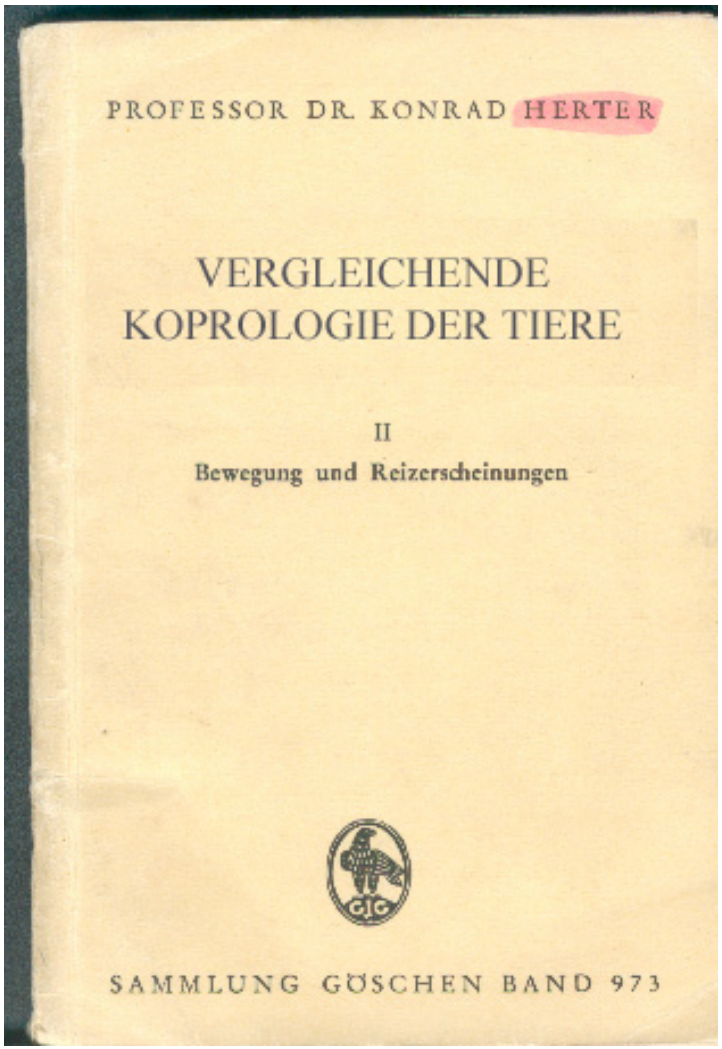
Viikon genetiikkateot

Genetiikka-alan ällistyttävimpiä
suorituksia
peruskurssin mausteeksi

Molekyylikoprologiaa



Skatologia = koprologia

skatologi, koprologi

skatonomi, kopronomi

koproskopia, koproskooppi

koprografi, koprogrammi

koprofilia, kopromania

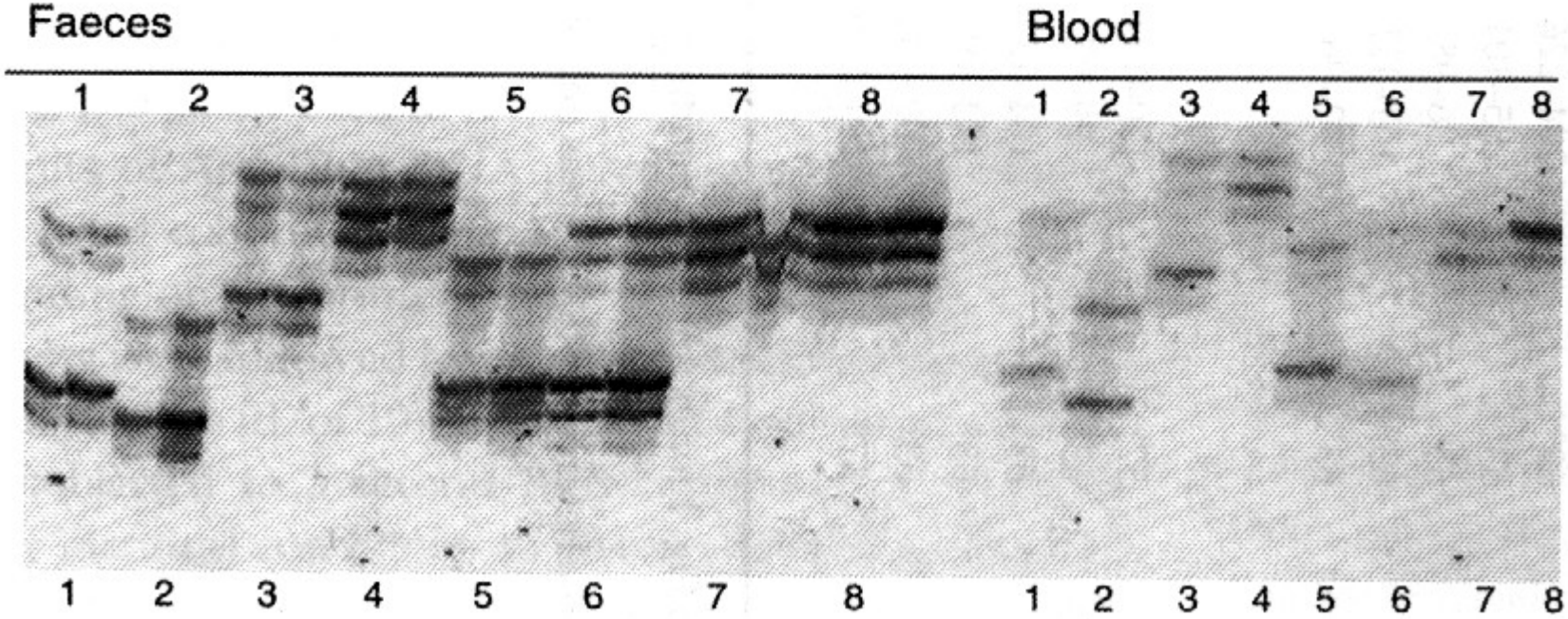
koprofagia, koprovoria

koprolalia

Flagstad, Roed, Stacy & Jakobsen (1999) Reliable noninvasive genotyping based on excremental PCR of nuclear DNA purified with a magnetic bead protocol. -Molecular Ecology 8: 879-883

Norjalaiset tutkijat keräsivät tuoreita lampaan- ja poronpapanoita ja antoivat niiden kuivua aamuun asti! Aamulla he pesivät papanoiden pinnasta hellävaroen jotakin ihan pientä ja eristivät siitä DNA:n. Sitten he osoittivat pystyvänsä tunnistamaan *mikrosatelliittiDNA*:n avulla yksilön tarkuudella, kuka laitumen lampaista tai poroista oli kunkin papanan kakkinut!

Sheep - SMHCC



Reed, Tollit, Thompson & Amos (1997) Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. -Molecular Ecology 6: 225-234

Hylkeet ja kaupallinen kalastus ja kalankasvatus ovat vihamielisiä. Nämä Cambridgen ja Aberdeenin tutkijat kehittivät menetelmän, jolla hylkeenpaskoista (harmaahylje ja kiehkuraishylje) saatiin määritettyä yllä mainitut asiat: laji, sukupuoli ja yksilökin.

Ulosteesta saadaan myöhemmin selville sekin, mitä se hylje on syönyt. Ehkäpä voidaan osoittaa joku yksilö syyttömäksi!

