Facts from feces revisited

Michael H. Kohn and Robert K. Wayne

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.

Michael Kohn and Robert Wayne are at the Dept of Biology, University of California, 621 Circle Drive South, Los Angeles, CA 90095-1606, USA.

In 1984, Putman[1] remarked in his review *Facts from Faeces* that 'for any frustrated field mammalogist seeking observational data on their elusive study animals, dung may represent the most readily-available and easily-collected source of information upon which they may fall back in despair'. Despair is frequent these days because many mammals are becoming increasingly elusive as their numbers decline. Rarity makes direct observation difficult but the need for genetic, demographic and life-history information for species facing extinction is more pressing than ever. Additionally, the great expense and extensive number of permits required to capture and transport samples makes studies of endangered species unpalatable to many biologists[2–6].

Even in abundant species, large-scale surveys of populations are logistically difficult if they depend on opportunistic sampling (e.g. Ref. 6). Moreover, 'any study of a natural population has the unavoidable side effect of altering the dynamics of the population in ways that may be difficult to predict'[7] or that may change some characteristics that a researcher is attempting to measure[1,8].

Must we lose hope if observation and biological sampling are not possible? Perhaps not; in this article we discuss applications of recently introduced genetic techniques for the analysis of feces (molecular scatology) when studying free-ranging mammal populations. These techniques allow for the purification of DNA from feces, which is then followed by amplification of specific DNA sequences using the polymerase chain reaction (PCR). An array of genetic markers with different properties is available[9–14] and can be used to address taxonomic questions, individual identification through 'genetic fingerprinting', and sex determination with gender specific markers (Box 1). Moreover, specific sequences from other passengers through the gut such as food and pathogens can be determined by using PCR primers specific for their amplification. Consequently, molecular techniques applied to feces allow the identification of animal species, individuals, their sex, pathogens and food habits. Furthermore, comprehensive sampling of an area for feces, or systematic fecal transects of populations, may allow estimates of home range, reproductive patterns, kinship structure and population size. This information may be augmented by techniques for analysis of feces that do not use DNA[1,15–25] (Box 2). Analysis of feces will potentially provide a means to study genetics, life history and population dynamics of mammals.

A history of molecular scatology

PCR can produce large quantities of specific DNA sequences from small, degraded and impure samples[26]. Stimulated by this property of PCR, early attempts of isolation and amplification of genes from fecal material were aimed at developing new tools for clinical diagnosis[27]. Similar techniques allowed parasitological studies of domestic and wild populations; for example, the amplification of DNA from tapeworm (*Echinococcus multilocularis*) in feces of red foxes (*Vulpes vulpes*) showed this parasite had a high prevalence[28].

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 Brenta mountains of northern Italy[29–31]. 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 Brenta 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 Brenta 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[10] (mtDNA) is a maternally inherited, haploid, non-recombining and extra-nuclear genome. Each cell has multiple mtDNA copies (range 10–2500 copies) and thus mtDNA sequences are more easily amplified than single copy, nuclear genes. Because the mtDNA cytochrome *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, phylogeography, assignment to management units, and forensics. Chloroplast DNA[13] (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[9,11] [also referred to as simple sequence length polymorphism (SSLP), or simple sequence repeats] 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[9,11]. Microsatellites identified in one species can usually be employed to study a related species[14].

PCR primers for the *SRY* gene on the Y chromosome can be used to distinguish male from female DNA[12].

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 taxon, genomic locus or sequence of interest.

 Copyright © 1997, Elsevier Science Ltd. All rights reserved. 0169-5347/97/$17.00 PII: S0169-5347(97)01050-1

Box 2. Conventional studies on feces

Mammal species can often be identified based on their feces, allowing studies of species composition[1]. However, without additional information feces are assigned correctly in only about 50–66% of the cases[15]. 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[15]. Relative abundance and population size have been estimated from fecal counts[1,16]. However, the resampling of individuals constitutes a problem. Census data based on feces can be supplemented by techniques described elsewhere[1,16,17].

Habitat use and range size may be investigated by examining spatial variation in feces abundance. Similarly, territory boundaries may be marked by fecal aggregations[1]. These studies often require individuals to ingest colored plastic markers or radioactive bait, which uniquely mark their feces.

