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Laura R. Prugh, Stephen M. Arthur & Carol E. Ritland


Faecal genotyping has been proposed as a method to examine the diets of individuals, but this application has been virtually unexplored by wildlife biologists. We used faecal genotyping and conventional scat analysis to determine the diets of 42 coyotes Canis latrans belonging to nine social groups in Alaska. We use rarefaction to examine the effect of scat sample size on the accuracy and precision of individual diets, and we simulate diets from scats to determine how diet richness and evenness affect sample size requirements. We then demonstrate the utility of this technique by examining variation in diet among individual coyotes and social groups in relation to prey availability. Estimates of diet diversity and composition were highly variable when < 10 scats were used to construct the diet. Diets simulated with a uniform (i.e. even) distribution of prey items required generally smaller sample sizes to estimate diet diversity and richness than diets with exponentially distributed items; however, items in actual scats were exponentially distributed. We found moderate dietary variability among individuals in our study area, and diet overlap was higher among coyotes within social groups than between groups. As predicted by optimal foraging theory, the niche widths of all coyote groups expanded as their primary prey (the snowshoe hare Lepus americanus) became scarce during our three-year study. Despite increased niche width, diet overlap among groups remained constant, suggesting that coyotes selected differing alternative prey. Spatio-temporal variation in snowshoe hare availability explained 70% of the variation in hare consumption among groups, indicating that variation in local prey availability may be the primary cause of diet variation among coyotes. Although faecal genotyping can be used to address ecological questions at the individual level, studies should be designed specifically for this purpose so that sufficient numbers of faeces can be obtained.

Key words: Alaska, canid, faecal genotyping, individual specialization, noninvasive genetics, rarefaction, resource use

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Resource utilization is often studied at the population level, and variation in resource use among individuals is commonly ignored. Each individual may utilize only a subset of the resources exploited by its population, and the degree of dietary variation within a population can have implications for ecology, evolution and conservation (Bolnick et al. 2003). For example, dietary variation among individuals may reduce intraspecific competition (Kohda 1994), facilitate sympatric speciation (Schluter & McPhail 1992), and lead to lags in predator responses to changing prey abundance (Werner et al. 1981). Theoretical models have shown that variation among individuals can lead to outcomes that are not predicted when all individuals are assumed to be 'average' (Lomnicki 1978, Huston et al. 1988, Judson 1994). Although complexity is increased when individual variation is considered, this approach provides a direct link between natural selection and population-level processes (Wilson 1998).

The diets of individuals are typically studied by directly observing foraging animals (Gese et al. 1996, Estes et al. 2003) or by capturing animals to obtain stomach contents or samples for stable isotope analysis (e.g. Angerbjörn et al. 1994, Bridcut & Giller 1995, Ben-David et al. 1997). However, these methods are not possible when animals are too secretive or sensitive to observe or capture, or when hair snag stations are not an option. We used a recently developed method to obtain such data (Taberlet et al. 1996), in which individual coyotes Canis latrans were identified based on DNA extracted from intestinal cells in faeces (Prugh et al. 2005) and matched with diets constructed from prey remains in the faeces.

Non-invasive genotyping reviews have highlighted the potential use of faecal genotyping as a tool to study individual diets (Kohn & Wayne 1997, Waits & Paetkau 2005), but only one study (Fedriani & Kohn 2001) has used this method to date. Fedriani & Kohn (2001) examined variation among coyote diets in southern California, and they used cluster analysis to show that individual variation existed among coyotes. However, they were not able to adequately determine the effect of scat sample size on the accuracy of individual diets because too few scats were collected per individual (range: 3-11 scats). In population-level analyses, at least 50 scats are recommended to estimate the average diet of a predator population (Windberg & Mitchell 1990, Mukherjee et al. 1994, Trites & Joy 2005). If sample sizes are inadequate, diet diversity will be underestimated and the composition (proportion of each item in the diet) will be inaccurate, potentially leading to spurious differences among individuals.

In this paper, we use our larger data set, in which as many as 49 scats were collected per individual, to determine the effect of scat sample size on the accuracy and precision of individual diets. We also simulated scat data sets to examine how diet richness and evenness affect sample size requirements, because the richness and evenness of diets likely vary among species, regions and years. Using these analyses to guide sample size requirements, we then investigate how dietary variation among coyotes in our study population changed in relation to prey availability.

