

Scent-Matching Dogs Determine Number of Unique Individuals From Scat

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ABSTRACT Noninvasive scat sampling methods can generate large samples sizes, collected over vast landscapes, ideal for addressing wildlife conservation and management questions. However, the cost of genotyping scat samples limits the accessibility of these techniques. We describe detection-dog methods for matching large numbers of scat samples to the individual, reducing or eliminating the need for sample genotyping. Three dogs correctly matched 25 out of 28 samples from 6 captive maned wolves (*Chrysocyon brachyurus*) of known identity. Sample scent-matching can increase overall accessibility and breadth of applications of noninvasive scat-collection methods to important landscape scale problems in wildlife sciences. (JOURNAL OF WILDLIFE MANAGEMENT 73(7):1233-1240; 2009)

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Fecal samples are the most accessible animal product in nature, can be collected over large landscapes without disturbing subjects, contain considerable information on the physiological health of wildlife, and can be genotyped to assign species, sex, and individual identities (Wasser 1996; Wasser et al. 1997, 2004; Millspaugh et al. 2001; Gobush et al. 2008). Sampling using scat-detection dogs makes such studies even more powerful, typically providing ≥ 5 times more samples compared to human collectors (Wasser et al. 2004, Rolland et al. 2006, MacKay et al. 2008, Wasser 2008). However, the utility of these methods has been limited by the high cost of genotyping fecal samples to assign individual identities (\geq US\$160/sample, depending on genetic diversity of the population). Sample degradation inflates costs further by reducing the number of samples that amplify DNA at enough loci to reliably genotype individuals.

Canine-based scent-matching methods can also be used to assign individual identities to fecal samples. This method is similar to that used by law enforcement, whereby dogs match scent evidence from one or more crime scenes to an individual criminal. Dogs match scent with sufficient accuracy to hold up as evidence in a court of law (Schoon 1996, 2005; Kaldenbach 1998). Properly applied to wildlife studies, this canine-based method could reduce the need for expensive and occasionally error-prone DNA analyses of wildlife scat (Taberlet et al. 1999). Scent-matching requires less time and money than does genotyping all samples in a data set. It appears to be virtually unconstrained by low genetic diversity of the population under study and far less susceptible to impacts of sample degradation (Kerley and Salkina 2007; see also below). If genotypes are still needed, only one sample from each individual would need to be analyzed, although method accuracy can be further verified by genotyping random samples identified by dogs as being

from the same individual. We validated a canine-based scent-matching method to assign individual identities to multiple fecal samples from 6 captive maned wolves (*Chrysocyon brachyurus*).

METHODS

All dogs selected for this work have a highly focused, excessive play-drive (Wasser et al. 2004). Because their reward for a correct response is approximately 90 seconds of play with their ball, only dogs with an extreme play-drive are able to sustain the focus and motivation required to make the many comparisons in our study design. The number of comparisons was a function of sample size and number of unique individuals in the data set and all matches needed to be compared multiple times to assure their reliability. The 3 dogs we used were 2 Australian cattle dogs, aged 2 years and 7 years, and a 2-year-old border collie, all rescued from animal shelters and selected for their excessive play-drive. The 7-year-old dog had worked for several years in our scat-detection-dog program, locating wildlife scat over large remote wilderness areas (Wasser et al. 2004). The other 2 dogs had no such prior experience.

We housed dogs individually in pens at our kennel facility in Seattle, Washington, USA, exercised them several times daily in an outdoor yard, and ran them 5 km 4-5 times/week. We performed all matching in an 8 m \times 13 m room in the facility that was well-ventilated, free of other distractions, and between 18° C and 20° C (Kaldenbach 1998).

We presented samples to each dog in 2 separate platforms made of plastic for easy cleaning (Fig. 1). One platform had one hole to deliver scent of the target sample. The second platform had 12 holes with a 9-cm diameter, each spaced 53 cm apart. This design allowed us to deliver scent from up to 12 samples to be matched to the target in one trial.

