®, Goodson and Associates, Incorporated, Lenexa, Kansas) was installed 60 cm above ground at the open side of each feeding site to continually monitor the number of deer intrusions as an index of activity and avoid recording non-target species (e.g., raccoon [*Procyon lotor*], fox squirrel [*Sciurus niger*]). A single strand of 15.5-gauge, four-point barbed wire was attached across the enclosure and above the leading edge of the feed trough such that deer attempting to feed would contact the wire. Wire height was assigned randomly to sites (n = four sites/height treatment) such that barbed wire strands were 70 or 80 cm above ground representing 20 or 30 cm above the feed trough. Sites were maintained for two weeks and monitored every two to three days; corn was added as necessary. During each inspection we recorded the number of events displayed on TrailMaster units and removed hair samples from each of the nine barbs that were directly above the feed trough. Each hair sample, defined as the total number of deer hairs on an individual barb, was placed in a separate envelope and air dried until analysis. To determine if relative deer activity at bait sites was associated with the number of hair samples obtained, we compared event counts recorded on TrailMaster units with number of hair samples collected during each site visit.

Within 15 m of each bait site we also established snares along active deer trails by placing a single strand of barbed wire across one to two trails leading to the bait site. We placed wire over trails which received the greatest apparent deer use. Wire heights (80 or 90 cm) were assigned to trails similarly to feed sites (n = four sites/treatment). At two sites we placed 80-cm high snares across two trails; remaining sites received snares over one trail. Wire heights allowed deer to pass under the wire and snare hair from the neck or back. We collected hair samples from trail snares every two to three days during the two-week period that bait sites were sampled and at one to three day intervals for two additional weeks. Although multiple deer trails entered each area, we used recorded events displayed on TrailMaster units as a general index of deer activity. We recorded the total number of barbs available for snaring hair as the number of barbs directly above the impacted trail plus one additional barb on either side of the trail. No attractants were used at trail snares. As with baited snare sites, we compared event counts recorded on TrailMaster units with the number of hair samples collected from each trail snare during each visit. Also, whenever conditions were suitable (e.g., snow was present), we searched for deer tracks on trails near snares to determine whether deer avoided or walked under snares. We combined trail snares by snare height and week to calculate the percentage of trail snares used and avoided by deer.

We established 12 hair snares at SBDNL during 1-3 May 2005; two on the mainland, six on NMI, and four on SMI. Snares were located in areas thought to maximize deer encounters but not directly on trails to avoid potential animal injury. Each snare consisted of a single strand of barbed wire with four sides 60-65 cm long and 46 cm above ground (Fig. 1) and was intended to snare hair from the throat or neck of a deer. Wire was typically attached to the outside of a tree and with stakes (76 cm length) containing washers welded on one end that supported remaining corners. Snares were constructed by driving stakes into the ground, passing the barbed wire through the washers, then stapling the wire ends to the tree. We applied about 1.9 L of BuckJam® (Evolved Habitats, New Roads, Louisiana 70760, USA) onto logs positioned in the center of each snare. BuckJam is a combination scent and mineral attractant. Commercial skunk essence was applied to trees at bait sites on North Manitou Island. Snares were checked every two weeks through July and an additional 1.9 L of attractant was added to each site during mid-June.

During late June and July 2005, we established 20 hair snares at PRNL and three hair snares at GINRL. As at SBDNL, we constructed snares near recent deer activity but avoided placing snares directly on game trails. Snares consisted of single strands of barbed wire attached to the outside of three to four trees using fence staples similar to Belant et al. (2005) but positioned 80 cm above ground. We removed leaf litter or added woody debris as necessary to ensure consistent wire height. We similarly applied 1.9 L BuckJam to logs placed in the center of the enclosure. Snares were checked on three to four occasions at one- (PRNL) or two-week (GINRA) intervals.

For all trials, during each snare check we placed hair samples from each barb in separate envelopes. Each hair sample was classified as Category 1 or 2, which represented the number of guard hairs with follicles collected. Follicles from four underfur hairs contain about the same amount of DNA as one guard hair and were included in Category classification assignments. Thus, Category 1 samples contained $\geq$1 but <3 guard hairs of DNA material and Category 2 samples contained $\geq$3 guard hairs. Category 2 samples represent at least a 90% probability of determining individual identity of a white-tailed deer. To assess suitability of samples for DNA extraction, we processed 53 samples (winter hair) from four baited sites at PBS separated by 0.9-1.0 km. All mean and standard deviations were calculated using SAS (SAS 1988).

