SMALL CARNIVORE CONSERVATION

The Newsletter and Journal of the IUCN/SSC Mustelid, Viverrid & Procyonid Specialist Group

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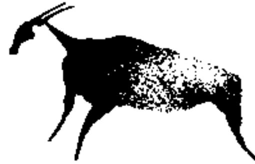

SPECIES SURVIVAL COMMISSION

Malagasy civet *(Fossa fossana)* in the Ranomafana National Park, Madagascar - Photo: Mario Perschke

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The aim of this publication is to offer the members of the IUCN/SSC MV&PSG, and those who are concerned with mustelids, viverrids, and procyonids, brief papers, news items, abstracts, and titles of recent literature. All readers are invited to send material to:

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Mongooses in the Tsimbazaza Zoo and the Ranomafana National Park, Madagascar

Mario PERSCHKE

In September 1995, two Malagasy broad-striped mongooses (*Galidictis fasciata*) came into the botanical-zoological garden 'Tsimbazaza' in Antananarivo, the capital of Madagascar. The couple was caught in the eastern rain forest of Zahamena (north-east Madagascar). Clearly, this is the first time this species has been kept in a zoo (R. Wirth, pers. comm.). The richly contrastingly-coloured animals lived in a double-paned glass case in the vivarium, which was provided with tree branches, sleeping boxes, stones, and a floor covering of dried leaves. They were fed with pieces of beef and freshly killed one-day-old chicks (two chicks per mongoose). The male died after a short time, but the female was obviously healthy and I could observe and photograph her in October and November 1995. She was quite friendly, and was active during several parts of the day. Unfortunately, I heard that the female has also died.

The picture of the broad-striped mongoose from Tsimbazaza Zoo appeared in the German journal *Arbeitsplatz Zoo* (No. 3, 1995). It is probably the first time that a photograph of a living individual of this attractive species has been published (R. Wirth, pers. comm.).

In October and November 1995, there were also other species of mongooses living at Tsimbazaza Zoo: Malagasy ring-tailed mongoose, *Galidia elegans elegans* (1,1) caught near Anjozorobe, north-east of Antananarivo, also *Galidia elegans occidentalis* (1,1) caught near Morondava, western Madagascar, and Malagasy narrow-striped mongoose (*Mungotictis decemlineata decemlineata*) (2.3), also caught near Morondava. These were also kept in double-paned glass cases in the vivarium, each in two outer enclosures (modelled concrete structures with caves, tree branches, and small islands of plants). It seems that both species have been bred at the zoo for several years. Offspring of the ring-tailed mongoose were born at the end of last year, but were probably eaten by their parents.

Broad-striped mongoose, *Galidictis fasciata*, in Tsimbazaza Zoo. Photo: Mario Perschke.

Free-living ring-tailed mongooses, *Galidia elegans elegans*, and Malagasy civets, *Fossa fossana*, can both be observed well and easily in the Ranomafana National Park (south-east Madagascar, north-east of Fianarantsoa), which was founded in 1991. During the day, several ring-tailed mongooses stay in the surroundings of a small camp site, close to its entrance. With the beginning of darkness, up to two Malagasy civets appear, which are attracted by fried pieces of meat, offered by the Madagascan tour leaders. The animals have lost their shyness of humans, and even of large groups of tourists, and will come very close.

With a bit of luck, in the early morning hours you may even encounter the fossa, *Cryptoprocta ferox*, along one of the paths in the 41,600 ha-large rain forest reserve.

Egon-Kisch-Strasse, 55, 13059 Berlin, Germany

Narrow-striped mongoose, *Mungotictis decemlineata*, in Tsimbazaza Zoo. Photo: Mario Perschke.

Molecular techniques and small carnivore conservation

Isabelle SCHÖN and Huw I. GRIFFITHS

Introduction

Zoological research has been transformed with the advent of new molecular based techniques, particularly those incorporating the polymerase chain reaction (PCR)(Mullis & Faloona, 1987; Saiki *et al.*, 1985). However, the zoological community is very much divided between those who have first hand experience of the use and application of these techniques and those who are aware of their significance, but have a limited understanding of the ways in which they can be applied to conservation biology. The aim of the present article is to provide a basic level review on laboratory molecular techniques, to explain the terminology used, and to provide examples illustrating their application to conservation problems. The review is divided into three parts: providing a historical background, explaining some of the key DNA techniques used (chronologically, by order of first widespread use by zoologists), and some examples of their application.

The basic problem facing non-molecular biologists is understanding the simple principles behind DNA-based techniques, coupled with difficulties in knowing the technical jargon commonly employed. To many the basic terminology used in molecular work is completely incomprehensible, and provides an insurmountable barrier to appreciation of findings presented in research reports, journal papers, and even research proposals. We here assume the majority of readers are familiar with the basic structure of deoxyribonucleic acid (DNA). Those who are not, we refer to the various student textbooks available which offer detailed descriptions (e.g. Alberts *et al.*,1989; Watson *et al.*, 1987).

Historical background

Prior to the 1980s, the most sophisticated technique available to zoologists for genetic analyses was allozyme electrophoresis. This operates on the principle that variants of the same protein (allozymes) migrate at different rates across a starch gel when a current is applied. Although allozyme electrophoresis provides a certain amount of information at the alpha-taxonomic scale (especially at species level), it is of less use for intrapopulation studies because of the often limited degree of variation (polymorphism) involved. A more general limitation is the necessity to use fresh or frozen material. With DNA based molecular techniques the major advantages are that much more variation may be available, the sample need not be fresh and that only very limited quantities of DNA are required.

