Facts from feces revisited
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Obtaining information on wild mammal populations has been a long-standing logistical problem. However, an array of non-invasive techniques is available, including recently developed molecular genetic techniques for the analysis of feces (molecular scatology). A battery of non-invasive, molecular approaches can be used on feces, which in conjunction with conventional analyses are potentially useful for assessing genetic structure, demography and life history of mammals. Several technical problems remain before large-scale studies of feces can be undertaken productively, but already studies are providing insights into population subdivision, food habits, reproduction, sex ratio and parasitology of free-ranging populations.

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and amplification of genes from fecal material were aimed at developing new tools for clinical diagnosis. Similar techniques allowed parasitological studies of domestic and wild populations; for example, the amplification of DNA from tape-worm (Echinococcus multilocularis) in feces of red foxes (Vulpes vulpes) showed this parasite had a high prevalence.

Feces contain cells shed from the intestinal lining; thus DNA from the host itself could potentially be isolated and analyzed. The application of this idea to an animal population involved a small population of elusive, highly endangered brown bears (Ursus arctos) in the Bresta mountains of northern Italy. The population size and sex composition were unknown, but anecdotal observations suggested that only a few bears remained. A single mitochondrial DNA control-region sequence was found in bear feces collected from the Bresta mountains suggesting that the population was small and isolated. This sequence was present also at high frequency in bear populations from Slovenia, Croatia and Bosnia, indicating that the Bresta bears formerly were in contact with these populations. A Y-chromosomal nuclear marker SRY was used to show that at least one male and one female bear were present in the area. These results also implied that other nuclear markers such as microsatellites may be used.

Box 1. Genetic markers in feces
Mitochondrial DNA (mtDNA) is a maternally inherited, haploid, non-recombining and extra-nuclear genome. Each cell has multiple mtDNA copies (10–2500 copies) and thus mtDNA sequences are more easily amplified than single-copy nuclear genes. Because the mtDNA cytchrome b (cyt b) gene has only moderate intraspecific variation, it is well suited for use in species identification. The mtDNA control region is commonly variable on the intraspecific level and is suitable for studies of genetic variability, phylogenetics, assignment to management units, and forensics. Chloroplast DNA (cpDNA) and mtDNA can be used to amplify plant and animal sequences from feces to study diet. Moreover, a vast number of pathogen sequences (viruses, bacteria, protists and macroparasites) are available for amplification from fecal material.

Microsatellites have also been used as simple sequence length polymorphism (SSLP), or simple sequence repeats (SSR) are nuclear, single-copy DNA consisting of tandemly repeated short sequence motifs such as (CA)n. They are scattered throughout the genome and are highly polymorphic. By assaying several microsatellite loci, a multilocus genotype can be obtained: a 'genetic fingerprint', which is unique to individual animals. These markers are codominantly inherited (alleles from both parents are traceable in the offspring) and are useful for studying paternity and kinship, genetic variation, population genetic structure and gene flow.

Microsatellites identified in one species can usually be employed to study a related species. PCR primers for the SRY gene on the Y chromosome can be used to distinguish male from female DNA.

Genetic marker sequences and PCR primers are published or accessible through GenBank or EMBL, which are electronic databases available on the Internet that can be searched for sequences by entering the genus, genomic locus or sequence of interest.
Box 2. Conventional studies on feces

Mammal species can often be identified based on their feces, allowing studies of species composition. However, without additional information feces are assigned correctly in only about 50–66% of the cases. This uncertainty is owing to considerable size-overlap of feces in related taxa. In some cases, the pH value and bile acids of feces have been useful to identify species. Relative abundance and population size have been estimated from fecal counts. However, the resampling of individuals constitutes a problem. Census data based on feces can be supplemented by techniques described elsewhere.

Habitat use and range size may be investigated by examining spatial variation in feces abundance. Similarly, territory boundaries may be marked by fecal aggregations. These studies often require individuals to ingest colored plastic markers or radiative bait, which uniquely mark their feces. Analysis of food habits and dietary quality have frequently used feces. In carnivores, undigested prey items can be recovered and identified. Herbivore dung often contains plant remains. In general, this approach has shortcomings. For herbivores, indices of dietary quality can be derived through chemical analysis of fecal nitrogen (N) and dietary nitrogen (D), although the interpretation of results is controversial.

Reproductive status (pregnancy, ovulation, luteal function) and physiological stress (adrenocortical activity) can be derived from fecal steroid hormone measures. Moreover, fecal steroid analysis provides information on sex and age of animals. The physical, microbiological and chemical examination of feces also provides information on parasite infestation, bacterial flora and environmental contamination (PCBs and pesticides).

on feces to identify individual bears. Finally, a glimpse of the feeding habits of Brenta bears during late summer (when feces were collected) was provided by amplifying plant chloroplast sequences of the rbcL gene from bear feces. Fecal plant sequences were identified as belonging to the hawknurn genus Phohtina, though to be important in the diet of brown bears. Although animals were never seen during the study, feces provided answers to questions that usually require direct observation and the handling of animals. The results also had conservation implications: the presence of a male and female bear left open the possibility that the small population might increase. Furthermore, the fecal studies showed that the source for reintroduction should be bears taken from the Slovenian population.