Analysis of food habits and dietary quality have frequently used feces[1,17]. In carnivores, undigested prey items can be recovered and identified. Herbivore dung often contains plant remains. In general, this approach has shortcomings[1,15,17,18]. For herbivores, indices of dietary quality can be derived through chemical analysis of fecal nitrogen (FN) and dietary nitrogen (DN)[1], although the interpretation of results is controversial[19,20].

Reproductive status (pregnancy, ovulation, luteal function) and physiological stress (adrenocortical activation) can be derived from fecal steroid hormone measures[21]. Moreover, fecal steroid analysis provides information on sex and age of animals[22].

The physical, microbiological and chemical examination of feces also provides information on parasite infestation[23], bacterial flora[24] and environmental contamination[25] (PCBs and pesticides).

on feces to identify individual bears[30]. 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 *rbc*L gene from bear feces. Fecal plant sequences were identified as belonging to the hawthorn genus *Photinia*, thought 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*)[30,31] and dugong (*Dugong dugong*)[32]. 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*)[33], bonobos (*Pan paniscus*)[34], brown bears (*Ursus arctos*)[35] and pinnipeds[45]. Although some technical problems were encountered (Box 3), these studies present convincing evidence that such problems can be overcome[27–36] (see also Refs 26,37,38), and feces may some day 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 analysis[3,26]. However, these sources 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 paternity and to document relatedness in natural populations[8,9,11,39,40] and have been amplified from feces[33–35]. However, depending on polymorphism and levels of heterozygosity of microsatellite loci, about 20 microsatellite loci may be needed to determine paternity and kinship and to distinguish relatives from non-relatives. This estimate is based on a study in 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 or full-sib pairs with better than 80% accuracy[39] (see also Ref. 40).

Importantly, the number of loci to be scored increases as population heterozygosity decreases[39], which may impair our ability to determine kinship reliably in small and inbred 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 difficulty 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 analysis, sometimes very few loci suffice to exclude most of the males of a troop of primates, especially when this troop has been extensively observed[8,10].

Census population size

A common method to estimate population size involves extrapolation from animal counts on designated transects[16]. 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

Box 3. Technical notes on molecular scatology

Inhibition

Feces contain cells shed from the gut and a complex mixture of other compounds (microorganisms, undigested food, digestive enzymes, mucus, bile salts and bilirubin)[27]. In addition, herbivore feces contain plant polysaccharides[36]. Initial studies encountered inhibition of the *Taq* polymerase during PCR by substances in the extract[29–31,34]. Results were improved when extraction methods relying on DNA adsorbing beads were employed[29–31,45]. Furthermore, addition of bovine serum albumin (BSA), cellulose or other substances to DNA extraction and PCR circumvented inhibition[29,30,36]. Hexadecyltrimethyl-ammonium bromide (CTAB) is commonly employed to remove plant polysaccharides from DNA extracts.

Inconsistency

Preliminary data show that DNA in feces is present in low copy numbers and is degraded[30,34]. Amplification of short DNA segments resulted in more consistent amplifications than longer fragments[30]. Of special concern in the amplification of microsatellites is the mistyping of individuals owing to PCR artifacts and/or stochastic events when pipetting dilute and degraded DNA template[34,35,37]. Consequently, multiple extractions and PCR amplifications from fecal samples should be done[30,34,35,37].

Specificity

It is important to select and test carefully PCR primers and to optimize PCR conditions[26,38]. Specificity is increased when species-specific primers (designed from published sequences) are used[38]. Nonetheless, PCR products should be sequenced to verify authenticity of results[30,31]. When amplifying microsatellites from feces, non-specific products often obscure results. Southern blotting[36] or hot-start PCR conditions[38] are useful to circumvent this problem.

Contamination

During DNA extraction and PCR setup, contamination is a potential source of error[26]. Thus, with degraded or dilute DNA, a separate facility dedicated to DNA extraction and PCR setup should be used as well as inclusion of blank extractions and PCR blanks in all steps of the experiment[26,30].