However, the first DNA based techniques were not quite so flexible. "Restriction fragment length polymorphisms" (RFLPs) and "DNA fingerprinting" were among the first available (all these terms will be fully described later). Both suffered to a degree in that they required fairly complex protocols and the information that they provided was often difficult to interpret. Since the late 1980s two key developments have enabled researchers other than specialized molecular biologists to use DNA techniques. The first was the advent of previously mentioned "polymerase chain reaction" (PCR), and the second was the development of so-called "universal (properly, "versatile") primers". Both techniques together enabled DNA analysis from poor quality specimens, with no prior sequence knowledge and, more importantly, no conventional "cloning". PCR and subsequent DNA sequence analysis have also made possible the DNA equivalent of allozyme electrophoresis, i.e. "microsatellites", and have facilitated the improved use of RFLPs for large scale screening programmes.

DNA techniques

DNA extraction

The majority of DNA techniques require a very small tissue/DNA sample that need not necessarily be of good quality. Nonetheless, steps should be taken to prevent sample degradation as much as possible, e.g. storing in 95% ethanol or a -20°C freezer. A range of non-lethal sampling regimes are possible and should always be considered. Ear punches or blood samples may be collected (e.g. from the tails of voles), but since mammalian red blood cells have no nucleus, the volume of sample must be larger than a corresponding sample taken from other vertebrate groups (birds, Amphibi), etc.).With DNA techniques it may not even be necessary to capture the animal. Hairs (from "sticky tape" traps) or even faecal remains (Taberlet & Bouvet, 1992) contain sufficient DNA for PCR based techniques. Finally, it is worth noting that large numbers of specimens are available in museums and other archival resources and these may also be used as sources of DNA. However, museums receive frequent requests for tissue specimens and will often require a research proposal before they are willing to release particularly important specimens. Specific technical problems are associated with so-called "ancient DNA", particularly when the sample is formaldehyde preserved, so that specialized extraction protocols are required.

Tissues selected as DNA source-material are processed to yield relatively pure DNA. Extraction must be undertaken with a certain amount of care, primarily to prevent cross-contamination

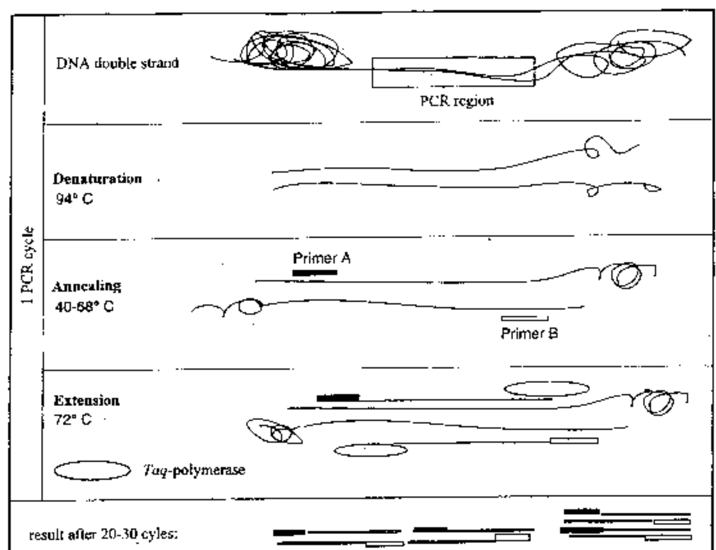


Fig. 1 Single steps of the "polymerase chain reaction" (PCR)(after Schön, 1995a)

of the samples. For instance, a recent "dinosaur" DNA sequence turned out to be contamination by the researcher (Zischler *et al.*, 1995). The extraction method varies depending on the source of the tissue sample analysed. Traditionally, DNA purification has involved proteinase digestion, followed by phenol/chloroform extraction to lyse cells, denature intracellular proteins, and free the DNA. DNA extraction in now better achieved through the use of simpler, safer techniques: silica (Höss & Pääbo, 1993), chelex (Walsh *et al.*,1991), commercial kits (Qiagen, Pharmacia) or simply by boiling an aliquot of tissue in buffer (Schön, 1995a).

In practice, different research aims are fulfilled by the examination of different DNA types: mitochondrial DNA (mt DNA), chloroplast DNA, and also various nuclear DNA regions that evolve at different rates (e.g. ribosomal or rDNA, and microsatellite DNA). The different scales at which these types of analyses operate are illustrated by Avise (1994). We will describe these in more detail later.

DNA-Fingerprinting

Perhaps the first DNA-based technique to attract widespread attention within the zoological community was "DNA fingerprinting", a technique developed by Jeffreys *et al.* (1985). The technique makes use of hypervariable parts of the nuclear genome, "minisatellites", which are multiple repeat units of DNA sequence. Minisatellite fragments are between 1 and 25 kilobases (kb=one thousand DNA bases) and highly polymorphic so that in theory, no two individuals (except identical twins) will produce the same DNA pattern. Their specificity and use in forensic work gave rise to their name. The technique is now considerably easier, and fingerprints have been obtained from a wide variety of species, but because minisatellites are so highly variable, their uses in conservation ecology have been limited outside of studies of paternity, mating system, and inheritance. Other limitations are that a relatively large and good quality DNA sample is required, and that it is difficult to apply the usual statistical analyses (such as testing for Hardy-Weinberg Equilibrium) because each DNA fragment is essentially anonymous (i.e. of unknown origin). As DNA fingerprinting is a multi-stage process, using techniques not applied to other types of DNA analysis, the methods are not discussed further here (but see review of Bruford *et al.*, 1992; Degnan, 1994).