The scent was delivered to the hole by a J-shaped apparatus made from polyvinyl chloride pipe attached to

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Figure 1. Sample-matching apparatus. The target sample is shown in the single sample rack to the right of the dog. The long racks contain the samples to be matched to the target. Data were collected on maned wolves during 2007 in Seattle, Washington, USA.

the underside of the hole at the short end of the J. We screwed the polypropylene vial containing the sample into this apparatus at the end of a y-shaped pipe, which branched downward away from the hole and connected at the middle of the horizontal section of the J. A small computer fan attached to the end of the horizontal pipe gently blew the scent out the opening of the hole toward the dog (Fig. 1). Keeping equipment clean and using experimental apparatuses that limit opportunities for the dog to contaminate the sample were critical (Schoon 1996, Kaldenbach 1998, Walker et al. 2006). The J apparatus prevented the dog from contaminating the sample by distancing the sample from the dog's nose. The entire apparatus was removable, allowing us to store it along with its capped sample at the end of each day. Each time we replaced a scat sample and pipe combination, we thoroughly wiped down the hole of the matching rack with 10% bleach followed by 90% ethanol. We also disassembled each J-apparatus and washed it weekly in a dishwasher.

We acquired 28 fresh samples from 6 known maned wolf individuals (4–5 samples/individual) from the captive population at the Smithsonian Conservation and Research Center in Front Royal, Virginia, USA. All 6 subjects had the same diet and all individuals but one were closely related.

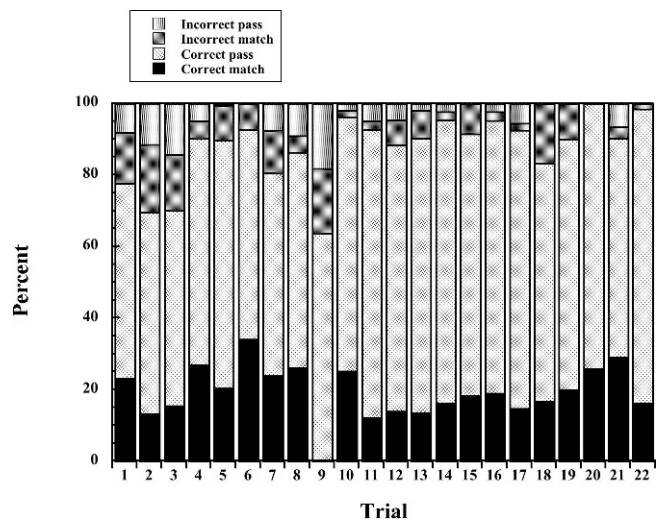


Figure 2. Gator's performance over time, based on proportions of correct and incorrect matches and passes of 16 wild grizzly bear (*Ursus arctos horribilis*) samples in consecutive trials. We freeze-dried and stored samples unfrozen for 4 years prior to testing during 2004 in Seattle, Washington, USA.

All samples were collected fresh and stored frozen until shipped overnight to Seattle. We then freeze-dried and sifted samples through a clean strainer to remove macro contents and isolate fecal powder, and we stored samples unfrozen in screw-top polypropylene vials. This method prevented sample degradation from repeated freeze-thaw cycles and reduced intersample variation in odor strength (Wasser et al. 2004, Kerley and Salkina 2007). We took care throughout this process to prevent cross-contamination of samples. We used latex gloves when processing new samples and washed and rinsed strainers between samples in 10% bleach followed by 90% ethanol.

Before adopting the above methods, we tested its efficacy on samples collected from wild grizzly bears (*Ursus arctos horribilis*) on a varied natural diet in Alberta, Canada (Wasser et al. 2004). Those samples were frozen for 4 years and then freeze-dried and stored unfrozen for the duration of the study. Our most senior dog matched samples from 16 genotyped individuals with 93% accuracy (Fig. 2). In fact, these successes are what prompted us to use animals that all had the same diet. Because diet should be most similar within versus between individuals, we wanted to make sure that the dogs could still make matches when diet was invariant.

We used a sample from study subject, Lucho, as the target in the initial training. We split the sample into 2 1-g halves, each stored in its own polypropylene screw-top vial. We attached the vial containing the target portion to its J-apparatus in the single sample rack (Fig. 1). The other half of the sample served as a definitive match, and we attached it to a J-apparatus at 1 of 12 holes in the sample-matching rack.

Matching dog training began by familiarizing the dog with the scent of that species' scat. We directed the dog to smell the hole emitting the target sample scent, while giving the command "target." We then directed the dog to the

matching rack and gave the command “match” as we presented each hole to the dog. We immediately rewarded the dog with the toss of a ball, followed by approximately 90 seconds of play, as soon as it sniffed the hole containing the matched sample.

