Chapter 6

Hair Collection

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The identification of species from hair samples is probably as old as humanity, but did not receive much scientific attention until efficient and relatively inexpensive methods for amplifying DNA became available. Prior to this time, keys were used to identify species through the microscopic analysis of hair shaft morphology (Moore et al. 1974; also see Raphael 1994 for a review of pre-DNA approaches to species identification). For North American carnivores, such analyses are reliable primarily at the family level. Canid hairs, for example, can consistently be differentiated from felid hairs (McDaniel et al. 2000), but hairs of closely related species are often difficult to distinguish. Indeed, for most species, DNA analysis is required to confirm species identification from hair samples, as well as to determine individual identification and population characteristics such as abundance (Woods et al. 1999), substructure (Proctor et al. 2002, 2005), movement (Proctor 2003; Proctor et al. 2004), relatedness (Ritland 1996), and population bottlenecks (Luikart and Cornuet 1998).

In this chapter, we describe methods for collecting hair with hair snagging devices that are positioned so that target animals either make contact with them naturally or via the use of attractants. Hair can also be collected opportunistically from den sites, snow track routes, or other areas frequented by the species of interest (McKelvey et al. 2006; also see chapter 3), but here we focus only on sampling devices specifically designed and deployed to collect hair. We assume that sampled hair will undergo genetic analysis.

A hair sampling method is inherently multitiered. At the most basic level, it typically comprises a hair collection device or series of devices—such as barbed wire, glue or adhesives, or brushes—forming or strategically situated within a collection structure, such as a corral or cubby. These structures are in turn sited within a sampling framework, thus permitting the acquisition of meaningful data. For the method to be effective, the collection structure must permit or promote use by the animal, the collection device must snag the animal's hair, and the resulting hair samples must contain useful DNA.

Hair collection methods can be broadly subdivided into *baited* and *passive* (unbaited) approaches. Although baited methods are most frequently used, passive approaches tend to be more effective for sampling certain species and for addressing fine-scale habitat use and a number of other survey objectives because behavior is not influenced by the draw of bait. Passive methods also have the advantage of requiring no induced response from the

target animal; samples are collected during normal behavior, and there is little risk of individuals becoming averse or habituated to a hair collection structure. We have further subdivided baited methods into four distinct types:

1. *Hair corrals* are structures that use at least one strand of barbed wire to encircle an attractant and are predominantly used to sample ursids.
2. *Rub stations* are structures saturated with scent lures to induce rubbing, and they typically use one of two types of hair collection devices:
   a. *Barbed rub pads* usually consist of a carpet pad with protruding nails (or, in some cases, stiff natural fibers) and are used primarily for felids.
   b. *Adhesive rub stations* typically consist of blocks of wood covered with adhesives and are used mainly for canids.
3. *Tree and post hair snares* are wrapped with barbed wire or fitted with alternative hair snagging devices and have generally been used to sample wolverines (*Gulo gulo*).
4. *Cubbies* are boxes or tubes containing attractants and fitted with snaring devices at the entries or along the inside walls and are used mostly for mustelids but can be effective for other small- to medium-sized species.

Finally, we have grouped passive methods into two categories:

1. *Natural rub objects* are objects found in nature (e.g., bear rub trees) that are fitted with hair snagging devices.
2. *Travel route snares* are hair snagging structures that target animal travel routes or other areas of concentration such as dens, burrows, beds, and latrines. Travel route snares employ one of three types of hair snagging devices:
   a. *Barbed wire* strands strung across travel routes are primarily used to sample ursids, but they have also been used for badgers.
   b. *Adhesives* (such as double-sided sticky tape) hung across travel routes are used to sample hairy-nosed wombats (*Lasiorhinus krefftii*) in Australia, and have been employed for some North American carnivores.
   c. *Modified snares and traps* are leg and body snares or traps that have been adapted to allow animals to escape but deposit hair samples in the process. These are used for a variety of species.

Background

Although the DNA analysis of animal hair dates from the early 1990s (Morin and Woodruff 1992), the monitoring of rare North American carnivores via noninvasively collected samples began more recently with the analysis of mitochondrial DNA to identify different species (Foran et al. 1997a, b; Paxinos et al. 1997; also see chapter 9). Foran et al. (1997a, b), for example, discussed reliable and inexpensive methods for identifying many species based on universal DNA primers (Kocher et al. 1989) from scats (Foran et al. 1997a) and hair (Foran, et al. 1997b).

The use of noninvasive hair collection methods to survey wildlife has expanded rapidly since the mid-1990s. Studies of high-profile, rare, and elusive species such as grizzly bears (*Ursus arctos*; Woods et al. 1999; Poole et al. 2001; Boulanger et al 2002; Paetkau 2003), American black bears (*U. americanus*; Boersen et al. 2003), Canada lynx (*Lynx canadensis*; McDaniel et al. 2000; J. Weaver, Wildlife Conservation Society, pers. comm.), and American martens (*Martes americana*; Foran et al. 1997b; Mowat and Paetkau 2002) were among the first to exploit DNA-based hair-snaring techniques in North America, and have generated the bulk of literature available in this field. Hair sampling is now common and has expanded to include numerous other carnivore species (table 6.1).

Target Species

The following section describes the primary carnivore species studied with noninvasive hair sampling

Table 6.1. References for hair collection surveys of North American carnivore species

Species	*Barbed wire corral*	*Barbed wire- wrapped tree or post*	*Barbed or adhesive rub pad*	*Cubby, enclosure, box, tube, or bucket*	*Natural rub object*	*Barbed wire or adhesive tape on travel route*	*Modified leg or body snares and traps on travel route*
Coyote			Harrison 2006[a]; Shinn 2002[a]; NLS[b]				
Gray wolf	Poole et al. 2001[a]	Fisher 2004[a]; Mulders at al. 2005[a]; Dumond 2005[a]	NLS[b]			Clevenger et al. 2005	
Gray fox			Harrison 2006[a]; Shinn 2002[a]; Downey 2005[a]	Bremner-Harrison et al. 2006			
Arctic fox		Fisher 2004[a]; Mulders at al. 2005[a]; Dumond 2005[a]					
Kit fox				Bremner-Harrison et al. 2006			
Red fox		Fisher 2004[a]; Mulders at al. 2005[a]; Dumond 2005[a]					
Ocelot			Shinn 2002; Weaver et al. 2005				
Canada lynx			McDaniel et al. 2000; NLS[b]				
Bobcat			NLS[b]				
Cougar			NLS[b]; Sawaya et al. 2005[c]				
Striped skunk				Belant 2003a			
Western spotted skunk				Zielinski et al. 2006			
Wolverine		Fisher 2004; Mulders at al. 2005; Dumond 2005					

Table 6.1. (Continued)

Species	*Barbed wire corral*	*Barbed wire- wrapped tree or post*	*Barbed or adhesive rub pad*	*Cubby, enclosure, box, tube, or bucket*	*Natural rub object*	*Barbed wire or adhesive tape on travel route*	*Modified leg or body snares and traps on travel route*
North American river otter							DePue and Ben-David,2007American marten						Foran et al. 1997b; Mowat and Paetkau 2002; Cushman et al., case study 6.1; Zielinski et al. 2006		
Fisher				Mowat and Paetkau 2002; Zielinski et al. 2006; Cushman et al.; case study 6.1; Belant 2003a			
Ermine				Mowat and Paetkau 2002			
Long-tailed weasel				Mowat and Paetkau 2002			
American mink							DePue and Ben-David 2007
American badger	Franz et al. 2004						

Ringtail			Zielinski et al. 2006		
Raccoon			Belant 2003a		DePue and Ben-David 2007
American black bear	Proctor 2003; Proctor et al. 2004; Kendall and Stetz, case study 6.2; Boulanger et al. 2006; Boulanger et al. 2008	NLS[a,b]; Long et al. 2007b[a]	Boulanger et al. 2008; Kendall and Stetz, case study 6.2 et al. 2005; Mowat et al. 2005	Clevenger et al. 2005; Beier et al. 2005; Haroldson	
Brown bear; grizzly bear	Proctor 2003; Proctor et al. 2004; Kendall and Stetz, case study 6.2; Boulanger et al. 2006; Boulanger et al. 2008		Boulanger et al. 2008; Kendall and Stetz, case study 6.2;	Beier et al. 2005; Haroldson et al. 2005; Mowat et al. 2005	DePue and Ben-David 2007

Note: The exclusion of certain species from this table reflects a lack of published accounts of their detection via this survey method.
[a]This survey did not target the given species, but was somewhat or quite effective at collecting hair from this species.
[b]National Lynx Survey (NLS; K. McKelvey, unpubl. data).
[c]Produced very low detection rates.

methods and the types of hair collection devices and structures that have most often been used for these species.

Ursids

A variety of hair collection techniques are effective for sampling American black bears and grizzly bears (also referred to as brown bears). Hair corrals are used extensively to sample bears (Proctor 2003; Proctor et al. 2004; Boulanger et al. 2006; Boulanger et al. 2008), and bears are also readily detected by hair collected from rub trees and other natural rub objects (Boulanger et al. 2008; case study 6.2). Numerous black bears and a few grizzly bears have been sampled as nontarget species at barbed rub pads deployed to detect Canada lynx and other species (Long et al. 2007b; K. McKelvey, USDA Forest Service, unpubl. data). Hair collection devices erected across travel and feeding routes, such as salmon spawning streams, have been employed to sample bears (Beier et al. 2005; Haroldson et al. 2005; Mowat et al. 2005) or have sampled them incidentally as nontarget species (DePue and Ben-David 2007). Barbed wire strung across highway underpasses and overpasses has been successful for sampling both black bears and grizzly bears (Clevenger et al. 2005). Polar bears (*Ursus maritimus*) have not been surveyed with hair collection methods. In some studies, clearly damaged and disturbed cubbies targeting mustelids were a sure sign that black bears were present (Zielinski et al. 2005).

Felids

A landmark effort to detect lynx in the United States, dubbed the *National Lynx Survey* (NLS; K. McKelvey, unpubl. data), employed barbed rub pads as hair snares. Barbed rub pads were originally designed to sample lynx and are fairly effective at detecting their presence (McDaniel et al. 2000). Along with 42 lynx identified during the first three years of the NLS, 166 bobcats (*Lynx rufus*) were also detected in the northern United States—even though the rub pads were deployed in preferred lynx habitat at elevations higher than those generally frequented by bobcats (K. McKelvey, unpubl. data). The NLS also detected numerous cougars (*Puma concolor*) and a few domestic cats (*Felis catus*). A rub pad survey of ocelots conducted in southern Texas detected twenty-nine bobcats, as well as eight ocelots (*Leopardus pardalis*) and a single cougar (Shinn 2002). Apparent detection rates were also high in another rub pad study of ocelots in Texas, with three of four radio-collared ocelots having been detected (Weaver et al. 2005). Finally, Ruell and Crooks (2007) successfully sampled hair from bobcats with ground-mounted natural fiber pads that did not contain barbs.

Some attempts to use barbed rub pad methods for detecting felids have been less successful. For example, even though the NLS obtained many samples from bobcats (K. McKelvey, unpubl. data), results from rub pad-based bobcat studies have been largely unsatisfactory (Harrison 2006; Long et al. 2007b). Although the NLS collected almost as many hair samples from cougars as from lynx, other studies using this method have either failed to detect cougars known to be present (P. Beier, Northern Arizona University, pers. comm.) or experienced lower than expected detection rates (Sawaya et al. 2005). A study targeting margays (*Leopardus wiedii*) in an area where they reportedly occurred also did not succeed in collecting margay hair on rub pads (Downey 2005). In contrast, barbed wire strung across highway underpasses obtained hair from three of five cougars documented with remote cameras (Clevenger et al. 2005).

Canids

There are relatively few published hair sampling surveys that include canids as one of the primary target species. Hair from gray foxes (*Urocyon cinereoargenteus*) and San Joaquin kit foxes (*Vulpes macrotis mutica*) has been collected with cubbies (Bremner-Harrison et al. 2006), however, and adhesive rub stations (see *Rub Stations*) have been used to sample dingoes (*Canis lupus dingo*) in Australia (N. Baker, University of Queensland, pers. comm.). Canids have more routinely been detected during surveys for other tar-

get species. In New Mexico, for example, a rub pad study of bobcats collected fifty gray fox, eighteen coyote (*Canis latrans*), and sixteen dog (*Canis lupus familiaris*) hair samples compared with only a single bobcat sample (Harrison 2006). Similarly, rub pads made of natural fiber carpeting affixed to wooden boards and placed on the ground to sample bobcats were highly successful at collecting hair from coyotes and gray foxes (Ruell and Crooks 2007). Of hair samples collected during a rub pad study targeting margays (*Leopardus wiedii*), 44% were genotyped as gray fox and some samples were from dogs (Downey 2005). A survey of ocelots conducted in southern Texas collected ten coyote, three dog, and two gray fox hair samples (Shinn 2002). Nontarget species sampled by the NLS included numerous coyotes, and wolves (*Canis lupus*) or dogs (K. McKelvey, unpubl. data).

In British Columbia, a bear inventory employing hair corrals detected wolves at fourteen sites (Poole et al. 2001). At Banff National Park, three of five wolves that were observed via remote cameras using a highway crossing structure deposited hair on barbed wire strung across the underpass (Clevenger et al. 2005). During three wolverine studies that employed barbed wire-wrapped posts, nontarget species sampled included arctic foxes (*Vulpes lagopus*), red (silver) foxes (*Vulpes vulpes*), and wolves (Fisher 2004; Mulders at al. 2005; Dumond 2005). The foxes were able to climb the post and reach the bait perched on top (figure 6.1), while wolves were sampled when they stood on their hind legs and braced against the post with their front legs to explore the bait (which they could not reach).

Figure 6.1. By-catch resulting from a hair collection survey. A red fox climbs a post wrapped with barbed wire and baited for wolverines. Photo by R. Mulders.

Wolverines

Trees or posts wrapped with barbed wire are currently the most effective hair collection method for sampling wolverines (but see *Cubbies* under *Baited Hair Collection Methods* later in this chapter; Mulders et al. 2005; Dumond 2005). Hair corrals are not effective with this species (Fisher 2004). The NLS detected only a single wolverine with rub pads (K. McKelvey, unpubl. data), and Mowat et al. (2003) found that rub pads were ineffective with wolverines (although a few wolverine hair samples were collected in box traps fitted with barbed wire across the entrance).

Smaller Mustelids and Other Mesocarnivores

Cubbies have been used for many years to trap small and mesocarnivores, particularly mustelids. In recent years, these structures have been modified and employed for noninvasive sampling of martens and fishers (*Martes pennanti*) using both track plates (Zielinski 1995; Zielinski and Truex 1995) and hair snares (Mowat and Paetkau 2002; Zielinski et al. 2006). Mesocarnivore studies using cubby-type hair

snares have detected long-tailed (*Mustela frenata*) and short-tailed weasels (*Mustela erminea*; Mowat and Paetkau 2002), ringtails (*Bassariscus astutus*), gray foxes, and western spotted skunks (*Spilogale gracilis*; Zielinski et al. 2006).

