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Noninvasive sampling of mountain lion hair using modified foothold traps

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T and E

Abstract

Although genetic analysis is an increasingly affordable option for wildlife studies, obtaining high-quality samples from cryptic carnivores remains difficult. To address this, we modified and tested 20.3-cm (8-inch) foot snares in unbaited trail sets for noninvasive collection of hair samples from mountain lions (*Puma concolor*). We deployed 22 hair traps in the Black Range in southern New Mexico from May to November 2017, monitored by remote cameras, at 66 locations for 1,618 trap nights ($\bar{x}$ = 24.5 nights, SD = 7.2 nights). Photos indicated 20 instances of mountain lions passing within 2 m of a hair trap and we collected 7 mountain lion hair samples, which averaged >20 hairs/sample. All samples contained hair with visible roots and were identifiable to species; 6 of the 7 (85.7%) yielded sufficient DNA for individual identification. We attributed failure to obtain samples to 3 primary causes: individual trap saturation (2 instances), trap failure (2 instances), and non-trigger events (9 instances). Black bears (*Ursus americanus*) and heavy rains were the primary sources of disturbance to hair trap sets, contributing to individual trap saturation and trap failure. We speculate that low trigger rates were associated with pan tension having been set too high in the first month of the study, as well as disturbance of hair traps or leading foot placements by nontarget species. We discuss strategies to increase hair sample collection rates, including seasonal use of hair traps, more selective placement on the landscape, and altering physical attributes of the hair traps. The quality of hair samples we collected and

subsequent amplification rates indicated that, along with proper deployment strategies, hair traps are a viable tool for non-invasively collecting genetic material for individual identification of mountain lions and other elusive species.

KEYWORDS
hair trap, mountain lion, New Mexico, noninvasive genetic sampling, *Puma concolor*

Studies of scarce, cryptic, and wide-ranging species are often limited by the quantity of data that can be obtained under financial and logistical constraints (Smallwood and Fitzhugh 1995, Beier and Cunningham 1996, Balme et al. 2009). For studies of large carnivore population dynamics, including mountain lions (*Puma concolor*), the ideal approach is to capture, anesthetize, and fit individuals with GPS or VHF enabled collars to monitor movement, home ranges sizes and overlap, dispersal, and survival (Sweanor et al. 2000). Nonetheless, live capture and collaring of even a subset of a population is time consuming, expensive, and logistically difficult; few projects can afford to capture and tag a large proportion of the study population. Increasingly, researchers employ noninvasive sampling strategies, including camera surveys, track transects, and genetic analyses aimed at obtaining high quality data with lower cost and reduced personnel hours.

Perhaps the most efficient and commonly used means of detecting wildlife presence is by remote cameras. Remote cameras are useful for species with individually identifying marks, such as bobcats (*Lynx rufus*) and leopards (*Panthera pardus*), because data can be collected at the individual level. For species without individually unique markings, cameras only provide data on species-level detections. Balme et al. (2009) reported that population-level data from track transect surveys offer biased but precise abundance estimates of leopards sufficient to monitor population trends, however use of individual-level detection data resulted in less-biased abundance estimates of the same population. For mountain lions (*Puma concolor*), cameras are a more expensive but less time-intensive alternative to the track transect surveys that have long been used to monitor large-scale trends in populations (Smallwood and Fitzhugh 1995, Beier and Cunningham 1996). Photographic data for an unmarked population is similarly better suited to detecting trends in abundance than to estimating abundance, and can do little to answer questions about movement, survivorship, population density, and other demographic parameters that require data to be obtained at the individual level.

To obtain individual-level data in naturally unmarked species, the only alternative to capturing individuals and applying artificial marks is to collect and analyze genetic material. The collection of genetic material can be done less invasively than capturing and marking animals; it can additionally provide data applicable to questions of heterozygosity, gene flow, breeding structure, connectivity, dispersal and migration, and metapopulation dynamics (Ernest et al. 2003, Onorato et al. 2011, Miotto et al. 2012). Due to the myriad applications of genetic data to scientific questions, as well as falling costs and improved techniques for genetic analyses, application of genetic advances to wildlife research is now common.

A suite of noninvasive sampling methods has been used to collect genetic material from carnivores and other mammals, typically in the form of scat or hair. Collection of scat samples often occurs with the aid of specifically trained scent-dogs (Harrison 2006, Davidson et al. 2014). Harrison (2006) reported scat detection dogs as the most effective means of obtaining genetic samples from bobcats. Nonetheless, he did not conduct genetic analyses at the individual level, and utility of this technique may be decreased because the DNA in scat is often degraded and scat contains more chemical inhibitors than other sources of DNA, thus reducing amplification rates (Waits and Paetkau 2005, Schwartz and Monfort 2008). Ruell and Crooks (2007) obtained individual identities from 87% of their bobcat scat samples; however, Davidson et al. (2014) yielded individual identification from only 42.7% of confirmed mountain lion scats. Detection dog surveys conducted in New Mexico reported that 32% of confirmed mountain

lion scats yielded individual identification, qualifying the technique as cost prohibitive for the species in arid environments (New Mexico State University, unpublished data).