Once the dog was able to locate the sample independently we taught it to sit prior to receiving its reward. This served as an unambiguous signal to the handler that the dog had detected the match. We presented no other samples with that match until the dog reliably sat on its own at the correct hole as soon as it detected the match. We then increased the number of samples presented to the dog, in increments of 3, until eventually all 12 holes of the rack were filled with 11 nonmatching and 1 matching sample, each randomly distributed in the matching rack. We then replaced the matching sample that was split from the target with a different scat from the same animal.

To ensure that dogs were selecting the individual as opposed to specific samples, we placed the remaining 2 Lucho samples in the apparatus, one sample at a time. We delayed rewards until the dog sat at the correct sample, unprompted by the handler. If the dog showed no reaction to the correct sample after 2 passes, we instructed it to sit at the sample and rewarded it. If the dog sat at an incorrect sample, we gave it a verbal correction along with a sharp tug on the leash. The dog then proceeded, and we instructed it to sit at the correct sample, rewarded it, and removed it from the room. We repeated this process until the dog reached our criterion, successfully matching any sample from the same animal as the target when randomly included among the group of 12 samples.

We placed a 1-g subsample of the selected target in the target apparatus (Fig. 1). We randomly selected 11 samples from the remaining sample pool and placed them, along with a subsample split from the target, in the 12-hole apparatus for presentation to the matching dogs. The split sample assured that the dog had the opportunity to be rewarded in every trial, because errors increase when dogs are not offered a correct choice in the trial (Schoon 1996). Dog-handler teams were blind to identities of all samples placed in the matching racks. The experimenter placing the samples in the racks was also blind to the true identity of all samples except for the randomly placed, known split.

Because we used Lucho’s samples for the training period, we also used him as the first target, which prevented dogs from having to pass over a sample that they remember receiving multiple rewards for hitting in the recent past. We arbitrarily selected all remaining targets from the sample pool.

Only one dog was in the room at any time. We directed the leashed dog to the target and then down the row of potential matches, consecutively checking each sample. If the dog chose a sample as a match, we noted its position, rewarded the dog, and returned it to its kennel. We then repeated the process separately for the second and third dogs. Once all 3 dogs made their selections, we removed all preceding samples passed by all 3 dogs from further comparisons to that target.

We set aside samples selected by ≥ 1 dogs for ≥ 3 sessions and then put them into a box of prior-selected samples. Keeping those samples out for a few trials reduced the likelihood of the dog keying in on a potentially wrong sample. We then put a new set of 12 samples in the rack, one from the box, the known-match sample (taken directly from the target sample) and the remainder from the still to be checked box, all in random order. As the test for each individual progressed we removed the known split sample and only used samples from the box of prior selected samples.

We based match and nonmatch assignments on the mean number of times 1) all 3 dogs, or 2) the 2 most certain dogs selected a sample as a match to the target (samples chosen 100–83% of time = high-certainty match, 67–82% = medium-certainty match, 51–66% = low certainty, 34–50% = low-certainty pass, 18–33% = medium-certainty pass, 0–17% = high-certainty pass). We completed all comparisons to a given target when we presented all samples in the box of prior-selected samples ≥ 3 times to each dog ($\bar{x} = 4.9$ times). At that point, we permanently removed without replacement the target sample and all of its confirmed matches from the sample set. We added the samples in the pass box from the previous target back into the sample pool. We randomly selected a new target from the remaining samples and continued the process until we tested 5 of the 6 possible targets. We then designated all remaining samples as a match to the sixth target by default.

Using 3 dogs in these trials provided 3 independent opportunities to flag an incorrect match or nonmatch (i.e., pass). Allowing dogs multiple opportunities to make a correct match and having multiple dogs as a cross-check were important for achieving the high precision found in this and other studies (e.g., Pickel et al. 2004, Kerley and Salkina 2007). If the same dog consistently disagreed with the other 2 dogs’ designation of samples as a match or nonmatch, we presumed that the disparate dog was having sampling problems that required corrective training. Such cross-checks are vital when dogs and handlers are blind to sample identities. Having 3 dogs also breaks up the tedium for any given dog, maintaining the dog’s motivation for work. We typically conducted 3 sessions at 3-hour intervals on any given day, with each session involving all 3 dogs.

We examined cost differences between canine sample-matching versus genotyping samples to obtain individual identities. Dog cost estimates per sample incorporated capital costs (e.g., construction costs of kennel and matching rooms) amortized over 10 years and 20 dogs (including field dogs); apparatus costs amortized across samples over 5 years; costs for locating dogs and their initial training and maintenance costs (including food, veterinary care, kennel upkeep, and exercise) all amortized over 5 years for the working life of the dog.