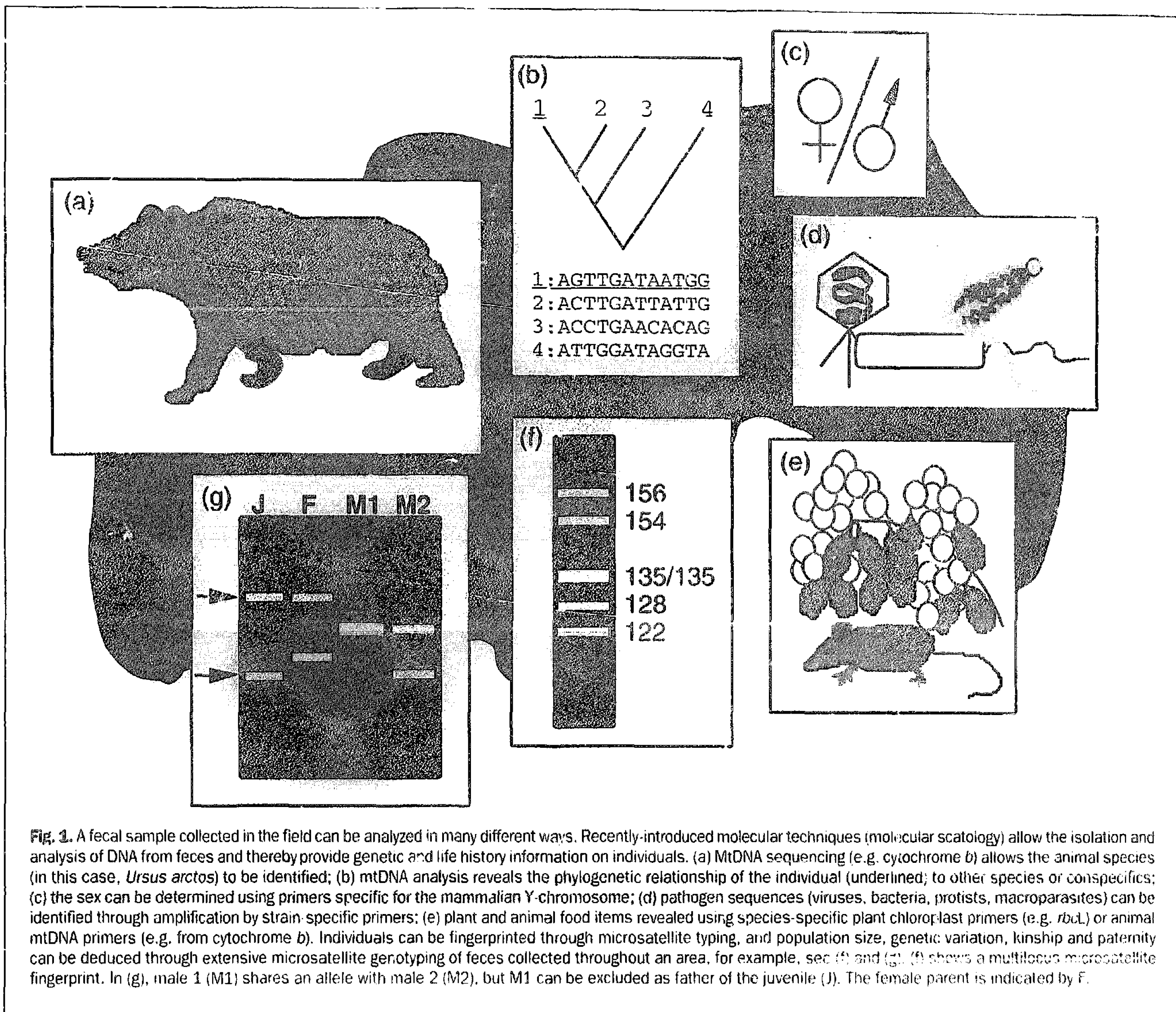

Fig. 1. A fecal sample collected in the field can be analyzed in many different ways. Recently-introduced molecular techniques (molecular scatology) allow the isolation and analysis of DNA from feces and thereby provide genetic and life history information on individuals. (a) MtDNA sequencing (e.g. cytochrome *b*) allows the animal species (in this case, *Ursus arctos*) to be identified; (b) mtDNA analysis reveals the phylogenetic relationship of the individual (underlined; to other species or conspecifics; (c) the sex can be determined using primers specific for the mammalian Y-chromosome; (d) pathogen sequences (viruses, bacteria, protists, macroparasites) can be identified through amplification by strain-specific primers; (e) plant and animal food items revealed using species-specific plant chloroplast primers (e.g. *rbcL*) or animal mtDNA primers (e.g. from cytochrome *b*). Individuals can be fingerprinted through microsatellite typing, and population size, genetic variation, kinship and paternity can be deduced through extensive microsatellite genotyping of feces collected throughout an area, for example, see (f) and (g). (f) shows a multilocus microsatellite fingerprint. In (g), male 1 (M1) shares an allele with male 2 (M2), but M1 can be excluded as father of the juvenile (J). The female parent is indicated by F.

such as roads or territorial boundaries[1,16]. 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[1]. 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[29].

