DGGE Analysis for Noninvasive Studies of Primate Diet: A Prototype for At-Distance Diet Analyses of Natural Populations

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Introduction

The applications of denaturing gradient gel electrophoresis (DGGE) to the investigation and diagnosis of human genetic diseases are well known (Fodde and Losekoot 1994). More recently, however, DGGE has also become a valuable tool for addressing questions pertaining to natural history. It has been applied to studies of population genetics (Lessa and Applebaum 1993) and phylogeography (Lessa 1993), and it has been particularly useful in analyses of microbial communities (Muyzer et al. 1993, Murray et al. 1996).

We demonstrate the use of DGGE as a molecular technique that can be employed in the absence of behavioral data for examining the diet of free-ranging howler monkeys (*Alouatta pigra*) (Figure 1). The difficulties associated with the collection of behavioral data for diet studies are well known. These include problems related to observing monkeys through thick foliage, the large distances separating observer from subject, and the difficulty of identifying tree species with very similar leaf morphologies. In order to circumvent these problems, we investigated the potential of a polymerase chain reaction (PCR)/DGGE method for augmenting knowledge of the howler diet. We hope that the development of a noninvasive technique for examining diet will facilitate studies of wild populations, particularly those for which behavioral data are not available.

Methods

Genomic DNA was extracted from fecal samples and reference plants, and the chloroplast *rbcL* gene amplified via the PCR with one GC-clamped primer. PCR mixtures contained 1x PC2 PCR buffer (Ab Peptides), 0.1 units/µl KlenTaq DNA polymerase (Ab Peptides), $MgCl_2$ (5.0 mM), 0.25 µM each primer, and 1.5% dimethyl sulfoxide. Thermal cycling conditions were: an initial denaturation at 94°C for 4 min; 3 cycles of 94°C, 1 min; 62°C, 1 min; 80°C, 45 sec; 4 cycles of 94°C, 1 min; 64°C, 1 min; 80°C, 45 sec; 32 cycles of 94°C, 1 min; 68°C, 1 min; 80°C, 45 sec; and a 5 min final extension at 80°C. PCR products were subsequently subjected to DGGE, using the DCode™ universal mutation detection system from Bio-Rad, to resolve the various plant sequences produced by PCR amplification.

To determine the melting temperature of PCR products, they were first analyzed on a perpendicular denaturing gradient gel poured with Bio-Rad's Model 475 gradient former. The gel was 6.5% 37.5:1 acrylamide/bis-acrylamide, and contained a gradient of 1–100% denaturant. Approximately 100 µl of PCR product were loaded on a gel, and electrophoresis performed at 130 V and 60°C for 3 hr (Figure 2).

Optimal resolution of fragments on subsequent parallel gels was obtained with gradients ranging from 30–40% denaturant, with electrophoresis at 130 V and 60°C for approximately 4 hr (Figure 3). Gels were stained with ethidium bromide, and visualized by UV fluorescence.

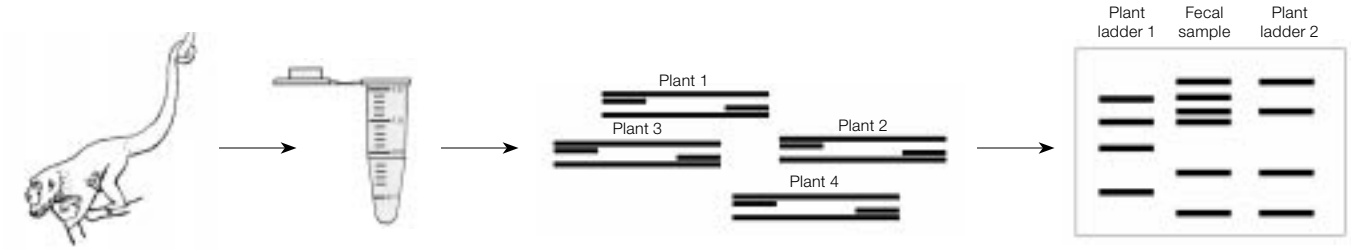

Fig. 1. Schematic outline of the molecular method used to examine the howler monkey diet. The technique produces PCR products from different plants that are identical in size, but may differ in sequence composition.