Coyotes are known to consume a wide variety of prey items and have long been regarded as a prototypical generalist species (Young & Jackson 1951, Bekoff 1977). However, several studies have shown that, at the population level, coyotes can be selective predators, and changes in resource availability can strongly affect their patterns of resource use (Windberg & Mitchell 1990, O’Donoghue et al. 1998). The primary prey of coyotes in Alaska, the snowshoe hare Lepus americanus, sharply declined in abundance during our study. Optimal foraging theory predicts that diet niches of individuals will broaden to include less profitable prey items as food shortage increases (MacArthur & Pianka 1966, Charnov 1976, Krebs 1978). Thus, we predicted that dietary niche widths and overlap of coyotes would increase in response to the hare decline. Because coyotes often live in social groups and hunt together, we also tested the hypothesis that packmates would have higher diet overlap than non-packmates.

**Material and methods**

**Study area**

Our study was conducted during January 2000 - March 2002 in a 1,000 km² area of the central Alaska Range (63°57'N, 147°18'W). Winter temperatures
averaged -14.7°C and snow cover occurred from October to April. Elevation ranged within 600-2,100 m a.s.l., and major habitat types included boreal forest, subalpine shrub and alpine meadow. Other carnivores in the area included grey wolves Canis lupus, red foxes Vulpes vulpes, American martens Martes americana, wolverines Gulo gulo, river otters Lutra canadensis, lynx Lynx canadensis, grizzly bears Ursus arctos, and black bears U. americanus.

Faecal genotyping
Frozen coyote faeces were collected at frequent intervals (almost every day) during each of three winters (January-March) along 150-200 km of snowmobile trails. Faeces were also collected while following coyote tracks on foot. We recorded the GPS location and estimated maximum age of each scat (based on snowfall history and the time between collections). A total of 1,237 faeces were collected, of which 850 were randomly selected for genetic analysis. DNA samples were obtained from 834 scats (16 samples were contaminated during DNA extraction).

Approximately 100 mg of frozen faecal material was collected from each scat, and DNA was extracted using QIAamp DNA Stool Mini-Kits (Qiagen, Valencia, CA, USA). We screened each sample with a mitochondrial DNA test to ensure that the isolated DNA was from a coyote (Prugh & Ritland 2005). Samples that did not amplify or showed non-coyote products were removed from the data set. Nuclear DNA was amplified from confirmed coyote samples at six microsatellite loci to establish individual identity (for methodological details, see Prugh et al. 2005). Because genotypes obtained from faeces can be unreliable (Taberlet et al. 1999), we assessed the accuracy of our genotyping methods in a pilot study and replicated each genetic fingerprint 2-5 times based on the results (after Frantz et al. 2003). The probabilities of obtaining identical genotypes for different individuals and of creating false individuals through genotyping errors were very low (0.004-0.007; Prugh et al. 2005). Sex was determined by amplifying a region of the SRY gene on the Y-chromosome with primers designed specifically for canids (Prugh et al. 2005). Based on preliminary evaluations of diet variability, we excluded coyotes with <4 genotyped scats from this study, unless they were identified as a member of a known social group (see below).

Diet analysis
After obtaining DNA from each sample, scats were sterilized and then washed in nylon mesh bags using a clothes washing machine on gentle cycle. Air-dried samples were examined and all food items present were recorded. We compared hairs, teeth and claws to reference specimens and guide books (Moore et al. 1974) for identification. Occurrences of moose Alces alces and caribou Rangifer tarandus in scats were assumed to be the result of scavenging and referred to as carrion, because studies in this area found that coyotes rarely killed these species (Boertje et al. 1996). The following 12 food items were recorded: snowshoe hares, voles Clethrionomys rutilus and Microtus spp., shrews Sorex spp., porcupines Erethizon dorsatum, sciurids (squirrel Spermophilus parryii, northern flying squirrel Glaucomys sabrinus and hoary marmot Marmota caligata), Dall sheep Ovis dalli, carrion (moose and reindeer), birds (usually unidentifiable to species), predators (lynx and river otter), arthropods and vegetation (berries and grass). Frequency of occurrence was calculated as the number of scats containing an item divided by the total number of scats; frequencies of different items are therefore independent of one another and can sum to greater than one.