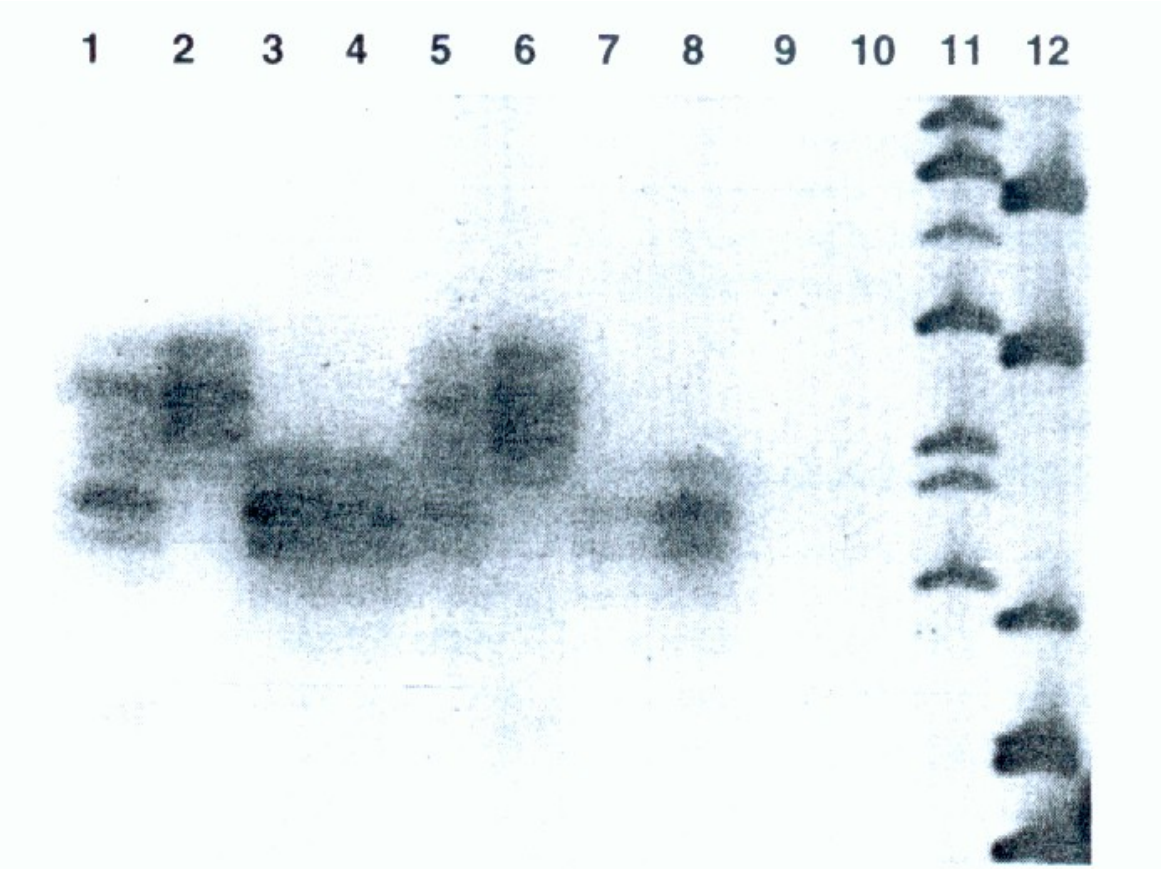


Fig. 2 Example of PCR amplifications from the scat–blood paired control samples. Lanes 1–4, PCR products corresponding to four individual blood samples; lanes 5–8, PCR products corresponding to the four matched scat samples; lanes 9–10 negative controls for extraction (amplification of an extract processed at the sample time but without scat sample) and PCR amplification (without template); lanes 11–12 marker sequence M13.