Other hair collection methods have also been successful with these species. For example, modified body snares and foot-hold traps have collected hair from North American river otters (*Lontra canadensis*), American mink (*Neovison vison*), and raccoons (DePue and Ben-David 2007). Short-tailed weasels have been sampled at barbed wire-wrapped posts (Fisher 2004), and hair from striped skunks (Shinn 2002) and long-tailed weasels (Downey 2005) has been found on barbed rub pads. Barbed wire strung across travel routes has collected hair from mink and raccoons (DePue and Ben-David 2007). Finally, work by Franz et al. (2004) suggests that hair corrals erected around burrow entrances may be effective for sampling American badgers (*Taxidea taxus*).

Nontarget Species as Bycatch

Hair collection methods are, to one extent or another, "omnibus" sampling methods that frequently sample nontarget species along with target species (table 6.2). Sometimes this bycatch can provide useful information. Simply knowing that a given species is in the area, for instance, is often of interest. Additionally, genetic monitoring is an expanding field (Schwartz et al. 2007), and samples that vary in quality or degree of population representation can yield a variety of insights into population status. A sample might be of insufficient quality to permit population enumeration, for example, but may allow estimation of effective population size (Schwartz et al. 2007). Further, if a method consistently collects hair from a particular nontarget species, it could potentially be used in more formal surveys of this species.

Strengths and Weaknesses

Noninvasive hair collection provides a means to obtain genetic samples from animals at known locations and has been especially transformative for the conservation and management of species that are reclusive, potentially dangerous, or that inhabit thick vegetation. For capture-mark-recapture (hereafter capture-recapture) studies, DNA marks offer the advantage that they cannot be lost. Because the rate at which hair sheds (and therefore capture probability) differs with age class, species, and season, however, these factors must be considered when designing studies to estimate population size. Despite the noninvasive nature of hair collection devices, some animals may avoid hair collection structures simply because human odors are present, resulting in detection heterogeneity, although any avoidance effect is likely to be much smaller than with animals that have been live-captured.

While the genetic analysis of hair samples can render hair collection more expensive than other survey methods, it is also generally more reliable. Further, if the enumeration of individual animals is required to meet survey objectives, costs are comparable to or lower than that of other methods. Genetic analyses associated with hair collection are expensive not only due to the high price of labor, materials (e.g., DNA polymerase), and equipment, but also because noninvasively collected hair samples are inherently uneconomical. A hair sample can fail to produce useable information if it is too small or degraded or contains hair from more than one animal (for projects seeking individual identification) or species (for projects seeking species identification). On the other hand, DNA from hair is often less degraded than DNA extracted from scat, and generally provides more consistent results at far lower cost (see chapter 9).

Hair collection methods can yield information about a large number of individual animals representing a significant proportion of the population from vast study areas. Furthermore, hair sampling can lead to reliable detections of rare animals where live-capture and other methods fail and can provide population-level metrics such as abundance, isolation, dispersal rate, and origin that are often only accessible through DNA-based methods (Proctor et al. 2004, 2005; Schwartz et al. 2007). Genetic analysis of

Table 6.2. North American carnivore species sampled by various hair collection methods

	Hair sampling method						
	Baited				*Unbaited*		
Target species	*Barbed wire corral*	*Barbed wire- wrapped tree or post*[a]	*Barbed or adhesive rub pad*[b]	*Cubby, enclosure, box, tube, or bucket*	*Natural rub object*	*Barbed wire or adhesive tape on travel route*[c]	*Modified leg or body snares and traps on travel route*[d]
Canids							
Coyote			B	B			
Gray wolf	B	B				T	
Gray fox			B	B			
Arctic fox		B					
Kit fox				T			
Red fox		B					
Felids							
Ocelot			T				
Margay			N				
Canada lynx		B	T				
Bobcat			T[e]				
Cougar		B	N			T	
Mephitids							
Striped skunk			B				
Western spotted skunk				B			
Mustelids							
Wolverine	N	T	B	N			
North American river otter						T	T
American marten		B	B	T			
Fisher		B		T			
Ermine		B		B			
Long-tailed weasel			B	B			
American mink						B	B
Procyonids							
Ringtail				B			
Raccoon							B
Ursids							
American black bear	T	T	B		T	T	
Grizzly bear	T	B	B		T	T	T

T = Method used to target this species.
B = Bycatch species detected with this method.
N = Method tried on this species but not effective.
[a]Requires animals to climb.
[b]Includes barbed rub pads and adhesive hair snare devices baited to elicit rubbing behavior.
[c]Includes barbed wire strung across animal trails (for bears, Eurasian badgers) and double-sided sticky tape hung across travel routes of hairy-nosed wombats.
[d]Leg/body snares and foothold traps modified to collect hair and allow animal to escape easily.
[e]Produced very low detection rates (Harrison 2006; Long et al. 2007b).

Box 6.1

Strengths and weaknesses of hair collection methods

Strengths	Weaknesses
• Representative sampling can often be achieved. • Can survey large, remote areas and locate rare, cryptic animals. • Allow discrimination between closely related species, individuals, gender. • Genetic analysis of samples enables many population metrics to be calculated. • Applicable in a broad diversity of habitat types. • Often capable of collecting hair from more than one species. * Snagging devices are generally lightweight and inexpensive. • Baited and passive methods can be mixed, improving sample quality and minimizing bias.	• DNA analysis typically required for species and individual identification. • Amount of DNA in hair samples varies widely between species. • Baited methods require a response from the target animal. • Effective hair snagging methods have not been developed for all species. • DNA degradation may be rapid in warm, wet environments. • Hair snares may become snow covered in the winter. • Most designs are largely effective only for the target species and others of similar size and behavior.

hair cannot, however, furnish information about an animal's age, reproductive status, body condition, or daily movement rates or patterns and is a relatively weak tool for investigating habitat use.

Passive hair sampling methods generally lack spatial representativeness. By definition, hair can only be collected in those places where animals leave it during the course of their normal activities (in areas of high concentration where the chances of obtaining a sample are high), or where it is feasible for people to find it—such as along trails. In most applications, passive sampling is better suited to detecting presence or assessing *minimum number alive* (MNA) than to estimating population size. But sometimes passive methods can be used in tandem with baited methods in a capture-recapture framework (chapters 2 and 11) to estimate population abundance (Boulanger et al. 2008).

Baited methods have different limitations and strengths. Baits can be set out systematically (as on grids) or randomly. This design flexibility allows the application of a variety of approaches to estimate population size, and spatial analyses are enhanced by the regular distribution of sampling locations (see chapter 2 for further discussion). Baited methods, however, must elicit a behavior from an animal to obtain a hair sample; at rub stations, lynx must rub against a baited, barbed pad (McDaniel et al 2000), and hair corrals require that bears cross a strand of barbed wire (Woods et al. 1999). Individual animals that do not engage in these behaviors (e.g., subordinate animals that are less likely to scent mark on rub pads or adhesive blocks) will not be sampled.

Most carnivores are highly mobile, and individuals can be drawn to bait from relatively long—but typically unknown—distances. In studies using attractants, wolverines have been live-trapped 20 km from the boundaries of putative home ranges defined through subsequent relocations (J. Copeland, USDA Forest Service, pers. comm.). Thus, the area surveyed via methods employing bait or lures can be problematic to define, and habitat associations inferred from sample locations are suspect, at least at the local scale. Such complexities must be carefully investigated or considered when attractants are incorporated into hair collection surveys. A summary of strengths and weaknesses associated with hair collection methods is provided in box 6.1.

Treatment of Objectives

Study objectives that can be successfully addressed by noninvasive hair sampling vary among species (table 6.3). Many factors affecting hair collection methods are species- or survey-specific, such as the thickness of hair, whether hair is readily pulled by snagging devices or only shed hair can be collected, the temperature and moisture of the environment, and the type of snagging device used. These factors will all affect study design and limit potential analyses.

Occurrence and Distribution

Species presence and broad-scale distribution are the most general and least demanding objectives of hair collection studies. Most species identification is based on mitochondrial DNA (mtDNA; Foran et al. 1997a, b; Mills et al. 2000; Riddle et al. 2003; see chapter 9 for further background on DNA types and approaches), which occurs in many copies per cell and is more durable than nuclear DNA because it is protected from enzymatic action within the cell (Foran 2006). The majority of hair samples thus contain sufficient DNA for species identification, even when hair samples are small or weathered. Because most hair samples from most species can be reliably identified to the species level using mtDNA, standard repeat-visit protocols (MacKenzie et al. 2002; see chapter 2) can be used to estimate detection probability and occupancy. The development of universal mammal primers (Kocher et al. 1989), and the fact that all published DNA sequences must be stored in GenBank (see details in chapter 9) allows virtually any species to be identified rapidly with minimal initial development costs (see Mills et al. 2000 and Riddle et al. 2003 for examples).

Relative Abundance

In hair collection-based studies of relative abundance, microsatellite DNA is usually examined to

Table 6.3. Study objectives addressed by noninvasive hair sampling methods for carnivore families

Study objectives	*Canids*	*Felids*	*Mephitids*	*Mustelids*	*Procyonids*	*Ursids*
Population status						
Occurrence and distribution	S	S	S	S	F	S
Relative abundance		S	F	S	F	S
Abundance and density			F	F	F	S
Monitoring						F
Population genetics/structure						
Effective population size, evolutionary significant unit, genetic variation	S	S	F	S	F	S
Connectivity between populations: barriers to movement, interbreeding, recolonization	S	S	F	S	F	S
Detection of hybridization	S	S				
Ecology						
Niche or diet via chemical/stable isotope analysis						S
Identify individuals for management/forensics						
Livestock predation	S					S
Incidents with human injury or property damage		S				S
Harvest rate and illegal take	S	S		S		S

Note: This is a current list that likely will change with advances in sampling and DNA technology.
S = Successfully applied.
F = Appears feasible but to our knowledge has not been attempted.

identify individuals (see chapter 9). Relative abundance is estimated using a systematic, or rarely a random, distribution of hair sampling stations. Methodologically, relative abundance estimates fall between well-developed occupancy statistics (MacKenzie et al. 2002) and abundance estimation using capture-recapture approaches (Otis et al. 1978) in terms of certainty. In general, we discourage the use of relative abundance to monitor population trends (chapter 2), but we believe that MNA assessments are often useful for management when detection effort is well documented. Particularly for rare and cryptic species whose presence in an area is subject to speculation, the ability to state with high reliability that multiple individuals are present can be powerful and useful. In northern Minnesota, for example, genetic analyses of hair and scat samples yielded an MNA estimate of twenty lynx (Schwartz et al. 2004). Coupled with anecdotal information that breeding was indeed occurring there, this MNA was sufficient to infer that a breeding population of lynx inhabited the area.

Abundance and Density

Abundance and density estimation from data acquired via hair collection surveys require reliable individual identification based on nuclear DNA (see chapter 9), and—if capture-recapture methods are used—that a substantial proportion of the total population be both captured and recaptured (see chapter 2). In many cases, hair collection methods may not be efficient enough to provide a capture-recapture sample size sufficient for meeting this objective, and extracted DNA may be of too low quality to reliably identify individuals (see chapter 2 for a more detailed discussion of capture-recapture considerations). Thus, for most species, hair sampling is currently less effective for estimating population size than for estimating occupancy.

Monitoring

Population trends can be obtained by periodically repeating capture-recapture population size estimates using hair snare grids, and changes in distribution or relative abundance can be assessed by repeatedly monitoring occupancy if detection probabilities can be estimated (MacKenzie et al. 2006). Alternatively, purely genetic indices of population status can be derived from much smaller and erratically collected groups of samples (Schwartz et al. 2007). Genetic indices of population status may be a desirable objective when the quality of DNA acquired through hair sampling is relatively high but representatively sampling a large proportion of the population is not feasible. Trends in population health can be tracked with statistics such as effective population size (N_e), expected heterozygosity (H_e), and allelic diversity (A). In a retrospective study of brown trout (*Salmo trutta*) in Denmark, for example, changes in H_e and A were examined between 1944 and 1997; older samples were acquired from museum-scale collections (Østergaard et al. 2003). Similarly, N_e was estimated for grizzly bears in Yellowstone National Park using samples from the 1910s, 1960s, and 1990s (Miller and Waits 2003). The ability to use museum specimens to accomplish such analyses demonstrates the utility of irregularly collected samples in producing these types of statistics and points to the tremendous potential of using noninvasive hair samples to achieve similar objectives.

Population Genetics

When DNA quality is high, hair can be used to answer questions about population genetics and structure, thereby providing guidance to conservation measures. For instance, hair sampled from either side of transportation corridors has been analyzed to determine if highways and rail lines pose barriers to grizzly bear movement and breeding (Proctor 2003; Proctor et al. 2004) and to document wildlife use of highway crossing structures (Clevenger et al. 2005). Further, hair samples can be used to identify the source population of individuals recolonizing historic species ranges (e.g., grizzly bears in Montana), and to define *distinct population segments* that help

identify and prioritize populations for conservation efforts. Vinkey et al. (2006), for example, found that Montana fishers contained unique mtDNA haplotypes (see chapter 9), indicating that native fishers—formerly thought to have been extirpated—had survived and formed a population in west-central Montana. Basic research on the genetic characteristics of populations (e.g., effective population size, evolutionary significant units, amount of genetic variation) can also be addressed with hair collection-based sampling (Schwartz et al. 2007; see chapter 9).

Habitat Assessment

Some hair collection studies have successfully assessed habitat relationships with data collected via attractant-based methods. Apps et al. (2004) applied grid-based hair sampling and the identification of individual animals through genotyping to evaluate relationships of grizzly bear detections with habitat and human activity variables. The resulting predictive model of the spatial distribution and abundance of grizzly bears was used as a strategic planning tool for large (11,000 km^2) regions of British Columbia and Alberta. Mowat (2006) examined coarse-scale habitat selection by martens using detection-nondetection data collected via hair snares.

Diet

While most survey objectives utilizing hair samples rely on DNA analyses, questions regarding ecological niche and differences in diet between and among species can be addressed through stable isotope and elemental analysis of hair. In a study of brown bears, stable isotope analysis documented one population segment that fed upon salmon (*Onchorhynchus* spp.) and another that fed on berries at higher elevations and did not frequent spawning streams (Mowat and Heard 2006). In Yellowstone National Park, the presence of naturally occurring mercury in fish was used to estimate the amount of cutthroat trout (*Salmo clarkii*) ingested by bears through the mercury concentration in bear hair (Felicetti et al. 2004).

Description and Application of Survey Method

As discussed earlier, a variety of noninvasive hair sampling methods have been used to study carnivores. To be effective, most methods need to be designed or adapted for a particular species or group of animals with similar body size, hair characteristics, and behavior (table 6.4).

Overview of Hair Collection Devices and Structures

Hair snagging devices vary in effectiveness among species due to differences in hair length and texture. In general, barbed wire is most useful for collecting hair from bears, canids, and wolverines because the hair of these animals is long enough to get pinched between the twisted wires of the barbs. Aggressive barbed wire—four prongs per set of barbs and 6–12 cm spacing between barbs—is the wire of choice for hair snagging and is available in a range of gauges. Ideal between-barb spacing varies with the size of the target species, with smaller animals requiring tighter spacing. Typically, all hair collected on one barb is considered one sample, regardless of the number of hairs present (figure 6.2).

