

## BRIEF REPORT

# Genotyping Aids Field Study of Unhabituated Wild Chimpanzees

W.C. MCGREW<sup>1,2\*</sup>, A.L. ENSMINGER<sup>1,3</sup>, L.F. MARCHANT<sup>3</sup>, J.D. PRUETZ<sup>1,4</sup>,  
AND L. VIGILANT<sup>5</sup>

<sup>1</sup>Department of Zoology, Miami University, Oxford, Ohio

<sup>2</sup>Department of Anthropology, Miami University, Oxford, Ohio

<sup>3</sup>Department of Biology, University of Kentucky, Lexington, Kentucky

<sup>4</sup>Department of Anthropology, Iowa State University, Ames, Iowa

<sup>5</sup>Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

Prolonged habituation times for wild great apes delay the collection of behavioral and environmental data, sometimes for years. However, genotyping of noninvasively collected feces can provide useful socio-ecological information in the meantime. We tested this premise on an unhabituated wild population of western chimpanzees (*Pan troglodytes verus*) at Mont Assirik, Senegal. Genotyping yielded information on kinship, group size, party size and composition, sex ratio, and ranging. *Am. J. Primatol.* 63:87–93, 2004. © 2004 Wiley-Liss, Inc.

**Key words:** chimpanzee; genotype; habituation; Assirik; *Pan troglodytes verus*; noninvasive

## INTRODUCTION

Few field studies of wild chimpanzees (*Pan troglodytes*) have involved subjects that were fully habituated to close-range observation. Many such studies require habituation of the animals, but this may take years to accomplish if the subjects are wary or elusive [Williamson & Feistner, 2003]. Field primatological knowledge increases in proportion to the degree of habituation, but what are most field researchers to do until it is achieved? Here we report an alternative strategy for collecting data that can be used while the apes remain unhabituated.

Contract grant sponsor: Rebecca Jeanne Andrew Memorial Award (Miami University); Contract grant sponsor: Hampton Fund (Miami University); Contract grant sponsor: National Geographic Society; Contract grant sponsor: Primate Conservation Inc.; Contract grant sponsor: Max Planck Gesellschaft; Contract grant sponsor: National Science Foundation; Contract grant number: BSC-0122518.

\*Correspondence to: W.C. McGrew, Dept. of Zoology, Miami University, Oxford, OH 45056.  
E-mail: mcgrewwc@muohio.edu

Received 12 July 2003; revision accepted 13 April 2004

DOI 10.1002/ajp.20041

Published online in Wiley InterScience (www.interscience.wiley.com).

The classic method for habituating chimpanzees is to provision them with prized food items, especially cultigens; however, this has been rejected on ethical and health grounds. Radiotracking has apparently never been tried in this regard, perhaps because it also entails ethical and logistical constraints. Meanwhile, habituation is sought from repeated neutral contact between observers and apes, so that tolerance for humans is gradually established. However, this method requires that such encounters occur frequently enough for the apes to learn that the primatologists are harmless. Such encounter rates depend at the very least on the observers being able to *find* the chimpanzees.

Various factors can increase encounter rates, such as skillful trackers, noisy ape activities (e.g., nut-cracking), open habitat with good visibility, etc. Researchers have employed all of these factors to facilitate habituation of forest chimpanzees, and much of what we know about wild chimpanzee behavior comes from direct observation of chimpanzees living in forested habitats [Boesch & Boesch-Achermann, 2000; Goodall, 1986; Nishida, 1990]. However, a sizable number of chimpanzees live in arid, open savannah environments, and direct, sustained observations of these chimpanzees have proven difficult [Moore, 1992]. This has hindered our understanding of how chimpanzee behavior, which has been shown to exhibit cultural variation among forested field sites, differs in the savannah [Whiten et al., 1999]. A notable problem that confronts students of savanna chimpanzees is widespread ranging. While the home ranges of forest-dwelling chimpanzees typically cover a few tens of kilometers, savanna-dwelling chimpanzees may range over a few hundreds of kilometers [Baldwin et al., 1982]. It is probably impossible for human observers on foot to cover such big areas and to meet wild chimpanzees often enough to habituate them fully.

So, what are researchers of savanna chimpanzees to do? One possibility is to enlist the help of recent advances in modern molecular genetics. Genetic analyses of habituated wild chimpanzees have been successfully applied to determine genetic relationships among known individuals [Constable et al., 2001; Morin et al., 1994; Vigilant et al., 2001], as well as for long-term monitoring of orphan chimpanzees released into the wild [Goosens et al., 2003]. The aim of this study was to show how genotyping of wild chimpanzees by noninvasive means [Vigilant, 2002] can enable the tracking of individuals through repeated identifications, sex determination, and identification of related dyads. This approach can provide valuable socioecological data long before either identification or habituation is achieved by standard observational methods.