Genotyping costs included labor and supplies (including extraction and DNA clean-up kits, primers, and other materials); capital equipment (e.g., thermocyclers, centrifuges, genetic analyzers) amortized over 5 years; and annual

Table 1. Samples chosen as matches to a given maned wolf target (i.e., selected ≥ 3 times by ≥ 2 of 3 dogs), broken down by percent of times each sample was selected by each dog in a series of repetitive trials. All samples from the same individual as the target are listed first and have the same name. Any nonmatching sample that was mistakenly matched to the target is listed next. “All others” are nonmatching samples that were correctly passed over in most comparisons. *N* is number of times the sample was compared to the target by all 3 dogs combined for matched samples, and number of nonmatched samples compared to the target for “All others.” Data were collected during 2007 in Seattle, Washington, USA.

| Target | Sample | <i>N</i> | % chosen by dog | | | | 3-dog mean | 2-dog mean |
|-----------|-----------------------|----------|-----------------|-------|---------|-----|------------|------------|
| | | | Alli | Gator | Frehley | | | |
| Lucho 5 | Lucho 1 | 17 | 100 | 100 | 100 | 100 | 100 | |
| | Lucho 3 | 12 | 100 | 100 | 50 | 83 | 100 | |
| | Lucho 4 | 18 | 100 | 100 | 83 | 94 | 100 | |
| | All others | 24 | 1 | 2 | 3 | 2 | 2 | |
| Chucho 5 | Chucho 1 | 16 | 60 | 80 | 33 | 58 | 70 | |
| | Chucho 2 | 9 | 100 | 67 | 67 | 78 | 83 | |
| | Chucho 3 | 15 | 50 | 100 | 80 | 77 | 90 | |
| | Chucho 4 | 9 | 100 | 67 | 33 | 67 | 83 | |
| | Rambo 3 ^a | 15 | 75 | 50 | 88 | 71 | 81 | |
| | All others | 18 | 4 | 2 | 3 | 3 | 3 | |
| Reynita 2 | Reynita 1 | 22 | 67 | 56 | 43 | 55 | 61 | |
| | Reynita 3 | 33 | 50 | 86 | 82 | 73 | 84 | |
| | Reynita 4 | 19 | 86 | 33 | 50 | 56 | 68 | |
| | All others | 15 | 15 | 6 | 8 | 10 | 7 | |
| Louise 5 | Louise 1 ^b | 14 | 50 | 60 | 40 | 50 | 55 | |
| | Louise 2 | 13 | 100 | 100 | 80 | 93 | 100 | |
| | Louise 3 | 14 | 100 | 100 | 75 | 92 | 100 | |
| | Louise 4 | 6 | 100 | 100 | 100 | 100 | 100 | |
| | All others | 10 | 21 | 17 | 9 | 15 | 13 | |
| Ibera 2 | Ibera 1 | 10 | 50 | 100 | 100 | 83 | 100 | |
| | Ibera 3 | 10 | 100 | 100 | 100 | 100 | 100 | |
| | Ibera 4 | 7 | 100 | 33 | 67 | 67 | 83 | |
| | Ibera 5 | 18 | 100 | 100 | 100 | 100 | 100 | |
| | Rambo 5 ^a | 15 | 80 | 80 | 80 | 80 | 80 | |
| | All others | 3 | 26 | 0 | 26 | 17 | 13 | |

^a Sample matched to wrong target.

^b Sample was selected too infrequently to be designated a match using the 3-dog mean.

service contracts amortized over the average number of samples analyzed in the lab each year.

RESULTS

There were 18 samples that could have been correctly matched to 1 of the 5 targets. Seventeen of 18 samples were correctly matched to their respective target (Table 1). The 18th sample (Louise 1) was correctly matched using the 2-dog but not the 3-dog mean. Two additional samples (Rambo 3 and 5) were incorrectly matched to 2 different targets (Chucho and Ibera, respectively). The 3 remaining samples of the 28 at the end of all trials were correctly matched to each other by default. “All others” were rarely selected (2–17%, Table 1).