When sampling kit fox hair in cubbies, Bremner-Harrison et al. (2006) found that dog brushes snagged more hair during molting, but lint roller tape was better at sampling hair from winter coats. Short-bristled wire brushes, such as gun-cleaning brushes (case study 6.1), curry combs (Belant 2003a), and glue pads designed to capture mice (Zielinski et al. 2006), are more efficient at snagging the shorter hair of small- and medium-sized mustelids such as martens and fishers. Adhesives and glue work well for both short and long hair, but because they are messy to deal with and time is required to remove the hair from them prior to analysis, alternative collection

Table 6.4. Devices determined effective (Y) and ineffective (N) for collecting hair from North American carnivore species

Species	*Barbed wire*	*Barbed nails*	*Sticky tape*[a]	*Adhesives*[b]	*Tree bark*	*Combs, brushes*[c]	*Modified*[d] *snare cable*
Canids							
Coyote	Y	Y					
Gray wolf	Y						
Arctic or red fox	Y	Y	Y			Y	
Felids							
Margay		N					
Canada lynx		Y					
Bobcat		Y					
Cougar	Y						
Mephitids							
Skunk				Y			
Mustelids							
Wolverine	Y	N				Y	
North American river otter						Y	Y
American marten	N	N	Y	Y		Y	
Fisher	Y		Y	Y		Y	
Short- or long-tailed weasel				Y			
American mink						Y	Y
Badger	Y		Y	Y			
Procyonids							
Ringtail			Y				
Raccoon	Y						Y
Ursids							
American black bear	Y	Y	Y	Y	Y		Y
Grizzly/brown bear	Y	Y	Y	Y	Y	N	

[a]Types: duct tape, gaffer's tape (similar to duct tape but leaves no residue), commercial lint roller, double-sided carpet tape.
[b]Types: commercial plastic or cardboard-backed glue traps for entangling mice and rats.
[c]Includes gun brushes, curry combs, and dog brushes.
[d]Snares modified by inserting short pieces of wire perpendicular to the cable.

devices are generally preferred if they have been proven effective for the target species. Zielinski et al (2006) report that hair removal from glue pads and subsequent cleaning with xylene requires twice as much handling time in the lab as removing hair from wire. Less toxic, citrus-based solvents, such as Goo Gone (www.magicamerican.com), are as effective as xylene at cleaning the glue from hair, hands, and equipment (D. Paetkau, Wildlife Genetics International, pers. comm.) and do not require a ventilation hood. Adhesives can be rendered ineffective in wet weather because wet animal fur fails to adhere, and glue can lose its ability to stick to hair when wet (Fowler and Golightly 1994; Mowat and Paetkau 2002). Glue pads, however, remain effective hair collectors at temperatures as low as –28°C (Mowat 2006).

Hair collection structures can be open, such as barbed wire corrals, or can be enclosed, as with cubbies. In addition, collection structures can be designed to become inaccessible after a sampling encounter (referred to as a *single-catch* configuration) or to remain accessible, thus allowing multiple animals to deposit hair. Choices between open versus enclosed and single- versus multiple-catch structures depend on many factors, including the social dynamics of the species, environmental conditions, and study goals (see *Practical Considerations*).

Figure 6.2. A bear hair sample is collected from barbed wire. Photo by Northern Divide Grizzly Bear Project, US Geological Survey.

Baited Hair Collection Methods

The following section describes hair collection methods that employ baits or lures to attract animals to detection devices and to elicit the response necessary for sampling hair.

Hair Corrals

Hair corrals typically consist of a perimeter of barbed wire supported by trees or posts and centered around a lure or bait (figure 6.3; box 6.2). Wire height is adjusted to the size of the target species with the goal of snagging hair when animals cross under or over the wire (figure 6.4A). To prevent the target species from crossing the wire without touching it, the optimal wire height is maintained throughout the corral by filling in low spots on the ground surface, and by using brush to block high terrain. Wire position is further ensured by securing it as tightly as possible—one person stretches the wire while another hammers the staples. Placing staples just in front of the barbs prevents the wire from loosening through slippage. If corrals are erected in areas without trees to support the wire, metal or wood fence posts can be used instead; steel T-posts work well, especially if the corners are braced with guy-wires. As bears often step on the wire when entering and exiting hair corrals, two or more staples should be used to attach wire to each tree, and staples should be long enough to penetrate the outer bark.

Wire and attractants should be positioned so that animals are compelled to cross the wire—rather than lean across it—to investigate. For grizzly bears, the attractant should be at least 2 m from the wire. Typically, one strand of wire is used per corral, but some studies have found that using two parallel strands for bears (positioned at 25 and 50 cm above

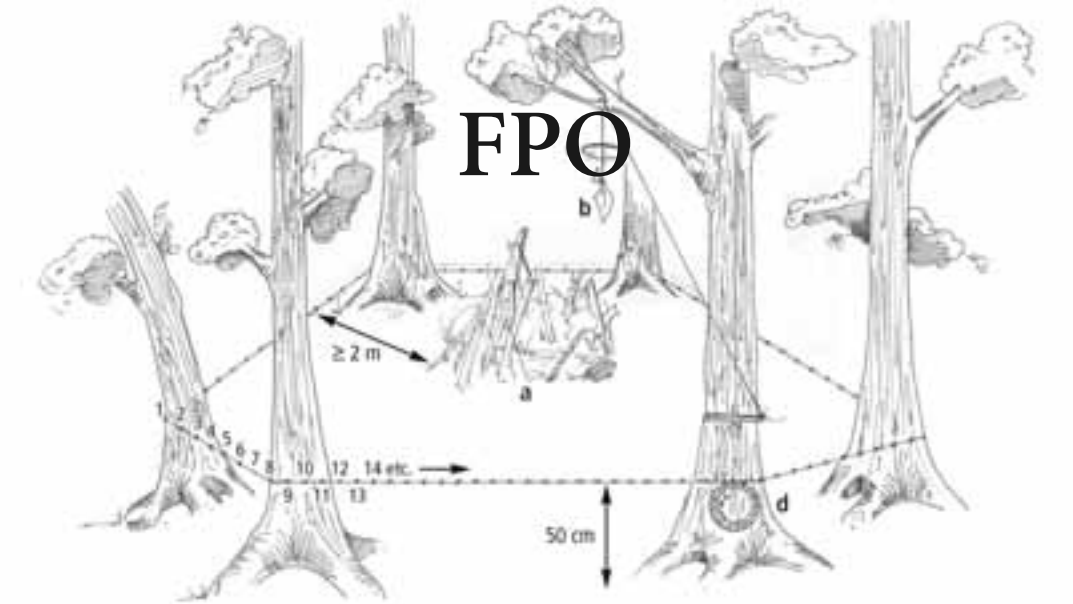


Figure 6.3. Components and layout of a barbed wire bear hair corral, showing the (a) debris pile treated with scent lure; (b) scent lure-soaked rag; (c) paper plate or aluminum pie pan hung to protect the rag from rain; and (d) coil of excess barbed wire. Note that barbs are numbered sequentially beginning at one of the trees. Illustration by S. Harrison.

A B

Figure 6.4. A grizzly bear (A) passing over barbed wire (photo by S. Himmer, Arctos Wildlife Services and Photography), and (B) depositing hair on debris by rubbing its neck on a lure pile (note the hair on the barbed wire in the foreground). Photo by M. Maples.

the ground) yields larger hair samples, presumably by forcing more contact between the bears and the wire (T. Eason, Florida Fish and Wildlife Conservation Commission, pers. comm.). Tredick (2005), found no benefit from using a second wire, however. Although the use of two wires should theoretically increase the sampling of young bears and other smaller species, Boulanger et al. (2006) determined that a single wire placed 60 cm from the ground successfully captured grizzly bear family groups, and

Hair corrals for sampling bears

Hair corrals can be formed with a perimeter of barbed wire wrapped around trees or posts and encircling a central bait or lure (see figure 6.3). You'll need

- 20–30 m of barbed wire, placed at a height of 50 cm for grizzly bears, 45 cm for black bears (be sure to maintain optimal wire height throughout the corral). Use four-pronged barbed wire with a 7–12 cm barb interval.
- Fencing staples (3 cm in length for most trees; ≥4 cm for thick bark) to securely attach wire to trees so that it can support the weight of bears when they step on it.
- Nonconsumable, liquid scent lure to apply to the debris pile on the ground, or bait to suspend out of reach from above (see chapter 10 and figure 10.3). For lure placed on debris, the corral should be large enough that the lure is at least 2 m from the closest wire.

concluded that single-wire sampling suitably targeted all bears in the population. These researchers also found that adding a second wire increased field and lab costs substantially but did not change population abundance estimates or improve estimate precision. Note that bears often rub or roll on or near debris treated with lure (figure 6.4B). The lure pile can therefore be a productive source of additional hair samples, and hair can often be found on the ground beneath the wire.

Hair corral microsite selection is usually based on habitat quality and species activity patterns. In locations with grizzly bears, we recommend that baited sites be situated ≥100 m from roads and trails and ≥500 m from developed areas for human and bear safety. If hair corrals are deployed in areas frequented by cattle, they must be surrounded by a livestock exclusion fence to prevent trampling. In several Montana studies, most unprotected hair corrals exposed to cattle produced no bear hair because cattle trampled the wire or knocked the bear hair off, and further masked the presence of bears by filling the barbs with their own hair (K. Kendall, unpubl. data; R. Mace, Montana Fish, Wildlife, and Parks, pers. comm.).

Hair corrals have been used with variable success to detect carnivores other than bears. Corrals with three wire strands, intended to survey wolverines in Alberta, failed to sampled wolverines but collected some hairs from martens and lynx (Fisher 2003). Eurasian badgers (*Meles meles*) were sampled with 20 cm-high barbed wire corrals baited with peanuts and deployed less than 10 m from communal burrow systems (Frantz et al. 2004). Because previous studies suggested that Eurasian badgers would be difficult to attract, bait was placed near burrows up to four months prior to the construction of the corrals. Even though 33% of the hair samples contained only a single guard hair, 93% produced reliable individual genotypes after a single round of amplification. This approach may be useful for sampling American badgers, but their solitary habits suggest that each corral would only have the potential to sample a single individual or a female with young.

Rub Stations

Rub stations exploit the natural cheek-rubbing behavior of many small felid species (Weaver et al. 2005; figure 6.5A) and the neck-rubbing behavior of canids. McDaniel et al. (2000) provided the first published description and test of this method, the prototype for which was developed by J. Weaver (unpubl. data). Rub pads basically consist of small carpet squares embedded with nails and treated with a scent lure (figure 6.5B; see box 6.3 for details).

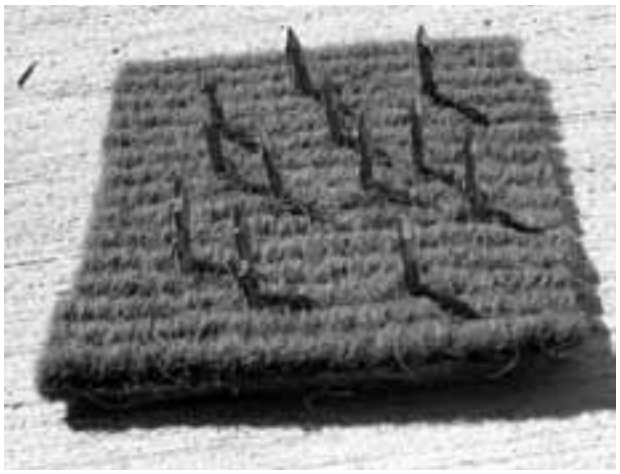
The NLS, described earlier, represents the most

Barbed rub pads for sampling felids

To create a barbed rub pad station for collecting hair from felids:

- Cut 10 x 10 cm pads from short, closed-loop carpet of a uniform color that contrasts with the hair of the target species.
- Stud the rub pads with eight to ten nail-gun nails, 3.2–3.8 cm in length—depending on the thickness of the carpet. Barbs consist of short lengths of copper connector wire.
- Nail the pad to the tree trunk at a height of 0.5 m from the forest floor for lynx, 0.3 m for ocelots. Trees should be selected for long sight distance.
- Apply 2 tbsp. of liquid lure to the pad, then sprinkle it with 3 tbsp. of crumbled dried catnip. The recipe for the lure is a 1:1:6 ratio of propylene glycol, glycerin, and beaver castoreum. Add six drops of catnip oil per oz. of castoreum/preservative mixture.
- Hang a second small carpet pad baited with liquid lure 0.5 m above the pad on the tree.
- For a visual attractant, mold an S-shaped undulation into an aluminum pie plate (see figure 10.1A) and attach it to a nearby tree limb with a fishing swivel (see figure 10.1D) at a height of 1 m from the ground.

A



B

Figure 6.5. (A) Canada lynx rubbing on a barbed pad (photo by P. Nyland). (B) Close-up of a rub pad with barbed nails protruding through the carpet (photo by K. McKelvey).

extensive use of rub pads to date and yielded a number of valuable lessons. For example, carpet pads of a uniform color (such as red or green) that contrasts with animal hair make it easier for field researchers to determine if hair has been deposited on the pad. Closed-loop carpet with tight, short loops eases hair collection and holds liquid lure best, and nails (designed for use in nail guns) should be long enough that barbs are fully exposed and not buried in the carpet. Last, nails snag hair most efficiently when the connecting wire is cut and the nails are pushed through the carpet by hand. This retains the wire barbs and bends them to approximately a 45° angle (figure 6.5B). Otherwise, if nails are fired through

the pad with a nail gun, the barbs (as the primary hair collection device) flatten against the nail shaft or are broken off.

The NLS used pie plates as visual attractants and found that twisting them into an undulated form (figure 10.1A) increased their movement in light breezes and reduced entanglement in vegetation. Further, reinforcing pie plates with grommets and fastening them to fishing swivels (figure 10.1D) increased the length of time the plates remained hanging above the rub pad.

At least one published study has extensively tested the effectiveness of various scent lures for use with rub pads. McDaniel et al. (2000) tested five lures in Kluane National Park, Yukon during a period of high lynx abundance. These tests generated high capture rates, with lynx hair collected on 45% of transects. Although all lures attracted lynx, a simple mix of beaver castoreum and catnip oil was most effective. This lure yielded 39% of lynx detections and was used at only 20% of the stations.

Adhesive rub stations—blocks of wood covered with sticky-side-out tape and treated with commercial canine lures to induce rubbing—were very effective at snagging large quantities of hair from dingoes in Australia (N. Baker, pers. comm.) and could potentially be effective for sampling North American canids. This method worked particularly well in the breeding season but continued to collect hair all year if lures were refreshed frequently. To ensure that nondominant animals were not missed, Baker also sampled DNA from epithelial tongue cells deposited when animals licked blocks of wood wrapped with sand paper and baited with rotting meat.

