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# Accurate DNA-based sex identification of apes using non-invasive samples

Brenda J. Bradley[1,2,*], Karen E. Chambers[1] & Linda Vigilant[1]
[1]*Max Planck Institute for Evolutionary Anthropology, Inselstrasse 22, Leipzig D-04103, Germany;* [2]*Anthropological Sciences, State University of New York, Stony Brook, NY 11794-4364, U.S.A. (*Author for correspondence: E-mail: bradley@eva.mpg.de)*

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Reliable DNA-based sex identification of wild individuals is becoming a key component of current research in molecular ecology and conservation genetics (Griffiths et al. 1998; Schliebe et al. 1999). In particular, sex identification using DNA from non-invasively collected samples provides new opportunities to improve census methods, determine the sex composition of social groups, and incorporate sex data into ongoing macro-analyses of fecal or hair samples (e.g. parasites: Landsoud-Soukate et al. 1995; diet: Marriott et al. 1996). One demonstration of DNA-based sex assignment in primates focused on the zinc-finger protein (ZFX/ZFY; Wilson and Erlandsson 1998), but the target fragments are of lengths (Y: 729 bp, X: 1151 bp) that cannot typically be amplified from the fragmented template DNA isolated from noninvasive samples. Similarly, amplification of the SRY locus, which is commonly used for sexing mammals (Griffiths and Tiwari 1993), is also not ideal as it is difficult to design controls to verify that non-amplification of the Y marker truly represents a female determination, rather than merely a PCR or primer problem. We describe here the results obtained with an amelogenin assay (Sullivan et al. 1993), commonly used in forensic studies of humans (Cotton et al. 2000), to assign sex to field-collected samples from several ape species. Alleles from the X chromosome are 6 bp shorter than those derived from the Y, a difference distinguishable by capillary or even conventional agarose gel electrophoresis. In addition, since the PCR amplicon is short in total length (X: 106 bp and Y: 112 bp) amplification is feasible using degraded DNA from noninvasive samples.

Faeces from identified individuals of known sex were collected by field researchers studying chimpanzees (*Pan troglodytes verus*) at Taï National Park, Côte d'Ivoire; mountain gorillas (*Gorilla gorilla beringei*) at Karisoke Research Center, Rwanda; and gibbons (*Hylobates lar*) at Khao Yai National Park, Thailand. Sample storage and extraction methodology is described in Bradley et al. (2000). The amounts of amplifiable genomic DNA obtained in extracts were measured using a real-time quantitative PCR assay as previously described (Morin et al. 2001). Due to the high frequency of allelic dropout when amplifying microsatellite loci from reactions containing less than 25 pg of DNA, we avoid using the 5–10% of extracts containing less than 5 pg of DNA per ul. PCR amplification was carried out in a total volume of 15 $\mu$l consisting of 2–5 $\mu$l DNA extract (minimum of 25 pg DNA), 2 mM $MgCl_2$, 12 $\mu$g BSA, 250 $\mu$M each dNTP, 200 nM each primer (AmelA 5′-CCCTGGGCTCTGTAAAGAATAGTG-3′, AmelB 5′-ATCAGAGCTTAAACTGGGAAGCTG-3′; Sullivan et al. 1993), 0.375 U Amplitaq® Gold and 1× PCR buffer (Perkin-Elmer). Amplification conditions were: initial denaturation at 95 °C for 3 minutes; 45 cycles of 30 s at 95 °C, 30s at 60 °C (chimpanzee and gorilla) or 55 °C (gibbon), 30s at 72 °C, final extension of 30 min at 72 °C. The Amel A primer was fluorescently labeled (NED), and amplification products were separated using capillary electrophoresis (ABI 310 PRISM). Allele sizes were scored relative to an internal size standard (HD400) using Gene Scan 2.0 (Perkin-Elmer Applied Biosystems).