Hair follicles are another common source of genetic material (Waits and Paetkau 2005). Noninvasive techniques for obtaining hair samples must be tailored to each study species. Bears (*Ursus* spp.), fishers (*Martes pennanti*), martens (*Martes americana*), and other scent-driven carnivores are typically drawn by bait or lures past barbed wire or other collection material assembled to snag hair from individuals (Waits and Paetkau 2005, Zielinski et al. 2006, Wegan et al. 2012, Stetz et al. 2014). Yeager (2016) adapted the use of barbed wire for hair collection from mountain lions in the Front Range of Colorado by stringing the wire across stick-cubby openings in conjunction with baits and auditory calls. Subsequent work on the same study area by Alldredge et al. (2019) achieved individual genotypes from 33.3% of hair samples collected and 20.3% of cubby set visits. Sampling success rates were reduced due to a lack of hair pulled with follicles intact, degradation of samples, or the failure of mixed samples to provide accurate genotypes. Researchers have also used backtracking individuals through snow to collect hair samples from bed sites and natural snags. Sawaya et al. (2011) obtained individual genotypes from 49% of mountain lion tracks backtracked in Yellowstone National Park. Similarly, Russell et al. (2012) obtained sufficient DNA for individual identification on 23% of tracks and 13% of hair samples collected by this technique. Whereas low rates of genetic amplification and identification are problematic for both cubby set and backtracking methods, backtracking is regionally and temporally limited by the necessity of snow.

Felid hair samples have been more widely gathered using a rub-pad method developed by McDaniel et al. (2000), which takes advantage of felid cheek rubbing behavior. Bobcats were visually attracted by an aluminum pie plate to squares of carpet saturated in castoreum, catnip oil, and dried catnip with roofing nails poking through from the back to collect hair when bobcats rubbed against them (McDaniel et al. 2000). Rub pads have been replicated for various felids with varying success, both due to low species-specificity and combining of genetic material from multiple individuals. Both Harrison (2006) and Downey et al. (2007) noted that the behavioral response of rubbing on the scent-lured carpet pads is more common in other species than in the targeted felids. Ruell and Crooks (2007), for example, determined that 78% of hair samples were from coyotes (*Canis latrans*; 47%), grey fox (*Urocyon cinereoargenteus*; 27%), and other nonfelid species. Sawaya et al. (2011) reported low rub rates by mountain lions in Yellowstone National Park, even when tracks were apparent within 1 m of the pads and mountain lion-killed ungulates were within 40 m of the rub pads. Castro-Arellano et al. (2008) reported good success detecting the presence of many carnivores, including mountain lions, in Mexico; but they highlighted weakness of the rub stations in that multiple species, and multiple individuals per species, could leave hair on any given rub pad. While multiple individual samples is not a problem for species-level identification, it may necessitate analysis of single hairs from a larger sample or lead to the culling of mixed samples if >2 alleles are detected at a single locus when attempting to resolve individual genotypes (Paetkau 2003, Kendall et al. 2009). Mixed samples containing hairs from multiple individuals are therefore better suited to occupancy-type modeling or studies in which high detection probability and a large sample size are not vital.

Beier et al. (2005), DePue and Ben-David (2007), and Stricker et al. (2012) constructed single-catch hair sampling devices for brown bear (*Ursus arctos*), river otters (*Lontra canadensis*), and bobcats, respectively. The single-catch devices were modified body or neck snares with break-away mechanisms and a hair-retaining surface (typically small strands of barbed wire), which only allow hair from one individual to be left on the device and thus reduce false or irreconcilable genotypes when DNA is amplified. A modified Belisle foothold cable-loop thrower (Belisle Enterprises, Quebec, Canada), was used to collect mountain lion hair samples on the Uncompahgre Plateau in Colorado (R. S. Alonso and K. A. Logan, Uncompahgre Plateau Puma Project, unpublished data). Initial testing indicated that the modified Belisle devices had some utility in capturing hair from mountain lions in western Colorado. We became familiar with the technique and associated site-specific logistics through a small pilot study on the Ladder Ranch, New Mexico, in November 2015. Here we describe a more concerted effort in May–November 2017 to explore the efficacy of a modified Belisle device (hereafter referred to as a hair trap) as a tool to noninvasively obtain hair samples for individual identification, with mountain lions as our target species.

STUDY AREA

Our study area was located on lowlands adjacent to the Black Range Mountains in Sierra County, New Mexico. The study area consisted of a 528-km^2 grid delineated for a study of mountain lion density on parts of the Ladder Ranch and adjacent Gila National Forest (Figure 1; Rossettie 2019). Elevation within the study area ranged from 1,300 m to 2,400 m, increasing from east to west. Rainfall in the county during 2013–2017 averaged 31.93 cm annually (SD = 2.15 cm) with 40% to 84% falling in a monsoon season from July–September (https://www.ncdc.noaa.gov/cag/, accessed 16 Feb 2022). Snow rarely accumulated except in the western extreme of the study area, but an average of 18.3 cm fell annually during 1893–2016 (https://wrcc.dri.edu/cgi-bin/cliMAIN.pl?nm4009, accessed 16 Feb 2022). Mean daily high and low temperatures during summer (May–August) were 31.4°C and 13.4°C, respectively. Winters (December–March), were cool, with mean daily high of 14.9°C and mean daily low of −2.3°C.