Tree and Post Hair Snares

Barbed wire can be wrapped spirally around a tree or wooden post, and bait attached above the wire, to entice the target animal to climb (box 6.3; figure 6.6; Fisher 2004; Mulders et al. 2005). These types of hair snares are potentially useful for any species that climbs trees but seem to work best on medium to large species—probably because barbed wire is more

A

B

Figure 6.6. (A) Barbed wire-wrapped post showing wolverine hair samples covered with rime ice. (B) Wolverine climbing a baited, barbed wire post. Photos by R. Mulders.

effective at snagging samples from animals with long, coarse hair (case study 6.1).

Wolverine sets, typically baited with meat and sometimes treated with secondary scent lures, have been shown to be highly effective during winter for both live-trapping (Copeland et al. 1995) and hair collection (Mulders et al. 2005). Scent lure alone is not effective for drawing wolverines to posts (Fisher 2004). Various baits have thus far proven ineffective for attracting wolverines in summer (J. Copeland, pers. comm.), and summer sampling can result in bear damage to the hair collection structure (Dumond 2005). For tree setups, if hair snagging devices do not encircle the bole, sheet metal can be mounted on the tree to prevent climbing on surfaces that are not fitted with devices.

The results of the first substantive trial using hair sampling for a capture-recapture-based wolverine population estimate are encouraging. Mulders et al. (2005) report that 284 baited rub posts were deployed in a 3 x 3 km grid for four sampling occasions in the tundra habitat of the Northwest Territories. Capture probabilities were above 0.5 for both sexes, suggesting a high degree of attraction to posts baited with caribou meat and scent lures. The sampling density they used (i.e., one rub post per 9 km^2 cell) was extremely high considering the large daily movements and home range sizes documented for these vagile animals. Given the high capture rate, it is likely that this population could have been adequately estimated with fewer sampling occasions or lower snare post density (Mulders et al. 2005).

Cubbies

Cubbies (referred to as *enclosures* in chapter 4) were one of the earliest structures used for noninvasive hair sampling (Foran et al. 1997b). Cubbies designed for hair sampling are long, thin boxes or tubes containing hair snagging devices and an attractant (figures 6.7, 6.8, 6.9; see chapter 4 for enclosure design

A

B

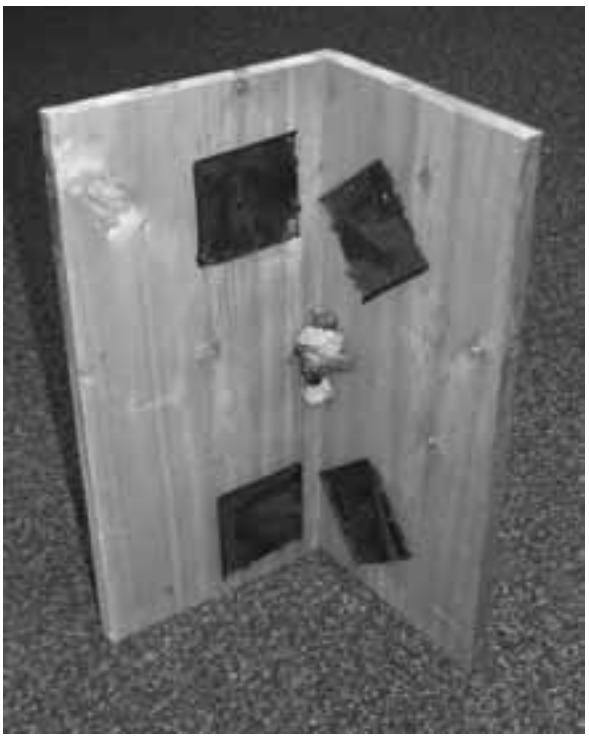


Figure 6.7. Marten cubby that can be accessed from both ends. (A) Cubby is vertically mounted on a tree with a roof installed above the top end (photo by J. Stetz). (B) Glue traps are fitted inside to collect hair on either end of the bait attached at the center of the cubby (photo by K. Kendall).

A

B

Figure 6.8. Triangular marten cubby (with track plate) installed on the ground, showing (A) the placement of gun brushes serving as hair collection devices, and (B) a close-up of a gun brush mounted on a mechanical lug (with fisher hair). Photos by P. MacKay.

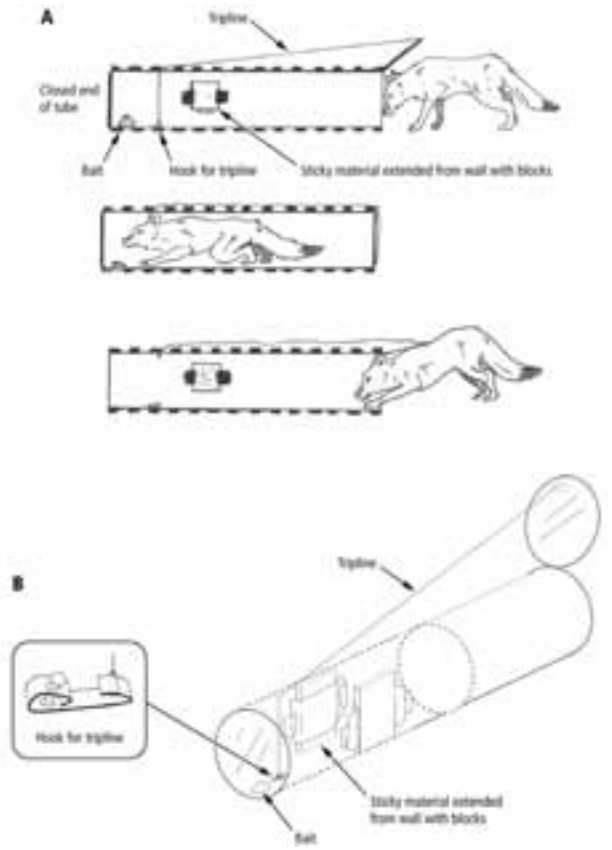
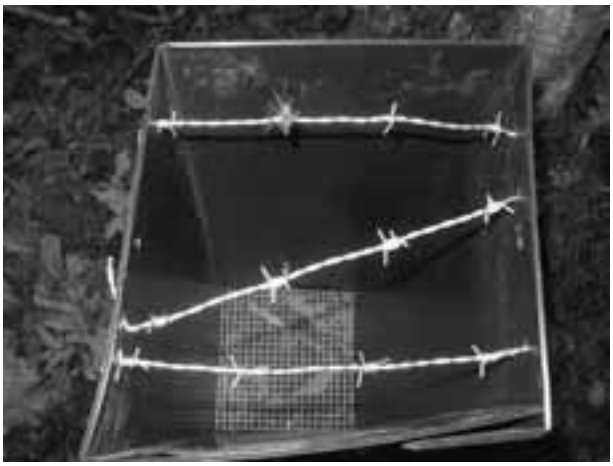


Figure 6.9. Single-capture cubby trap for kit foxes, illustrating (A) a side view of a fox entering and exiting and (B) the location of the sticky material and (inset) details of a tripline hook. Figure 6.9A is reprinted from Bremner-Harrison et al. (2006) with permission from The Wildlife Society. Illustrations by S. Harrison.

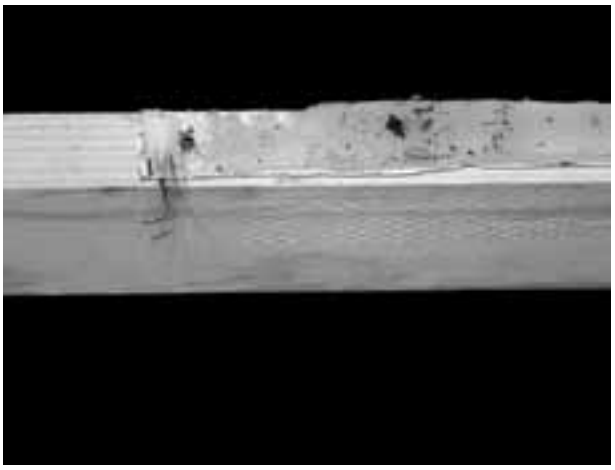
details) and offer two primary advantages for hair collection. First, they improve the reliability of hair capture by orienting the target animal. Further, certain species (e.g., martens and fishers) are detected at highest frequencies when bait is enclosed within a structure (Foresman and Pearson 1998). In North America, hair snaring cubbies have mainly been used to increase the attractiveness of hair snare devices for martens (figure 6.7; Foran et al. 1997b; Mowat and Paetkau 2002), kit foxes (figure 6.9; Bremner-Harrison et al. 2006) and fishers (figure 6.8; Zielinski et al. 2006; R. Long/P. MacKay, pers. comm.).

Until recently, published methods for the detection of fishers focused on track identification at track plates (Zielinski and Truex 1995; also see chapter 4) versus hair collection. Zielinski et al. (2006), however, tested the effectiveness of modified cubbies containing both hair snagging devices and sooted track plates for detecting fishers and martens. Some cubbies were modified by placing three strands of

barbed wire across the opening in a Z formation (figure 6.10A). Barbed wire with four-prong barbs every 7.6 cm was used to prevent target animals from slipping between the barbs unsampled. Other cubbies were modified with glue-impregnated cardboard sheets (originally designed to catch mice) attached to wooden slats and placed in front of the bait near the rear of the cubby at a height of 6 cm from the floor (figure 6.10B, C). The authors concluded that glue was preferable to the wire configuration for snagging hair—particularly from martens. Mowat and Paetkau (2002) also found glue to be highly effective for collecting marten hair. Likewise, in tests with captive wild martens and in a field trial in



A



C

B

Figure 6.10. Rectangular cubbies showing (A) barbed wire mounted in a Z pattern to collect hair from fishers; (B) wooden slats fitted with glue traps to snag marten hair; and (C) a close-up of a glue strip on a slat, with marten hair. Photos by F. Schlexer.

Michigan, 61 cm-long pieces of 10 cm-diameter plastic French drain tile, with glue pads attached to the top half of both ends, successfully detected martens (J. Belant, National Park Service, pers. comm.). Given that cardboard-backed glue pads can fall apart when wet (Mowat and Paetkau 2002), plastic-backed glue traps (Foran et al. 1997b) should be considered in wet environments.

It is important to note that, because box-type fisher cubbies were originally designed to obtain tracks using sooted plates and sticky paper (Zielinski and Truex 1995) and have only recently been modified to collect hair (Zielinski et al. 2006; case study 6.1), some published design features have been constrained by the requirements for collecting tracks. Since the target animal needs to walk across soot and then paper before reaching the bait, for example, fisher cubbies are very long relative to the size of the animal and are constructed to allow easy removal of both soot plates and paper. In addition, the rectangular cubbies used by Zielinski et al. (2006; figure 6.10A) required hardware cloth and stakes for stability. Such features are likely unnecessary—and may be counterproductive—if the sole goal is hair collection.

Triangular cubbies (figure 6.8A) have also been used to capture hair from fishers and martens (see case study 6.1). The primary advantages of the triangular design are that it does not collapse or need stakes for stability, and it requires less material than box-type cubbies. In the Idaho survey described in case study 6.1, single entry cubbies were fitted with barbed wire at the entrance, behind which three 30-caliber (7.62mm) gun brushes were attached to mechanical lugs (threaded metal connectors that provided support for the brushes) projecting from the walls of the cubby approximately 30 cm from the other entrance (figure 6.8B). Like Zielinski et al. (2006), this survey had no success sampling martens with barbed wire; of the forty-eight marten samples collected at 158 cubbies, all were on gun brushes. Triangular cubbies with gun brushes, based on the same design but with a single entrance, were combined with track plates (as in the Zielinski et al. [2006] surveys) to collect both hair and tracks from fishers during summer surveys in the Adirondacks of New York (figure 6.8A; R. Long/P. MacKay, pers. comm.).

Gun brushes have proven effective for sampling captive wolverines (J. Copeland, pers. comm.) as well. In general, gun brushes offer benefits in terms of ease of use. Lugs can easily be attached to a variety of surfaces, and because brushes are secured by a set-screw (figure 6.8B), they can be removed and replaced with very little handling. And brushes can be deposited directly into desiccant-filled vials, thus eliminating the need to handle hair in the field.

As opposed to setting cubbies on the ground (e.g., Zielinski et al. 2006; case study 6.1), Foran et al. (1997b) and Mowat and Paetkau (2002) mounted cubbies vertically on trees (figure 6.7A). The primary advantages of tree-mounted cubbies are that they are less likely to be covered with snow than are cubbies on the ground, and they may reduce unwanted bycatch. The main disadvantage of vertical mounting is that water can enter the cubby and expose both bait and hair to moisture. This can be addressed by placing a roof above the cubby (figure 6.7A; Mowat and Paetkau 2002) and is likely less of an issue if hair snagging devices other than glue are used.

It is important to size the cubby opening and the distance between snagging devices appropriately for the target species. Structures should also be tested to ensure that certain segments of the population—such as large males—are not excluded, and that smaller animals cannot slip through undetected. Devices must be placed in the cubby such that they make physical contact with the target animal, and there should be no space between them large enough to allow the target species to enter the cubby without contacting at least one device. Mounting devices to the sides of the cubby, as is common with gun brushes, will help to control the maximum width of the entry. Blocks can be used to extend adhesives away from the cubby walls (figure 6.9B; Bremner-Harrison et al. 2006). Pointing gun brushes away from the entrance minimizes resistance to entry

while providing a more aggressive snag when the animal exits.

To maximize effectiveness, a combination of hair snagging devices should be considered. The length of the cubby allows for several different devices between the entrance and the bait. Zielinski et al. (2006) found that some animals entered cubbies but failed to leave hair samples. Not only should multiple snagging devices decrease the number of undetected visits, but this approach provides an experimental context for testing the relative efficacy of various devices.

We suspect that as interest in and experience with collecting hair via cubbies increases, the diversity of cubby structures and the types and arrangements of hair snagging devices will expand. For example, J. Belant (pers. comm.) sampled raccoons by attaching barbed wire with 6 cm spacing between barbs in an inverted V at the entrance to a five-gallon bucket lying on its side. He baited the structure with a chicken wing or strip of bacon in a small mesh bag attached to the top rear of the bucket and braced it against a tree or with logs to prevent it from rolling or being moved. The optimal size for cubbies will also continue to be refined. Smaller enclosures can increase hair snagging efficiency and decrease unwanted bycatch, but may discourage entry (see chapter 4). Although most cubbies to date have either exclusively allowed entry from one end or are set up such that entry is primarily limited to one end, there is no intrinsic reason for this design—bait can be located in the center of the structure with hair collection devices at each opening to allow access from either end.

Passive Hair Collection Methods

This section describes hair collection methods that do not use baits or lures. Again, these methods rely on hair deposited by animals engaged in natural behavior (e.g., rubbing on trees), or snagged as animals pass by devices deployed on travel routes.

Natural Rub Objects

Although many species rub or roll on natural objects, opportunistic hair collection associated with rubbing behavior has largely been limited to rub trees for bears. Rubbing by bears has not been studied rigorously, but it is thought to represent a form of chemical marking for social communication (Green and Mattson 2003). Surveys in Montana and Wyoming found that grizzly bears and black bears commonly rubbed on trees (figure 6.11A), as well as power poles, sign and fence posts along forest trails and roads, and other structures (K. Kendall, unpubl. data; Green and Mattson 2003). Wolverines and wolves also rub on trees and various other natural and manmade objects and could potentially be sampled with this approach.