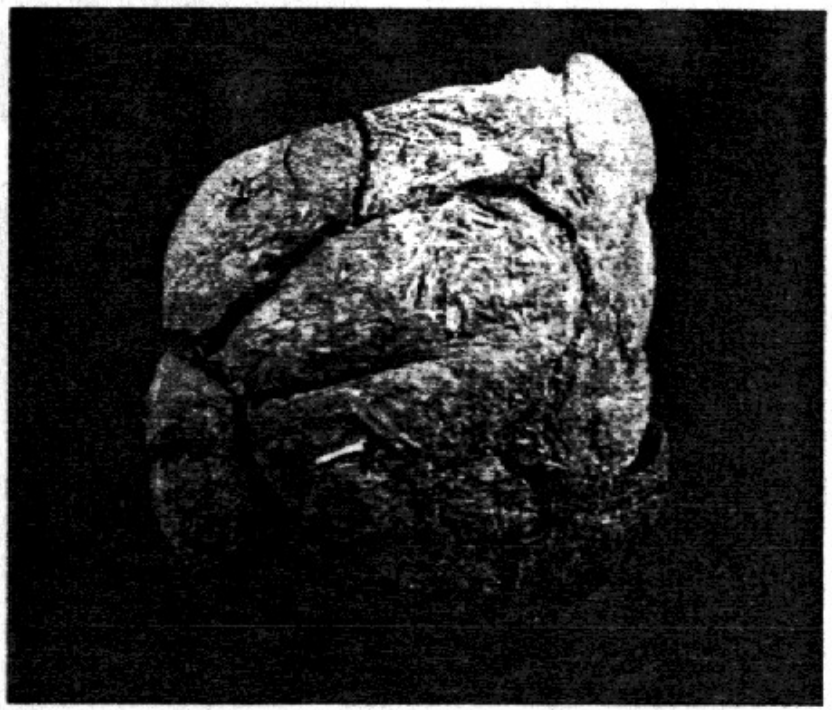
1,5 veri/ kakka
2,6
3,7
4,8

9,10 negat.kontr.

11, 12 molekyyli-painostandardit

Poinar, Hofreiter, Spaulding, Martin, Stankiewicz, Bland, Evershed, Possnert & Pääbo (1998) Molecular coproscopy: Dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. - Science 281: 402-406

Monikansallinen huippuryhmä iski kätensä **19 875** vuotta vanhaan jättiläislaiskiaisen koproon! Tämän laiskiaisen fylogeneettinen sukulaisuus nykyisten ja yhden toisenkin sukupuuttoon kuol-leen lajin kanssa selvitettiin *DNA*:n avulla.



Data dump. This 10-centimeter fossil dropping preserves DNA from the sloth's diet of greens.

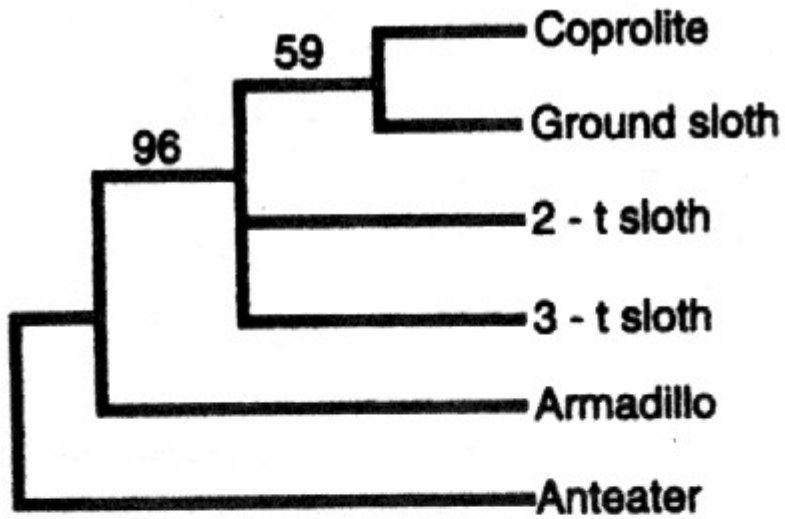


Fig. 3. Phylogenetic reconstruction of 12*S* rDNA sequences from the coprolite and representatives of modern edentates—an anteater (*Tamandua tetradactyla*), armadillo (*Cabassous unicinctus*), two-toed sloth (*Choloepus didactylus*), and three-toed sloth (*Bradypus variegatus*)—and from the extinct ground sloth *Mylodon darwinii*. The anteater is used as an outgroup. Numbers on internal branches refer to quartet puzzling support values (*21*).

Ko. kakka

Mylodon darwini

2-varpainen laiskiainen

3-varvaslaiskiainen

vyötiäinen

muurahaiskarhu

Kaliforniassa San Joaquin -laaksossa elelee tällainen söpö pieni kettu, endeeminen kit fox (*Vulpes macrotis mutica*)

Paxinos, McIntosh, Ralls & Fleischer (1997) A noninvasive method for distinguishing among canid species: amplification and enzyme restriction of DNA from dung. -Molecular Ecology 6: 483-486

Uhanalainen *Vulpes macrotis mutica*, kalifornialainen kettu joutuu elelemään punaketun, harmaaketun, kojootin ja koirien asuttamilla seuduilla. Kakkaläjät ovat samanlaisia, mutta tutkijat haluaisivat saada selville, mikä läjä on tämän uhanalaisen. Monistamalla 412 bp *mitokondrion sytokromi*-b *geeniä* kakoista ja digestoimalla se sitten restriktioentsyymeillä voitiin laji varmuudella tunnistaa!