FIGURE 1. Snare configurations used to obtain hair samples from free-ranging white-tailed deer. Top panel is a 60 x 60 cm barbed wire hair snare positioned 46 cm above ground. Bottom panel is a barbed wire snare stapled to three trees 80 cm above ground level.

Genetic Analyses

To increase probability of determining individual genotype, all DNA analyses were performed using Category 2 hair samples. We used 10 guard hairs for extraction when possible to reduce the probability of genotyping errors (Gossens et al. 1998). We used 12 microsatellite loci for analyses of individual identity: Rt07, BL42, Rt05, OhP, OvA, BM6506, Rt24, Rt13, OhD, OhN, BM4107, and OvH. Locus BL42 was described by Bishop et al. (1994); remaining loci have been deposited on Genbank (www.ncbi.nlm.nig.gov). We conducted DNA extractions using QIAGEN DNeasy Tissue kits (Qiagen Inc., Mississiauga, Ontario, Canada), following the manufacturer's instructions.

We used the software GENEPOP (Raymond and Rousset 1995) to calculate observed and expected heterozygosity and the number of alleles present at each locus. We examined distribution of genotype similarity to estimate the probability of two or more sampled individuals having identical genotypes at the six loci we examined. The observed numbers of pairs of similar genotypes were used to estimate the expected number of pairs of identical genotypes (0 mismatching marker [MM] pairs; Paetkau 2003). The typical pattern reflects an order of magnitude decline with each successive decrease in number of mismatching markers. Thus one would expect a single error for every 10 1MM pairs (Paetkau 2003).

RESULTS

Hair Trapping

At PBS, the number of Category 2 and total hair samples obtained from baited snare sites increased with deer activity (Fig. 2). Rates of increase appeared similar for both snare heights; however, the number of Category 2 samples obtained from snares 80 cm above ground appeared to increase at a greater rate. Hair samples were collected from baited snare sites during 98% of checks. Overall, 59% of hair samples collected were classified as Category 2.

At PBS, no relationship was observed in the number of Category 2 or total hair samples obtained on trails relative to snare height or deer activity (Fig. 3). Hair samples were obtained from trail snares during 31% of checks. Overall, 76% of hair samples collected were Category 2. Deer avoidance exceeded use of trail snares during week one (Fig. 4). Evidence of avoidance continued at about 50% of sites through week four in contrast to deer use increasing to 70-85% during weeks two through four. Combined activity exceeded 100%, as some sites had evidence of use and avoidance of snares.

The mean number of hair samples collected at SBDNL decreased from about 3.5 per snare in May to about one per snare during July (Fig. 5). Although the number of barbs available for snagging hair at PRNL was greater than at SBDNL, the mean number of hair samples collected in July was 1.4 per snare and then increased to 2.1 per snare in late August. The overall percentage of Category 2 samples at SBDNL was 72% during May-June and 27% during July; percentage of Category 2 samples at PRNL was 27%.

The mean number of hair samples collected at two-week intervals GINRA was 4.4 per snare, slightly greater than twice the rate samples were collected at PRNL at one-week intervals. Twenty-six percent of hair samples collected at GINRA were Category 2.

At several sites in Michigan study areas, slight cratering of the soil was observed apparently from deer attempting to ingest minerals from the attractant. Although no evidence of injuries was observed for deer or other wildlife entering snare sites, the wire from one side of a snare at PRNL was pulled from the tree. Barbed

wire was bent on several occasions at hair snares at SBDNL and required straightening. Additionally, 19 samples at snares from SBDNL were collected from the ground.

DNA Analyses

Of the 53 winter hair samples analyzed from PBS, only one (2%) lacked adequate DNA for determining genotype. Degradation of this sample in the field was suspected as a suitable number of guard hairs (n = four) for extraction were collected. Twelve additional samples (23%) produced clear evidence of ≥3 alleles, suggesting these samples contained hair from ≥2 deer. The remaining 40 samples produced good genetic data and comprised 23 distinct genotypes. The most similar pairs of genotypes differed at four of the six markers (4MM pairs) with greatest H_t (Table 1), suggesting it is highly unlikely that we sampled any single pair of individuals with identical six-locus genotypes.