The height of the hair deposited on bear rub trees and limited photographic evidence suggest that, although bears sometimes rub the sides of their bodies while positioned on all four feet, they typically stand on their hind feet and rub their back, neck, and head. The most heavily used bear rub trees can be easily spotted by smooth or discolored patches of bark (figure 6.11B), bear trails (track-like depressions worn into the ground by bears repeatedly scuffing or grinding their feet in the same locations) leading to them (figure 6.11C), bare ground at the base, or the presence of large amounts of bear hair. But most rub trees are more subtle, and careful inspection is required to find them. Rub trees that do not occur along human trails or roads are often found on short game trails that become more distinct near the rub trees (Burst and Pelton 1983).

Bear hair naturally accumulates on rub objects, but samples from barbed wire attached to the rub area (figure 6.11D) tend to be of higher quality, require less time to collect, and define discrete samples that help prevent mixed samples containing hair from more than one individual (K. Kendall, unpubl. data). All hair should be cleared from rub trees before sampling to ensure that the period of hair accumulation is known and that genotyping success rates are optimized. Barbed wire should be mounted low enough to sample young bears and bears that stand on four feet when they rub.

Rub tree surveys are problematic in areas heavily used by cattle or horses. Cattle and bears tend to rub on the same trees, making it very difficult to find

A

B

C

D

Figure 6.11. Bear rub trees. (A) Grizzly bear rubbing on a tree in Glacier National Park (photo by J. Stetz). (B) Bear rub tree illustrating the discolored bark and damage that usually results from bear rubbing behavior (photo by A. Macleod/J. Stetz). (C) Bear trail leading to a rub tree (photo by A. Macleod/J. Stetz). (D) Wire (with bear hair) mounted on a rub tree to enhance hair collection (photo by W. Blomstedt).

deposited bear hair. When rub trees are located on trails used by horses, they are often bumped by pack stock. As barbed wire can damage packs, alternative hair snagging devices may be required. In the Bob Marshall Wilderness, Montana, where 15% of rub trees surveyed along trails were bumped by pack stock, the most effective alternative hair device tested was barbless fencing wire mounted vertically on trees (figure 6.12; K. Kendall, unpubl. data). Hair was snared between the split ends of the twisted wire strands and where staples attached the wire to the tree.

It may be possible to capitalize on the curious nature of bears by installing posts to serve as rub objects. A post-based hair collection survey for wolverines, conducted in a treeless area, found that grizzly bears rubbed and deposited hair on posts that had not been baited for several months (Dumond 2005; see case study 6.2). Abandoned apple orchards and feral crab apple trees that dot rural landscapes in northeastern North America attract black bears in late summer and early fall, providing additional opportunities for the passive collection of bear hair (Hirth et al. 2002). When black bears climb these trees to feed, their claws leave identifiable damage and hair accumulates on the dense, prickly branches and rough bark. Barbed wire can be wrapped around tree boles to increase the amount of hair snagged and to decrease the amount of time required for hair collection.

Figure 6.12. Smooth fencing wire can be mounted vertically on a tree to collect bear hair when the use of barbed wire conflicts with horse use on trails. Photo by Northern Divide Grizzly Bear Project, US Geological Survey.

Travel Route Snares

Unbaited hair collection devices including body snares, foot-hold traps, and lengths of barbed wire can be positioned on travel routes and runways to sample hair from select species. Such travel route snares are most effective in areas that feature dense concentrations of animals, and therefore single-catch methods (see *Multiple- Versus Single-Catch Structures* later in the chapter) are often employed to circumvent genetic lab costs associated with analyzing mixed samples (Paetkau 2003).

Beier et al. (2005) developed an efficient, single-catch method for collecting hair from brown bears using a modified wolf neck snare (figure 6.13). Snares were hung across bear trails along salmon spawning streams. Short pieces of barbed wire were attached to the snare cable, and a piece of inner tube was inserted to complete the loop and provide the breakaway component. To protect the target animal and enable the recovery of samples, the snare was firmly anchored so that the bear immediately broke the inner tube and the snare dropped where it could be later found. Snares effectively collected hair from bears representing a variety of coat conditions, both sexes, and a wide range of sizes, and they were inexpensive and easy to deploy. Substituting wire brushes for barbed wire increased hair sample sizes and may be worth the investment when maximum capture rates are required (Beier et al. 2005). This

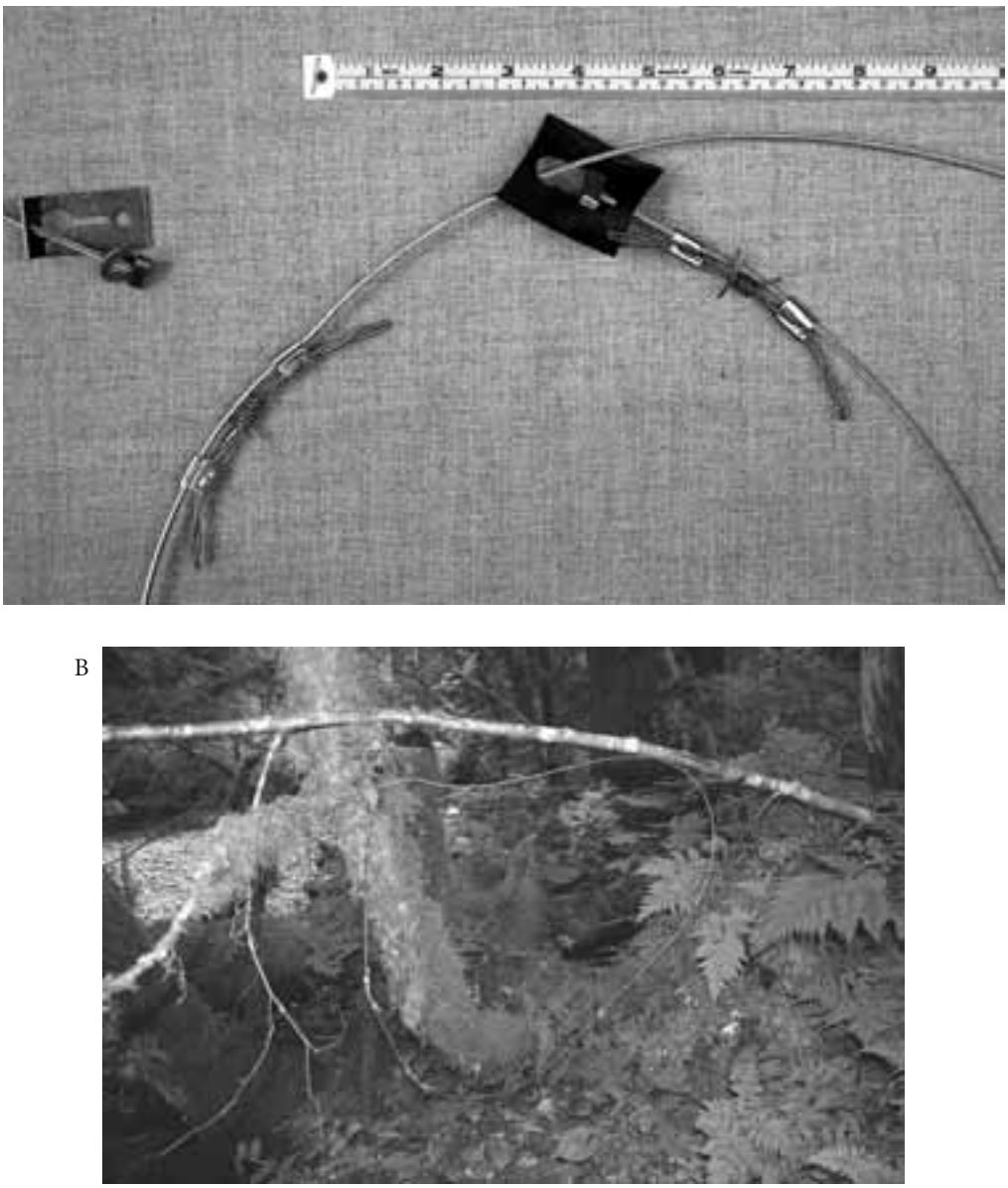


Figure 6.13. Modified, self-releasing body snares used to collect hair. (A) Close-up of a bear body snare with a break-away component (photo by S. Lewis). (B) A body snare hung across a trail to snag brown bear hair (photo by L. Beier).

method should be used judiciously in areas where other species frequent travel routes and is obviously inappropriate for trails used by people (see *Safety* later in this chapter).

Bears congregating to feed on fish can also be sampled by suspending barbed wire between trees (or posts in treeless areas) across bear trails. Hair snares were set up near spawning streams in Wyoming's Yellowstone ecosystem to assess the importance of cutthroat trout for grizzly bears (Haroldson et al. 2005). Approximately half of the sampling effort consisted of unbaited barbed wire stretched diagonally across bear trails or fishing sites; the other half comprised baited hair corrals near spawning streams. During four years of hair sampling, seventy-four grizzly bears were identified, and many

black bears were detected but not genotyped to distinguish individuals. Although unbaited wire sets were less efficient at obtaining hair samples than baited sites, they were useful in areas where it was not appropriate to use bear attractants (see *Safety* for measures to prevent human injuries from snares).

Similarly, a pilot study monitoring highway crossing structures designed for wildlife employed two strands of barbed wire spanning the width of underpasses to collect hair for identifying animals by species, gender, and individual genotype (Clevenger et al. 2005). The strands were respectively suspended 35 cm and 75 cm above the ground to target large carnivores (figure 6.14). Initially, a sticky string or webbing (Atlantic Paste & Glue Company, Quebec, Canada) was intertwined with the barbed wire. Although this method captured hair, many of the samples did not contain DNA—leading the researchers to conclude that hairs collected on the string were largely shed hairs (A. Clevenger, Western Transportation Institute, pers. comm.; see *DNA Quality and Hair Storage* in this chapter for a discussion of shed versus plucked hairs).

Two types of single-catch, unbaited hair snares have been used to sample river otters at river- and ocean-side activity sites (DePue and Ben-David 2007). The first involved setting modified body-snares on otter trails. Two to four microstrands of cable were inserted perpendicularly through the snare cable, with 4 mm lengths protruding at various angles from either side (figure 6.15A), and the snare locking mechanism was replaced with a paper clip to allow the snare to cinch around the target animal and then break free. In the second application, modified foot-hold traps (figure 6.15B) were set at otter latrine (i.e., scent-marking) sites. Hair capture success for otters was three times higher with body snares than foot-hold traps, but foot-holds may be useful should animals develop an aversion to entering body snares. As otter feces are difficult to locate in the field, hair sampling may be superior to scat collection for the DNA identification of individual river otters (DePue and Ben-David 2007); but see chapter 7). Modified foot-hold traps and body snares could potentially be effective for hair sampling coyotes and other canids (DePue and Ben-David 2007).

Eurasian badgers that did not respond to baiting were sampled by barbed wire strung 20 cm above the ground between stakes set on both sides of a clearly visible badger run (Frantz et al 2004). With a similar

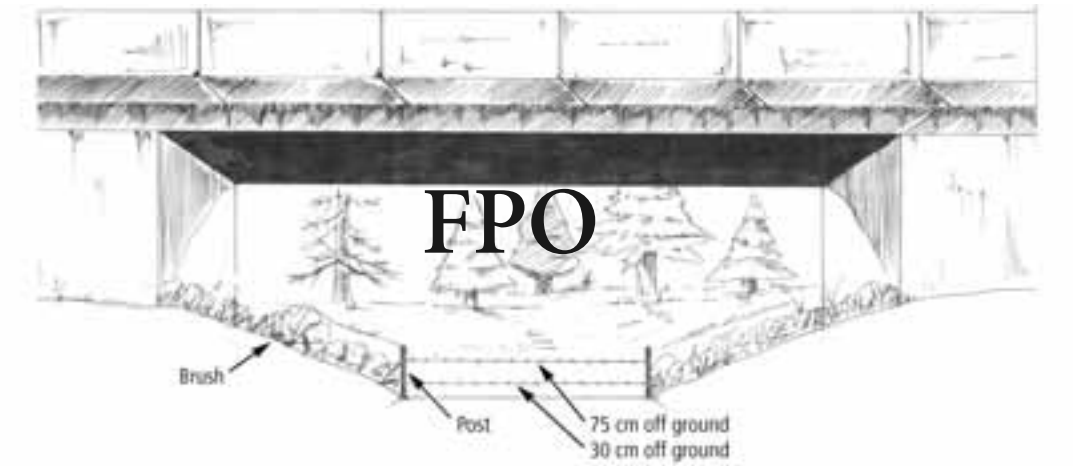


Figure 6.14. Ground-level view of a hair sampling method for detecting carnivore movement through a highway underpass (adapted with permission from Clevenger et al. [2006]). Note the brush placed over page-wire material and used to funnel animals toward the wire structure. Illustration by S. Harrison.

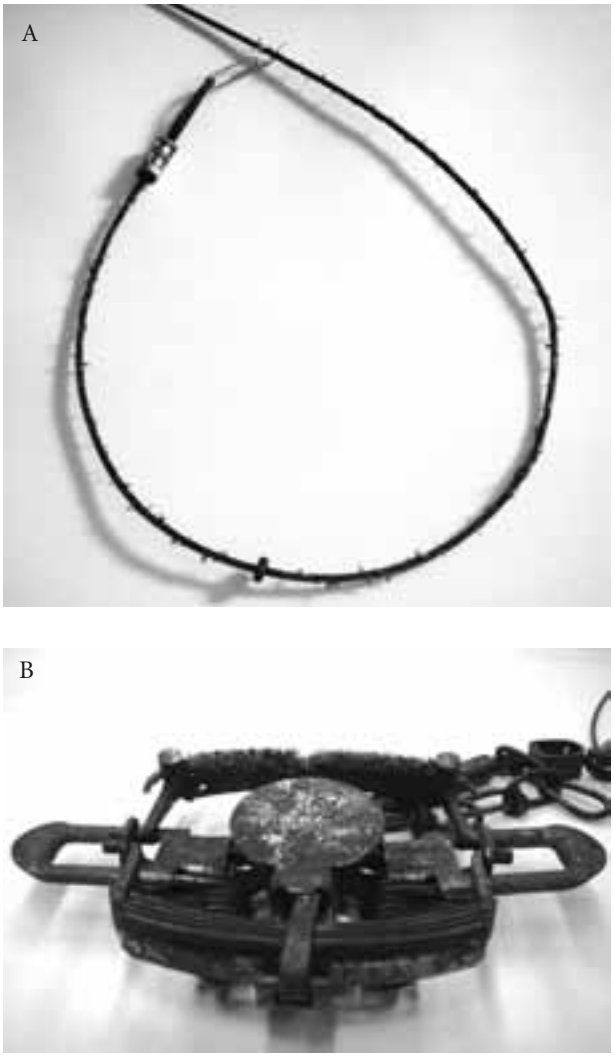


Figure 6.15. Single-catch structures modified from live capture traps designed to collect hair from a single individual and then instantly release the animal. (A) A body snare with a break-away component (i.e, paper clip) used to sample river otter hair; wires that serve as the hair snare device are inserted into the cable. (B) A foot/leg trap fitted with hair collection brushes modified to press brushes against the animal's leg and then allow it to escape. Photos by J. DePue; reprinted from DePue and Ben-David (2007) with permission from the The Wildlife Society.

design, hair from hairy-nosed wombats was collected by suspending strong double-sided sticky tape between metal posts placed on both sides of burrow runways and entrances (Sloane et al. 2000). Because this method was likely to produce mixed samples, single rather than pooled hairs were genotyped to identify individuals. Given that the distal end of the hairs usually stuck to the tape and the follicles held clear, the samples air-dried rapidly and were easily clipped for extraction (Banks et al. 2003). Unbaited sticky tape corrals and runway barbed wire or adhesive hair snares appear to function best when sampling small species whose travel routes, dens, or nest sites are well defined.