PCR (Polymerase Chain Reaction)(Fig. 1)

PCR essentially mimics in vitro the process of DNA replication (DNA making a copy of itself). This process was first achieved in the laboratory by the use of polymerase enzymes to amplify bacterial DNA. Originally, this was a painfully slow and laborious process. To make multiple copies of a specific region of the DNA molecule (such as a gene selected at the start of the research programme), you had to use a chain of reactions at specific temperature conditions, each cycle of which would double the DNA content. The process itself involves three thermally assisted components, together termed a cycle. The first step is the "denaturing" step, in which the DNA double strand is separated into two single strands at a temperature between 93° and 95°C. This produces two single stranded DNA templates upon which the polymerase enzyme can act. For this purpose, it needs a small part of double stranded DNA to begin its polymerisation reaction. The second stage therefore is termed "annealing" in which a "primer", a short DNA fragment which is artificially synthesized binds to the DNA fragment in the reaction. The primer binds at this position, where its base sequence fits with the sequence of the DNA fragment. The reaction solution now consists of two templates with primers attached, in solution with appropriate salts, four free nucleotide bases (from which the new strand can be built) and the polymerase enzyme. Annealing takes place at a range of temperatures between 37° and 65°C. In practice, the optimum annealing temperature for each reaction must be sought by experimentation. The third stage is known as "extension". It is during this stage that the polymerase enzyme causes synthesis of a new, complementary DNA strand from the template and the free nucleotides. However, at the end of a single cycle such as this, there is only a doubling of the DNA content of the original reaction. For analytical purposes, this is insufficient, so the reaction cycle must be repeated 20 to 35 times, each with an associated doubling of DNA contents. After 35 cycles, the amount of original DNA has been copied by several order magnitude, and this abundance of DNA allows easier laboratory handling and treatments. However, the size of a typical PCR product would not normally exceed 2 kb, due to the polymerisation limit of the enzyme. If working with large sized DNA fragments, special conditions must be used e.g. cloning vectors and yacs (yeast artificial chromosomes) both of which are beyond the scope of this article. The major breakthrough in the harnessing of DNA analysis in routine laboratory studies came with the discovery of the enzyme *Taq*-polymerase. The enzyme itself is derived from a hot spring-dwelling bacterium *Thermus aquaticus*, and is now commercially available. The advantage of *Taq*-polymerase over the other types of polymerase enzymes available is its high resistance to elevated temperatures (in the "denaturing step" of the cycle) that would cause the others to denature and lose their function. Therefore, instead of adding a fresh amount of polymerase after each PCR-cycle, *Taq*-polymerase is included in the reaction mix from the beginning.

The result of PCR is a highly concentrated, high purity solution of the amplified DNA fragment. Its size can be assayed by the migration of a small amount of the PCR product across an electrically charged gel. This process is very similar to that of allozyme electrophoresis, but the gel is made of agarose, not starch. Visualization of the DNA is achieved by staining with ethidium bromide solution which can be seen under UV light. Size can be assessed by comparison with a mixture of DNA fragments of known size (often known as a "ladder"). These are usually constructed on a logarithmic scale which gives higher accuracy to measurements of small fragments (see Fig. 3).

Primers

The specificity of DNA polymerisation reaction is largely a product of the primer decided on. Primers are usually short (15 to 25 basepairs long) and synthesized for specific purposes. The choice of primer will depend on the target DNA region. This itself will often be selected on the basis of availability of published sequences (either in research literature or in particular DNA sequence data bases). If, for example, the DNA fragment of interest lies within mitochondria, data bases can be searched for mitochondrial sequence that is likely to have a high degree of similarity (homology) with that under investigation. Once a suitable target region has been chosen, a short sequence of bases is selected for use as a primer. Primers are synthesized automatically, following a base "recipe". Ideally, there should be sufficient homology between DNA sequences available on the data base and that to be investigated to allow amplification to occur. Sufficient difference is also required so that the DNA samples can be compared and differences between them identified. If no appropriate DNA sequence data are available, it is also possible to use "random" primer for DNA amplification. These are very

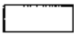
1. DNA double strand

AACGTGACTGCTTAAGCGTCGTACGGGGCCATATACTG

2. Separation of the double strand, primer annealing

(only one DNA strand is shown now)

AACGTGACTGCTTAAGCGTCGTACGGGGCCATATACTG

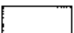


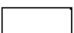
primer

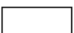
3. Polymerisation of the new synthesised strand

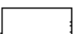
AACGTGACTGCTTAAGCGTCGTACGGGGCCATATACTG

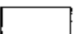
CTG**A**CG**AA**TTCGCTGC***T***

 CTG**A**CG**AA**TTCGCTGCT***G***

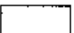
 CTG**A**CG**AA**TTCGCTGCTG***C***

 CTG**A**CG**AA**TTCGCTGCTGC***C***

 CTG**A**CG**AA**TTCGCTGCTGCC***C***

 CTG**A**CG**AA**TTCGCTGCTGCCC***C***

CTG**A**CG**AA**TTCGCTGCTGCCCC***G***

 CTG**A**CG**AA**TTCGCTGCTGCCCCG***T***

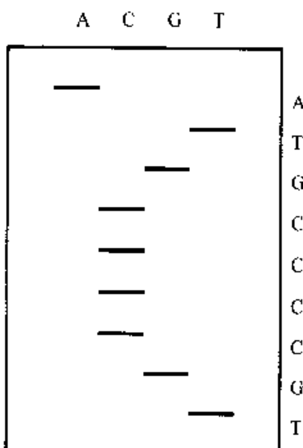
CTG**A**CG**AA**TTCGCTGCTGCCCCGGT***A***

A, C, G, T = "normal nucleotides" (dNTPs)

A, C, G, T = chemically modified nucleotides (ddNTPs), their incorporation results in termination of the polymerisation-reaction

A = radioactive labelled nucleotide

4. Electrophoretical separation of the DNA-fragments



sequence is: TGCCCCGTA

Fig. 2 Single steps of a "manual" sequencing reaction

short primers (10 to 15 basepairs long) which bind to random regions in the DNA. This procedure is known as "RAPD" (or verbally, "rapids") ("randomly amplified polymorphic DNA").