Coniferous forests and montane scrub dominated by ponderosa pine (*Pinus ponderosa*), alligator juniper (*Juniperus deppeana*), and mountain mahogany (*Cercocarpus montanus*) transitioned to pinyon-juniper savanna comprised of pinyon pine (*Pinus edulis*), one-seeded juniper (*Juniperus monosperma*), and grama grasses (*Bouteloua* spp.) at the foot of the Black Range Mountains. Three perennial streams flowed east, providing riparian corridors of cottonwoods (*Populus* spp.), willows (*Salix* spp.), and alder (*Alnus oblongifolia*) through what was otherwise largely Chihuahuan desert scrub and desert grassland dominated by creosote (*Larrea tridentata*),

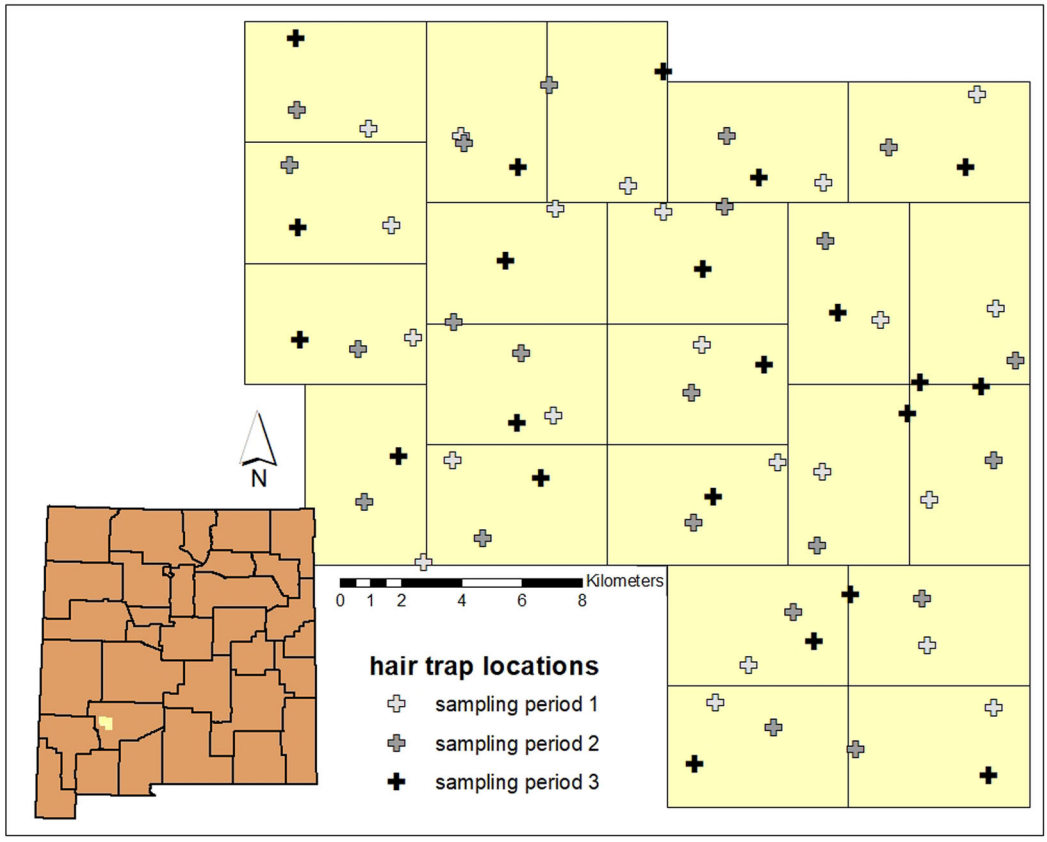

FIGURE 1 Inset of the study area and 66 mountain lion hair trap locations across a 528-km^2 study area in western Sierra County, New Mexico, USA (latitude: 33.1°N, longitude: 107.6°W), USA, May–November 2017.

cacti (*Opuntia* spp. and *Cylindropuntia* spp.), ocotillo (*Fouquieria splendens*), Apache plume (*Fallugia paradoxa*), and grama grasses in the eastern part of the study area. A managed population of bison (*Bison bison*) grazed the majority of the Ladder Ranch and all game species present, including mountain lions, received hunting pressure during seasonal hunts. Abundant elk (*Cervus canadensis*) and mule deer (*Odocoileus hemionus*) provided the majority of the prey base for mountain lions on the study area. Sympatric predators included black bear (*Ursus americanus*), coyote, bobcat, and smaller mesocarnivores.