Practical Considerations

When planning and implementing a hair collection survey, practical considerations abound. Here, we discuss those pertaining to safety, DNA quality and hair storage, single- versus multiple-capture devices, target species-specific behavior, and basic materials and cost estimates for a number of methods.

Safety

There are a number of safety concerns related to stringing barbed wire across game trails. To protect nontarget species—especially ungulates—a thin, strong pole can be nailed above the wire; ungulates tend to step over the pole while bears duck under the pole and wire (G. Mowat, British Columbia Ministry of Environment, pers. comm.). The pole prevents fast-moving elk (*Cervus elaphus*) and moose (*Alces alces*) from breaking the wire and reduces the collection of hair from nontarget species. In areas where people use game trails, such as along fishing streams or mountain biking and jogging trails, the use of poles—or alternatively, flagging and signing the wire—can prevent human injury and may promote human tolerance of the wire's presence. Careful consideration should be given to employing barbed wire snares in places frequented by people.

DNA Quality and Hair Storage

Hair must be collected in a manner that allows for subsequent analysis and stored such that DNA

degradation is minimized. Successful surveys require detailed planning that takes into account seasonal pelage changes, animal behavior, and the climate and circumstances under which hair will likely be collected (also see chapter 9 for details on obtaining DNA from hair).

As a source of DNA, hair varies in quality and quantity depending on the particular species, the environment in which samples are collected, and whether the method plucks hair or collects shed hair. Follicles are the best source of DNA, and plucked hairs have follicles attached more frequently than shed hairs (Goosens et al. 1998). Hair that is still growing is more difficult to pull out than shedding hair, however, so its collection should be timed to optimize competing trends in hair sample size and quality. For example, bear projects typically collect larger samples in spring and summer because the hair is preparing to shed and is looser than in fall and winter, but samples snagged during cold weather contain more DNA per hair (D. Paetkau, pers. comm.; T. Eason, pers. comm.).

There is no nuclear DNA in hair shafts, but shafts with no follicle can provide useful DNA contributed by dander, saliva, or DNA-containing tissue that adheres to hair as it grows (Williams et al. 2003). Using mitochondrial DNA, Mills et al. (2000) successfully obtained species identification from 84% (91/108) of hair samples without follicles. Occasionally, problems can arise when genotyping hair with no roots from family groups or social animals such as wolves, because it is difficult to discern if the DNA originated from the animal that deposited the hair or from saliva or dander contributed by conspecifics.

Coarse guard hairs yield more DNA than fine underfur because the follicles are larger. Compared with scats, DNA from hair is "cleaner" (i.e., contains few polymerase chain reaction [PCR] inhibitors) and less degraded, but hair produces a relatively tiny DNA sample. Thus, in many cases, only a single extraction can be made from any given sample, and it is possible to exhaust the sample before analysis is complete (Paetkau 2003).

The number of hairs available and the amount of DNA obtained from them varies by species due to differences in density of the coat and fineness of the hair (Goosens et al. 1998). The smaller follicle of fine hair contains less DNA than that of coarse hair and provides less surface area for the surrounding DNA-carrying tissue to adhere to. For example, because felid hair is finer than bear hair, noninvasively collected felid hair samples only sporadically contain DNA sufficient in quality to reliably identify individuals. Genotyping success rates from bear guard hairs are much higher, while success rates for finer bear underfur are similar to felid hair (D. Paetkau, pers. comm.)

The climate at hair collection study sites influences the amount of useful DNA obtained. Ultraviolet light and moisture degrade DNA, with the degree of deterioration increasing with length of exposure. When sampling with cubbies or in forests with dense canopies, however, ultraviolet exposure is limited and moisture is the chief concern. For best genotyping results, hair should not be left in the field for longer than three to four weeks in dry, sunny climates, and should be collected more frequently in wet climates (D. Paetkau, pers. comm.). It is possible to obtain useable data from some older, weathered hairs, but genotyping success is lower and lab costs are higher than for fresh hair.

Hair is uniformly stored dry, and two approaches have been widely used. For bears, which often provide many samples, hair is pulled from barbs and generally stored in small paper envelopes—with silica gel desiccant if the climate is damp. A second approach is to place each hair sample in an air- and water-tight plastic vial containing desiccant. The advantages of vial storage are that it is more secure and typically minimizes sample handling in the field. For gun brushes and barbed wire used in cubbies, the brush or barb (cut from of the wire with pliers) can be dropped directly into a vial, obviating the need to handle hair. The disadvantages of vials include bulk and expense (of materials and because many labs will charge extra to remove hair from the sampling device)—both of which are important considerations for bear surveys. When glue is used to collect hair, glue pads can be covered with clean plastic (the manufacturer's cover works best), and placed

in a paper envelope or bag to protect hair samples until they reach the DNA lab (see chapter 9 for hair removal methods).

Multiple- Versus Single-Catch Structures

Multiple individuals or multiple species depositing hair on a single hair sampling device can result in a failure to identify individuals or species, respectively. These problems can be eliminated by analyzing single hairs, but single hairs often provide too little DNA for analysis. In a study of shed hair from chimpanzees (*Pan troglodytes*), Gagneux et al. (1997) showed that 31% of all single-hair amplifications produced allelic dropout (see chapter 9 for a discussion of genotyping errors). Goosens et al. (1998) found that error rates fell from 14% to 4.9% to 0.3% as numbers of alpine marmot (*Marmota marmota*) hairs increased from one to three to ten hairs, respectively. It is therefore advantageous to design hair collection structures and sampling approaches to minimize mixed samples.

Open hair collection methods—such as barbed wire hair corrals—are capable of detecting multiple individuals at a single set, but because there are many barbs available and animal movement is not concentrated, there is a low probability of two animals leaving hair on the same barb. Mixed hair samples are more likely to result when animal movement is either concentrated, such as with hair snares stretched across travel routes, or channeled to relatively few snagging devices, as in cubbies. Thus, in these situations, collection intervals should be shorter than with hair corrals if individual identification is needed. With canids and other social species that overmark, even open methods can yield many mixed samples. If the device is intrinsically likely to collect mixed samples, the only way to reduce their proportion is to reduce the total number of samples collected at each device. This can be accomplished by employing shorter intervals between checks, or by providing an easily removable, single-serving size of bait that is consumed during the first visit by an animal (Foran et al. 1997b; Mowat and Paetkau 2002).

Single-catch structures terminate sampling after one animal visit. Kit fox cubbies can be modified so that a trip wire attached to the bait frees a door that closes the trap after the animal backs out (Bremner-Harrison et al. 2006; figure 6.9). A similar idea was successfully implemented for fishers and martens using a modified box trap in which the door was prevented from locking (Belant 2003a). This allowed captured animals to push the door open to escape, but prevented any other animals from entering. In another application, a mechanism for sampling black bears consisted of an arm that pressed tie plates (used in wood construction) against the target animal when the animal pulled on hanging bait (Immell et al. 2004), and was unlikely to be activated once the bait was gone. Modified body snares, described above in this chapter, have also been employed as successful single-catch structures for bears (Beier et al. 2005) and river otters (DePue and Ben-David 2007).

Behavioral Considerations

Given that all baited hair collection methods seek to induce a behavioral response, understanding the biology underlying this behavior is important for successful sampling. For instance, if the induced behavior is related to territorial marking, the sex or age of samples may be biased. Alternately, food baits are more effective during seasons when the target animals are hungry and might fail at other times of the year. If the response to bait is linked to its novelty, then initial response rates can provide misleading information when used to design a multisession capture-recapture study. Additionally, interspecies interactions may affect sampling success. Downey (2005), for example, postulated that gray fox visits to rub pads might have interfered with felid marking.

While there is no doubt that induced behavior lies at the core of baited methods, behavioral studies designed to elucidate how animals respond to bait are exceedingly difficult to execute. Most frequently, these studies use captive facilities to study the reactions of a few animals. Captive animal responses, however, may differ from responses of animals in the

wild. Captive animals are generally bored, well fed, and situated only a short distance from the bait. Also, captive facilities are saturated with animal scent, and territorial behavior is often absent. For these reasons, results from captive studies are probably most valuable for addressing physical versus behavioral issues. For example, the use of captive animals to determine whether a given hair collection device will reliably produce DNA if an animal enters a cubby will probably be more reliable than testing a variety of baits to determine which bait elicits cubby entry. Due to the logistics and costs of field testing, most baited hair collection methods rely on modified trapper sets whose baits and capture methods have been refined through centuries of trial and error. In fact, the evolution of hair snagging methods and protocols has benefited as much from the rich tradition of trapper lore as it has from controlled experimentation.

Materials and Costs

The bulk of expenses for hair collection-based population studies are associated with genetic analyses and field technician salaries. Nonetheless, the cost of materials and equipment necessary for hair snaring devices can, in some cases, determine the collection method, the sampling intensity, and the size of area that can be studied. Although space precludes an exhaustive list, we have provided a summary of the materials and costs associated with three commonly used hair collection methods.

Bear Hair Corrals

Materials. Barbed wire (30 m), fencing staples (0.23 kg), lure, Rite in Rain paper for warning signs, twine to hang lure-soaked cloth, cloth, paper envelopes for hair. Cost: $4.50 per corral.

Equipment. Fencing pliers (1), leather gloves (2), global positioning system (GPS) (1) per two-person crew.

National Lynx Survey—Detection Protocol

Materials. Carpet pads, pie pans, dried catnip, liquid lure, forceps, surgical gloves, nails, stove pipe wire for hanging pie pans, swivel hooks, desiccant vials, plastic bags, flagging. Cost: $2.50 per set.

Equipment. Hammer, GPS, magnifying glass (to look for hair).

Cubbies for Marten/Fisher

Materials. Corrugated plastic sheeting (0.5–10 m^2 per cubby), barbed wire, gun brushes, or glue pads, hardware cloth, meat or chicken wing, commercial scent lure, duct tape, gloves, pliers with wire cutters (if barbed wire is used), desiccant vials, plastic bags, flagging. If the cubby is to be mounted on a tree, add wood screws and nails. Cost: $4 per cubby with barbed wire, $7 per cubby with gun brushes.

Equipment. Hammer, pliers, GPS.

Survey Design Issues

The efficacy of various hair collection methods is governed by the biology of the target species, the physical characteristics of the hair, and the ability of the devices to collect hair. Method effectiveness in turn determines the types of analyses that can be conducted. Capture-recapture methods, for example, require that a significant proportion of the total population be captured more than once. For sparsely distributed carnivores, achieving this level of capture typically requires a very desirable attractant capable of "pulling" animals from long distances. Species that have an acute sense of smell, like bears and wolverines, can presumably be drawn from great distances to visit bait or scent stations. Thus, high capture rates can be achieved with bears (Boulanger et al. 2002, , 2005a, b) and winter-surveyed wolverines (B. Mulders, Northwest Territories Department of Resources, Wildlife, and Economic Development, pers. comm.) using widely spaced detection stations. Felids, in contrast, are thought to be difficult to attract from long distances because they respond primarily to visual stimuli. Therefore, lynx detection stations include visual attractants (see *Rub Stations* and box 6.3) and are set in closely spaced transects (McDaniel et al. 2000). Audio attractants

can also be used to enhance detection rates (see chapter 10); Chamberlain et al. (1999) reported that bobcat (*Lynx rufus*) detection was higher at track stations equipped with a mechanical cottontail rabbit distress call than at stations containing a fatty acid scent, bobcat urine, or a visual lure.

Ultimately, the questions that can be addressed using hair collection data are related to the overall detection rate, the recapture rate, and the quality of the DNA extracted. Therefore, a clear understanding of detection rates and the expected quality of the DNA to be collected must be developed prior to designing the study. This will also help ensure that anticipated analyses are consistent with the data.

Bias

When designing noninvasive surveys based on hair collection, there will almost always be the strong potential for sampling bias in captures, recaptures, or both. Because many carnivores are difficult to sample, however, detection rates may not be high enough to quantitatively assess these biases. Potential sources of bias should be carefully considered, and survey designs should attempt to minimize these biases even if bias has not been demonstrated in previous studies.

As discussed in chapter 10, scent-based attractants can consist of either consumable food or a scent lure that provides no food reward. When animals receive food at sampling structures, they may develop a trap-happy response—thus exhibiting higher recapture rates. Attractants lacking a food reward may have the opposite effect: once the animal determines that there is no reward, it might not be interested in revisiting the site—a situation referred to as trap-shyness. For capture-recapture estimates of population size, models that accommodate a trap-happy or trap-shy response (i.e., behavioral variation) are less precise than simpler models. Hair corrals for bears are commonly moved between sessions to increase novelty and thereby discourage trap-shy behavior (Boulanger et al. 2006).

Unbaited methods are also prone to biased captures. Collecting hair from natural rub objects, for instance, may bias capture toward those sex and age classes that are engaged in territorial marking. For grizzly bear population estimates using combined rub tree/hair corral data (e.g., Boulanger et al. 2008), we advise modeling males and females separately if sample size allows; rub tree samples are biased toward males when collected prior to midsummer (K. Kendall, unpubl. data). Similarly, because ursid social hierarchy dictates that adult male bears exclude other sex and age classes from the most favorable fishing sites (e.g., in Yellowstone National Park, male grizzly bears consume five times more trout than females [Felicetti et al. 2005]), less productive sampling locations must be sought to ensure that subadults and females with cubs are adequately sampled. Furthermore, barbed wire heights that are best for sampling adults often miss juvenile animals. And where grizzly bears and black bears are sympatric, rub trees detect more grizzlies than black bears—even if black bears substantially outnumber grizzlies (K. Kendall, unpubl. data). This phenomenon must be kept in mind when estimating survey effort in studies targeting both black and grizzly bears.

Power and Precision Considerations for Capture-Recapture Sampling

When estimating population size, power analysis based on the expected population size and desired precision of the estimate can be used to determine the density of hair snares (Boulanger et al. 2002, 2004b). As most of the published, hair-based capture-recapture studies have been directed at bears, we primarily use bear studies as examples in this section.