Sequencing (Fig. 2)

Sequencing is a very complex procedure which is now made considerably easier by the availability of automated sequencing machines. Unfortunately, such facilities are only available in the largest institutes and universities and are very expensive to install. In practice, most workers must still sequence "by hand". This is a time consuming procedure that can bring investigators into contact both with noxious chemical reagents and radioactive isotopes. In short, a sequencing reaction is quite similar to PCR, but the end result of sequencing is a photographic image of bands representing individual nucleotides in their order of assembly. (Some of the basic uses of DNA sequence analysis in ecological research are discussed by Butlin & Griffiths, 1995).

The first step in DNA sequencing is separating the double stranded PCR product. This can be done by various techniques e.g. incubating at a high temperature or magnetic separation using "magnetic, chemical labelled beads" to which one DNA strand will bind. In a second step, similar to the PCR cycle, a primer, the same as in the original reaction or one specifically designed for the purpose of sequencing, binds to the single stranded DNA. Also in this technique, a polymerase synthesises a complementary strand. It has a greater accuracy than the *Taq*-polymerase to ensure that only a small amount of sequence divergences arises due to mismatching. The great difference, compared to the reaction mix for PCR, is the mixture of nucleotides. In the sequencing reaction, equal sized measures of PCR products are incubated with different nucleotides individually (*i.e.* there are four different complementary reactions; one for each base). To visualise the reaction products at the end of the process, a radioactively-labelled nucleotide is included, which will provide an image on an X-ray film. Specific amounts of chemically modified nucleotides are also included, one being used in each one of the four separate reaction mixes. Every time one of these nucleotides is incorporated by the polymerase, the enzyme stops further polymerisation. As this happens randomly, the reaction ends with a mixture of DNA strands of different length, allowing their eventual visualisation electrophoretically. These mixtures rich in the four bases (G, A, T, C) are separated on acrylamide gels which have a resolution of single bases. After exposure of the gel to an X-ray film, a pattern of four lanes is revealed. Each lane represents one of the four bases with the presence of the base being indicated by a dark spot on the lane. Thus, the presence of Guanine will be shown by a dark spot in the guanine lane, but nothing in the other three. Therefore, by looking downwards at the pattern of spots on the lanes of the gel, it is possible to determine the DNA sequence from which they derive.

The process of sequencing needs to be done in "both directions", *i.e.* on both strands of the DNA, with the sequences running in opposite direction from one another. In the case of automatic sequencing, the four bases are labelled with fluorescent dyes, which are detected after gel electrophoresis by a laser in the machine. Eventually, this provides a printout indicating the position of the various bases within the sequence.

Sequence data analyses

DNA sequence information provides the "raw" data from which analyses are made. Such data consist of "chains of letters", which refer to the four bases in assembly order. It is necessary to

make a comparison of both strands (directions) from the same DNA sample. This helps to guard against errors in the sequencing process. Once this is done, it is possible to compare sequences between individuals, populations, and species. Specific algorithms allow computerised sequence analyses, which make the process much easier than the previous method in which sequences were compared by "eye and hand". In computer analyses the most commonly used package is "gcg" ("genetics computer group"), which is available, free of charge, from Daresbury, UK. The use of these programs allows the ready alignment of similar sequences and also the reversal of sequence orientation (if necessary). Data thus generated can be compared with previously published DNA sequences in various data bases. Newly generated sequence data should be submitted to a formal DNA data base such as Genebank (US National Academy of Sciences) or EMBL (European Molecular Biology Laboratory, Heidelberg, Germany).

Wright's F-statistics also can be applied to DNA sequence data, in a similar manner to that used for the analysis of information from allozyme studies.

Microsatellites

The analysis of microsatellites (also often confusingly referred to as VNTRs and SSR) is the most recent and most fashionable DNA technique that has been developed. Exactly as in an ordinary PCR reaction, a small region of the genome is amplified, but the region selected will contain highly polymorphic, short (typically dimeric) DNA repeats (e.g. ACACACACA). These microsatellites have such high mutation rate that they are useful for fine-scale studies of population differentiation and in studies of very closely related lineages. The advantages over allozymes are that often many more alleles are present, and that good quality specimen preservation is not required -museum preserved material or DNA from faeces will suffice. However, the problem associated with microsatellites is that new markers commonly have to be developed for each species under investigation, a process that may take from three months to one year.

What can molecular data tell us ?

Population size and subdivision

Allozymic techniques are weakest when examining divergence within species as the levels of variation observed within studied populations are often too low (i.e. beyond the limits of resolution of the analytical technique). This is precisely the type of problem to which molecular genetics data are ideally suited. The techniques actually utilised in phylogeny reconstruction are discussed by Swofford & Olsen (1990). A wide variety of species has thus far been investigated from different points of view. Byrne *et al.* (1990) describe various polymorphisms found amongst populations of woodmouse, *Apodemus sylvaticus*, and bank vole, *Clethrio glareolus*, in Ireland. Schön (1995 a,b) uses similar polymorphisms to examine the effects of habitat fragmentation on vole (*Microtus*) population substructures. Similarly, species regarded as being highly endangered have been " profiled" for their population variation, particularly when population sizes are restricted. Taylor *et al.* (1994) investigated the microsatellite variation within the northern hairy-nosed wombat, a species which has gone through a population bottle neck.