METHODS

We developed hair traps from 20.32-cm (8-inch) Belisle cable loop throwers (Figure 2A) designed similarly to traditional leg hold traps but intended to be used in conjunction with a cable-loop leg snare. The cable thrower has smooth wire jaws that temporarily detain an animal under low closing pressure while the cable loop tightens securely around the animal's leg. Aside from the low closing pressure, breakaway measures are in place to release the jaws if the device were to remain attached to a struggling animal. A metal spacer allows easier opening of the trap during the process of setting the device and we reduced this spacer to a width of 1.25 cm for use in regular deployment of the traps to prevent the jaws from fully closing. The resulting 2.5 cm gap at the top of the otherwise closed jaws allows for easy egress of a captured paw. We covered the wire jaws with removable vinyl tubing sheaths wrapped in 2.5 cm width hook side of hook-and-loop adhesive wrap and strips of 100 grit drywall sandpaper (Figure 2B). The vinyl tubing serves both to create interchangeable units and to increase surface area for hair retention; the hook wrap and 100 grit drywall sandpaper remove and retain hair with follicles still intact. Approximately 10 kg of pressure is required to pull the paw of an adult mountain lion free from fully modified hair traps when tested on sedated mountain lions (R. S. Alonso and K. A. Logan, Uncompahgre Plateau Puma Project, unpublished data), which is sufficient to retain hairs with visible follicles on the collection surface.

We set pan tension to >8 kg in an effort to exclude smaller nontarget species. The Belisle devices have a screw-style pan tensioner, but we found this to be unreliable and used instead 2.5 cm compression springs set between the baseplate and pan of the traps to set pan tension. Traps deployed in the first month of sampling had 2 compression springs to increase pan tension, but these were reduced to a single compression

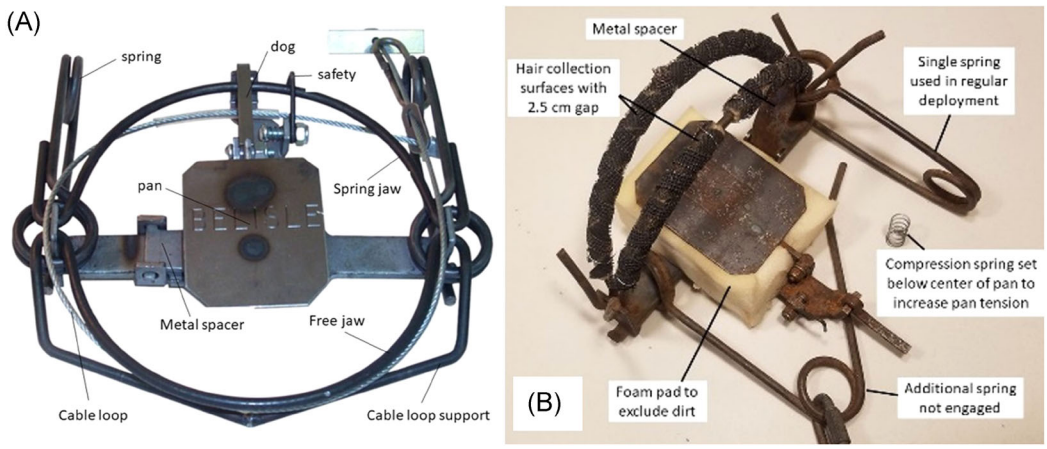

FIGURE 2 Modifications of the Belisle snare thrower (Belisle Enterprises, Quebec, Canada) used to sample mountain lion hair in Western Sierra County, New Mexico, USA, May–November 2017.

spring when tracks and photos indicated that mountain lions stepped on the pan but failed to trigger hair traps on at least 2 occasions. We enveloped the compression springs in a 4 × 9 × 11.5-cm foam pad, which prevented dirt and debris from filling the space underneath the pan and maintained trap functionality through marginal disturbance by animals and rain. Other modifications include the removal of the secondary wire on the free jaw of the Belisle (used to support a cable loop) and the use of only 1 of the 2 springs used to force the jaws of the trap closed (Figure 2B). Each measure allowed us to reduce the total pressure on the jaws while simultaneously reducing the likelihood that the extra metal on the free jaw would accumulate dirt or mud and slow the closure of the jaws.

We deployed hair traps, monitored by remote cameras (Cuddeback models C-1, Attack, or Ambush with white flash; Non Typical, Inc., Park Falls, WI, USA), at a total of 66 locations across a 528-km^2 study area in conjunction with a density estimate study between 30 May and 25 November 2017 (Figure 1; Rossettie 2019). Up to 22 hair traps were active at any given time in each of 3 sampling periods to allow for greater coverage of the study area within a feasible maintenance schedule. The typical detection station was used for 30 days, for an expected 1,980 total trap nights minus those nights where the single-catch mechanism of the devices, flash flood events, and other disturbances temporarily rendered the hair trap nonoperational. We placed the hair traps in unbaited trail sets with 3 leading foot placements on each side (negative spaces delineated by breaks in rocks, sticks, and other coarse debris covering the trail to direct the footfalls of mountain lions on either side of the devices). Jump sticks were placed 60–70 cm directly above the hair trap to exclude ungulates and other large nontarget species (Logan et al. 1999). We checked the hair traps every 5–7 days as weather and logistics allowed to collect hairs, reset or replace traps that had been triggered or disturbed, and to prevent rust build-up from locking trap components in place. Notably, the date at which a trap was scheduled to move to another location was pushed back in the event that the set was washed out by heavy rain for an extended period of site inaccessibility; if the wash-out took place between regular 5–7 day maintenance checks, no extension was allotted and trap nights were lost as they would be for a trap that had been triggered and closed.