The Shannon index (H') was used to estimate diet diversity and niche width (Krebs 1999). Two measures, detailed in Bolnick et al. (2002), were used to examine diet variation. First, we used Petraitis’ W, a robust maximum-likelihood index, to estimate the similarity of individual diets to the average population diet (Petraitis 1979, Bolnick et al. 2002). A value of '1' for this index indicates that an individual diet is identical to the population diet, and a value near '0' indicates extreme deviation from the population diet. The population diet was calculated as the average frequency of prey occurrences across individuals. Second, we calculated the pairwise diet overlap among individuals (Schoener 1970), which also varies from '0' (no overlap) to '1' (identical diets). We used the program IndSpec1 (Bolnick et al. 2002) for these analyses.

Rarefaction and simulations
We used rarefaction of actual scat data and simulated scat data sets to estimate sample size requirements for accurate estimation of individual diets. Random subsamples of increasing size (N = 1 scat to the maximum available for each set) were drawn with replacement from each individual’s pool of
scats, with 1,000 repeated draws for each sample size. Diets were constructed for each subsample as the mean frequency of prey occurrences in scats. H' was also calculated for each subsample. Bootstrap estimates of variation were calculated for each diet diversity and composition estimate.

Data sets of 1,000 scats each were simulated using either a uniform distribution of prey items, which maximizes dietary evenness, or an exponential distribution, which is highly uneven and closely matched the distribution of prey items in our real scat data set. Using each distribution, diets were simulated with a total richness of 5, 10, 15 or 20 items. Each item had an equal probability of occurrence when diets were simulated with a uniform distribution, whereas the probability of occurrence increased exponentially across items from 0.01 to 0.90 when scats were simulated using the exponential distribution. As with the actual scat data, we drew random subsamples of increasing size, N = 1-200, from the pool of simulated scats. Each subsample was drawn 1,000 times, and the mean and variation of H' were calculated. For each data set, we determined the sample size required to obtain a diversity or richness estimate that was 95% of the true diversity or richness, as well as the sample size required to include the true diversity or richness value in the 95% confidence intervals of the estimate. Programs were written and run in program R (R Development Core Team 2005) for these analyses.

Social group composition
Social groups generally consisted of a mated pair and one or more offspring born the previous spring (radiotelemetry data; Table 1). We assigned coyotes to social groups using a combination of genetic analyses, radiotelemetry and snow-tracking. The program Kinship (version 1.3.1, Goodnight Software) was used to obtain pairwise relatedness coefficients (r) for all coyotes, and simulations were used to generate likelihood ratios testing the hypothesis that r = 0.5 (parent or full sibling) for each pair (1,000 simulations per pair; Goodnight & Queller 1999). Potential parents and offspring identified by these analyses were examined to confirm relationships using the following four criteria: 1) each parent contributed one allele at each locus to the offspring’s genotype, 2) the parents were of the opposite sex, 3) the locations of collected faeces from each parent overlapped spatially, and 4) the parents were detected in the study area prior to the offspring.

Coyote tracks were followed each winter to further resolve social group composition (N = 167 track sessions; total distance = 309.5 km). Fresh coyote tracks were located by traveling on snowmobile trails after fresh snowfalls, and they were backtracked on foot. We recorded the number of coyotes traveling together, and individuals were identified by genotyping scats collected during the tracking session. This helped to identify probable adult pairs that had no detected offspring (e.g. the N.W. Fork social group; see Table 1).

Coyotes were captured using immobilizing darts fired from low-flying helicopters and immobilized with a mixture of equal parts tiletamine hydrochloride and zolazepam hydrochloride (Telazol™; Fort Dodge Animal Health, Fort Dodge, IA, USA) administered at dosages of 9.0-10.0 mg/kg. Each coyote was fitted with a radio-collar weighing 0.25 kg (MOD 400, Telonics Inc., Mesa, AZ, USA). Ear punches and blood samples were collected for DNA analysis, and samples were genotyped twice to ensure accuracy. Collared coyotes were located by aerial telemetry daily in spring, weekly in summer, and bi-weekly in winter. GPS locations were recorded and denning activity was monitored.