Similar studies have been undertaken upon species such as the European brown bear (Randi *et al.*, 1994), the black rhino (O'Ryan *et al.*, 1994), the Ethiopian wolf (Gotelli *et al.*, 1995), Spekes gazelle (Butler *et al.*, 1994), and humpback whales (Baker *et al.*, 1994).

Hybridization

Some of the most striking results to emerge from molecular studies are those in which apparently well known species have been found either to be hybrids between the taxa, or composites of several species of convergent appearance. The most well characterised of these cases is the now notorious dispute arising from Wayne & Jenks' (1991) study of the red wolf. Here, molecular data revealed the red wolf, a species that has attracted considerable attention and funding from the conservation community, was in fact a hybrid between the grey wolf and the coyote. The potential for hybridization to occur between related species either following human habitat change or the introduction of exotic or domesticated species, is also widely appreciated. Abernethy (1994) demonstrated extensive hybridization between red deer and the introduced sika deer (itself possibly a hybrid). Hughes & Carr (1993) reported a similar case amongst American white-tailed deer (*Odococilus virginianus* and *O. hemionus*). Recent DNA sequence analyses have revealed a prior hybridization event between American bison and cattle (Polziehn *et al.*, 1995).

Similar techniques are now currently being applied to investigate the introgressive hybridization between European polecat *Mustela putorius* and feral ferrets *M. p. furo* in Britain (Davison *et al.*, in prep.).

Phylogenetic reconstruction

Until recently it was commonly thought that the burst of mammalian radiations ca. 65 million years BP would prove a stumbling block to resolving mammalian phylogenetics from morphological features (all groups having been derived at approximately the same time [Gaur, 1993]). Molecular data are thus perceived as providing a robust means of reconstructing the evolutionary history of the various mammalian groups by the use of traits that are less prone to subjective interpretation and evolutionary convergence. Attempts to address the phylogeny of different mammalian groups have been made by many authors, e.g. Kuma & Miyata (1994, mammalian phylogeny), Cao *et al.* (1994, Eutheria), Vrana *et al.* (1994, higher level of arctoid groups), Masuda & Yoshida (1994, Mustelidae), Hoelzel *et al.* (1991), Milinkovitch *et al.* (1993, Cetacea), and Graur *et al.* (1996, Lagomorpha). At a lower phylogenetic level, Beltran *et al.* (1996) have looked at the relationships of lynx and relatives, showing the uniqueness of the pardel or Iberian lynx, *Lynx iberica*, in comparison to Canadian and European lynx. Veron & Catzeflis (1994) have similarly examined the relationships of the aberrant Malagasy viverrid *Cryptoprocta ferox*. Taberlet *et al.* (1994) compare the evolutionary histories of lineages of common shrew, *Sorex areneus*, by combining cytological and molecular approaches. In some cases, it has even been possible to investigate these types of relationships for extinct forms such as the Tasmanian wolf, by the extraction of DNA from museum material (Thomas *et al.*, 1989). A more detailed review of molecular techniques in phylogenetic work is given by Avise (1994). It is important to note however, that different DNA systems (e.g. mtDNA versus nuclear DNA) may give different phylogenetic results. For this reason, it is best to utilise two (or more) types of analysis in tandem.

Animal welfare implications

Aside from the advantages offered by molecular work from a purely scientific point-of-view, DNA technology has also

Fig. 3 Electrophoretic separation of DNA in a 1% agarose gel with ethidium bromide staining. "+" indicates smaller-sized PCR-fragments. A "ladder" (indicated by the letter M) was loaded into the last lane of the gel. The exact sizes of the different DNA fragments within the ladder are given in basepairs (bp)

some implications that are ethically beneficial. For example, the ability to use DNA from road-killed animals, and from material such as hair and faeces for analysis negates the need to trap. This obviously reduces any problems associated with animal handling, and removes any need to kill animals, or to use invasive tissue sampling techniques, both of which are undesirable if populations are threatened.

However, a word of warning is necessary here. Not all DNA techniques can be applied to samples such as these. For example, in the case of faeces, the large amounts of other DNA present contraindicates the use of mitrochondrial primers, although microsatellites, or species-specific primers, may prove useful.

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Columbian Weasel

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Introduction

The Colombian Weasel Project was initiated in 1993 to realise a recommendation in the the IUCN/SSC Action Plan for the Conservation of Mustelids and Viverrids (Schreiber *et al.*, 1989). This was to search for and study the ecological requirements of the Colombian weasel *Mustela felipei*.

[N.B. Unless otherwise stated, all sites mentioned are in Colombia. Abbreviations: RNP = Regional Natural Park; NNP = National Natural Park.]