Most nonmatches occurred with high certainty—93% of cases using 2-dog means (Fig. 3B) and 83% using 3-dog means (Fig. 3A; $n = 70$). By contrast, 78% of matches occurred with high certainty using 2-dog means and 50% using 3-dog means ($n = 18$). Low-certainty choices were rare in all cases (0% of cases for nonmatches and 11% for matches using 2-dog means, Fig. 3B; 1% of cases for nonmatches and 28% [including Louise 1] for matches with 3-dog means, Fig. 3A). The 2 exceptional low-certainty nonmatch samples (Fig. 3A) were the match sample (Louise 1), selected too infrequently to be categorized as a match to its target using the 3-dog mean, and the nonmatch sample (Ibera 4), which the dogs matched to its mother, Louise, 44% of the time.

We estimated that canine sample-matching methods may reduce individual identification analysis costs from 20% to 4,600% relative to that required using fecal DNA, assuming it takes 6 loci to genotype a sample (Table 2). The savings may be even greater if >6 loci are required. Scent-matching costs vary by sample size and mean number of samples per individual, as well as whether matching uses aggregate (see below) or nonaggregate designs (Table 2). Number of required loci and, hence, genotyping costs increase with genetic homogeneity of the population.

DISCUSSION

We found that dogs were consistently able to match samples from the same individual, while generally avoiding matching closely related individuals in our trials. This suggests that scat dog matching is unlikely to be constrained by low genetic variability in a wild population. Because all maned wolf subjects had similar diets, our results also occurred independent of diet. Successful matching of sample from wild grizzly bear (Fig. 2) and tigers (Kerley and Salkina 2007) also demonstrates that scent-matching is not restricted to subjects in the family Canidae.

Two types of errors can occur in matching work, a false match (match error) or a false pass (nonmatch error). Nonmatch errors increase the number of unique individuals, which would overestimate population size. Match errors either leave population estimates unchanged, or underestimate population size if the mistakenly matched individual