Coyote age at time of first detection (i.e. capture or first genotyped scat) was categorized as juvenile (<1 year old), adult (>1 year old) or unknown. The age of each radio-collared coyote was determined by subjectively assessing tooth wear and body condition during capture. Uncollared coyotes identified by faecal genotypes were categorized as 'juvenile' if they were identified as the offspring of an identified pair, 'adult' if they were identified as a parent, and otherwise as 'unknown'. Only one juvenile (S3) remained in the study area for >1 year, and this coyote was categorized as a juvenile during both years because he remained with his parents in his natal territory. The probability of detecting coyotes present in the area for ≥1 year was approximately 100% (all radio-collared coyotes were also detected by genotyping scats; Prugh et al. 2005), so it is unlikely that adult offspring residing in the study area were falsely categorized as juveniles. We used Mantel tests to examine the correlation between social group affiliation and diet overlap among individuals (Manly 2005). Analyses were conducted in program R.

Hare abundance
Faecal pellet counts were used to monitor changes in snowshoe hare abundance. Pellet counts have been...
shown to be accurate indices of population change for snowshoe hares (Krebs et al. 2001, Murray et al. 2002). Faecal pellets were counted on 12 grids, each with \( i \) 50 circular plots spaced 15 m apart. Plots were counted and cleared once per year (for details of the pellet count protocols, see Prugh & Krebs 2004). Each grid was within or adjacent to the home range of a resident coyote social group. To examine the influence of hare abundance on social group diet, pellet grids within a group territory were averaged (1-3 grids per territory) and matched with the social group diet each year.

### Results

#### Genotyped scat data set

Of the 56 coyotes identified in the study area during 2000-2002 (Prugh et al. 2005), 36 were identified as members of eight resident social groups, and one resident lone coyote was identified (see Table 1). An additional four coyotes of unknown social group affiliation met our minimum criteria for inclusion in the study (at least four genotyped scats and/or known social group affiliation). Thus, our data set consisted of 513 scats collected from 42 coyotes,

<table>
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<th>Individual ID</th>
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14 of which were radio-collared (see Table 1). The average size of social groups fell from 2.9 coyotes in 2000 to 2.7 coyotes in 2001 and 2.3 coyotes in 2002. The number of scats collected per individual ranged within 1-49, with an average of 21 scats collected per social group per year (range: 7-87).

**Sample size requirements**

Estimates of the Shannon index ($H'$) were substantially underestimated and highly variable for individual coyotes when the sample of scats used to construct the diet was $< 8-10$ (Fig. 1). Estimates of $H'$ tended to stabilize when 10-20 scats were used (see Fig. 1A), and the variability dropped exponentially as sample sizes increased (see Fig. 1B).

Examination of specific dietary items from four individuals with large samples indicated that non-overlapping bootstrap confidence intervals could be obtained when items differed in frequency by at least 30% and 10-15 scats were used to construct the diet (Fig. 2A-D). As few as five scats per individual were sufficient to obtain non-overlapping intervals when differences in occurrence exceeded 50% (see Fig. 2C). In contrast, 30-50 scats were required to obtain non-overlapping confidence intervals when the scats of all 27 coyotes present in 2002 were pooled and items differed in frequency by 25-30% (see Fig. 2E).

Rarefaction of simulated scats showed that confidence intervals were wider around diversity and richness estimates when an exponential distribution was used, and curves approached their true values more gradually for estimates of richness than estimates of diversity (Fig. 3). When items were distributed uniformly, the sample size required to obtain an estimate within 95% of the true diversity or richness value increased with richness: 6-10 scats were required when five items were in the diet, whereas 28-29 scats were required when 20 items were in the diet (Table 2). Interestingly, the reverse pattern occurred when items were distributed exponentially, and far more scats were required to estimate richness: 34-160 scats were required when five items were in the diet, whereas 16-96 scats were required when 20 items were in the diet (see Table 2).

**Diet variation among individuals**

We limited our analyses of individual diet variation to the 18 coyotes in our data set with at least 10 scats, based on the above rarefaction results. Although the proportions of specific items in the diet varied markedly among individuals (Fig. 4), overall measures of diet variability showed that individual coyote diets were fairly similar to the average population diet, with a mean Petraitis' W similarity index of 0.81 (Table 3). The average pairwise overlap in diet was 0.68, and diet overlap was significantly higher among coyotes within social groups than between groups (Mantel $Z = 16.4, r = 0.17, P = 0.001, N = 18$; within-group overlap was 0.74 and between-group overlap was 0.67).