Materials and methods

The project used wooden box live traps of dimensions 60 x 15 x 14 cm, based on the design used by Sleeman (1987) for *M. erminea hibernica*, which adapts the Whitlock mechanism used for *Mustela nivalis* by King (1973). Non-absorbent cotton wool was provided in the trap as insulating nest material. For the majority of the study, the traps were baited with sardines or tuna from a can. The vegetable oil in which they were preserved, which smelt particularly strongly, was applied to the trap entrance. Maize or wheat grain was also provided to sustain trapped small rodents (since some appeared to avoid eating the fish). An initial trial employed live five-day old chicks as bait, but none survived the cold of the first night, so this was not continued. Attempts were later made to use live small adult chickens as bait, but this too was soon abandoned, since the traps were not really of a suitable design to accomodate them. For a limited period near the end of the study, as an additional attractant to the sardines inside the trap, the gut contents of domestic rabbits were mashed and applied around the entrance area of each trap: an English gamekeepers' tactic employed successfully by King (1989).

The traps were set in transects at a spacing of approximately 100 metres, usually in forest, amongst or beside tree roots, logs, branches, rocks and banks of earth. (The exception to this was the last month of field effort, when traps were widely distributed around a farming area. Two to five traps were set - sometimes in gaps under buildings - at each farm previously visited by weasels). Several pieces of branch were often arranged as a "funnel" towards the trap entrance. Large leaves were placed on the roof of the trap to deflect rain. Traps were checked daily and left set at a given location for one week on average (varying from a couple of days to three weeks).

Results and discussion

Captures were dominated by small rodent species, which are of interest here particularly as potential prey for weasels. Our trapping efforts yielded only one weasel, a subadult male *Mustela frenata*, caught in Ucumarí RNP on 20 February 1994. The trap was baited with a dead 5-day old chick, and set between the Río Otún and a mule trail (2.5 km from El Cedral en route for La Pastora, altitude 2,300 m) on the ground under low thin tree branches. The immediate habitat appeared to be secondary growth forest, (with estimates of cover: canopy 0%, understorey 60%, herb layer 5%, fallen trees <5%, leaf litter and root mass 90%, bare soil 5% and woody stem densities: 4.5/m^2 of diameter <1 cm, 3.8/m^2 of diameter 1-3 cm, 0.3/m^2 of diameter 3-10 cm, 0.3/m^2 of diameter >10cm).

Table 1. Study sites

N°.	Site name	Co-ordinates	Department(s)	Approx. altitudes trapped (m ASL)
1	Ucumarí RNP	04° 42' N, 75° 31' W	Risaralda	2,050 - 2,550
2	Rio San Rafael in Tatamá NNP	05° 08' N, 76° 02' W	Risaralda	2,150 - 2,250
3	Alto de Galápagos	04° 51' N, 75° 12' W	Valle/Chocó	1,800 - 2,050
4	Cueva de los Guácharos NNP	01° 32' N, 76° 04' W	Huila	1,700 - 2,150
5	Farmland adjacent to site 4	01° 42' N, 76° 08' W	Huila	1,700 - 2,100

Table 2, Trapping effort and captures

Site	Dates	N° trap nights	N° capture events	Capture rate %	N° species
1	15/2/94 - 4/3/94	679	50	7.4	6
2	19/3/94 - 27/3/94	402	78	19.4	2
3	23/4/94 - 17/5/94	1,072	54	5.0	4
4	23/5/94 - 24/6/94	861	6	0.7	2
5	28/6/94 - 18/7/94	366	2	0.5	2
Total		3,380	190	5.6	11

Table 3: Species caught at each study site

Species caught	N° individuals (N° recapture events in brackets)				
	Site 1	Site 2	Site 3	Site 4	Site 5
Didelphis albiventris	2 (1)			1 (0)	
Mustela frenata	1 (1)				
Heteromys sp.			2 (0)		
Oryzomys albigularis	10 (1)	53 (15)	24 (20)		
Oryzomys minutus			1 *		
"Oryzomine" sp.	14 (1)				
Melanomys caliginosus				5 (0)	1 (0)
Aepeomys fuscatus		8 (2)	1 (0)		
Thomasomys aureus	3 (0)				
Thomasomys cineriventer			7 (0)		
Akodon affinis	13 (3)				
Rattus rattus					1 (0)
Total individuals	43	61	34	6	2
Total capture events	50	78	54	6	2

* captured by hand, so not included in trapping data

This weasel was restrained with an intramuscular injection of ketamine hydrochloride (100 mg/ml in Vetalar) at a dose of 25 mg/kg, for examination and the attachment of a radio tag (Biotrack SS-2 with a cable-tie collar, 3.5g). Measurements in mm were: total length 433, tail 175, hind foot 49, ear 19; body weight 250 g. Two ticks were removed from an ear and the back of the neck. The individually unique outline of the lightly coloured area on underside was traced directly onto a sheet of transparent acetate using a permanent marker pen. (A reference collection of these could be reliably used for individual identification should collars be shed or as an alternative to ear-tagging.)

As far as we were aware, radio tracking of this species was previously limited to three individuals in North America (King, 1989, cites DeVan, 1982 and Vispo pers. comm.). Tracking *Mustela frenata* was also intended to aid future tracking of *M. felipei* in similar habitat. The main aim was to try and identify hunting sites and den sites (through "homing"). Fixes were triangulated continuously (at intervals typically less than five minutes) during periods of tracking effort, which were maintained part-time, to enable trapping for *Mustela felipei* to continue. Following release at the point of capture, the weasel was tracked for three hours, first travelling rapidly to a half-hectare flat area of open wet meadow on the other side of the mule trail, dominated by herbaceous vegetation such as long grasses (there was low grazing pressure from a single horse), and surrounded by shrub-dominated scrub on three sides, and by forest up steeper slopes to the north. The weasel remained active in and around this open area (perhaps hunting) before entering an adjacent den, located 250 m from the point of capture, under an epiphyte-laden understorey tree with a very high density of thin, low branches protruding in all directions. This was growing on soil packed with large rocks, in a grove of similar trees and shrubs interspersed with patches of low scrub and herbaceous plants. This gave an overall impression of regrowth after previous clear-felling (almost coppice-like). During continuous daytime tracking the weasel's time was split between the same hunting area and den on the second day, but spent entirely in the den on the third day. On the fourth morning the weasel was tracked on a one-hour sortie in the opposite direction from the meadow, passing through a comparably sized area of mixed scrub, before crossing the mule track to riparian forest beside the Río Otún (approx ½ km upstream from the point of capture) and eventually returning to the den via the same route. A seven-minute sortie was made into the same scrub area mid-afternoon. Towards evening the weasel left the den and contact was lost as it headed beyond the meadow into the steep forested slopes below Cerro Buenavista. It was later tracked in that area, travelling constantly for over one hour (perhaps hunting or looking for alternative den site) but contact was lost despite overnight tracking. The weasel was briefly found back at the original den at the start of the fifth day, before disappearing up the slopes where it had been lost the night before.