The cameras, associated photos, and often tracks at each detection station provided evidence for the effectiveness of our trail sets when a mountain lion was present at a detection station. When we failed to obtain a hair sample from a photographed mountain lion, we categorized the missed opportunity as 1 of 3 primary problems: individual trap saturation, trap failure, and simple misses by non-trigger events. Individual trap saturation (hereafter, trap saturation) describes the condition of a hair trap that has already been triggered and closed, rendering it incapable of collecting more mountain lion hair. Trap failure occurs when a mountain lion stepped on the pan of the trap with sufficient pressure to trigger the trap, but the jaws of the trap failed to close properly. Simple misses include any event in which the mountain lion did not step on the pan of the trap or did so without committing enough force to engage the release mechanism.

When the hair traps collected samples, we pulled the hairs with tweezers, counting them both as they were removed from the trap and as they were placed in a white coin envelope. When clusters of interlaced hairs could not be counted precisely, we recorded the number of hairs as the highest interval of 5 that both counts fell into (e.g., ≥25, ≥30). We maintained hair count records to track the effectiveness of the jaw surface material at collecting hair and, with enough samples, to examine the relationship between hair count and genetic amplification rates (Paetkau 2003). We labeled all sample envelopes with date, location, suspected species, and number of hairs, and stored them in plastic bags with silica desiccant. We replaced all hair collection surfaces or sterilized them with bleach after removing hairs and prior to deploying the trap again.

Upon conclusion of the field season, we sent all mountain lion hair samples to the University of Idaho for genetic analysis. The DNA was extracted from the hair follicles using a DNeasy Blood and Tissue Kit (Qiagen, Inc. Germantown, MD, USA). Laboratory personnel included a negative control test for contamination in each sample during a mitochondrial DNA fragment-based test for species identification (Waits and Paeatkau 2005, Davidson et al. 2014, De Barba et al. 2014). The subsequent PCR multiplex for individual identification of

confirmed mountain lion hair samples utilized 10 microsatellite loci (Menotti-Raymond et al. 1999) and a single sex ID locus (Pilgrim et al. 2005). The PCR products were visualized using a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and fragment sizes were scored using Genemapper 5.0 (Applied Biosystems). The hair samples were amplified a minimum of 2 times and a maximum of 4 times to account for possible allelic dropout.

RESULTS

From 30 May through 25 November 2017, the hair traps were operational for 1,618 total trap nights ($\bar{x}$ = 24.5 nights/trap, SD = 7.2 nights) across 3 sampling periods. The remote cameras at our detection stations operated for 2,012 trap nights and recorded 20 instances of mountain lions passing within 2 m of a hair trap. Of these 20 occasions, 7 yielded hair samples averaging >20 hairs/sample (min–max = 9–≥35); in addition, traps failed on 2 occasions, 2 samples were missed due to trap saturation, and there were 9 simple misses in which the trap was not triggered. Following the removal of a compression spring from beneath the pan of traps at the end of the first sampling period, non-trigger events were reduced (Figure 3). Sets were triggered or otherwise disrupted on 136 occasions, 61 of which were instances of bear damage (Figure 4).

We achieved individual identification with 6 of the 7 hair samples we sent to the laboratory, representing 6 unique individuals. Two of these were ear-tagged mountain lions from which we previously sampled hair during live captures (Rossettie 2019); genetic signatures aligned with photographic identification of these individuals in each case. The sample that failed to amplify for individual identification was comprised of only 11 hairs. This hair sample was, however, successfully analyzed at the species level and confirmed genetically as a mountain lion hair sample. No known individuals were detected more than once during the survey.

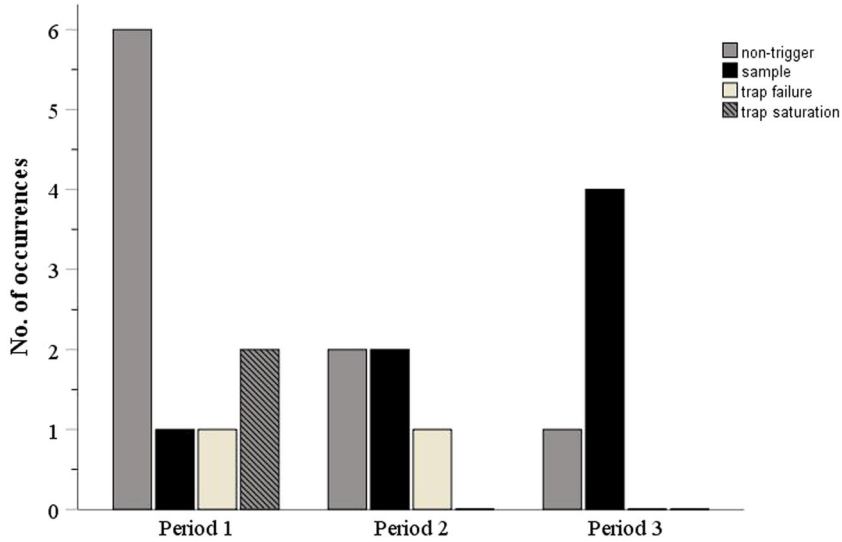

FIGURE 3 Performance of 22 hair traps associated with mountain lion occurrence during each of 3 sampling periods in western Sierra County, New Mexico, USA, May–November 2017. Pan tension was reduced between sampling periods 1 (30 May–14 July) and 2 (1 July–22 August) in an effort to decrease simple misses by non-trigger events. This change was maintained in sampling period 3 (4 August–25 November) due to decreased non-trigger events in sampling period 2.