Mitochondrial DNA has been amplified also from feces of the Indian elephant (Elephas maximus), European bison (Bison bonasus), polar bear (Ursus maritimus) and durgon (Dugong dugon) . Four studies have now confirmed that individuals could be 'fingerprinted' from their feces; these studies successfully amplified microsatellites from feces of wild baboons (Papio cynocephalus), bonobos (Pan paniscus), brown bears (Ursus arctos) and pinnipeds. Although some technical problems were encountered (Box 3), these studies present convincing evidence that such problems can be overcome and may someday be as useful as blood or tissue samples for genetic analysis. In part, our intent in this article is to provide incentive for additional technical research.

Previously, a variety of other potential sources for DNA such as hair, bones, feathers, saliva, skin and nails have been collected for non-invasive genetic analyses. However, these approaches may be more difficult to obtain than fecal samples and may provide less information. The genetic and life history information that potentially can be extracted from fecal samples is summarized in Fig. 1.

The information feces can provide is diverse

Behavioral biology

Two essential parameters that need to be assessed in behavioral studies are the number of offspring produced by individuals and the relationship of individuals in a social group to each other. This information allows behaviors that lead to reproductive success to be identified and provides an assessment of the kinship component of cooperative behaviors (e.g. Ref. 11). Microsatellite loci have been used to establish kinship by their degree of relatedness in natural populations and have been amplified from feces. Depending on polymorphism and levels of heterozygosity of microsatellite loci, about 20 microsatellite loci may be necessary to determine kinship of multiple relatives. This estimate is based on a study of wild-caught, outbred mice (Mus musculus) where 20 unlinked loci were sufficient to discriminate between unrelated and full-sib dyads with about 97% accuracy, and to discriminate half-sib pairs from unrelated sib pairs with better than 80% accuracy (see also Ref. 40).

Importantly, the number of loci to be scored increases as population heterozygosity decreases, which may impair our ability to determine kinship reliably in small and inbreed populations. However, in small populations fewer fecal samples have to be analyzed, which may compensate for the effort of scoring more loci. Because of the difficulties in reliably amplifying many single-copy loci from feces (Box 3), the study of large social groups may not be logistically or economically feasible at present through analysis of feces alone. Even so, limited studies of small social groups, especially when augmented by blood or hair samples, are feasible. Moreover, when conducting paternity exclusion analyses, sometimes very few loci suffice to exclude most of the males of a troop of primates, especially when this troop has been extensively observed.

Census population size

A common method to estimate population size involves extrapolation from animal counts on designated tracts. However, this technique generally assumes a uniform density of animals within their habitat and that individuals are not double counted. In many species of large mammals, feces are deposited in concentrations along established routes.
such as roads or territorial boundaries. Consequently, a large proportion of the population may leave feces at specific points that can be sampled repeatedly. The point where additional collection over a large area does not reveal any new multilocus microsatellite genotypes may indicate that all individuals have been sampled and the census size is then the number of unique multilocus genotypes.

Even in species that deposit feces in a more uniform or random distribution across the landscape, population size may be estimated using a transect method. Feces collected along transects would be typed for microsatellite loci and the relationship of feces collected to new multilocus genotypes should define a curve whose asymptote represents the census population size. Especially for cryptic species, systematic recovery of feces may allow more accurate estimates of population size than observation alone. Moreover, DNA analysis of feces may be the only way to prove the presence of a cryptic, rare or putatively extinct species in an area.

Home range and territory size

In territorial species, the approximate dimensions of territories and core-use area can potentially be uncovered by documenting the distribution and density of multilocus microsatellite genotypes found in feces. Presumably, areas of highest density of feces from a given multilocus genotype may mark the core area used by an individual. Quantitative methods similar to those used to deduce core areas and home range from radiotelemetry observations could be applied to these kinds of data from fecal studies (e.g. Ref. 41).

Effective population size

Three variables are critical to accurate assessments of effective population size: (1) the fraction of individuals that reproduce, (2) the sex ratio of those individuals, and (3) the variance in family size. In small populations, for which feces can be obtained from all individuals, such variables can be estimated (Fig. 2). The number of individuals can be tabulated from the number of unique multilocus microsatellite genotypes (see above), the sex ratio determined by use of sex specific probes (Box 1) and some information about family size deduced from paternity analysis (see Refs 8, 39, 40). In endangered species, often just any evidence of reproduction is very important information that microsatellite-based fecal analysis can provide.

Genetic variation

The extent of genetic variation depends on effective population size and in declining populations is an important variable to monitor because a goal of many conservation programs is to maintain a certain fraction of genetic variation
analysis of feces may be the only viable means to infer diet.

Conclusions
Relief from sampling despair has an unexpected source in feces. The combination of data from conventional analysis of feces with data from newly emerging DNA-based techniques may provide a much more comprehensive picture of the hidden life of elusive and rare mammals. Conventional analysis can provide a portrait of reproductive cycles, pathogens and diet, which can be augmented with data from DNA-based techniques. Additionally, analysis of DNA sequences from feces may provide information on paternity, kinship, sex ratios, census and effective population size, gene flow and phylogeography. In conservation management, the lack of such genetic, demographic and life history data has been a notorious problem that non-invasive population assessment potentially can eliminate. The challenge of the future is to develop better methods for dealing with the problems of inhibition, inconsistency, specificity and contamination such that molecular scatology can be routinely applied to large population samples.

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