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Excess of non-parental bands in offspring from known primate pedigrees assayed using RAPD PCR

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The random amplified polymorphic DNA (RAPD) method allows the detection of DNA sequence polymorphisms using single primers of arbitrary sequence in the polymerase chain reaction (1). We have attempted to use this technique to assess paternity in a troop of chacma baboons (*Papio cynocephalus ursinus*). We have also examined individuals from known pedigrees of this species and humans. Our results demonstrate that this technique leads to an unacceptable number of non-parental bands within a pedigree, thus raising a serious concern regarding its use in paternity analysis.

Twenty different RAPD oligomers obtained from Operon Ltd were used in the initial screening with the five most variable chosen for further analysis: A16, A17, A18, A19, and A20. A total of 18 field-collected baboon samples and 24 pedigree individuals, including 10 baboons and 14 humans from CEPH pedigree 1468 were examined. PCR was carried out as described by (1) with 0.4 mM $MgCl_2$ using 5–10 ng of genomic DNA and the PCR products separated on agarose gels. Controls were run with either no Taq Polymerase or with no template DNA and produced no visible bands (Figure 1).

Each RAPD primer produced a distinct pattern of amplification products consisting of 3–18 bands ranging in size between 0.25 kb and 6 kb in both humans and baboons (Figure 1). All primers reproducibly amplified products in some offspring which were not found in either parent (i.e., non-parental bands). We examined two nuclear families of olive baboons (*Papio cynocephalus anubis*) each of which included both parents and three offspring (two females, one male). The sires and dams for each family were unrelated. In the two baboon pedigrees, the average number of novel bands per parent–offspring combination was 4.4 and ranged from 1–9 for the five primers. Similarly, a high frequency of non-parental bands was found in the CEPH pedigree for which there is no question of parentage. The average number of novel bands per parent–offspring combination in the human pedigree was 2.7 with a range of 2–4.

Explanations for these results include PCR artifact and genomic mutation. Contamination of samples or reagents is unlikely given our control results and repeatability of novel bands. Polymerase slippage during replication, non-template directed addition of nucleotides by Taq polymerase and the amplification of *in vitro* recombinants may also generate artifactual product bands (4). Genomic mutation could also produce novel bands, but a very high rate of mutation (7–9% per band per generation) would be necessary to generate the number of non-parental fragments we observed. A mutation rate as high as 5% per locus per gamete generation has been reported for the human minisatellite locus D1S7 (5) but most loci show mutation rates at least an order of magnitude lower. The high average band-sharing probabilities we observed for both presumably unrelated humans and baboons (62.8% and 75.9%, respectively) are not consistent with mutation being the sole source of novel bands.

The RAPD technique has proven to be useful in constructing linkage maps and detecting genetic markers in a variety of organisms (1–3). Our results, however, raise serious concerns about the use of the current RAPD technique for paternity assessment. Whether due to mutation or PCR artifact, the high frequency of occurrence of non-parental bands make these genetic markers unsuitable for paternity analysis as they will lead to false exclusions. It is possible that modifications to this method may be forthcoming that will eliminate the problems we have encountered.

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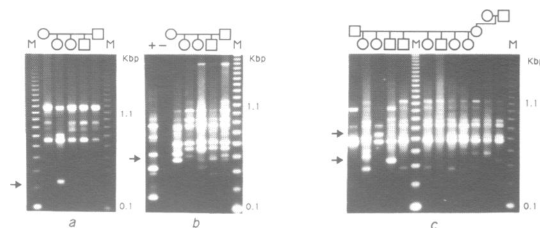

Figure 1. Arnpffication products of primate genomic DNA which demonstrate non-parental bands detected in two baboon and one human pedigree designated a, b and c, respectively. RAPD primers which produced the banding patterns are as follows; A16(a), A19(b) and A20(c). Band sizes are in kilobases (Kbp) as determined by a 123 bp ladder (BRL) run in the lane marked 'M'. + and – designate positive and negative controls. Arrows indicate the position of non-parental bands.