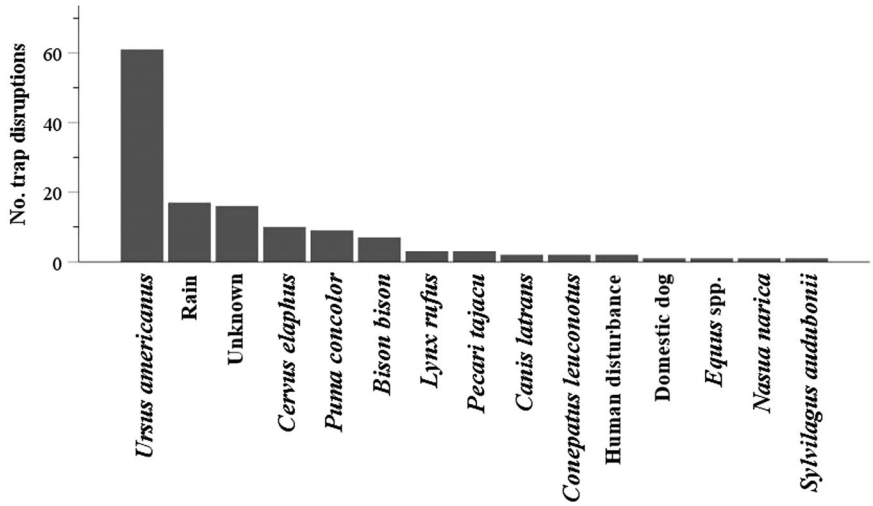

FIGURE 4 Instances of hair trap saturation and disruption by source in western Sierra County, New Mexico, USA, May–November 2017.

DISCUSSION

Efficacy of hair traps

Our cameras indicated that the hair traps did not sample hair from every passing mountain lion; nevertheless, when functional hair traps were triggered by mountain lions the resulting samples tended to contain intact follicles and DNA that amplified well during genetic analyses. Acknowledging low sample size and the need for replicate tests of and surveys using this technique, we successfully identified to the species and individual levels 100% and 85% of mountain lion hair samples, respectively. Our amplification rate was substantially higher than the 10% amplification rates of bobcat hair (Ruell and Crooks 2007), 62.6% for mountain lion fecal samples (Miotto et al. 2012), 49.6–68% for bear hairs (Beier et al. 2005, Gould et al. 2018), and 33.3% for mountain lion hair samples (Alldredge et al. 2019). We speculate that, in addition to pulling hairs with visible follicles intact, our comparatively high amplification rate is related to the regular collection of hair samples from the field every 5–7 days and associated brief exposure of those samples to DNA-damaging moisture and UV light. Successful genetic amplification was countered by missed samples, which brought genotyping success relative to photographic detection of mountain lions down to 30%, as compared to the 20.3% of detections at cubby sets that yielded individual genotypes in Colorado (Alldredge et al. 2019). We hypothesize that placing >1 hair trap at each detection station, as Stricker et al. (2012) did for body snares adapted to sample hair from bobcats, would improve hair sample rates. Increasing the number of hair traps at each location would have minimal effects on personnel hours and should reduce the likelihood of animals passing through sets unsampled due to trap failure, non-trigger events, or saturation at the detection station.

The single-catch nature of the hair traps is desirable in preventing the mixing of genetic material of multiple individuals in a single hair sample. Hair samples could be discarded or divided into single hairs and analyzed if known to be mixed in advance (increasing cost and potentially decreasing amplification success) or else culled if >2 alleles appear at a single locus (Beier et al. 2005, Kendall et al. 2009). The trade-off is that individual traps are easily saturated by both target and nontarget species and unavailable for further sampling until reset. After a pilot study to gain familiarity with the hair traps on our study area in November 2015, we suspected that most failures to obtain hair would result from mountain lions encountering traps that had already been triggered and not yet reset. Javelina (*Pecari tajacu*), which are distinctly lighter in weight than mountain lions, frequently triggered the hair traps

in 2015, so we decided to increase pan tension slightly for the 2017 study. Two compression springs were imbedded in the foam of each trap rather than one, such that the step of a mountain lion would still trigger the hair traps but javelina would be less likely to trigger them. By the second night of the study, a mountain lion had triggered a trap with this increased pan tension; several javelina passed through sets without triggering traps in the first 2 weeks. Nonetheless, as we gained evidence of mountain lions failing to trigger traps in the first sampling period, we reverted to the lower pan tension with a single compression spring in the second and third sampling periods and had fewer simple misses. We additionally recommend maintaining a low pan tension (approximately 8 kg), as 83% of undesired trap disruptions were caused by bears, elk, bison, and other sources such as rain, that cannot be mitigated by increased pan tension.