Metodien nimi on **PCR** (polymerase chain reaction) ja **RFLP** (restriction fragment length polymorphism)

M = molekyylipainotikkaat

C = kojootti
D = koira
G = harmaa kettu
K = se kit fox, joka haluttiin
R = punakettu

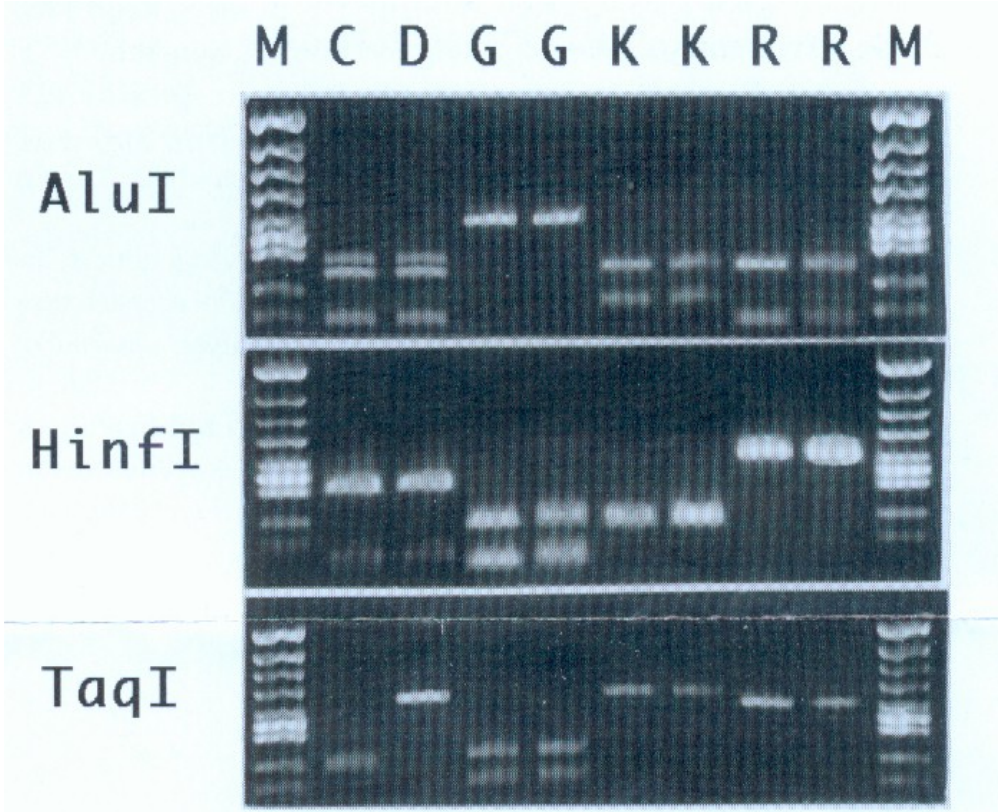


Fig. 2 Restriction enzyme digested cytochrome-*b* amplification products of five canid species electrophoresed in a 2.0% agarose gel: coyote (C), domestic dog (i.e. greyhound; D), gray fox (G), kit fox (K), and red fox (R). DNA size marker (M) is *Hinc*II-digested ϕx 174 [fragment sizes from the top are 1057 bp, 770 bp, 612 bp, 495 bp, 392 bp (345–341–335 bp combined) (297–291 bp combined), 210 bp, and 162 bp]. (a) *Alu*I digests. (b) *Hin*fI digests. (c) *Taq*I digests. The second lane of each fox species is a digestion of a product amplified from scat. Coyote and domestic dog had identical patterns for *Alu*I.

