CHROMATOGRAPHIC (TLC) DIFFERENTIATION OF GRIZZLY BEAR AND BLACK BEAR SCATS

HAROLD D. PICTON, Department of Biology, Montana State University, Bozeman, MT 59717
KATHERINE C. KENDALL, Science Center, Glacier National Park, West Glacier, MT 59936

Abstract: While past work concluded that thin-layer chromatography (TLC) was inadequate for the separation of grizzly (*Ursus arctos horribilis*) and black bear (*U. americanus*) scats, our study found differences adequate for species separation. A key was constructed using 19 of 40 data points recorded on each ($N = 356$ profiles of 178) known-species scat. Accuracy was best for late summer scats (94%). Methods for specimen preparation, analysis, and reading the TLC profiles are discussed. Factors involved in scat variation were tested.

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The lack of an effective method to discriminate the species of bear scats in areas where grizzly bears and black bears are sympatric is a major impediment in studies of these species. Field sign of the species is often absent when scats are collected days or weeks after deposition. While scat diameter has been widely used as an indicator of species, it inherently confuses black bears with small grizzly bears (Hamer et al 1981). Calder (1984) indicates that the allometric slope of intestinal diameter is low in mammals suggesting that the larger body size of the grizzly would be reflected in only a small increase in intestinal diameter. Scat diameter is also affected by the nature and quantity of food consumed. Scat volume also does not appear to be a reliable character for species separation (Hamer et al. 1981). Since grizzly bears dig more than black bears it might be expected that their scats would contain more soil and mineral matter than those of black bears. However, this expectation was not supported by an exploratory study of the mineral ash content of scats as a method of species discrimination. Electrophoretic methods of detecting species from the proteins shed from the intestine into the scat appear to be possible but have not been used for bears. These fecal proteins can be expected to be degraded by moist conditions and exposure to the elements (Schribner and Warren 1984). DNA has been extracted from bear scats by E. Vyse (per. commun., January 1989 and 1992) but it is not clear if bear DNA from the intestinal cells can be separated from the large amounts of bacterial DNA present in fecal material.

Bile acids are very stable compounds having been extracted from 2,000-year-old human coprolites (Lin et al. 1978) and apparently from dinosaur coprolites (per. commun., J. Horner, January 1992). Major et al. (1980) and Johnson et al. (1984) reported that species-specific separation of carnivore scats could be obtained by use of thin-layer chromatography (TLC) to identify the array of bile acids contained in the scats. Goodwin (1984) in Alaska and Picton (1986) in Montana attempted to apply this technique to the separation of grizzly and black bear scats without success. Thin-layer chromatography bile acid profiles were obtained from both scats and bile from gall bladders (Welsh and Picton 1983). Both study groups agreed that TLC assessment of bile acids did not provide a reliable means of differentiating scats of the 2 species. The removal of plant pigments that obscured the desired TLC profiles was a major problem. Another possible problem with the use of bile acids is that bears may eat the livers of prey animals and thus bile acids in the scats may represent this exogenous source rather than those of the bear itself. Chemical extraction procedures used in chromatogram preparation extract many neutral lipids and related materials in addition to bile acids. Johnson et al. (1979) reported finding steroid sex hormones in scats and being able to distinguish sexes. Evaluation of data from Goodwin's (1984) and Picton's (1986) studies suggested that discrimination of scats might be possible if all spots on the chromatograms were used instead of just the presumed bile acids. Here we report an evaluation of the use of TLC to identify bear scats to species.

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METHODS

A modified TLC method was developed from that used by Major et al. (1980), Goodwin (1984), and Welsh and Picton (1983). Plant pigments were

extracted from the ground sample by soaking in 1:1 hexane-methanol. The filtered extract was then spotted at the origins of 6 lanes on a TLC plate divided into 9 lanes. Three lanes were used for a control mixture containing cholesterol, cholic acid, lithocholic acid, chenodeoxycholic acid, and deoxycholic acid. After spotting, the plate was developed in Petcoff's solution and then visualized with a 50:1:0.5 v/v solution of acetic acid:sulfuric acid:p-anisaldehyde. The visualized plate was photographed within 15 minutes under visible and ultraviolet light. The photographic slides were then projected and the chromatographic locations converted to computer data. Two concentrations of each scat were spotted on the TLC plates. Each of these lanes were read under both visible and ultraviolet light. Thus 80 data points (2 data sets of 20 points from each of 2 different concentration replicates) was obtained for each scat. The pattern of observed spots in each lane was designated the profile for that lane. These lane profiles were then analyzed to develop species scat profiles. The data from 356 lanes on TLC plates for 178 scats of known species were used to develop a key scat identification. Separate interpretations of the plates were made from the appearance of the plate under visible and ultraviolet light. Three lanes containing bile acid standards were included on each plate. STATA 2.1 (1990) was used to analyze data and to assist in the development of the key. An initial key was developed from 240 lanes of data from known scats (67% of the known scat data). The key was tested by using known samples not included in the key. It was then modified after each test to include additional known material. This process was repeated over 20 times until all 356 lanes of data were incorporated into the key.