**Diet variation in relation to prey abundance**

Because scat sample sizes of individuals were generally too small to examine annual diets, and because diets of individuals within social groups were non-independent, we examined annual changes in the pooled diets of social groups in relation to snowshoe hare availability. Hare abundance declined >10-fold during the study ($F_{2,31} = 19.8, P < 0.001$;
As predicted by optimal foraging theory, social group diet niches expanded as hare abundance declined ($F_{2,20} = 12.1$, $P < 0.001$; see Fig. 5). Coyote social groups included 2-7 items in their diets in 2000, 5-8 items in 2001, and 5-10 items in 2002. Despite the increase in niche width, diet similarity and overlap among groups did not change over time (Petraitis' $W = 0.70$, $P = 0.51$, pairwise overlap $F_{2,74} = 0.35$, $P = 0.71$; see Fig. 5). Spatiotemporal variation in hare abundance explained 70% of the variation in hare occurrence in the diets of social groups ($F_{1,21} = 50.1$, $P < 0.001$; Fig. 6).
Figure 3. Effect of sample size on estimates of diet richness and diversity. Data sets of 1,000 scats were simulated using a uniform distribution of prey items (A and C) and an exponential distribution (B and D), with 5 (solid line), 10 (short dash), 15 (dash dot dot), or 20 (long dash) items in the diet. Mean richness (A and B) and Shannon diversity indices (C and D) from 1,000 bootstrap runs per subsample (from \( N = 1-200 \) scats) are shown with 95% confidence intervals. Horizontal grey lines indicate the true value of richness or diversity (\( H' \)).

Table 2. Rarefaction results from simulated scat data sets. Data sets of 1,000 scats containing a total of 5, 10, 15 or 20 diet items were simulated using either a uniform (even) or exponential (highly uneven) distribution for the proportion of items in the diets. Each data set was resampled with replacement 1,000 times from \( N = 1-200 \) scats, and the mean and variance of diet richness and diversity (Shannon index, \( H' \)) were recorded. The number of scats required to obtain an estimate within 95% of the true richness or \( H' \), and for the true richness or \( H' \) to be within the 95% confidence intervals, are shown. The true \( H' \) was always within the confidence intervals using an exponential distribution because they were very wide at low sample sizes.

<table>
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<th>Richness (N items)</th>
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<th>True ( H' )</th>
<th>95% true ( H' )</th>
<th>( H' ) in CI</th>
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Discussion

Diet variation among individuals and social groups

Using a combination of faecal genotyping and conventional diet analysis of scats, we determined that coyotes in our Alaska Range study area exhibited a low-to-moderate level of diet variation among individuals. As snowshoe hares declined during the study, coyote social groups expanded their diet niches, but this expansion did not increase dietary overlap among groups. Thus, consumption of alternative prey varied among groups, and levels of competition may have remained stable. Most of the variation in consumption of hares was explained by variation in hare abundance, indicating that local prey abundance is the main driver of individual consumption.
diet variation among generalist predators such as coyotes.

**Causes of diet variation**

Although coyotes in this study had fairly broad diets, overlap was as low as 0.42 between some social group pairs. The fact that individuals of the same species living in the same population can have highly divergent diets has intrigued evolutionary ecologists for decades (Van Valen 1965, Bolnick et al. 2003). We found that spatial and temporal variation in hare abundance explained a majority (70%) of the variation in hare consumption among coyote social groups. This suggests that fine-scale heterogeneity in the distribution of prey is an important cause of intraspecific diet variation, particularly for spatially-structured predator populations. Studies of other territorial species, including arctic foxes *Alopex lagopus*, martens, striped surfperches *Embiotoca lateralis*, brent geese *Branta bernicla*, and turnstones *Arenaria interpres*, also found that prey availability was the main factor influencing diet, with dominant individuals controlling the best territories (e.g. Whitfield 1990, Holbrook & Schmitt 1992, Angerbjörn et al. 1994).