Canine Assistants for Conservationists

ALTHOUGH SOME FORENSIC USES OF SCENT-following dogs may be questionable, as Brisbin, Austad, and Jacobson point out in their letter "Canine detectives: the nose knows—or does it?" (10 Nov., p. 1093), the scent discrimination abilities of canines are still impressive. Controlled behavioral tests indicate that dogs can distinguish the odors from different species of animals, male and female dogs, and even different individuals within a species (*1*). These abilities are proving invaluable in conservation programs for endangered and difficult-to-distinguish species (*2*), particularly when combined with molecular techniques for the analysis of DNA extracted from the sloughed intestinal cells contained in feces (*3*).

Conservationists might have a new way to identify the calling cards of endangered species such as this San Joaquin kit fox.

We have obtained molecular genetic confirmation of the ability of trained scenting dogs to distinguish among the scats (feces) of sympatric canid species. We trained dogs (*4*) to detect scats of the endangered San Joaquin kit fox (*Vulpes macrotis mutica*) and ignore those of coyotes (*Canis latrans*). One German shepherd recovered 435 presumed kit fox scats along 140 kilometers of transects in the Carrizo Plain Natural Area, California, in 16 days. We were able to isolate DNA from 329 of the scats. Mitochondrial DNA tests developed in the National Zoological Park's Molecular Genetics Laboratory (*5*) revealed that all 329 scats were indeed from kit foxes. Thus, the dog was 100% accurate in identifying kit fox scats in the presence of coyote, skunk (*Mephitis mephitis*), and badger (*Taxidea taxus*) scats along the transects. Species identification based on mitochondrial DNA (*5*) costs about $40 per sample. Hence, use of scent-detection dogs to distinguish scats from species of interest could provide a cost-effective alternative to laboratory methods in some conservation applications.

Fecal DNA analysis is potentially a powerful method for identifying species, population size, sex ratio, home range, paternity, and kinship (*6*). However, finding enough samples for this approach is difficult and time-consuming in many habitats, such as dense vegetation. Along transects through vegetation, our trained dog found about four times as many kit fox scats as an experienced person searching for scats visually. Thus, trained scent dogs can greatly increase the utility of DNA techniques in conservation and might be the only way to obtain information on the presence or absence of some endangered species around the world.

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References and Notes

1. H. Shiroma and H. Ukuta, in *Problem Snake Management: The Habu and the Brown Treesnake*, G. H. Rodda *et al.*, Eds. (Cornell Univ. Press, Ithaca, NY, 1999), pp. 348–352; B. J. LeBoeuf, *Behavior* **29**, 268 (1967); V. I. Krutova *et al.*, *Sens. Syst.* **12**, 211 (1998).
2. Dogs have been used successfully to find feces of grizzly bears (*Ursus arctos horribilis*) (S. Wasser, personal communication), black bears (*Ursus americanus*) (S. Wasser, personal communication; P. Paquet, personal communication), wolves (*Canis lupus*), and coyotes (P. Paquet, personal communication).
3. M. H. Kohn and R. K. Wayne, *Trends Ecol. Evol.* **12**, 223 (1997).
4. Our training methods were similar to those used when training dogs to detect narcotics.
5. E. Paxinos *et al.*, *Mol. Ecol.* **6**, 483 (1997).
6. M. H. Kohn *et al.*, *Proc. R. Soc. Lond. Ser. B.* **266**, 1429 (1999); H. B. Ernest *et al.*, *Mol. Ecol.* **9**, 433 (2000).

Vielä ihmeellisempää seuraa!

Kun läjät oli opittu tunnistamaan DNA:avulla, $40 kappale, opetettiin koira tunnistamaan ne.

Yksi shäfer löysi 435 kitfoxin läjää 140 kilometrin matkalta 16 päivässä, tekemättä yhtään virhettä! Tarjolla oli myös kojootin, skunkin ja mäyrän läjiä.

Koirahan tietysti osaa erottaa läjät opettamatta, mutta koiranohjaajaa pitää vähän opettaa!

Mahtikopro (karhun), jonka voisi yksilöidä