The number of alleles for the 12 markers used to identify these 23 individuals ranged from five in marker OvH to 11 in Rt07; mean allelic diversity was 8.2 alleles per locus (Table 1). Mean observed heterozygosity (0.75) was similar to mean expected heterozygosity (0.77).

The number of samples obtained from individual deer ranged from one to six. Twenty-one deer were identified from one baited site and two deer were identified from the two baited sites located 1.0 km apart. This suggests that deer use of sites, and likely movements between sites, was low during the period that hair samples were collected.

DISCUSSION

Barbed wire snares were effective for non-invasively obtaining hair samples from free-ranging white-tailed deer under a wide range of densities (3-54/km²). We obtained many samples of sufficient quantity and quality for determining genotype. Several aspects of this technique could potentially be enhanced to improve efficacy. The attractant we used at SBDNL, PRNL, and GINRA was a combination of food scent and minerals. The premise was that deer would be attracted initially to the food scent and encounter the mineral component which would result in repeated use. An advantage of this type of attractant was that sites did not require reapplication of bait during each check session, in contrast to studies of other species where attractant was reapplied during each check session (e.g., Belant et al. 2005). Indeed, in some cases we did not

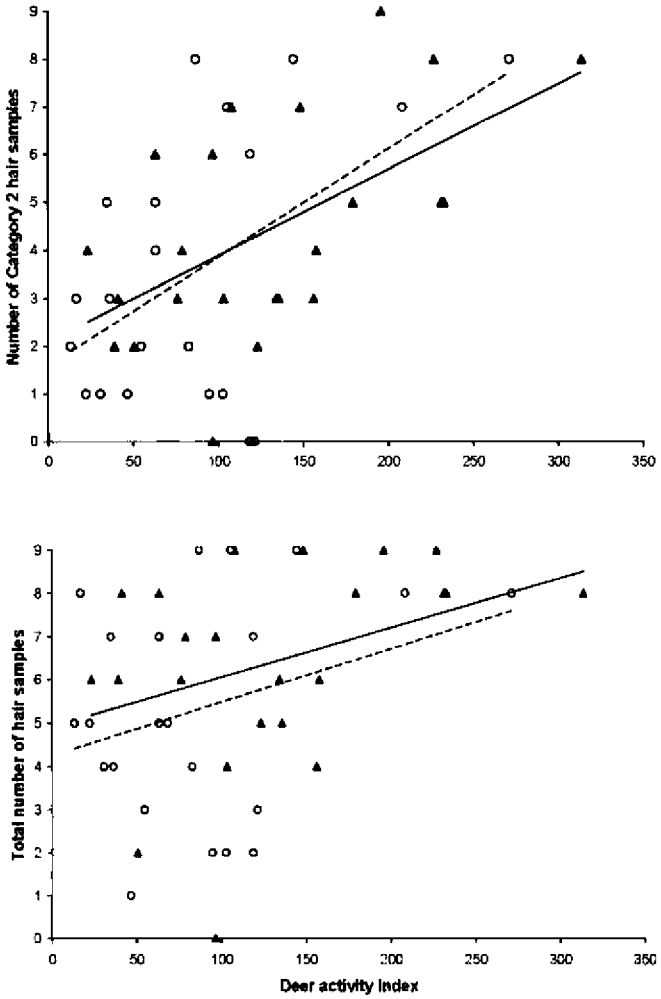

FIGURE 2. Number of category 2 and total number of deer hair samples collected from barbed wire hair snares at baited sites positioned 70 (triangles) and 80 (circles) cm above ground relative to deer activity, Plum Brook Station, Ohio, December 2004. Solid and dashed lines represent trends for 70- and 80-cm high snares, respectively.