Several studies have suggested that factors difficult for researchers to measure, such as learning, play a significant role in individual diet variation, particularly among social foragers (West 1988, Gu et al. 1997). For example, Estes et al. (2003) found that dietary preferences in sea otters *Enhydra lutris* were unrelated to prey availability and were passed along matrilines. Likewise, differences in experience and learning may have contributed to variation among coyote social groups in the selection of alternative prey. For example, the S.W. Fork, Sheep Creek and S. Dry Creek social groups all had access to similar numbers of Dall sheep (Alaska Department of Fish and Game, unpubl. sheep surveys), but in 2002 only the S.W. Fork group utilized this resource to an appreciable extent (10% of the diet vs 0-3% for other groups). Specific individual predators and family groups can thus have a disproportionate impact on prey populations (Ross et al. 1997, Williams et al. 2004), which highlights the importance of incorporating intraspecific diet variation in predator-prey management programs.

**Niche width and overlap**

In a recent review of individual resource specialization, Bolnick et al. (2003) reported evidence for significant levels of intraspecific diet variation for many species and highlighted the importance of this variation as a target for natural selection. In our study, dietary overlap was moderate, and each coyote’s diet was similar to the average population diet. Additionally, the diet niches of all coyote groups expanded when hares declined. Dietary flexibility is likely favoured in systems characterized by high levels of resource variability, and strong intraspecific differences in diet choice are therefore unlikely to develop.

Despite the increased niche widths of coyote social groups during the hare decline, diet overlap among groups remained constant. This pattern implies that the mean diets of social groups diverged as niches widened; i.e., all coyote groups used more prey types when hares became scarce, but relative use of these types differed among coyote groups. This pattern could occur either in response to intraspecific competition or increased spatial patchiness of prey. Intraspecific competition among coyotes may have increased during the hare decline, but territoriality should have limited its intensity. The coefficient of variation among the 12 hare pellet grids increased from 24.9% in 2000 to 33.1% in 2001 and 81.2% in 2002, indicating that patchiness in the hare distribution increased as the hare population declined. This increased patchiness is consistent with the ‘refugia’ hypothesis, which states that hare populations contract to patches of high quality habitat during decline phases of the cycle (Wolff 1980). Prey patchiness was likely the dominant factor contributing to dietary divergence among social groups.

**Sample size requirements**

Although we successfully utilized faecal genotyping to address ecological questions about diet variation...
among individuals and social groups, we have reservations about the utility of this method. Most importantly, it may be difficult to obtain sufficient sample sizes of scats to accurately estimate the diets of individuals. Rarefraction of actual and simulated scat data sets showed that, for estimation of diet diversity and composition, a minimum of approximately 10 scats should be obtained per individual, and in cases where use of food types differs greatly from a uniform distribution, a sample size of 20-35 would be preferable. These sample sizes are lower than those recommended for estimation of population-level diets, most likely because the between-individual variation has been removed. Nonetheless, intensive sampling would be required to meet these requirements. To our knowledge, our data set of genotyped faeces is the largest reported, and our sample was still insufficient to calculate annual diets for more than a handful of individuals. Based on our results, we believe that ecological conclusions drawn from studies in which individual diets are constructed using <10 scats (e.g. Fedriani & Kohn 2001) should be interpreted with caution.

**Role of richness and evenness**

Despite the fact that increased evenness leads to higher values of the Shannon diversity index (Krebs 1999), diets simulated using an even (uniform) distribution of prey items required generally fewer scats to accurately estimate diversity and richness than did diets simulated using an exponential distribution. Additionally, diets with exponentially-distributed prey and low richness required more samples than diets with higher richness to achieve a similar level of accuracy. Unfortunately, actual diets are most likely to have exponentially-distributed prey items, in which some items are highly preferred and others are rarely consumed (e.g. Corbett & Newsome 1987, Windberg & Mitchell 1990, Ben-David et al. 1997). In our data set, coyote diets were distributed exponentially and individuals had relatively low diet richness (2-9 items). Thus, our estimates of diet diversity may have been underestimated when <35 scats were used to create the diet.

**Faecal genotyping as a tool for individual diet analysis**

If a faecal genotyping study is designed *a priori* with the purpose of determining individual diet, such that sampling maximizes the number scats collected per individual, this approach can be used successfully. For example, collecting scats at natal dens of predators yields a large number of scats and could be used to determine individual variability in consumption of prey species of interest. It is less likely that studies designed exclusively to estimate population size, currently the most common goal of faecal genotyping projects (e.g. Banks et al. 2002, Eggert et al. 2003, Frantz et al. 2003), would yield large enough sample sizes to rigorously examine individual diets.

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**References**


