
<NOTE>**Highly Successful Non-Invasive Collection of DNA from Wild Chimpanzees**

*Linda F. Marchant, Amanda Ensminger,
Jill Pruett, and William C. McGrew*

*Miami University, Departments of Anthropology
and Zoology, Oxford, OHIO, USA*

In the last decade, field primatologists studying apes have worked hard to extract DNA from biological materials recovered non-invasively from their subjects. For *Pan* spp., hair, feces, and wadges have been the principal foci of investigation (1,2,3,4). These techniques yield new lines of evidence about individuals' reproductive choices, kinship and community lineage structure, and population genetics at the

subspecies level for the chimpanzee. From a practical standpoint, however, the extent of successful recovery has sometimes been frustratingly low. Here we report on a recent field season (February – April 2000) at Mt. Assirik, Parc National Niokolo-Koba, Senegal (5), where we studied the far West African subspecies of chimpanzee (*Pan troglodytes verus*). One of the research aims was to examine chimpanzee diet via fecal analysis and to collect fecal samples for DNA extraction. Given our criteria for fecal collection (see below), we obtained 54 samples; of these, 98% (53 of 54) yielded chimpanzee DNA. In hopes that this success may help other field workers, we provide some specific details of collection procedures and laboratory analysis. See also Wasser *et al.* 1996 (6) for more details of the collection protocol used.

The decision to collect a fecal specimen required that at least one of several criteria be met: The feces needed to be beneath or nearby a chimpanzee nest judged to be fresh, i.e. the preceding night's bed; or the sample needed to be associated with fresh chimpanzee feeding remains; or we had a sighting of a chimpanzee, and then scrutinized the surrounding area. To collect a sample, we used sterile gloves, sterile tongue depressor, and a 50 ml screw-top, plastic test-tube filled to the 35 ml mark with silica beads, overlaid with a small square of Kim-wipe. The Kim-wipe acted as a divider between the beads and the feces. Wearing a glove and using a tongue depressor, the researcher removed about 5 g of feces from the dung (taking as much from its outer surface as possible, to maximize capture of gut epithelial cells). This was placed in the test-tube, the tube was closed, and it was assigned a unique identification number. Upon return to camp, all test-tube samples were logged in and stored at ambient temperatures (of up to 32 degrees Celsius in shade) in Ziploc bags. The first sample was logged in on 14 Feb. and the final sample entry was 4 April. The samples were taken as hand luggage on the return airline flights to the U.S. They were stored at room temperature at Miami University until early July, and then taken to Germany for DNA analysis.

Extraction and amplification of DNA was carried out under the instruction and direction of Dr. Linda Vigilant, Department of Primatology,

Director, Dr. Christophe Boesch, at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. Fecal samples were processed in groups of 10, along with two negative controls for each set. We weighed out 80-120 mg of each of the 54 Assirik chimpanzee fecal samples. The DNA was extracted using the Qiagen QIAamp DNA stool kit, with protocol modifications made by Dr. Vigilant and her laboratory. We successfully amplified a fragment of the amelogenin gene (7) in 53 of the 54 fecal samples. The sex of each sample was identified by capillary electrophoresis on an ABI 310 PRISM with Gene Scan 2.0 software (Perkin-Elmer Applied Biosystems). We found that of the 53 samples from which DNA was successfully extracted and amplified, 11 were from males. In order to determine community sex composition, one would need to identify the independence of the samples, i.e. which samples came from the same individuals. This could be accomplished by using a combination of highly variable microsatellites (8).

In summary, this simple collection technique outlined above worked well. This method can easily be taught to field assistants; it requires no addition of preservatives to the sample in the field; and it has no special storage constraints. We believe that the relatively short time between collection and laboratory analysis also helped the success rate. Equally important were the expertise, advice, and help rendered by Drs. Linda Vigilant and Karen Chambers, and Brenda Bradley, and Heike Siedel of the Primatology Department mentioned above. We are most grateful to them all.

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