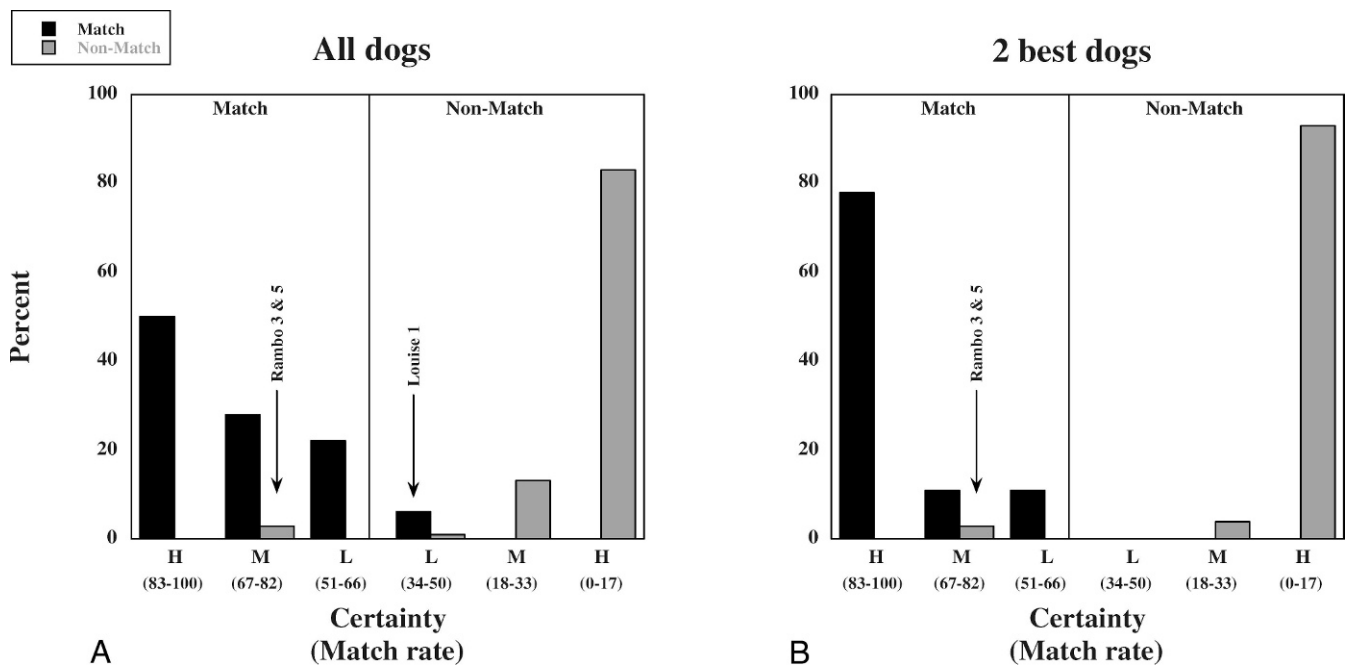


Figure 3. Percent of match (solid) and nonmatch (shaded) maned wolf samples that were assigned as a match (left panel) or a nonmatch (right panel) with high (H), medium (M), or low (L) certainty, averaged across (A) all 3 dogs, or (B) the 2 dogs with the highest certainty scores. Samples matched 100–83% of time = high-certainty match, 67–82% = medium-certainty match, 51–66% = low certainty, 34–50% = low-certainty pass, 18–33% = medium-certainty pass, 0–17% = high-certainty pass. $N = 70$ possible nonmatches and 18 possible matches to 5 target samples. Testing occurred during 2007 in Seattle, Washington, USA.

was not yet represented in the data set. Although nonmatch errors tend to be more problematic for population estimates, they are far less likely to occur than are match errors (Fig. 3).

The high certainty in assignments of nonmatching samples also widens the gap between selection of matching versus nonmatching samples, allowing greater tolerance in the selection criteria required to declare a sample a match. Averaging certainty across the selections of the 2 most certain dogs further increases assignment reliability because it increases certainty for both match and nonmatch samples. Thus, even the Louise 1 and Ibera 3 samples became distinguishable using the 2-dog mean criterion (Fig. 3B). Regardless, low-certainty samples (e.g., Louise 1, Ibera 4) should be genotyped or discarded.

The Rambo 3 and 5 samples that were mistakenly matched to Chucho and Ibera, respectively, appear to be exceptions to the above. Those samples were mismatched when they should have been passed. One of the 2 incorrectly matched samples was particularly troubling (Rambo 5, incorrectly matched to Ibera) because all 3 dogs matched the Rambo sample to Ibera 80% of the time. We subsequently learned that these 2 animals were housed together during sample collection at the National Zoo and that it was possible that this sample could actually have been from Ibera. (Too little sample remained to genotype them.) Alternatively, cross-contamination may have occurred prior to collection because maned wolves commonly scent-mark other individual's samples or common defecation areas. Chucho and Rambo were housed separately.

We found that the more samples used for training the easier it was for the dog to understand that it is looking for

individual differences between samples. The tendency for dogs to erroneously select nonmatch samples as a match increased as the remaining samples to choose from became low (Table 1). Supplementing the low number of remaining samples at the end of a study should alleviate this problem (e.g., adding samples from another population or from animals in captivity). Having too few samples to discriminate can also cause dogs to lose motivation. Our dogs proved capable of comparing up to 32 samples/trial, which also increases sample throughput.

When introducing dogs to a new target, we found that repetitions help lock the dog into the scent. It is also important to give the dog time to reflect between repetitions. It is striking how a dog's performance can improve after it has had time to consider the task set before it (Kaldenbach 1998). Reducing the number or duration of trials also helps to avoid oversaturating the dog's olfactory system.

Dogs vary in aptitude and even the best dogs have bad days, owing in large part to the tediousness of the work (Settle et al. 1994). Using 3 dogs generally resolved this problem because the odd dog was most likely incorrect. When in doubt, it is important to stop and test the dogs with known (e.g., genotyped) samples, providing corrective training as needed. Problematic samples can also be tested against other targets and thrown out if the problem persists (e.g., Kerley and Salkina 2007).

High-drive dogs can make mistakes simply because they move through the trial too quickly. Handlers must control the speed of these intense dogs to prevent them from overshooting samples, sitting at the hole adjacent to the

Table 2. Cost comparisons between (A) sample-matching using scenting dogs versus (B) genotyping, based on total number of samples and average number of samples per individual maned wolf in the data set. Cost reductions are also shown if total number of samples is divided into multiple subgroups of 100 and one sample per individual from each subgroup is then aggregated into a final group for comparison (see text). Genotyping costs assume a US\$55.00 DNA extraction fee (done in duplicate) and US\$17.50/locus fee to amplify and analyze the sample. Dog costs assume 56 comparisons/hour and an hourly handler fee of US\$21/hour for 3 dog handlers. Data were collected during 2007 in Seattle, Washington, USA.