We believe that the modified Belisle hair-trapping technique, adapted to smaller or larger diameter traps, would be effective for collecting hair samples from other species, especially bobcats and bears. Avoiding disruption by bears as a nontarget species would require deploying hair traps in the winter (or on study areas with minimal bear activity). Taking this step should decrease both missed hair samples by trap saturation and project personnel hours, as sets would need to be rebuilt less frequently. Bison damage also dramatically increased personnel hours as a herd moving through would demolish the brushwork and foot placements associated with the hair trap, requiring a full rebuild (~90 min depending on the site). As our study occurred in conjunction with a density estimation study (Rossettie 2019), hair trap placement was largely dictated by the assumptions and goals of density estimation; nonetheless, future use of these hair traps could be coordinated with landowners to try to minimize placement in areas known to be frequented by livestock and other large ungulates.

Trap failure can result from debris lodged between the jaws of the hair trap or by buildup of rust at trap joints. Proper trap maintenance with dye and wax at the beginning of a season should prevent most rust, but traps must be tested for functionality both before placing them in the ground (trigger testing for proper closure even if the trap has been used previously) and at each maintenance check (trigger-testing with the safety on to observe proper release of the dog), even when the set appears undisturbed. We used only one of the trap springs designed to close the jaws of the trap when triggered as a compromise with our institutional animal care and use committee (IACUC), given the limited testing of this technique to date. It is possible that engaging both springs may provide adequate power to overcome rust or debris that might otherwise slow or stop jaw closure. Additionally, there is a learning curve to placing debris around the trap and other foot placements to entice mountain lions to step in the center of the pan without increasing the risk of that debris becoming lodged in moveable parts of the hair traps. Researchers considering the use of these devices should become familiar with them well before the study begins.

We missed more hair samples from mountain lions not triggering the hair traps than from any other issue. Missed-samples events could be further broken down into at least 3 separate scenarios: (1) foot placements being set such that the passing mountain lion never stepped on the pan of the trap, (2) foot placements being set such that the mountain lion could step on the edge of the pan or on the pan and spring jaw or dog simultaneously, preventing the dog from being released, or (3) the mountain lion stepping on the pan but not committing enough weight to that foot placement to trigger the hair trap, perhaps due to the incremental movement of the foam and spring under the pan as it is depressed toward the trigger-point. The first of the 3 aforementioned scenarios was observed on at least 2 occasions when a desert cottontail chewed on the exposed hair-collection surface of the free jaw, pulling the trap out of its sandy substrate and leaving it in a position far from ideal for a mountain lion to step on it (Figure 5). Movement of hair trap by nontarget species are occasionally unavoidable, but uncommon if bear and livestock disturbances can be reduced with strategic placement of hair traps. The second scenario was associated with leading foot placements being too wide and was easily mitigated with additional sharp rocks or sticks lining both leading foot placements and the inside of the jaws of the hair traps. The final scenario can likely be resolved with further modification of the Belisle devices, though we have not yet found an appropriate modification. In snaring for live capture with Aldrich-style spring arms, strips of retractable measuring tape are placed, convex side up, below the treadle-foot to create an all-or-nothing trigger release mechanism. The foam and spring combination used on the adapted Belisle hair traps, by contrast, is depressed gradually until the point at which the

FIGURE 5 Photograph of a mountain lion approaching a disrupted hair trap set during a survey of hair trap effectiveness in Sierra County, New Mexico, USA, May–November 2017.

dog releases. The gradual release of the dog caused by the use of foam and spring provides mountain lions the opportunity to react to the markedly softer ground beneath them and reposition their foot off the pan of the hair trap before applying full weight to that foot. In addition to reducing the pan tension with the use of a single compression spring below the pan, we also increased the thickness and density of the substrate covering the pan to make it feel more like solid ground. The increased substrate had the undesirable effect of mounding the critical foot placement well above the ordinary level of the jaws of the trap, which subsequently had to be positioned at higher angles from neutral to avoid being caked with mud and thus ineffective at retaining hair. Resolving the gradual release mechanism will therefore be of particular interest for future use of this technique.

