# Excrement analysis by PCR

SIR — Samples from endangered animals are hard to obtain for genetic analysis. To study a threatened bear population in the Pyrenees, Taberlet and Bouvet[1] used hair collected from wire netting attached to trees on which the bears scratch themselves. We have approached the problem by the use of bear droppings, which can be collected without disturbing the animals and can be used to amplify DNA sequences not only from the animals themselves but also from the foods they have ingested.

We are studying a population of European brown bears in the Brenta region of Northern Italy. Like the Pyrenean bears, this population is in decline and today may number less than 10 individuals. The introduction of animals from the large populations in the Balkans is contemplated. To evaluate the extent of genetic variation in the Brenta bears as well as their genetic relationship to Romanian bears, we looked at the sequence variation in mitochondrial DNA from the Italian bears. Nucleic acids were extracted from excremental remains, presumed from their morphology to be of bear origin. All extracts contained large amounts of bacterial DNA.

Two oligonucleotide primers were designed to span an 88-base-pair-long segment of the mitochondrial control region which has been sequenced in the American brown bear[2]. Using these primers in the polymerase chain reaction (PCR), we were able to amplify mitochondrial DNA from three droppings (Fig. 1*a*). Sequences from the Brenta droppings as well as from a liver sample of a Romanian bear are shown in Fig. 1*b*. The European bears differ at seven positions from the American brown bear. The three Brenta DNA samples are identical to each other and differ at three positions from the Romanian bear sample. Further work will clarify whether this indicates that the Brenta population is genetically depauperate compared with other bear populations. Statistical analysis will have to take into account that the samples are being drawn with replacement, because individual animals may be sampled several times.

We also used primers specific for the chloroplast *rbc*L gene to investigate whether nucleic acids of plants ingested by the bears can pass through the digestive tract. As can be seen in Fig. 1*a*, all three bear droppings produced bands. The direct sequencing of the amplification products showed that they contain one *rbc*L sequence. When this sequence was compared with 414 *rbc*L sequences (M. W. Chase, personal communication), it turned out to be identical to *Photinia*, a genus in the *Rosaceae*. A representative of this genus, *P. villosa*, is found in the Brenta region. Consequently, the fruits of this plant seem to be a dominant component of the bears' diet during late summer when the droppings were collected. Thus the feeding behaviour of animals can be investigated after amplification by PCR of DNA from their excrement. The collection of droppings may also be a useful way to screen large areas for the presence of rare animals.

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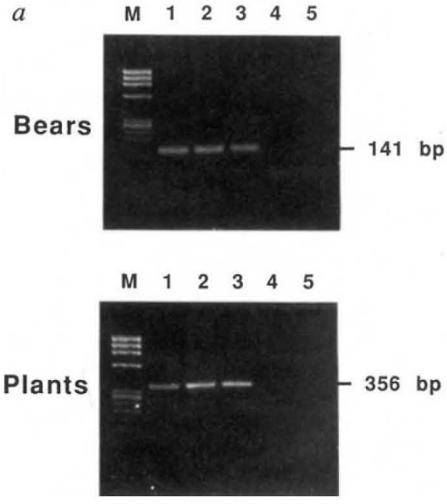


*a*, PCR amplifications of bear mitochondrial DNA from three droppings. Lane M, size marker (ΦX174 DNA digested with the restriction enzyme *Hae*III); lanes 1–3, three droppings from the Brenta population; lanes 4 and 5, extraction and PCR controls, respectively. Upper panel, amplification of a 141-base-pair (bp) fragment (including primers) from the mitochondrial control region. Lower panel, amplification of a 356-bp fragment (including primers) of the chloroplast *rbc*L gene. *b*, Nucleotide sequences of the 88-bp mitochondrial fragment from three individuals of the Brenta population (Brenta 1, 2 and 3). Sequences are compared with the American brown bear[2] and a brown bear from Romania. Droppings (0.5 g) were extracted as described in ref. 3, except that the phenol/chloroform and concentration steps were replaced by silica purification[4]. For PCR, we used 25-μl reaction volumes containing BSA (2 μg $mg^{-1}$) to overcome inhibition and 10 μl (10%) extract. Each of 40 PCR cycles consisted of denaturation at 92 °C for 40 s, annealing at 55 °C for 60 s and extension at 72 °C for 60 s. Primers for the mitochondrial fragment: 5′-CGTGCATTAATGGCGTGCCCCATGCAT-3′ and 5′-TGGTGATCAAGCTCCCGGACTAAGTG-3′, and for the *rbc*L fragment: 5′-ATGTCACCACAAACAGAAACTAAAGCAAGT-3′ and 5′-CCAAGACAATGATTGAACAATACTTC-3′. Amplified DNA was electrophoresed on 2.8% low-melting agarose, the bands were cut out and melted in 100 μl water and then reamplified in 50 μl using the same conditions, except for a 60 °C annealing temperature. The products of this second amplification were purified using GeneClean (Dianova) and directly sequenced[5].

*b*

| | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 |
|---|---|---|---|---|---|---|---|---|---|
| America | ATAAGCATGT | ACATACTGTG | CTTGATCTTG | CATGAGGACC | TACGTTCTGA | AAGTTTATTT | CAGGCGTATA | GTCTGCAAGC | ATGTATTT |
| Romania | ..... | .....T.... | .....G...A | .....G...T | .....C.... | .....G.... | ..... | .....T.... | ..... |
| Brenta 1 | ..... | .....T.... | .....G.T..A | ..... | .....C.... | .....G.... | .....T.... | .....T.... | ..... |
| Brenta 2 | ..... | .....T.... | .....G.T..A | ..... | .....C.... | .....G.... | .....T.... | .....T.... | ..... |
| Brenta 3 | ..... | .....T.... | .....G.T..A | ..... | .....C.... | .....G.... | .....T.... | .....T.... | ..... |

# Evidence for some hot dark matter?

SIR — The recent detection of the large scale microwave background anisotropy by the cosmic background explorer (COBE) satellite is in remarkable agreement with the predictions based on an inflationary scheme in which the dark matter is a mixture of cold and hot components. We briefly summarize how the COBE measurements, inflation, and the observed large-scale structure all seem to point to a flat Universe in which a fraction (~20–30 per cent) of the critical energy density resides in massive (~3–9 eV) relic neutrinos. A crucial test of this scheme comes from the anisotropies expected on smaller (~1°–2°) angular scales, which are estimated to lie within reach of current and planned experiments.

A generic prediction of inflation is that the Universe contains a critical density of matter. Primordial nucleosynthesis arguments imply that <90% of this matter is nonbaryonic, but inflation does not specify what the dark matter should be. Typically, investigators have assumed the dark matter takes one of two possible forms: cold dark matter (CDM; massive cold particles) or hot dark matter (HDM; relic light neutrinos). Although pure HDM has several well-discussed problems, models with pure CDM provide a basis for structure