| Sample size | A. Sample matching | | | | B. Genotyping cost/sample (US\$) | | | | |
|-----------------------------------------|---------------------------|--------------------------|--------------------------|---------------------------|----------------------------------|--------------------------|-------------------------------------|--------------------------|-------|
| | No. of comparisons | | Cost/sample (US\$) | | No. of comparisons | | Cost/sample in blocks of 100 (US\$) | | |
| | 10 samples/ individual | 5 samples/ individual | 2 samples/ individual | 10 samples/ individual | 5 samples/ individual | 2 samples/ individual | 10 samples/ individual | 5 samples/ individual | |
| 25 | 104 | 126 | 192 | 8.42 | 10.21 | 15.55 | 2.53 | 5.06 | 12.66 |
| 50 | 271 | 381 | 699 | 10.98 | 15.43 | 28.31 | 5.06 | 10.13 | 25.31 |
| 100 | 801 | 1,266 | 2,649 | 16.22 | 25.64 | 53.64 | 10.13 | 20.25 | 50.63 |
| 200 | 2,611 | 4,536 | 10,299 | 26.44 | 45.93 | 104.28 | 17.84 | 30.76 | 80.46 |
| 300 | 5,421 | 9,806 | 22,949 | 36.59 | 66.19 | 154.91 | 21.63 | 34.18 | 98.34 |
| 400 | 9,231 | 17,076 | 40,599 | 46.73 | 86.45 | 205.53 | 18.25 | 32.05 | 93.87 |
| 500 | 14,041 | 26,344 | 63,249 | 56.87 | 106.69 | 256.16 | 19.46 | 30.76 | 96.56 |
| B. Genotyping cost/sample (US\$) | | | | | | | | | |
| | | 6 loci | | | 12 loci | | | 20 loci | |
| | | 160.00 | | | 264.00 | | | 404.00 | |

correct one, or sitting at the last possible hole (Kerley and Salkina 2007). Presenting samples in a row appeared to exacerbate this problem in our study. Eager dogs often rushed past the first hole, or sat as they neared the end of the row hoping for a reward. Slowing the dog's movement helped prevent them from overshooting samples. However, we have since moved to an oval design (e.g., Kerley and Salkina 2007) to prevent these problems where there is no first or last hole. Once the dog selects a sample, we simply restart it at the next hole.

The range in matching dog costs (Table 2) and, hence, savings varies because matching costs depend on the number of comparisons that must be made, which is a function of the total number of samples to be compared, and mean number of samples per individual in the data set. In fact, when there is only an average of 2 samples/individual in the data set and the sample size exceeds 300, this method becomes more expensive than genotyping because the total number of comparisons becomes huge.

Because the number of comparisons increase 3–4-fold with each sample-size doubling (Table 2), total number of comparisons (and hence cost/sample) can be substantially reduced for larger sample sizes by dividing the total number of samples into smaller subsets and matching all samples within each subset (e.g., 500 samples = 5 subsets of 100 samples). An aggregate of these subsets would then be created by taking one sample from each of the matched groups per subset forming a newly aggregated subset of 50 samples (e.g., aggregating one sample from each of the 10 matched groupings for each of the 5 subsets, assuming an average of 10 samples/individual). This approach ultimately enables all unique sample groups (individuals) to be matched (e.g., 500 samples broken into [5 + 1] aggregate groups = 4,806 comparisons = US\$9,732.00 [\$19.46/sample] vs. 14,041 comparisons = US\$28,433.00 [\$56.87/sample] when all 500 samples are compared in one block; Table 2). Total genotyping cost for 500 samples is US\$80,000 (assuming \$160/sample for 6 loci/sample).

The calculations for the aggregate design (Table 2) assume that each subset is created in a manner that maintains the number of samples per individual in the original data set. Assuming that an individual is most likely to be recaptured in the same area, the best way to maintain the number of samples per individual in any given subset is to divide the entire field study area into contiguous blocks and draw all samples for a given subset from the same study block. Similarly, any additionally aggregated subsets should be comprised from contiguous study blocks. This aggregate design assumes that dogs can be trained to pass previously selected samples when the target is changed.

Another cost advantage of matching dogs stems from the dog's ability to match samples that are too degraded to be genotyped, markedly reducing the overall number of samples that have to be discarded. Such failed DNA analyses are essentially sunk costs because there is yet no reliable method for establishing that a fecal sample is too degraded to genotype prior to its analysis. In our initial studies (Fig. 2), dogs frequently matched samples collected

from wild grizzly bears in Alberta, Canada (Wasser et al. 2004) that failed to genotype at >4 loci required for a probability of 0.01 that the sibling of a particular individual would have the same observed genotype (p_{sib} ; Woods et al. 1999) for individual identification. Although we could not genetically confirm those matches, the loci that did amplify were consistent with the dog matches, as were the close proximities of these samples to each other when they were collected in the field.