We were limited in our ability to assess whether mountain lions develop any kind of trap avoidance behavior after an initial exposure to a modified Belisle hair sampling device. The lack of recaptures might indicate trap avoidance, except that the walk-throughs that failed to obtain hair samples occurred early in this study and with naïve individuals. The only evidence we have of a mountain lion avoiding a hair trap set was an old male mountain lion with increasingly severe cataracts who had been live captured and immobilized 3 times in snare sets of similar composition (Logan et al. 1999). This male had been observed to circumnavigate all live capture snare sets for over a year by the time the hair trap survey was initiated. As such, this animal's trap shyness tells us nothing of what may be expected of mountain lions that encounter multiple hair traps, yet the observed behavior anecdotally supports the superficial similarity between foothold-based trail sets for live capture and for passive genetic sampling. Although Logan et al. (1999) found that female mountain lions in the San Andres range occasionally developed shyness toward foothold cable loop snares, it was not specified whether the trap avoidance was cued by the softness of foam underfoot, visual patterns in set construction, human scent, or specific locations. We can speculate that trap shyness would be slower to develop with hair traps than live capture, as the experience is much shorter and less intense, but more observations will have to be made to substantiate this. Our only direct evidence against trap shyness comes from the original testing of these hair traps on the Uncompahgre Plateau, Colorado, in which at least one mountain lion triggered hair traps more than once with no apparent trap shyness. However, predator calls were used, possibly distracting the mountain lion from cues associated with the hair trap's presence (R. Alonso, Virginia Polytechnic Institute and State University, personal communication).

Materials and associated costs

Once purchased and modified, the hair sampling devices described here are reusable with only minor investments in replacement hook-and-loop wrap and drywall sandpaper as needed. We maintained a total of 32 hair traps to efficiently replace any of the 22 deployed in the ground that had been damaged or exposed to hair. The 10 extra hair traps allowed

hair removal, trap sterilization, and general maintenance to occur in the laboratory. Our study required approximately 70 person hours/week with 22 traps deployed across 528 km^2 and a high-frequency maintenance schedule of visiting each trap once every 5–7 days. After identifying a new detection station location, each set took 1.5 to 3.5 hours to establish. Maintenance checks typically ranged from 5 to 90 min at each detection station, depending on the level of disturbance. Full replacement of hair collection surfaces (vinyl, hook wrap, and sandpaper) took approximately 20 min/trap, although this was very rarely required after initial preparation of the devices; simple sterilization and replacement of worn sandpaper took closer to 5 min/trap. We employed cameras to monitor the hair traps and include this in the equipment cost summary of the technique (Table 1), but they are not essential for the use of this technique when operated by

TABLE 1 Summary of equipment costs (purchased September 2015–August 2017) associated with noninvasive sampling of mountain lion hair in Sierra County, New Mexico, USA, May–November 2017, for genetic analysis using modified Belisle hair sampling devices.

Equipment	Quantity	Cost/unit[a]	Total	Notes
Belisle traps	32	$60	$1920	One-time expense, undamaged by extended use as long as maintained to prevent rust
Vinyl tubing (3/8")	3 × 20 ft	$7	$21	Exceptionally infrequent replacement needed
1" hook-side wrap with adhesive backing	2 × 25 yds	$45	$90	Changed every ~45 days in the field, depending primarily on rodent activity
100 grit drywall sandpaper	1 × 12 sheets	$8	$16	Changed out with very ~25 days in the field
Compression springs	40	$0.95	$38	Very low rate of loss, do not seem to lose effectiveness
1.5" thick foam (mattress topper)	0.25	$50	$12	Changed out when fully caked with mud and no longer compressible or chewed extensively by rodents
18" rebar anchors (0.5" diameter)	25	$1	$25	One-time expense, undamaged by extended and repeated use
Quick-links (3/16")	25	$2	$50	One-time expense, undamaged by extended and repeated use
Coin envelopes	100	$25/500	$5	Fewer can be used if only target species hair is collected
Silica bead packets	20	$15/80	$4	Multiple envelopes with hair samples can be placed in a single plastic bag with silica desiccant
Genetic analysis	Up to 40	$50	$2000	Depends on laboratory and analyses required
Cuddeback cameras	25	$200	$5000	One-time expense, may need replacement every 2–5 years
Batteries (AA & D)	5 x 40ct AA 3 x 14ct D	$15	$120	Need to be replaced with every ~120 days, depending on photo rates/video length
SD cards	45	$20	$900	Two for every camera for easiest maintenance checks, one-time expense

[a]Costs are dependent on both number of devices and trap nights; here they are factored for operating up to 22 concurrent hair traps over a targeted 1980 trap nights with 10 surplus hair traps to increase efficiency in the field. Analysis of 40 hair samples at the individual level is included. Vehicle use and personnel hours are not included.

researchers familiar with track and hair identification. With an allowance for cameras monitoring all sites and up to 40 hair samples to be genetically analyzed, our equipment costs came to approximately $10,200 (or $464 for each of 22 detection stations that ran concurrently for 30 days). Without factoring in the expense of remote cameras, which many studies already have in place, this drops to $190/hair trap station.

The cost of genetic analysis varies according to the laboratory, number of samples to be analyzed, and types of analyses required; poor quality samples that fail to yield usable identification data represent sunken costs, especially for large-scale studies. Our results indicated that hair samples collected by modified Belisle devices are associated with high genetic amplification rates and that future use of the hair traps should focus on maximizing sample collection rates. We expect that familiarity with and intentional placement of hair traps on the landscape will allow for cost-effective collection of genetic data from mountain lions and that the technique would be similarly effective if adapted for bears, bobcats, and other species.

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CONFLICT OF INTEREST

The authors declare that there are no conflict of interest.

ETHICS STATEMENT

We conducted our work under New Mexico State University IACUC Protocol No. 2015-038.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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