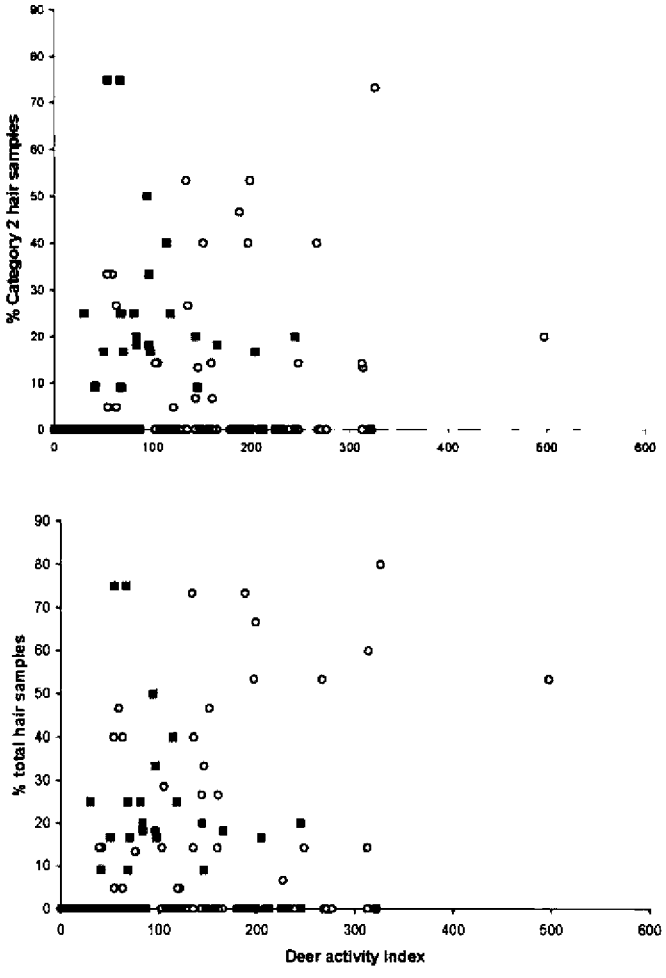

FIGURE 3. Percentage of category 2 and total number of deer hair samples collected from barbed wire hair snares on trails positioned 80 (circles) and 90 (squares) cm above ground relative to deer activity, Plum Brook Station, Ohio, December 2004-January 2005. Dashed and solid lines represent trends for 80- and 90-cm high snares, respectively.

reapply attractant for up to six weeks, yet deer continued using sites. This duration should be adequate for many field studies including population enumeration. However, numerous baits and scents are available to attract white-tailed deer, especially those developed by commercial manufacturers for sport hunting. Prebaiting sites until deer use is consistent may also facilitate obtaining hair samples. Improving attractiveness of bait used or bait delivery would increase deer activity at snare sites and consequently the number of hair samples obtained.

In general, the number of hair samples and amount of hair collected in individual samples was greatest during winter and spring and declined considerably during summer. The fewer number of hair samples obtained, particularly during summer, was attributed in part to working in areas of lower deer density at that time of year. Other factors that likely reduced the number of samples and amount of hair obtained during summer were availability of alternate foods and decreased effectiveness of barbed wire to snag and hold guard hairs from summer pelage. Although not quantified, the longer and larger-diameter winter guard hairs appeared to entangle more readily in the barbs than did the shorter, narrow diameter summer guard hairs. We suspect that largest hair samples would be obtained during spring when deer are shedding winter hair.

Conducting projects during spring also would be advantageous because herbaceous vegetation has not yet fully emerged and deer access to snare sites after snowmelt would be energetically easy, particularly in areas of high snowfall. Although a large number of samples can be obtained during winter, snowpack may hamper logistics and snowfall would cover bait, reducing efficacy. Changing snow depth during conduct of a study could also affect snare height and limit the number of samples obtained. However, winter projects in areas with little or no snowfall should yield good results. Because of decreased quantity and quality of hair samples collected, we do not recommend conducting large-scale projects after June or before winter hair is acquired.

Because of the substantial and consistent avoidance of hair snares on trails by deer, they may not be appropriate for some applications. It is possible to have bias relative to sex or age classes of deer as males, particularly mature males, are known to have movement patterns different from other cohorts (Marchington and Hirth 1984). However, collection of hair samples from trails may be appropriate for assessing genetic relatedness between populations or genetic dispersal rates. The comparatively high percentage of Category 2 samples would also facilitate DNA analyses.

