A Single Plucked Feather as a Source of DNA for Bird Genetic Studies

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Based on mitochondrial DNA (mtDNA) we recently differentiated two lineages of the Blue Tit (*Parus caeruleus*) in the French Alps, near Grenoble (Taberlet and Bouvet 1990). In an attempt to explain this unusual local polymorphism, we undertook a study of mtDNA variation of the *P. caeruleus–P. cyanus* complex. Sampling is a major difficulty as the distribution is broad (Vaurie 1957). Two nondestructive procedures for population genetic studies of birds have been published. For protein electrophoresis studies, Marsden and May (1984) used large amounts of pulp tissue extracted from growing feathers. A second procedure involved a blood sample (Arctander 1988), and is particularly used for DNA fingerprinting studies (Burke and Bruford 1987, Burke 1989).

The recent development of the polymerase chain reaction (PCR) using a thermostable DNA polymerase (Saiki et al. 1988) allows genomic analysis from a very small amount of tissue. It is now possible to extract DNA from nanogram samples of fresh specimen or microgram amounts of tissues preserved in ethanol, and to amplify mtDNA with conserved primers (Kocher et al. 1989). DNA amplification has also been reported from a single hair (Higuchi et al. 1988, Vigilant et al. 1989), and even from a single haploid or diploid cell (Li et al. 1988). Our studies require only very small amounts of DNA, and we developed a nondestructive sampling technique. The technique requires that a few feathers are plucked, and then preserved in 70% ethanol at room temperature. One advantage of this procedure is the ease with which samples can be collected and sent by mail. Growing feathers plucked from nestlings of *P. caeruleus* contain microgram amounts of tissue and are easily used for DNA extraction. Freshly plucked nongrowing feathers contain only a few nanograms of pulp cells. Enough DNA can be extracted from mature feathers to permit multiple amplification via the polymerase chain reaction. We developed a fast and simple procedure of tissue sampling and extraction that will facilitate the study of DNA sequence variation in natural bird populations.

Sampling procedure.—With clean forceps, a few feathers are plucked with a single motion from the breast or from the top of the head, and placed into a sterile cryotube containing 70% ethanol. One feather is used for the DNA extraction. To avoid possible contamination, the feathers are plucked only with forceps, and the base must not be touched. The use of disposable gloves is recommended during this step, although this is not always compatible with fieldwork. Samples can be stored for several months at room temperature (or, more preferably, at 4°C) before DNA extraction.

DNA extraction.—DNA extraction is carried out according to Kocher et al. (1989), Vigilant et al. (1989) and Thomas et al. (1990) with some minor modifications. One feather is cut transversely 2 mm from the base. The base is put into an Eppendorf tube to be digested with proteinase K (10 mM Tris-HCl, pH 8.0, 2 mM EDTA, 10 mM NaCl, 1% SDS, 10 mg/ml DTT, 0.5 mg/ml proteinase K). The digestion is carried out in a total volume of 400 μl, at 37°C with gentle mixing, for 1–2 h until the sample is dissolved completely. Then, the DNA is extracted twice with an equal volume of phenol/chloroform and once with chloroform (Sambrook et al. 1989). A Centricon-30 microconcentrator (Amicon) is used to desalt and concentrate the sample. The aqueous phase is made up to 1 ml with TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) in the upper portion of the microconcentrator, and centrifuged according to the manufacturer's instructions. This step is repeated twice. The retentate (50–100 μl) is stored at −20°C. When using growing feathers with microgram amounts of tissue, the microconcentration step can be replaced with an ethanol precipitation (Sambrook et al. 1989). It is essential that strong measures be taken during sampling and extraction to avoid contamination with foreign DNA. A negative control (a tube with no sample) must be taken through the entire extraction procedure. We also recommend consultation of Kwok (1990) for general measures to avoid PCR contamination.

Pulp cells are inside the lower part of the rachis of a freshly plucked nongrowing feather. From the number of visible nuclei in a longitudinal section through the base of such a feather, we estimated the total number of cells. A feather with a rachis <0.2 mm in diameter may contain several hundred pulp cells. This is roughly 10^5–10^6 copies of mtDNA, 10^4–10^5 copies of nuclear ribosomal DNA, and 10^2–10^3 copies of single locus nuclear genes. Therefore, amplification can be carried out on both mitochondrial and nuclear DNA. We routinely obtain mtDNA amplifications using 2.5–5% of the total extract. Although we have not tried to amplify nuclear DNA, the sensitivity of the polymerase chain reaction allows such amplification (Saiki et al. 1988). Our sampling and extraction methods do not yield enough material to perform DNA fingerprinting studies using the technique of Burke and Bruford (1987), but the recent paper of Williams et al. (1990) suggests that random amplified polymorphic DNA could be used as genomic fingerprinting.

Many field ornithologists manipulate various species of birds at nests or during banding. Our method may give them the opportunity to perform genetic studies using a very simple sampling technique.

We are grateful to M. Kieny and N. Cambonie for their assistance. We also thank P. Kaye for help with the English.

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Received 27 November 1990, accepted 7 March 1991.

Nitrogen Isotope Ratios Identify Deserted Seabird Colonies

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The breeding population of Rockhopper Penguins (*Eudyptes chrysocome*) on subantarctic Campbell Island (52°33'S, 169°09'E), New Zealand, in the Australasian quadrant of the Southern Ocean has experienced a dramatic decline (Moors 1986). Field observations and photographs taken intermittently since 1941 document the time course of desertion of some colonies. We found this situation ideal to test the ability of the nitrogen isotope ratio ($\delta^{15}N$) and other chemical parameters in determining the locations of past seabird colonies.

There are two stable isotopes of nitrogen: ^{14}N and ^{15}N. In various biogeochemical reactions, they react at different rates; this results in a range of values for the ratio, $^{15}N/^{14}N$, of various nitrogen reservoirs. Among these reactions, the fractionation during the evaporation of ammonia greatly enriches the remain-

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