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[42,43]. 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

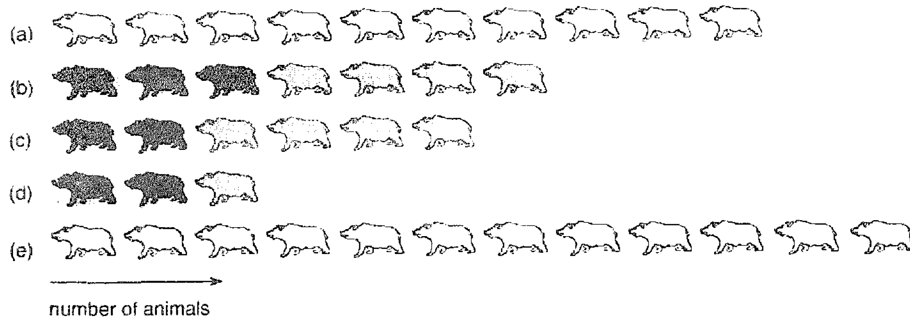

Fig. 2. The actual number of individuals in a population is often much greater than the genetically effective population size, N_e. Analysis of fecal DNA in conjunction with fecal steroid hormone analysis may allow the estimation of the number of reproducing individuals, which is related to N_e. This is illustrated using a hypothetical population of 10 bears: (a) individuals are counted by collection of feces followed by analysis using microsatellite loci; (b) the number of adults, and their respective sex (females, black; males, grey) is determined by hormone analysis and with Y-chromosome markers; (c) based on their estradiol and progesterone levels some of the adult animals are not reproductive; (d) paternity analysis using microsatellite loci find that only a few bears actually reproduce; and (e) the population one generation later.

over time (e.g. Refs 10,42–44). Molecular genetic analysis of feces can provide estimates of genetic variation in mitochondrial and nuclear genes and allows for the continued monitoring of populations without handling of animals. Moreover, an indirect method of estimating effective population size, the variance effective population size, involves determining the change in genetic heterozygosity over time[43]. Thus, data on temporal changes in heterozygosity based on programs of fecal sampling over time may provide a genetic estimate of population size to compare with those based on demographic factors.

Phylogeography

Two general considerations in population studies are the relationships of populations to each other and the degree of gene flow that occurs between them[8]. Recently, two DNA-based tools have been used to estimate gene flow and population relationships. The first involves mitochondrial DNA control-region sequences[10], and the second involves the analysis of microsatellite loci[9]. Both control-region sequence and microsatellite variation can be obtained from PCR analysis of feces[29–35]. A population genetic perspective on variation is important for conservation if historical patterns of gene flow are to be maintained or restored. Similarly, for populations that are becoming rare and inbred or that have gone extinct, a closely related source population can be identified by comparison of genetic markers.

Diet and disease

Diet and parasite infestation can be studied by conventional analyses of feces[1,15–19,23,24]. However, molecular techniques may increase the resolution of the analysis by allowing more precise identification of food or pathogen species. Diet can be approached through the use of plant or animal species-specific PCR primers. The amplification of sequences from food species gives proof of their consumption. However, the absence of amplification does not indicate that they have not been eaten, because the PCR may have failed for a variety of reasons (Box 3). Moreover, temporal sequences of fecal samples should be considered so that foods infrequently eaten are not missed. Importantly, the abundance of foods in feces cannot be quantified easily by DNA analysis; conventional identification of foods by inspection might be better for this purpose. In some species, where foods are too thoroughly digested for recognition or where food species cannot be diagnosed based on remains, PCR 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.

Acknowledgements

Funding to M.H.K. was provided by an academic senate grant, and non-resident tuition fellowship from the University of California, Los Angeles, Department of Biology. We are indebted to D. Tautz and U. Gerloff for pointing out references 27 and 37. For discussion of the manuscript we thank K.P. Koepfli and G. Roemer. Comments on the manuscript were provided by E. Hadly, F. Hertel, J. Maldonado and S. West.