For traditional capture-recapture studies, traps are typically placed in a grid—a model that has been followed in most hair corral surveys of bears (Woods et al. 1999; Triant et al. 2004 used hexagonal cells). In capture-recapture analysis, the precision of population estimates increases with the probability of capture, the number of sampling occasions, and the degree to which the capture rates follow "null model" expectations (i.e., equal capture probability for all individuals and across sessions,

no behavioral response, geographic and demographic closure; Otis et al. 1978; White et al. 1982). Capture-recapture-based bear studies typically conduct four to five sampling occasions (Boulanger et al. 2004b). To minimize individual capture heterogeneity, the ideal cell size is no larger than the smallest individual home range during sampling (White et al. 1982). A meta-analysis of seven DNA-based hair collection studies of interior grizzly bear populations examined tradeoffs between increasing the precision of the estimate and ensuring geographic closure (Boulanger et al. 2002). As cell size decreased, the recapture rate and precision of the estimate increased, but cost constraints mandated decreasing the size of the study area resulting in an increased likelihood of closure violation. Because monetary constraints often preclude sampling at the optimal intensity, we advise careful consideration of precision requirements before embarking on capture-recapture studies using hair sampling.

Another important design question is whether to move hair collection structures between sampling occasions. If attractants are used, moving structures to new locations between occasions may inhibit habituation to the attractant and decrease individual capture heterogeneity. Relocating structures is also thought to reduce conditioned behavioral responses to sites baited with food. When hair snare density is high (e.g., 4 snares per home range), and scent lures (as opposed to reward-type baits; see chapter 10) are used as attractants, moving snare sites between occasions is generally thought to be unnecessary (Mowat and Strobeck 2000; Boersen et al. 2003). In an empirical test of sampling strategies, however, Boulanger et al. (2006) compared moved and fixed site designs using the same sampling density and found that moving sites between sample sessions resulted in more captures and reduced capture heterogeneity.

Hair collection intervals will also be determined by balancing competing goals. Shorter sampling intervals (e.g., one- to seven-day sessions) minimize violations of demographic and geographic closure for closed population models, as well as exposure of hair to DNA-degrading UV radiation and moisture. But the number of individuals visiting a site, and therefore the probability that any given individual will be captured or recaptured during an interval, increases with interval length as long as the attractant remains effective. To complicate matters further, the effectiveness of scent lures and baits fade with time unless they are refreshed. For many hair collection studies, a fourteen-day sampling interval has been chosen as a reasonable compromise (Boulanger et al. 2005a, b; Proctor et al. 2007; K. Kendall, unpubl. data). These and other considerations for designing capture-recapture surveys are discussed further in chapters 2 and 11.

Assessing Occurrence and Distribution

For detection-nondetection sampling, the goal is to survey with sufficient effort to reliably detect at least one individual if the area is occupied, or to estimate the probability of detecting an individual, which enables occupancy to be accurately estimated when detectability is low (see chapter 2 for more details). Repeated visits (i.e., multiple independent sampling occasions) are the key to meeting either goal, as the resulting pattern of detections and nondetections furnishes the information necessary to compute detection probabilities (MacKenzie et al. 2002).

The NLS, designed to provide reliable presence-absence information for lynx across large administrative units such as national forests and national parks (McKelvey et al. 2006), presents a good example of design issues that should be considered when using hair sampling to document occurrence. The overall goal of the NLS was to define current lynx range at a relatively coarse scale, and to locate populations. Thus, the initial survey was the first step in a multistep process. The first step of the survey was to collect hair from at least one lynx in each occupied area and to do so with high reliability. To accomplish this objective, rub pad transects were designed to saturate a given area, with twenty-five transects placed on a 3.2 km grid. Each transect consisted of five collection stations 100 m apart, running perpendicular to the slope contour. To satisfy survey re-

quirements, pads were left in the field for at least one month (two sampling occasions) during the summer, and for three consecutive years. Additional grids were located in areas where lynx were known to be present, providing tests of survey effectiveness that ran concurrently with the general sampling. If lynx were detected in an area where they were previously undetected, intensive snow tracking surveys were initiated the following winter (Squires et al. 2004). This follow-up effort was designed to determine whether resident lynx were in the area and to look for evidence of reproduction (i.e., family groups). Further research, in turn, could entail live-capture/radio telemetry to evaluate survival, reproduction, and habitat use patterns.

Sampling Without a Representative Design

In contrast to surveys such as the NLS, where a grid-based design was used to equalize sampling effort, the objective of some surveys may be to simply confirm the presence of a single individual in an area where a species has been sighted or where putative tracks have been identified (see chapter 2 for pitfalls of such single-location efforts). In fact, much useful information can be gleaned from hair collection efforts even if fully representative sampling is precluded by logistical constraints or the use of passive methods. Establishing that a rare carnivore is present in an area, and particularly that both sexes are present, can be of tremendous importance for conservation. Such goals can often be achieved using nonrepresentative sampling. Further, in certain cases (case study 6.2), passive methods (e.g., rub trees that can be sampled opportunistically while traveling to and from baited survey structures) can increase the total number of individuals counted and contribute to MNA estimates.

Additionally, the genetic monitoring of effective population size and habitat connectivity, or the detection of hybridization (Schwartz et al. 2004) can often be based on small samples, and rules for representativeness may be relaxed when compared with occupancy or capture-recapture sampling (Schwartz et al. 2007). Perhaps the most important point to keep in mind when contemplating nonrepresentative sampling is that meaningful results are produced only if samples are obtained, and results generally become more meaningful as sample size increases. Negative results are not interpretable (i.e., nondetections do not mean that the species is not present).

The variable detection effort associated with passive hair collection methods is generally consistent with the above types of goals but not with the requirements of abundance or occupancy estimation. It may, however, be possible to use nonrepresentative samples in combination with representative samples to produce abundance estimates. The Lincoln-Petersen model, for example, requires only that capture *or* recapture achieve equal capture effort for all animals (Seber 1982). Thus, a rub tree survey can provide the recapture samples for captures made with a hair corral grid if the grid-based captures are uniformly distributed across the sampled population. Recently, more complex capture-recapture models that allow mixing of representative and nonrepresentative samples have been developed that yield estimates of similar magnitude but higher precision than those made with grid-based data alone (Boulanger et al. 2008).

Sample and Data Collection and Management

Extensive hair collection surveys using the methods presented in this chapter may result in hundreds to thousands of hair samples. With many individuals in the population, and possibly thousands of samples collected, there are countless ways in which errors can creep into a survey—potentially invalidating its results. Errors can be minimized, however, with careful sample and data management.

Subsampling Hair

Most noninvasive hair sampling methods provide redundant samples for many of the animals

sampled. For example, a bear visiting a corral may leave multiple samples upon entry and exit. While genotyping multiple samples from the same individual is one way to check for genotyping errors (through replication of multilocus genotypes; see chapter 9), analyzing all samples collected is seldom desirable. Not only does the analysis of redundant samples increase cost, but genotyping errors can lead to "inventing" spurious animals if stringent measures are not taken to guard against them (chapter 9). Thus, even when single-catch methods are employed, a subsampling scheme is often necessary to minimize analytical costs while maximizing the number of individuals detected. Although not all hair samples will be analyzed, it is important to collect and retain all samples as reserves in case problems arise with genotyping the initial sample.

There are two basic approaches to subsampling, with one rooted in design and the other in analysis. These approaches are not mutually exclusive. Design-based approaches seek to limit redundancy by taking advantage of known characteristics of the target species and hair snagging structure. With bear corrals, for example, two hairs found on adjacent barbs during a single sampling period are more likely to be from the same bear than hairs found on barbs 5 m apart. Most bear studies using barbed wire corrals thus subsample hair based on adjacency. Typically, the largest sample among adjacent barbs is analyzed (M. Proctor, Birchdale Ecological, pers. comm.; R. Mace, pers. comm.; G. Stenhouse, Alberta Fish and Wildlife Service, pers. comm.). This strategy is commonly thought to detect the maximum number of individuals at the least cost, but that assumption has not been tested. An alternative design-based approach employs systematic subsampling. Mowat et al. (2005) usually analyzed every third sample in a group of adjacent samples, and at least one sample from each group of adjacent samples. These researchers did not extract adjacent samples or samples separated by a single barb. Such corral-specific approaches can be adapted for most of the other hair collection methods described in this chapter.

Analysis-based approaches involve subsampling—either randomly or with design considerations—and analyzing samples until the desired output metric is stabilized. For instance, if the goal is to determine the number of individuals represented in a sample, one strategy would be to randomly analyze samples until the total number of individuals asymptotes. The advantage here is that the effects of subsampling are directly related to the desired output metrics. That is, one can estimate the likely change in the output had all samples been analyzed. The disadvantages associated with analysis-based approaches lie in the need for very close collaboration with the DNA lab, and lab-related inefficiencies and resultant higher costs due to running samples in multiple, smaller batches.

Tracking Hair Samples

For most studies, properly associating a particular sample with a specific time and place is critical. Mistakes in recorded time or location can be made in the field or in the lab. To avoid labeling errors, a recent, large-scale grizzly bear survey in Montana utilized bar-coded labels on hair sample envelopes, with duplicate peel-off sample number labels (i.e., piggyback labels) for field data forms (figure 6.16; K. Kendall, unpubl. data). This system allowed data entry via scanning of the bar codes and ensured that forms and data remained linked to the proper samples in the field and lab. Sample labels should include complete information on the date and location associated with the collection of each sample, so that even if the field data form is lost, the sample can be properly documented. In cases where survey results may be controversial or affect the management of rare or high profile species, hair samples need to be closely tracked and secured in limited-access, locked files.

As discussed, many hair sampling techniques produce multiple samples from one animal visit. For instance, with bear hair corrals, it is common to obtain multiple samples from adjacent barbs associated with a single bear crossing the wire, and when wolverines climb posts wrapped with barbed wire,

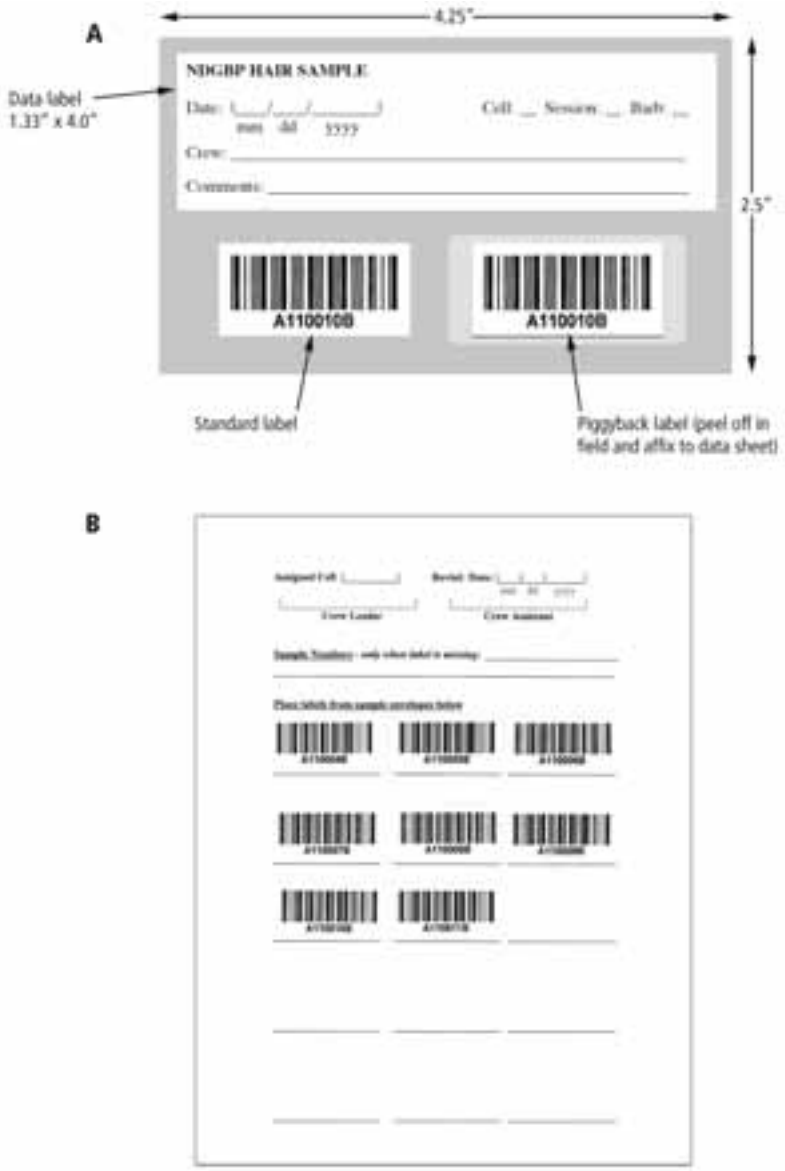


Figure 6.16. (A) Bear hair sample envelope with a removable piggyback barcode label and (B) field form with attached bar code labels (K. Kendall, unpubl. data).

they can deposit hair on adjacent rows of wire. To identify samples that are likely to be redundant, it is almost always useful to record the position of the hair sample on the device or within the collection structure.

For barbed wire hair corrals, barbs can be numbered sequentially beginning at any of the trees or posts supporting the wire (figure 6.3). If two wires are used, the barb number for a hair sample found on the lower wire should correspond with the number assigned to a sample found directly above it on the upper wire. To record sample position on wire-wrapped trees and round posts, it is helpful to divide the wire into four vertical sectors with permanent, waterproof paint (paint pens work well) after the wire is spirally wrapped. Then the row and barb

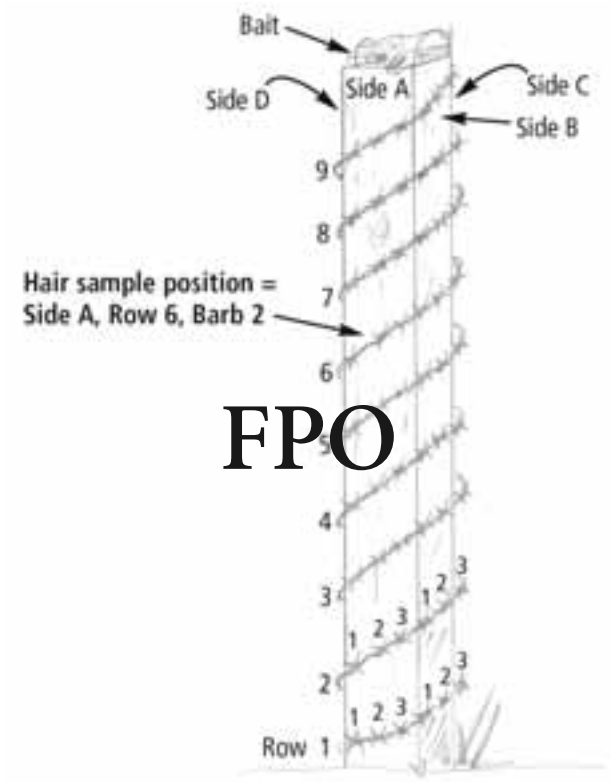


Figure 6.17. Method for numbering barbs on a wire-wrapped post to record the position of hair samples, for use in selecting a subset of samples for genetic analysis. Illustration by S. Harrison.

number can be recorded for each sector. If square posts are used, each side can be labeled and considered a sector (figure 6.17).

