# Paternity testing of endangered species of birds by DNA fingerprinting and random amplified polymorphic DNA fingerprinting

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## Introduction

Since 1985 DNA fingerprinting is the method of choice to solve forensic problems such as paternity testing (Jeffreys et al., 1986) or identification of immigration cases (Jeffreys et al., 1985 a, b). Its applicability became evident not only in man but also in many biological problems. Jeffreys'probes 33.15 and 33.6 or Epplen's oligonucleotide probes were used in DNA fingerprinting of dogs and cats (Jeffreys & Morton, 1987), non-human primates (Weiss, 1989), birds (Longmire et al., 1991, Miyaki et al., 1993), fishes (Schartl et al., 1993) or even plants (Weising et al., 1991). Especially its application in paternity testing turned to account in many species of animals (Lubjuhn et al., 1991, Mariat & Robert, 1993, Wickings & Dixson, 1992). The new method of random amplified polymorphic DNA fingerprinting (RAPD fingerprinting) allows determination of paternity with very small amounts of DNA such as in the case of insects (Hadrys et al., 1993).
The German Federal Ministry of Environment, Natural Protection and Reactor Safety subsidizes a project to apply DNA fingerprinting in the protection of endangered species, such as birds of prey or parrots (Wolfes et al., 1991, Máthé et al., 1993). The Washington Convention on International Trade in Endangered Species of Fauna and Flora (Cites) only allows selling of rare species by breeding success. These rare species are, however, hardly breeding within captivity. Therefore, dealers are often misled to get eggs or nestlings illegally by robbery to sell them with high profit margins. But now, those dealers could be convicted by DNA fingerprinting (Máthé et al., 1993). In case of getting only very small amounts of blood from nestlings or little birds or tissues from dead birds, the method of random amplified polymorphic DNA fingerprinting should be proved of its applicability.

## Material & Methods

We got more than 600 blood and tissue samples of 32 species of birds of prey and parrots, in most cases by confiscation. Peripheral blood (0,5-1,0 ml per sample) was diluted in two volumes of anticoagulant preservative solution (APS) buffer (Arctander, 1988). DNA was extracted by the salting out method of Miller (1988). The DNA was stored at 4°C, diluted in 1xTris-EDTA buffer. About 10 µg DNA of each sample was digested with 50 units *Hinf*I. After electrophoresis in 0,6% agarose gels (1xTBE) DNA was transferred to nylon membranes by Southern blotting or downward gravity blotting (Koetsier et al., 1993). Membranes were hybridized with digoxigenated oligonucleotide probes, mainly $(GGAT)_4$ and $(GACA)_4$. Detection of signals was carried out by an anti-DIG-AP-conjugate and a subsequent color reaction with NBT/BCIP or with the chemoluminescent substrates AMPPD and CSPD.
For RAPD fingerprinting it is useful to dilute DNA in 0,1xTris-EDTA buffer. We took different concentrations of DNA for each sample of 1 ng to 40 ng. As primer (10 bp) we used Operon kits O, E and R. The RAPD analysis was performed according to Williams et al. (1990).

## Results

In the last four years we tested more than 100 families of birds (peregrine falcon, lanner falcon, laggar falcon, saker falcon gyrfalcon, prairie falcon, kestrel, common buzzard, long-legged buzzard, sparrow hawk, goshawk, kite, snowy owl, eagle owl, Griffon vulture, Bonelli's eagle,

golden eagle, scarlet macao, cuban amazon, yellow-shouldered amazon, yellow-an-green lorikeet grey-parrot, yellow-crested cackatoo, blue-throated conure, golden-shouldered-parrot, imperial eagle, tucuman amazon, bald eagle, Granada amazon, rose-crested cackatoo, red-tailed amazon, Ecuador amazon) with 1 to 16 siblings for paternity. In more than 50% siblings had to be excluded as breeding success. The DNA fingerprints were mostly analyzed by hand. The patterns of nestlings were compared with the patterns of putative parents. The bandsharing frequency (bsf) was calculated according to the formula bsf%= 2n/(a+b), where n represents the number of common bands and a, b the total number of bands per lane (Jeffreys et al., 1985b). Normally about 20% of bands agree even with non-related individuals. This conformity can be affected either by chance or by sex or is typical for populations or species. In all cases of excluded paternity, we calculated a bandsharing frequency of 10% to 25%, whereas related individuals showed a bandsharing frequency of 55% to 85%.
With RAPD fingerprinting we tested different species of falcons (peregrine falcon, lanner falcon, saker falcon, gyrfalcon and their hybrids) with more than 60 primers. We got the best result with the primer Operon O 11 (5`-GACAGGAGGT-3`). The RAPD analysis of each species of falcon showed specific bands and the hybrid-falcon bands of both species. With many primers RAPD`s showed up to 15 patterns for each sample, but are not polymorphic for individuals and also not for species of falcon. In these cases we could only differ between falcons and eagles or parrots.

## Discussion

All species of birds, tested with DNA fingerprinting, polymorphic fragment patterns are detectable, especially with the probes $(GGAT)_4$ and $(GACA)_4$. In all cases of paternity testing we were able to get a clear result. As a consequence dealers which had obtained eggs by robbery or smuggling could be convicted on trial. As a result of our success in paternity testing, a significant reduction in "breeding success" of some dealers occurred.
With RAPD fingerprinting we tested some species of falcons and their hybrids. In some cases the hybrids are phenotypically indistinguishable from their parents. By performing RAPD fingerprinting using the Operon primer O 11 we detected species-specific patterns. As a result we are able to discriminate between the hybrid falcons and true-bred falcons.

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