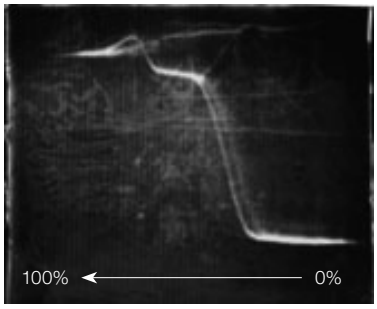

Fig. 2. Perpendicular denaturing gradient gel of fecal sample PCR product. The two inflection points visible in the gel represent two melting domains in the fragment. The existence of two melting domains was also evident from analysis with MacMelt™ software (Bio-Rad). The two bands visible in the first domain represent at least two plant DNAs amplified in the PCR.

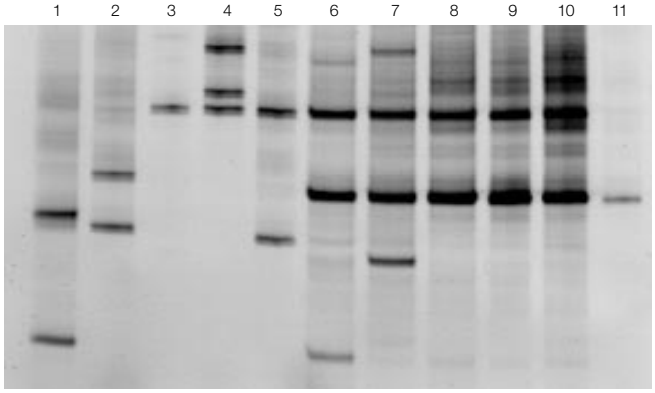

Fig. 3. DGGE of fecal samples from captive animals and the plants they are known to have eaten. The *rbcL* gene was amplified from individual plants, and the PCR products were combined as follows prior to loading: Lane 1, plants 10 and 11; lane 2, plants 12 and 13; lane 3, plant 14; lane 4, plants 15–17; lane 5, plants 18 and 19; lanes 6–10, correspond to PCR products from fecal samples 1–5 and from plant 20, respectively. The additional 7 plants eaten by the monkeys were not loaded on this gel, yet are distinguishable from each other and from plants 10–20. Note the identical migration of plants 14, 17, and 18.

Results and Discussion

Our results indicate that DGGE is an effective technique for resolving unique sequences from mixed template PCR amplifications. Analyses of eighteen plant species consumed by captive animals showed that DGGE permitted resolution of 15 of the 18 species, or over 80% (Figure 3). The three unresolved species possessed identical sequences (unpublished) in the melting domain under examination, and thus differentiation could not be expected.

Additional results from samples obtained from captive animals whose diets were known, indicate that, on average, only about 30% of all plants ingested by the monkeys were detected by PCR/DGGE. Further experiments on constructed plant mixtures confirmed that a moderate amount of amplification bias occurs in PCR amplifications of mixed templates (Figure 4). Experiments to assess the preferential amplification observed in both the control fecal samples and the constructed plant mixtures suggest multiple factors at play, including template concentration and differential primer fidelity to various templates.

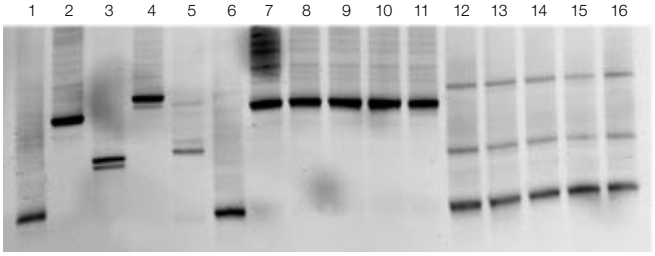

Fig. 4. PCR amplification of the *rbcL* gene from individual plants and from plant mixtures. Lanes 1-6, individual plants 1-6, respectively; lanes 7–11, replicates of mixture of plants 1–6; lanes 12–16, replicates of mixture of plants 1,3,4,5, and 6.

These data suggest that the PCR/DGGE approach tested in this investigation should be useful in situations where a universal detection system is not required, or where nothing is known about the diet of an animal. Further optimization of the technique should permit greater fragment resolution and enhanced sensitivity. Yet, any future investigations utilizing these methods should carefully consider the effects of PCR bias (Reysenbach et al. 1992, Ferris et al. 1996, Suzuki and Giovannoni 1996).

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