Reference plates for 13 experimental chemical standards were also run. These included a bile acid group: lithocholic, deoxycholic, chenodeoxycholic, ursodeoxycholic, and cholic acids. A group of sterol hormones and other compounds of physiological significance were also subjected to TLC analysis: estradiol, progesterone, pregnanolone, testosterone, androsterone, cortisone, cholesterol, and creatinine. These data were used to develop reference standards and to aid in the interpretation of TLC data from scats. In explanation, cholic and chenodeoxycholic acids are regarded as the primary bile acids formed from cholesterol. Deoxycholic acid is a secondary derivative from cholic acid and lithocholic acid is a secondary derivative of chenodeoxycholic acid (Martin et al. 1981). We did not test coprostanol, the principal sterol formed by bacteria in the lower intestine (Murray et al. 1993).

Forty-four samples of 11 common bear food plants in various phenological stages were analyzed for reference. These were used to examine the effects of diet, sample drying methods, and sample weathering on TLC profiles. These were also useful in resolving the problem of effective removal of plant pigments.

RESULTS

Data from 2,056 samples of scats (n = 1,828), bear foods, and other possible fecal chemicals on 707 TLC plates were analyzed. Multiple TLC analyses were performed on some scats to study the effect of sample age on TLC profiles and to assess the replicability of the technique.

Relation to Chemical Standards

Bile acids were weakly related to the TLC profiles of either species. Forty percent of the grizzly bear scats matched the TLC profiles of cholic acid and lithocholic acid. The lithocholic acid TLC profile matched 48% of the black bear scats. Fluorescent patterns typical of bile acids were frequently seen in bear scats but were of little value in distinguishing species. A synthetic profile combining the cholesterol and creatinine profiles matched many grizzly bear scat profiles. A synthetic profile combining cholesterol with estradiol was the best match for black bear scats. The patterns produced by these materials partially overlapped the most useful points for distinguishing species. High Performance Liquid Chromatographic (HPLC) runs of the scat extracts may contain up to 300 compounds and their variants, indicating that a simple match of TLC spots, such as seen in these synthetic profiles, is not sufficient for the definitive identification of a compound.

Diet Influence

Bear scats tended to have fewer spots in their TLC profiles than did the plant materials. Plant materials generally had an abundance of spots at the higher r/f values (r/f = distance traveled by the spot of interest from the origin divided by the distance traveled by the solvent front). Thus, bear scat profiles were not just a simple projection of their food materials.

The TLC profiles of 21 known grizzly bear scats were matched with the food residues contained in these scats. This permitted comparison of profiles for several levels of grass, cowparsnip (*Heraculum lanatum*), horsetail (*Equisetum* sp.), and moose (*Alces alces*) content in the scats. No clear patterns of difference were seen between these groups of scats. The TLC profiles from scats containing moose differed more

from scats containing vegetation than the vegetation containing scats did from each other. This suggests that a moose or meat diet resulted in fewer spots on the TLC profiles.

Influence of Scat Collection and Treatment Parameters

The type of drying treatment; air dry, oven dry, or freezing with oven drying did not have a major influence upon the TLC results. Grass samples subjected to weathering under typical good mountain weather conditions (clear skies, sun, diurnal temperature change with dew) for 1, 2, 4, and 7 days showed no major changes in the TLC data. A sample subjected to 24 hours of snow melt leaching showed a reduced number of spots as compared to 1 day samples.

The estimated age of the scats (time elapsed between time of deposition to time of collection) was recorded for a group of scats (n = 33). Correlation coefficients for scat profiles from scats 1, 2, and 3 weeks old compared to scats 1 day old were r = 0.7 or greater, suggesting that some information was lost due to weathering, which probably involved leaching by rain or snow melt. Scats retested after storage in the laboratory for up to 5 years showed no major changes in the TLC profiles. However, it was noted that runs of other unknown scats stored for several years seemed to give an increased percentage of unidentifiable scats. This seeming effect of age could have also been produced by having a larger proportion of black bear scats in the collections from earlier years. The scat reference library was skewed toward Yellowstone-area scats while the unknowns were from Glacier Park. Scat profiles showed seasonal differences but the small sample numbers for some months prevented clear definition of the variation. The patterns suggested that the TLC scat profiles reflected changes in food habits associated with the annual maturation and drying of the plant communities.