Management Implications

Cost-effective ways of gathering wildlife samples over large landscapes and assigning them to the individual are fundamental tools for addressing key questions in wildlife management and conservation biology. Fecal samples are ideal for such purposes because of their considerable availability in the wilderness and the amount of biological information they contain. Sample-matching by detection dogs increases overall accessibility of these important techniques to wildlife sciences by providing a reliable, cost-effective tool for assigning individual identities to the large sample sizes needed to address some of the most pressing problems in conservation and population biology.

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LITERATURE CITED

- Gobush, K. S., B. M. Mutayoba, and S. K. Wasser. 2008. Long-term impacts of poaching on relatedness, stress physiology, and reproductive output of adult female African elephants. *Conservation Biology* 22:1590–1599.
- Kaldenbach, J. 1998. K9 scent detection. Detselig Enterprises, Calgary, Alberta, Canada.
- Kerley, L. L., and G. L. Salkina. 2007. Using scent-matching dogs to identify individual Amur tigers from scats. *Journal of Wildlife Management* 71:1349–1356.
- MacKay, P., D. A. Smith, R. A. Long, and M. Parker. 2008. Scat detection dogs. Pages 183–222 in R. A. Long, P. MacKay, W. J. Zielinski, and J. Ray, editors. *Noninvasive survey methods for carnivores*. Island Press, Washington, D.C., USA.
- Millspaugh, J. J., R. J. Woods, K. E. Hunt, K. J. Raedeke, G. C. Brundige, B. E. Washburn, and S. K. Wasser. 2001. Fecal glucocorticoid assays and the physiological stress response in elk. *Wildlife Society Bulletin* 29:899–907.
- Pickel, D., G. P. Manucy, D. B. Walker, S. B. Hall, and J. C. Walker. 2004. Evidence for canine olfactory detection of melanoma. *Applied Animal Behaviour Science* 84:107–116.
- Rolland, R. M., P. K. Hamilton, S. D. Kraus, B. Davenport, R. M. Bower, and S. K. Wasser. 2006. Use of scat detection dogs to study reproduction and health in North Atlantic right whales. *Journal of Cetacean Research and Management* 8:121–125.
- Schoon, G. A. A. 1996. Scent identification lineups by dogs (*Canis familiaris*): experimental design and forensic application. *Applied Animal Behaviour Science* 49:257–267.
- Schoon, G. A. A. 2005. The effect of the ageing of crime scene objects on the results of scent identification line-ups using trained dogs. *Forensic Science International* 147:43–47.

- Settle, R. H., B. A. Sommerville, J. McCormick, and D. M. Broom. 1994. Human scent matching using specially trained dogs. *The association for the study of animal behaviour* 48:1443–1448.
- Taberlet, P., L. P. Waits, and G. Luikart. 1999. Noninvasive genetic sampling: look before you leap. *Trends in Ecology and Evolution* 14:323–327.
- Walker, D. B., J. C. Walker, P. J. Cavnar, J. L. Taylor, D. H. Pickel, S. B. Hall, and J. C. Suarez. 2006. Naturalistic quantification of canine olfactory sensitivity. *Applied Animal Behaviour Science* 97:241–254.
- Wasser, S. K. 1996. Reproductive control in wild baboons measured by fecal steroids. *Biology of Reproduction* 55:393–399.
- Wasser, S. K. 2008. Lucky dogs: dogs sniff out scat from endangered animals, trumping more technical tracking methods. *Natural History*, 117(8):48–53.
- Wasser, S. K., K. Bevis, G. King, and E. Hanson. 1997. Noninvasive physiological measures of disturbance in the Northern Spotted Owl. *Conservation Biology* 11:1019–1022.
- Wasser, S. K., B. Davenport, E. R. Ramage, K. E. Hunt, M. Parker, C. Clarke, and G. Stenhouse. 2004. Scat-detection dogs in wildlife research and management: applications to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. *Canadian Journal of Zoology* 82:475–492.
- Woods, J. G., D. Paetkau, D. Lewis. B. N. McLellan, M. Proctor, and C. Strobeck. 1999. Genetic tagging of free-ranging black and brown bears. *Wildlife Society Bulletin* 27:616–627.

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