The weasel was however recaptured on 26 February 1994, approx. 2 km south west of the original capture site, following the north bank of the Río Otún. (i.e. approx. ½ km along the path from El Cedral to La Pastora). The trap was baited with sardines, and set amongst large rocks 4 m from a small stream approximately 30 m into the forest between the path and the Río Otun. Estimates of habitat cover were: canopy 20%, understorey 40%, herb layer <5%, fallen trees 30%, leaf litter and root mass 80%, bare soil 5%, overhanging rock (moss-covered) 85%, and densities of woody stems: 2.5/m^2 of diameter <1 cm, 0/m^2 of diameter 1-3 cm, 0.3/m^2 of diameter 3-10 cm, 0/m^2 of diameter >10cm. The detectable range of the radio transmitter range was down to 60 m along line-of-sight (suggesting that the battery had failed - they do not store well). As we could only handle the weasel under anaesthesia once it became clear that the collar had been put on too tightly and the animal was in poor body condition. It did not recover from anaesthesia and later died.

After this an attempt was made to locate nest or latrine areas within the den site and find scats and other remains of prey e.g., fur or feathers used to line nest. No signs were found, despite extensive excavation, which suggested that it was not an established or well used den. Although some faeces had been deposited prominently on what appeared to be territorial "landmarks" such as rocks beside streams, it was not possible to identify any of these as weasel scats (they were often deformed by rain). Some elongated pellets found beside the Río Otún were identified as the faeces of the torrent duck *Merganetta armata*.

Inhabitants of areas adjoining study sites were asked which mammals they were familiar with. Almost all farm-dwelling people questioned had seen weasels, and usually thought of them as pests, since most encounters involved predation upon chickens or domestic guinea pigs (or in one case, a frog). The chickens were usually free-ranging, although a few people had night-cages on smooth bamboo legs, with small mesh chicken wire, designed to be inaccessible to weasels. It was not possible to substantiate many peoples' claims (unsolicited by us) that they could distinguish two kinds of weasels found locally, on the basis of size and/or colouration differences - usually one reddish-brown, one blackish-brown (all these could be accounted for by individual variation within *M.frenata* - see also below paragraph). Sometimes this included naming one of the "forms" *comadreja* and the other *chucurí* or *condumbí*. There was however, no apparent consistency in the combination of names with descriptions, perhaps because all three names are merely the word for weasel originating from a different language: the latter two coming from distinct indigenous linguistic families and the former coming from Castillian Spanish. No description was given of the main distinguishing features of *Mustela frenata* (the black tail tip) or *felipei* (the dark spot in the centre of the pale coloured chest/throat). Observation of these features had not been possible at a distance of 6 m during the one chance sighting we had of two weasels crossing our path, approximately 1km from El Cedral in RNP Ucumarí, shortly before dusk on 25 November 1993.

On close comparison of a specimen of *felipei* in the Universidad del Valle collection (Alberico, 1994) with our *Mustela frenata* specimen from Ucumarí RNP, the latter appeared darker all over the dorsum (whereas Izor and de la Torre, 1978, observed the opposite in specimens elsewhere), as well as on the head and upper neck (the dorsum of *felipei* was of a uniform colouration). This was due to the darker brown colour of the *frenata's* guard hairs, which contrasted strongly with paler duller brown underfur. The guard hairs of the *felipei* were much more rufous, as was the less contrasting underfur (both seemed more dense than in *frenata*). The venters of both specimens were mostly washed with a pale orange colour, being whiter towards the chin. It is possible that the *felipei* may have faded slightly since capture in 1988. A faded specimen of *M. africana* at the Escuela Politécnica Nacional in Quito was a lighter much redder brown above and yellowed below, although another caught in 1989 had maintained a very dark coffee (not rufous) brown dorsum and near-white venter. The interdigital webbing in the *felipei* seemed to be no more extensive than that in the *frenata*. As King (1989) points out, weasels in

general are good swimmers, whilst Alberico (1994) noted that hunting in water bodies by carnivore should not be taken as an indication of habitat restriction.

Following our visit to the Nutriovo chicken farm in La Suiza, outside RNP Ucumarí, its staff captured a male *Mustela frenata* in a sack on 11 March 1994, and took it to the local INDERENA centre, where it was examined and video filmed by Jorge Marulanda, Javier Bustos and Victor Avila, before being released alive. Measurements in mm were: total length 440, tail 125 (tip missing), hind foot 52; body weight 290 g. This individual was an adult, with testes descended and a high degree of scarring observed on the face.