Deer density and the time of year studies are conducted will influence the frequency hair snares should be checked. The seven- or 14-day check intervals we used appeared suitable for collecting hair samples in our Michigan study areas during spring-summer with low to medium deer densities. This is similar to the interval used for bears (*Ursus* spp.; Woods et al. 1999, Belant et al. 2005). However, more frequent check intervals are likely warranted in areas of high deer density. Using a check interval of one to three days at PBS with an estimated density of 54 deer/km^2, 23% of our samples were from >1 deer. Standardizing check intervals to one day would probably have reduced the percentage of mixed samples. Another alternative would involve analyzing an individual hair from each sample; however, the probability of determining genotype would be reduced. Finally, deer at PBS had restricted access to bait by being forced to enter from only one side of the site containing a 6.1 m length of barbed wire. Increasing the number of barbs available at each site by constructing a larger snare or having all sides of the area containing snare material may spatially separate deer when entering the site and reduce the number of mixed samples. Further investigations refining snare check intervals at varying deer densities

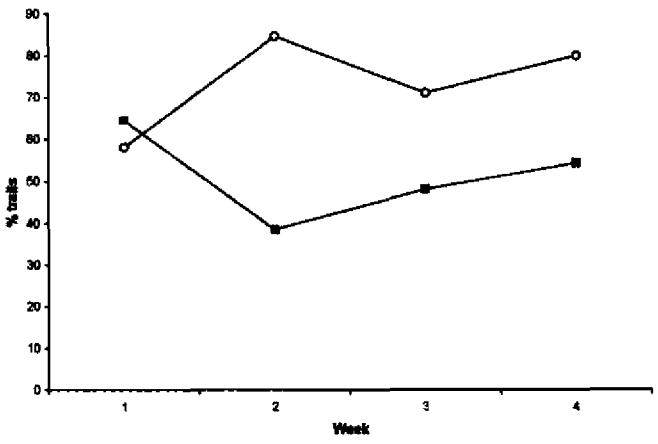

FIGURE 4. Percentage of trails with barbed-wire hair snares where white-tailed deer avoided passing under the snares (squares) and passed under the snares (circles), Plum Brook Station, Ohio, December 2004 through January 2005. Sums exceeded 100% as deer at some sites passed under and avoided snares.

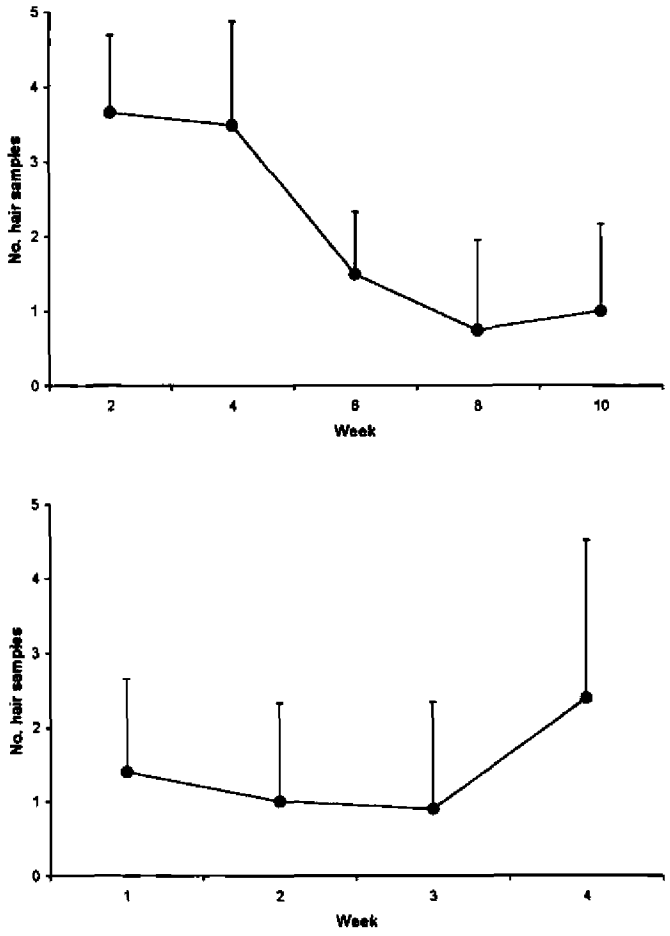

FIGURE 5. Mean (+ SD) number of deer hair samples obtained from barbed-wire snares at North Manitou Island, Sleeping Bear Dunes National Lakeshore (top panel) and Pictured Rocks National Lakeshore (bottom panel), May through August 2005.

to maximize the total number of hair samples while minimizing mixed hair samples collected are warranted.