References

1 Putman, R.J. (1984) **Facts from faeces,** *Mammal Rev.* 14, 79–97
2 Bowen, B.W. and Avise, J.C. (1994) **Conservation research and the legal status of PCR products,** *Science* 266, 713
3 Morin, P.A. and Woodruff, D.S. (1996) **Noninvasive genotyping for vertebrate conservation,** in *Molecular Genetic Approaches in Conservation* (Smith, T.B. and Wayne, R.K., eds), pp. 298–313, Oxford University Press
4 Morell, V. (1995) **Dogfight erupts over animal studies in the Serengeti,** *Science* 270, 1302–1303
5 Harper, J.L. (1995) **Editorial note,** *Proc. R. Soc. London Ser. B* 262, 247
6 Stevens, J. (1992) **Fighting the rules of the game,** *Nature* 355, 489
7 Harrison, S. *et al.* (1991) **Estimating the effects of scientific study on 2 butterfly populations,** *Am. Nat.* 137, 227–243
8 Morin, P.A. *et al.* (1994) **Kin selection, social structure, gene flow, and the evolution of chimpanzees,** *Science* 265, 1193–1201
9 Bruford, M.W. and Wayne, R.K. (1993) **Microsatellites and their application to population genetic studies,** *Curr. Biol.* 3, 939–943
10 Moritz, C. (1994) **Applications of mitochondrial DNA in conservation: a critical review,** *Mol. Ecol.* 3, 401–411
11 Queller, D.C., Strassmann, J.E. and Hughes, C.R. (1993) **Microsatellites and kinship,** *Trends Ecol. Evol.* 8, 285–288
12 Griffiths, R. and Tiwari, B. (1993) **Primers for the differential amplification of the sex-determining region Y gene in a range of mammal species,** *Mol. Ecol.* 2, 405–406
13 Cullings, K.W. (1992) **Design and testing of a plant specific PCR primer for ecological and evolutionary studies,** *Mol. Ecol.* 1, 233–240

14 O'Brien. S.J. *et al.* (1993) **Anchored reference loci for comparative genome mapping in mammals,** *Nat. Genet.* 3, 103–117

15 Halfpenny, J. and Biesot. E. (1986) *A Field Guide to Mammal Tracking in North America*, Johnson Books

16 Sutherland, W.J., ed. (1996) *Ecological Census Techniques, a Handbook*, Cambridge University Press

17 Mills, M.G.L. (1996) **Methological advances in capture, census, and food-habits studies of large African carnivores**, in *Carnivore Behavior, Ecology, and Evolution* (Vol. 2) (Gittleman, J.L., ed.), pp. 223–242, Cornell University Press

18 Carss, D.N. and Parkinson, S.G. (1996) **Errors associated with otter (*Lutra lutra*) fecal analysis. 1. Assessing general diet from spraints,** *J. Zool.* 238, 301–317

19 Hobbs, N.T. (1987) **Fecal indices to dietary quality: a critique,** *J. Wildl. Manage.* 51, 317–320

20 Leslie, D.M. and Starkey, E.E. (1987) **Fecal indices to dietary quality: a reply,** *J. Wildl. Manage.* 51, 321–325

21 Wasser, S.K., Monfort, S.L. and Wildt, D.E. (1991) **Rapid extraction of faecal steroids for measuring reproductive cyclicity and early pregnancy in free-ranging yellow baboons (*Papio cynocephalus cynocephalus*),** *J. Reprod. Fert.* 92, 415–423

22 Varley, J. (1991) **Monitoring ovarian function in captive and free-ranging wildlife by means of urinary and fecal steroids,** *J. Zoo Wildl. Med.* 22, 23–31

23 Phillips, M.K. and Scheck, J. (1991) **Parasitism in captive and reintroduced red wolves,** *J. Wildl. Disease* 27, 498–501

24 Hirayama, K. *et al.* (1989) **The fecal flora of the giant panda,** *J. Appl. Bacteriol.* 67, 411–415

25 Mason, C.F. and Ratford, J.R. (1994) **PCB congeners in tissues of European otter (*Lutra lutra*),** *Bull. Environ. Contam. Toxicol.* 53, 548–554

26 Arnheim, N., White, T. and Rainey, W.E. (1990) **Application of PCR: organismal and population biology,** *BioScience* 40, 174–182

27 Sidransky, D. *et al.* (1992) **Identification of *ras* mutations in the stool of patients with curable colorectable tumors,** *Science* 256, 102–105