Scat Key Development

Identification keys were constructed from 356 profiles from 178 known scats. Of this total, 14% were black bear scats from the Yellowstone area and 6% were black bears from the Glacier Park area. Grizzly bear scats represented 80% of the total with 47% from the Greater Yellowstone Ecosystem and the remainder from the North Continental Divide Ecosystem. The keys were used to identify the TLC data points containing information concerning species and to develop profiles for species identification. These profiles then formed a computer library for scat inference. A computer matching program by S. Martin (1989, unpubl. data) was devised to match the profiles of unknown scats against the identification library. Scatkey 11.0, the current version of this key, is available from the senior author. It consists of 2 separate keys, one based upon the appearance of the TLC plates under visible light and one using data from the plates viewed under ultraviolet light. When scatkey 11.0 was tested against unknown and known samples not used in the development data base, ambiguous identifications were given to 15% of the scats. These ambiguous judgments arose from tie vote given by the key when 4 identifications (2 different concentrations viewed under both visible and UV light) disagree, with 2 indicating black bear and 2 indicating grizzly bear. When this key was used in blind tests with scats of known species it correctly identified 66.7% of the samples collected from 1 June to 7 July but this identification rate was not significantly different from random choice (P = 0.1214; chi square test). The identification success for scats collected from 8 July to 31 August (Table 1) was significant (P = 0.0001; chi square test) and represented a success rate of 94%. These test groups of scats gave high rates (24-54% of ambiguous or no identification) of findings, much higher than for the 1,650 unknown scats run. These unknown scats were used in operational tests of the system and in other phases described above where the species identity of the scat was not critical.

Tests were also made using a library of identification profiles that had never produced an erroneous match. Unfortunately these "100 percent correct" profiles were typically able to identify only 30 to 40% of the scats in a test group. Thus there appears to be a tradeoff between accuracy and numbers of scats given an identity. Examination of the "100 percent correct" matching profiles from the visible light key indicated that grizzly bear and black bear scats differed most frequently at r/f's of 0.25, 0.95, and 1.0. Differences at r/f 0.45, 0.6, 0.65, and 0.7 were also common. The visible light key used 19 of the 20 possible spot locations in profile matching. The ultraviolet light key found differences most common at r/f 0.5 and 0.55. Differences at r/f 0.15, 0.2, and 0.7 were also common. The ultraviolet key used data from 18 of the data sites.

DISCUSSION

Any scat identification method must cope with a number of factors that can cause variation. Major scat-related variations include: differing compositions

Table 1. Test results of scat key 11.0 against 77 known grizzly and black bear scats collected from the North Continental Divide and Greater Yellowstone Ecosystems. Percentages in parentheses.

Date	*N*	Correct	Incorrect	Ambiguous
1 June to 7 July				
Group I (GB = 9; BB = 9)[ab]	17	3 (18%)	7 (41%)	7 (41%)
Group II (GB = 21; BB = 0)	21	15 (71%)	4 (19%)	2 (10%)
Combined[c]	38	18 (47%)	11 (29%)	9 (24%)
8 July to 31 August				
Group I (GB = 4; BB = 1)[d]	15	7 (47%)	1 (6%)	7 (47%)
Group II (GB = 24; BB = 1)[e]	24	10 (48%)	0 (0%)	14 (22%)
Combined[f]	39	17 (44%)	1 (2%)	21 (54%)

[a] GB = Grizzly bear scats, BB = black bear scats; Ambiguous, tie vote (2 BB and 2 GB).
[b] Correct category 2 of 3 scats were BB, Incorrect category 5 of 7 were BB and Ambiguous 2 of 7 were BB.
[c] For scats in the Correct and Incorrect categories, 66.7% of total (29) were correctly identified ($P = 0.1214$; χ^2).
[d] In Correct category 1 of 7 were BB.
[e] In Ambiguous category 1 of 14 were BB.
[f] For scats in the Correct and Incorrect categories, 94.4% of total (18) were correctly identified ($P = 0.0001$; χ^2)

among subsamples of the same scat; seasonal changes in scat composition; food-habit variation; the length of time that the scat was subjected to weathering before being collected; the method of drying and storage; length of time before final processing; and differing concentrations of the chemicals extracted from the scats. Laboratory controlled TLC method variations appear to be minor and include: variation in migration rates of the solvent front between plates and across the plate, minor variations in technique between technicians, inconsistencies in reading plates because of indistinct spots, spots spread over several *r/f* units, or excessively dark spots. The evidence suggests that the procedures are robust enough to compensate for these variations and allow successful identifications.

The identification procedure uses the presence or absence of spots at particular locations for identifications. There appear to be basic species differences that are partially masked by food-induced and other sources of variation. The use of spot-intensity data rather than just the presence and absence of spots would probably improve the method.

There is no single TLC profile that can serve as a reliable species indicator. The evidence suggests that there is sufficient difference between the scats of the 2 species to allow more reliable species separation than previous methods. Although it was noted that black bear scats seemed to match a profile containing cholesterol and estradiol, the method as it is presented, is inadequate for the determination of sex. High Performance Liquid Chromatographic or immuno-detection may be able to detect excreted hormones.

An increase in the size of the library of known scats is highly desirable. A larger number of black bear scats is needed. Scat profiles are "learned" by the key as new ones are discovered. The learning curve for grizzly bear scats of 1 new profile for every 8 new scats suggests that the data base for them is reasonably adequate, particularly for late summer and fall. The learning curve for black bear scats of a new profile for each 3 new scats indicates that expansion of the library of black bear profiles is needed.

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