We recommend use of barbed wire attached to ≥3 trees and positioned about 80 cm above ground in forested areas. Cost of materials (wire and staples) to construct a snare using trees was about US $1; lure was about US $4. Stakes could be used as supports for wire in place of trees in non-forested areas as we did at SBDNL. Alternatively, fence posts could be used to elevate wire 80 cm above ground. We do not recommend using the small snare we employed at SBDNL in areas of high density deer because of the limited number of barbs available and the increased likelihood of mixed samples.

Our use of Category assignments in the field was corroborated by the success of our DNA extraction from winter hair samples. Assigning Category class to samples based on the amount of hair/follicular material available can facilitate selection of samples to submit for analysis, which will improve success rate and reduce overall costs.

A previous limitation of this technique was our inability to determine gender from hair samples. However, Lindsay and Belant (2007) recently developed a simple sexing technique suitable for use with hair samples. Consequently, demographic aspects including sex-mediated gene flow (e.g., Paetkau et al. 1998) can now be addressed using this technique.

Although many techniques have been developed to assist wildlife practitioners in understanding deer ecology, hair snares may provide a practical alternative in situations where other methods are impractical. For example, extensive forested areas with limited roads that occur in many National Park Service units precludes the use of spotlight, infrared, or aerial surveys as monitoring techniques for white-tailed deer. Although study objectives will in large part dictate techniques used, cost is also an important consideration. Field costs of constructing and checking snares will typically be inexpensive relative to the costs of genetic analyses, which can easily exceed $40/hair sample. However, the number of commercial and university labs that perform DNA analyses has increased considerably in recent years and costs have actually decreased in some situations. We recommend that researchers conduct a cost-benefit analysis of relevant techniques before initiating a DNA-based study.

We demonstrated application of DNA-based non-invasive sampling of free-ranging white-tailed deer to assess degree of deer movements between research study sites at PBS. There are numerous additional applications in ecological field studies including species distribution, genetic lineage and population origin, and monitoring population abundance. As has been done with other species (e.g., Woods et al. 1999, Belant et al. 2005), repeated collection of hair samples at snare sites and use of mark-recapture methods or possibly

Table 1

Locus name, allelic diversity (alleles/locus), expected heterozygosity (H_E), observed heterozygosity (H_O), and Genbank reference for 12 microsatellite loci for white-tailed deer, Plum Brook Station, Ohio, December 2004.

Locus	Alleles	H_E	H_O	Species of origin	Genbank reference
Rt07	11	0.89	0.91	*Rangifer tarandus*	U90740
BL42	8	0.87	0.87	*Bos taurus*	[a]
Rt05	9	0.84	0.83	*Rangifer tarandus*	U90738
OhP	8	0.84	0.78	*O. hemionus*	AF102240
OvA	10	0.83	0.91	*O. virginianus*	L35576
BM6506	9	0.80	0.70	*Bos taurus*	G18455
6-locus mean	9.2	0.84	0.83		
Rt24	7	0.74	0.65	*Rangifer tarandus*	U90746
Rt13	7	0.71	0.70	*Rangifer tarandus*	U90743
OhD	8	0.71	0.70	*O. hemionus*	AF1022
OhN	9	0.68	0.74	*O. hemionus*	AF102244
BM4107	6	0.67	0.70	*Bos Taurus*	G18519
OvH	5	0.54	0.43	*O. virginianus*	L35583
12-locus mean	8.2	0.77	0.75		

[a] Bishop et al. (1994)

distance sampling could be used to enumerate deer population sizes that would include estimates of precision. We also believe this technique has application for other ungulate species. Modifications of wire height, size of snare enclosure, and attractant used may be necessary depending on the species studied.

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