One local inhabitant of the hamlet of Jerico (near Palestina, Huila), Alfredo Galindez, brought us a subadult male *Mustela frenata*. Measurements in mm were: total length 471, tail 203, hind foot 49, ear 23; body weight 265 g. He had caught it in a home-made trap of a style in widespread use. These traps are wooden boxes with a strong metal mesh cage on the back, in which a live chicken is housed as a highly attractive bait which weasels can see, hear and smell. Upon entering the wooden box to reach the chicken the weasel triggers a pivot on the roof of the trap, releasing the door which slides shut down channels in the box entrance. Safe behind the mesh, the chicken survives, whilst the weasel is liable to starve, (this was the unfortunate fate of the above mentioned individual, after feeding with raw egg failed to rescue it). Although many reports of weasels killed by this method or with dogs or guns were encountered, only a small number of skins seem to be kept (as curios or for sale for medicinal use e.g., for infertility) - three were found around Palestina, Huila, and one at Casanga, near Baeza, Ecuador - all *Mustela frenata*. Bodies were almost invariably discarded irretrievably - owing to their strong smell or superstition - even being burned to prevent their "return".

Small samples for DNA analysis were collected from three *Mustela frenata* skins (delivered to the Ministry of Environment collection in Bogotá), for comparison with *M. felipei* and *M. africana*. It has not however, yet been possible to secure material from these latter species for the research on mustelid molecular phylogenetics which is being carried out by Angus Davison. Anyone able to provide samples of these or any other poorly known mustelid species should contact him at: Department of Genetics, School of Biological Sciences, University of Leeds, Woodhouse Road, Leeds, LS2 9TJ, UK.

Opportunistic checking of museum specimen collections around Colombia and in Quito failed to reveal any further examples of *Mustela felipei* amongst specimens labelled as *frenata*. (Three of the five known specimens of *M. felipei* had originally been labelled *frenata* prior to knowledge of *felipei* existence).

Further exploration of Alto de Galápago, the site where Alberico (1994) reported *felipei* revealed that it is in fact at the headwaters of some streams, Quebrada Cristalina and Quebrada Pacífico, which drain northwards and southwards respectively . The flow of one branch of the latter seemed to have been affected by its diversion on construction of the road. Alto de Galápago is situated in the Serranía de los Paraguas mountain range, just to the south of where this leaves Tatamá NNP. In 1994 measures were initiated to develop protection along the full length of the Serranía, banning further felling or settlement of forest above 1,800 m ASL and developing sustainable management in surrounding zones. Action and Management Plans were being formulated by Municipal Committees, Autonomous Regional Corporations, the Environment Ministry and the Biopacífico programme.

Santa Marta, the holotypic locality of *Mustela felipei*, lies just outside Puracé NNP, at 2,700 m ASL in a steep-sided forested valley where the Río Magdelena leaves the park from the Páramo de las Papas. Weasels are frequently seen around Laguna San Rafael, in the north of the park, hunting rabbits in páramo habitat (high moorland in which *M. frenata* but not yet *felipei*, is known to occur elsewhere in the Andes). A trail passing through Santa Marta into the park from Quinchana, is an excursion from San Agustin, one of the main tourist sites in the country. This currently presents little pressure and could encourage protection of the forests and spectacular landscape currently lying outside the park. A proposal had already been made to extend the boundaries to include more of Río Claros basin. Further unprotected forest extends below the Páramo de la Soledad for about 50 km (equivalent to ¾ the length of Puracé NNP) from Santa Marta eastwards along the range of mountains where the eastern cordillera joins the central, to Cueva de los Guácharos NNP, where a specimen of *Mustela felipei* found under the adminstrative headquarters cabin was captured with a blanket by INDERENA staff in 1983 and preserved in alcohol in Unifem collection in Bogotá). A survey of this forest might identify a potentially important stronghold for *felipei*.

Baeza (0° 25' S, 77° 55' W, province of Napo, Ecuador), from which the American Museum of Natural History has a 1923 specimen of *Mustela felipei*, lies just outside two huge Ecological Reserves, Cayambe-Coca (which extends to the north) and Antisana (which extends to the west), both of which contain habitat of an appropriate altitude range for *felipei*. The area also has potential for comparative study of this species with its nearest relative, *M. africana*, which Dr Luis Albuja (pers. comm.) of the Escuela Politécnica Nacional in Quito captured in 1989 at Huaticocha, 1,200 m ASL, near Sumaco to the south east. This is yet closer to Baeza than the previous record of *M. africana* from the Jatun Yacu, a tributary of the Río Napo.

Further protected areas within potential geographical and altitudinal range of *felipei* include the Colombian NNPs of Sumapaz and Chingaza in the Eastern Cordillera, Nevado de Huila and Las Hermosas in the Central Cordillera, and Caramanta, Las Orquideas, Paramillo, Munchique and Los Farallones de Cali in the Western Cordillera (a proposal has been made to conserve habitat between the latter two as a "biological corridor" to maintain viable wildlife populations), and NGO-run Nature Reserves such as La Planada in the Nudo de los Pastos range. Additional National Parks of potential interest in Ecuador include Podocarpus and Sangay, which are already the subjects of Andean mammal conservation programmes (carnivores and mountain tapirs respectively). There also remain considerable areas of forest of appropriate altitude outside protected areas, and of course we still have no evidence that *felipei* cannot also survive in farming areas, as *frenata* clearly does, despite persecution.

Concluding remarks

The fact that we failed to trap *Mustela felipei* should not in itself be seen as an indication of rarity. After all, through our own trapping efforts we only encountered one individual of the widespread, common species *M. frenata*. Although we remain uncer-