We found reliable amplification of the appropriate X and Y allele segments from DNA of numerous individuals of each sex from chimpanzees (29 males, 55 females), gorillas (35 males, 39 females), and gibbons (18 males, 6 females) (Figure 1). Similar successful

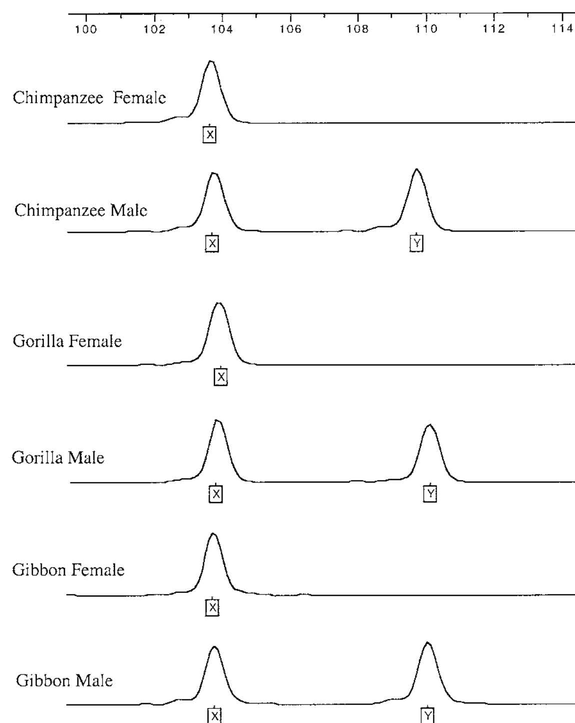


*Figure 1.* ABI electropherogram of amelogenin PCR products from male and female apes. Our system reads the X and Y alleles as 104 bp and 110 bp respectively (in apes and humans), rather than the 106 bp and 112 bp sizing of Sullivan et al. (1993). The 6 bp relative difference between X and Y is nevertheless consistent.

*Table 1a.* Amplification success rate of amelogenin PCR using DNA from faeces

| Species | PCRs attempted | Specific product observed | Reliability |
|---|---|---|---|
| Chimpanzees (*P. t. troglodytes*) | 248 | 234 | 94% |
| Gorillas (*G. g. beringei*) | 424 | 413 | 97% |

*Table 1b.* Accuracy of amelogenin amplification for sex identification

| Species | PCRs | Allele detected* | | | Accurate assignment |
|---|---|---|---|---|---|
| | | X only | Y only | XY | |
| Chimpanzees[1] (*P. t. troglodytes*) | 102 | 2 (2%) | 7 (7%) | 93 (91%) | 98% |
| Gorillas[2] (*G. g. beringei*) | 156 | 4 (3%) | 4 (3%) | 148 (95%) | 97% |

*Only amplifications using DNA from males are included as females score the same with and without allelic dropout.
[1]Using 50 faecal extractions from 29 individual chimpanzee males. The 2 X-only amplifications are from 2 males and the 7 Y-only amplifications are from 6 males.
[2]Using 50 faecal extractions from 35 individual gorilla males. The 4 X-only amplifications are from 4 males and the 4 Y-only amplifications are from 3 males.
All amplifications started from at least 25 pg of genomic DNA, the amount identified as the minimal level of DNA needed for accurate microsatellite genotypes (Morin et al. 2001).

results were obtained in amelogenin typing of gorillas and chimpanzees of other subspecies (*P. t. schweinfurthii, G. g. gorilla, G. g. graueri*) as well as bonobos (*P. paniscus*) (data not shown).

A high rate of amplification success was seen using a large number of independent PCR reactions from chimpanzee and gorilla faecal DNAs (Table 1a), with PCR products obtained in 94% and 97% of PCRs, respectively. Genotyping errors associated with nonamplification of the Y allele were infrequent (Table 1b), and males were properly identified (i.e. the sample amplified both the XY alleles or the Y allele) in 97–98% of PCRs. Spurious amplification of the Y allele in DNA from a female occurred in only 1 of 185 PCRs for chimpanzees and 2 of 178 PCRs for gorillas, giving an error rate of 0.5–1.1%. This can likely be attributed to sporadic lab contamination, as numerous (>6) additional PCRs from the same extracts amplified only the X allele. This low incidence of erroneous amplifications indicates that if PCR reactions are set up in duplicate or triplicate so that each extraction is scored at least twice, the probability of misassigning sex is extremely low.

Similar success rates were obtained by application of this assay to DNA derived from various sample types (e.g. single shed hairs and bones from chimpanzees) as well as to faecal samples from unhabituated groups of western lowland gorillas, eastern lowland gorillas and western chimpanzees. An unresolved question is the extent to which this assay will function in other species of Old and New World primates. Preliminary investigations in our laboratory reveal that this assay fails to distinguish male and female orangutans (*Pongo pygmaeus*), and redesign of primers or the choice of new target segments would likely be necessary for this and possibly other species.

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