28 Bretagne, S. *et al.* (1993) **Detection of *Echinococcus multicolaris* DNA in fox feces,** *Parasitology* 106, 193–199

29 Hoss, M. *et al.* (1992) **Excrement analysis by PCR,** *Nature* 359, 199

30 Kohn, M. *et al.* (1995) **Conservation genetics of the European brown bear – a study using excremental PCR of nuclear and mitochondrial sequences,** *Mol. Ecol.* 4, 95–103

31 Kohn, M.H. and Knauer, F. **Phylogeography of brown bears in Europe and excremental PCR – the new tool in the genetic analysis of animals in the wild,** *Int. Conf. Bear Res. Manage.* (in press)

32 Tikel, D., Blair, D. and Marsh, H.D. (1996) **Marine mammal feces as a source of DNA,** *Mol. Ecol.* 5, 456–457

33 Constable, J.J. *et al.* (1995) **Nuclear DNA from primate dung,** *Nature* 373, 393

34 Gerloff, U. *et al.* (1995) **Amplification of hypervariable simple sequence repeats (microsatellites) from excremental DNA of wild living bonobos (*Pan paniscus*),** *Mol. Ecol.* 4, 515–518

35 Taberlet, P. *et al.* (1996) **Reliable genotyping of samples with very low DNA quantities using PCR,** *Nucleic Acids Res.* 24, 3189–3194

36 Deuter, R. *et al.* (1995) **A method for preparation of fecal DNA suitable for PCR,** *Nucleic Acids Res.* 23, 3800–3801

37 Foulcault, F. *et al.* (1996) **Experimental limits of PCR analysis of $(CA)_n$ repeat alterations,** *Trends Genet.* 12, 450–451

38 Roux, K.H. (1995) **Optimization and troubleshooting in PCR,** *PCR Methods Appl.* 4, S185–S194

39 Blouin, M.S. *et al.* (1996) **Use of microsatellite loci to classify individuals by relatedness,** *Mol. Ecol.* 5, 393–401

40 Altmann, J. *et al.* (1996) **Behavior predicts genetic structure in a wild primate group,** *Proc. Natl. Acad. Sci. U. S. A.* 93, 5797–5801

41 White, G.C. and Garrott, R.A. (1990) *Analysis of Wildlife Radio-Tracking Data*, Academic Press

42 Nunney, L. and Campbell, K.A. (1993) **Assessing minimum viable population size: demography meets population genetics,** *Trends Ecol. Evol.* 8, 234–239

43 Harris, R.B. and Allendorf, F.W. (1989) **Genetically effective population size of large mammals: an assessment of estimators,** *Conserv. Biol.* 3, 181–191

44 O'Brien, S.J. and Evermann, J.F. (1988) **Interactive influence of infectious disease and genetic diversity in natural populations,** *Trends Ecol. Evol.* 3, 254–259

45 Reed, J.Z. *et al.* (1997) **Molecular scatology: the use of molecular genetic analysis to assign species, sex, and individual identity to seal faeces,** *Mol. Ecol.* 6, 225–234

Current trends

– articles of ecological or evolutionary interest in recent issues of other *Trends* magazines

- The evolution of ribonucleotide reduction *P. Riechard*, **Trends in Biochemical Sciences** 22, 81–85 (March 1997)
- Genetics of sex determination in flowering plants, *S. Lebel-Hardenack* and *S.R. Grant*, **Trends in Plant Science** 2, 130–136 (April 1997)
- Chromosomal duplication and the emergence of the adaptive immune system, *M. Kasahrar* et al., **Trends in Genetics** 13, 90–92 (March 1997)
- Worms as an evolutionary model, *S.W. Emmons*, **Trends in Genetics** 13, 131–134
- Evolution in fast forward: haplochromine fishes of the Lake Victoria region, *L.S. Kaufman* et al., **Endeavour** 21, 23–29 (March 1997)
- Evolutionary tuning knobs, *D.G. King* et al., **Endeavour** 21, 36–40 (March 1997)
- Canine distemper virus – a morbillivirus in search of new hosts? *T.C. Harder* and *A.D.M.E. Osterhaus*, **Trends in Microbiology** 5, 120–124 (March 1997)
- *Hox* genes in evolution: protein surfaces and paralog groups, *M. Sharkey* et al., **Trends in Genetics** 13, 145–151