Future Directions and Concluding Thoughts

Noninvasive hair sampling is increasingly being used worldwide to enhance our scientific understanding of an ever-widening array of taxa. With hair collection methods, questions can be addressed that have defied other sampling strategies, or that were not possible to tackle before the advent of methods to analyze small DNA samples (e.g., PCR; chapter 9). In this relatively young field, existing techniques are being continuously refined and new sampling approaches developed. For example, break-away body hair snares that have been used for brown bears and river otters impart unexplored potential as single-catch hair sampling methods for a variety of other species.

Hair will likely persist as a primary source of DNA for mammal studies, particularly where baits and scent lures are used to attract animals. But emerging avenues for the noninvasive acquisition of genetic material also offer promise. For example, saliva samples containing DNA have been collected from tree cambium fed upon by bears and from baited sampling discs (D. Paetkau, pers. comm.); snake, whale, and bird populations have been studied using sloughed skin or shed feathers; and dingoes have been identified from epithelial tongue cells (N. Baker, pers. comm.).

Improvements in DNA extraction and the development of better primers will undoubtedly enhance our ability to identify animals from hair and reduce the costs associated with genetic analyses. Nonetheless, hair collection will remain a multistage endeavor. The collection and subsequent DNA analyses of samples are only the last steps in a lengthy survey process; if no animals visit a collection structure, the effectiveness of the snagging device and the quality of the DNA lab will be of little importance. The utility of hair samples is tightly linked to the overall efficacy of the survey design, which in turn is linked directly to the behavior and biology of the animals. Analysis methods such as capture-recapture are critically dependent on rates of detection. For these reasons, we believe that the greatest advances in noninvasive sampling will likely be associated with better understanding of target species biology. Studies of an animal's behavior when presented with a bait stimulus are a vital and often undervalued component of noninvasive sampling design.

The field of noninvasive hair collection has developed rapidly in the last ten years and will continue to do so with innovations by field biologists. The rate of growth in the future will depend in part on how well experimental studies of new methods are designed, and on how widely the results are dissemi-

nated. We encourage experimentation with and adaptation of the methods described in this chapter to create new hair sampling approaches. We also recommend using domestic and captive animals in initial trials of hair snagging devices and structures, as well as testing the efficacy of novel techniques with pilot studies before launching larger projects. The notes sections of journals, and methods-oriented periodicals in general, should be fully utilized to make sure that the details of newly emerging hair collection methods are made available to other scientists and managers.

CASE STUDY 6.1: DNA SURVEY FOR FISHERS IN NORTHERN IDAHO

Samuel Cushman, Kevin McKelvey, and Michael Schwartz

Location: Northern Selkirk Mountains in northern Idaho.

Target species: Fisher.

Size of survey area: ~1,500 km^2.

Purpose of survey: Unique haplotypes indicating the presence of a residual native population of fisher were found in central Idaho (Vinkey et al. 2006). Fishers had been detected previously using camera sets in the Selkirk Mountains just south of the Canadian border, but their population status and genetic composition were unknown. The purpose of the study was to provide a comprehensive survey of the northern Selkirk Mountains and to determine the genetic makeup (and therefore population source) of detected fishers.

Survey units: Creek drainages ≥30 km^2 in area.

Survey method: This study used cubbies constructed from folded plastic sheeting. In 2003–4, the cubby design followed Zielinski et al. (2006). The cubbies used in 2005–6 were triangular by cross section, with sides 41 cm in length, and each contained three 7.62 mm gun brushes in addition to the Z of barbed wire described in Zielinski et al. (2006). Both years, the cubbies were baited with a carpet pad soaked in beaver castoreum and approximately 125 cm^2 cube of deer meat. These items were attached to hardware cloth (i.e., wire mesh) on the inside of the cubby. A sponge splashed with skunk essence was hung above the cubbies as a lure.

Survey design and protocol: The Selkirk Mountains are a granite batholith cut by deep canyons. As fisher habitat was located in the densely timbered valleys, surveys were concentrated in the valley bottoms, while the higher elevation areas were not surveyed. Surveys were conducted during the winters of 2003–4 and 2004–5. Cubbies were placed at approximately 1 km intervals along roads and trails in major creek drainages (figure 6.18), and were checked and rebaited once after a period of sixteen to thirty-six days. Total sampling periods varied from thirty to seventy-three days. Snowmobiles were used to set and check hair snare cubbies, with the exception of a single roadless area that was surveyed using snowshoes. Efforts were made to survey all drainages larger than 30 km^2, although there were some holes in the survey due to lack of access.

Analysis and statistical methods: Hair samples from mustelids were analyzed to the species level using restriction enzymes for all samples (Riddle et al. 2003). A small group of nonmustelid samples were sequenced and compared to published sequences in GenBank (www.ncbi.nlm.nih.gov/BLAST/).

Results and conclusions:

During both years combined, 344 cubbies (186 in year one, 158 in year two) were placed along roads and trails in twenty major creek drainages (figure 6.18).

2003–4 Field Season

- Of 300 hair samples, most were collected from the floor of the cubby versus from barbs.
- Only 55% of samples produced DNA of sufficient quality for analysis.
- Eighteen samples collected from eight cubbies were identified as fisher; twenty-two samples collected from fourteen cubbies were identified as marten.
- Of the eighteen fisher samples, one had a haplotype associated with native fishers. The other

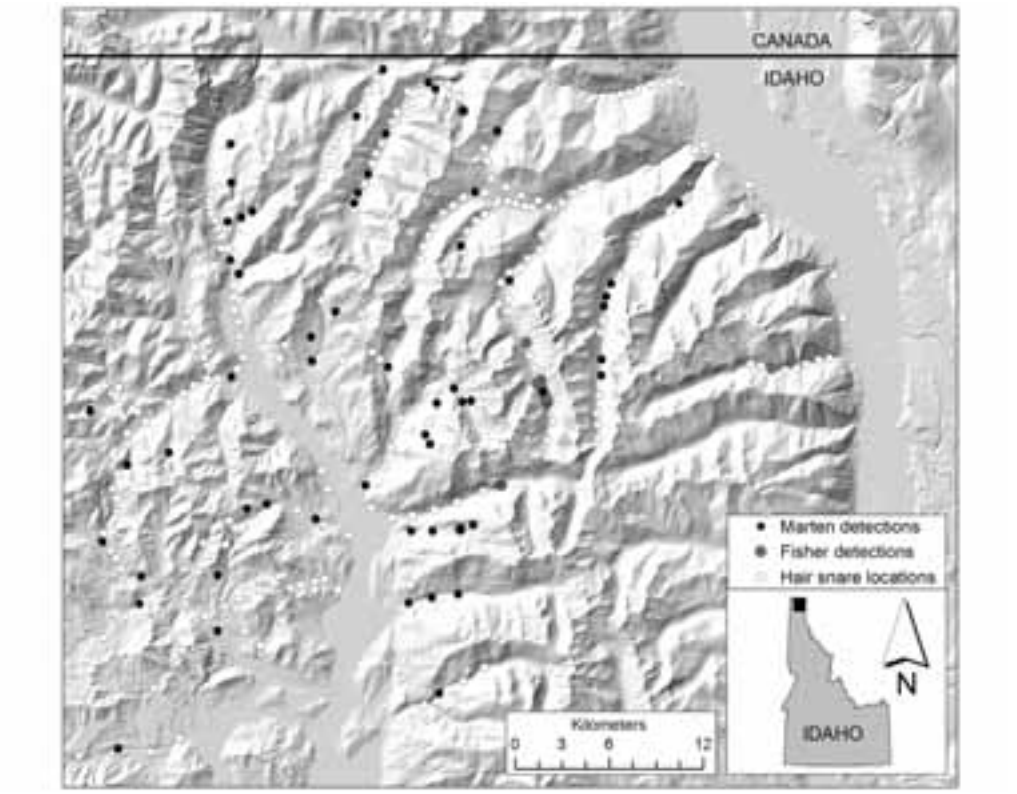


Figure 6.18. Hair sampling locations for fishers in the Selkirk Mountains of northern Idaho.

haplotypes were associated with fishers from Wisconsin and Minnesota (Vinkey et al 2006; Drew et al 2003).

2004–5 Field Season

- In all, 337 samples were collected; 6 of the samples were taken from barbed wire, 183 from gun brushes, and 148 from the bottom or sides of the cubbies.
- Of the 260 samples tested, 83% yielded sufficient DNA for species identification. The 77 untested samples were deer hair from the bait.
- Eight fishers were detected at three cubbies; all fisher haplotypes indicated Midwestern origin.
- Eighty-three marten samples and one wolverine sample were also collected.
- Other species detected included red squirrel (*Tamiasciurus hudsonicus*), striped skunk, short-tailed weasel, coyote, wolf or dog, and bobcat.

Synthesis

- At the time of the survey, a relatively small population of fishers occurred in the northern Selkirk Mountains.
- Most of the samples collected were likely associated with an introduction of Midwestern fishers into the Cabinet Mountains in 1989–91 (Vinkey et al. 2006), but at least one fisher was maternally descended from native fishers.

CASE STUDY 6.2: BEAR RUB TREE SURVEY

Katherine C. Kendall and Jeffery B. Stetz

Location: Glacier National Park, Montana.
Target species: Black bear, grizzly bear.

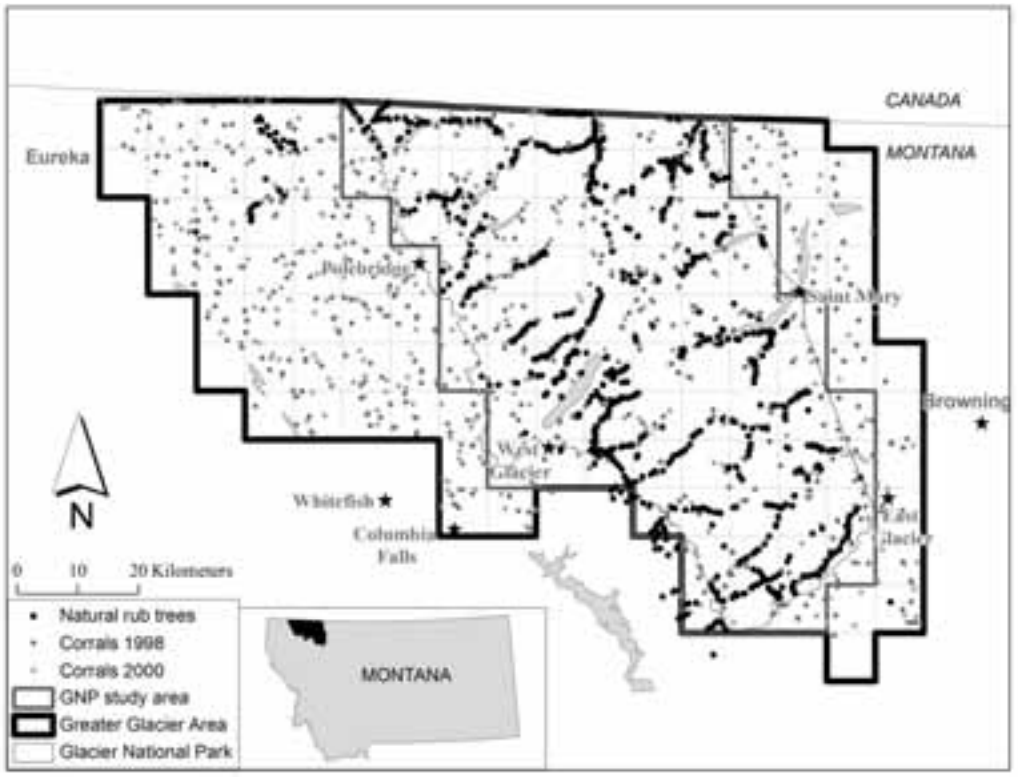


Figure 6.19. Locations of natural rub trees and baited hair corrals used to sample grizzly bear and black bear hair in and around Glacier National Park.

Size of survey area: 4,100 km^2.

Purpose of survey: To test rub tree survey methodology, compare detection bias between bear rub tree and barbed wire corral grid sampling methods, and compare the bias and precision of capture-recapture population estimates made using joint rub tree/hair corral data with hair corral-only detections.

Survey units: Hair was collected from rub trees identified on maintained trails in the Glacier National Park area. Hair corrals were distributed systematically on an 8 x 8 km grid with one corral per cell.

Survey method: Hair snagging devices comprising three to four short (~30 cm) pieces of barbed wire, totaling nine to twelve barbs, were stapled to each selected tree in a zigzag pattern on the rub surface.

Survey design and protocol: As part of a study to estimate density and distribution of grizzly bear and black bear populations in the greater Glacier area, 1,185 km of maintained trails were surveyed to identify bear rub trees bears. Based on the level of bear use and geographic distribution, 884 trees were selected for monitoring (figure 6.19). Rub trees were surveyed concurrently with hair corral surveys, which consisted of five, two-week sampling occasions on a grid of 126 baited hair corrals. Rub tree surveys were conducted on foot at approximately four-week intervals in 1998 and two-week intervals in 1999 and 2000. All hair from each barb was placed in its own sample envelope and sent for genetic analysis.

Analysis and statistical methods: Genetic analysis was initially attempted on all hair samples with at least five follicles. For those sites where no grizzly

bears were identified during the initial analysis, all hair corral samples with at least one follicle, and the two largest hair samples per rub tree survey, were analyzed. The bear species associated with a given sample was determined via analysis of mitochondrial DNA and confirmed with microsatellite analysis. The individual identity of grizzly bears was established using six highly variable microsatellite loci, and gender was determined using the Amelogenin system (see chapter 9). Population estimates using hair corral data alone and joint rub tree/hair corral data were compared using Huggins closed mixture models and the Lincoln-Petersen estimator in program MARK (Boulanger et al. 2008).

Results and conclusions:

- The mean number of surveys per tree ranged from 2.46 in 1998 to 6.10 in 2000.
- Two hundred thirty-eight grizzly bears were identified through rub tree sampling during three summers.
- Rub trees were more heavily used by grizzly bears than black bears; the grizzly to black bear ratio was 57:43 at rub trees and 30:70 at hair corrals.
- Male grizzly bears used rub trees more than females during the mid-May through September survey period, however, detection of females increased from virtually no samples in May to 50% or more of the samples from September and October. The male to female ratio of unique grizzly bears sampled was 70:30 at rub trees and 41:59 at hair corrals.
- Of the 231 individual grizzly bears identified in 1998 and 2000, when both hair corrals and rub trees were sampled, 28%were found only at rub trees and another 29% were found at both corrals and rub trees. Thus, including rub trees in the survey significantly increased the number of detected bears.
- The joint rub tree/hair corral data set produced population estimates of similar magnitude but greater precision than hair corral grid data alone (Boulanger et al. 2008).

Acknowledgments

Amy Macleod provided invaluable help by ferreting out information on the latest developments in the noninvasive hair snaring field and creating an electronic bibliography and library. We thank the many scientists around the world that presented us with information on new, unpublished hair snaring techniques in response to our listserve postings. The US Geological Survey and USDA Forest Service provided support for Kate Kendall and Kevin McKelvey, respectively.