## MATERIALS AND METHODS

While DNA can be obtained from many biological materials, the most useful of these (e.g., blood and tissue) are unavailable from living, free-ranging subjects unless invasive methods are employed [Morin et al., 2001; Woodruff, 1993]. To avoid altering the natural behavior of the study subjects, it is preferable to collect noninvasive samples consisting of materials left naturally by the apes, such as feces, hair, urine, or food-wedges. The challenge is to collect uncontaminated material in sufficient amounts, store it successfully under field conditions, transport it safely to the laboratory, and extract the DNA.

Although chimpanzees defecate several times a day, observers of unhabituated chimpanzees rarely see this activity. Field identification of chimpanzee feces is aided by indirect evidence of deposition that distinguishes ape dung from that of baboons, pigs, etc. Apes most predictably defecate early in the morning, just after they arise; therefore, fresh feces found below nests that were occupied

the night before is the best source of such evidence. Similar arguments apply to fresh feces found at sites where chimpanzees were recently seen or heard, or on the trail on which they were being tracked. We collected specimens found in such circumstances, and assumed that the specimens were a random sample of feces deposited by the focal population.

Three of the present authors (L.F.M., W.C.M., and J.D.P.) studied wild chimpanzees (*P.t. verus*) at Mont Assirik, in the Parc National du Niokolo-Koba, Republique du Senegal, between February and April 2000 [Pruetz et al., 2002]. A total of 54 putative chimpanzee fecal specimens were collected for genetic analysis, mostly in the two main valleys (Stella's Valley and Lion Valley) that drain from Mt. Assirik. Earlier studies there showed that these animals were the widest-ranging chimpanzees known, and were not fully habituated after 44 months of field research [Baldwin et al., 1982; McGrew et al., 1981]. From 1976 to 1979, Tutin et al. [1983] studied one community of chimpanzees of about 25 members at Assirik.

Three of the present authors (A.L.E., L.F.M., and L.V.) extracted DNA from the 54 samples, as detailed in Marchant et al. [2000]. In brief, 100 mg of feces that had been stored dry (by dessication over silica gel beads) were extracted with the use of a QIAamp DNA stool kit (Qiagen) according to the manufacturer's instructions [Bradley et al., 2000]. A polymerase chain reaction (PCR)-based amelogenin assay was used for sex determination, and positive results were obtained from 53 of 54 (98%) extracts [Bradley et al., 2001; Sullivan et al., 1993]. An assay measuring the amount of amplifiable nuclear DNA present in the extracts was conducted [Morin et al., 2001], and the 32 extracts containing adequate concentrations of DNA for reliable genotyping analysis were identified. These extracts were genotyped at nine microsatellite loci that were previously described as being highly variable in western chimpanzees [Bradley et al., 2000].

## RESULTS

Eleven of the 32 samples genotyped poorly, perhaps due to the presence of PCR inhibitors, and hence 21 genotypes were obtained. Of the 21 genotyped specimens, four were collected outside the two valleys: three (specimens 46, 47, and 50) were within the estimated range of the Assirik chimpanzees, and one (specimen 55) was outside that range. The 21 samples comprised 16 unique genotypes (i.e., 12 genotypes were obtained once, three different genotypes were obtained twice, and one genotype was obtained three times; Table I). The three different male genotypes were each obtained once, and there were 13 different female genotypes. To determine whether samples with identical genotypes were indeed from the same individuals, or were merely from individuals of the same population that happened to have identical genotypes, we calculated the probability of individual identification [Waits et al., 2001]. The unbiased probability of identity (i.e., the probability that two individuals drawn from this population would have the same genotype at all loci) was  $1.09 \times 10^{-8}$ . A more stringent case is to consider the probability that two siblings drawn from the same population have identical genotypes, but this was also very low with our data (0.00079). Thus, it is highly likely that all cases in which identical genotypes were obtained from different samples are cases of the same individual being recognized.

### Individual Genotyping

The results from individual genotyping are socioecologically informative in several ways, as described below.

**TABLE I. Identification of Individuals in the Chimpanzee Community at Mt. Assirik, Senegal, Genotyped From Fecal Specimens**

Individual	Fecal specimen no.	Collection date	Valley
F1	3	16 Feb.	S
F2	4	20 Feb.	S
F3	10	21 Feb.	L
(F3)	14	24 Feb.	L
F4	20	26 Feb.	L
F5	24	6 Mar.	L
(F1)	25	6 Mar.	L
F6	27	6 Mar.	L
F7	29	6 Mar.	L
(F2)	30	6 Mar.	L
M1	32	6 Mar.	L
F8	35	6 Mar.	L
M2	36	6 Mar.	L
(F5)	46	2 Mar.	A
F9	47	2 Mar.	A
F10	50	10 Mar.	A
M3	55	Mar.	O
F11	57	22 Mar.	L
F12	63	3 Apr.	L
F13	65	3 Apr.	L
(F2)	69	4 Apr.	S

F, female; M, male; S, Stella's Valley; L, lion Valley; A, Assirik but elsewhere in range; O, outside Assirik range.

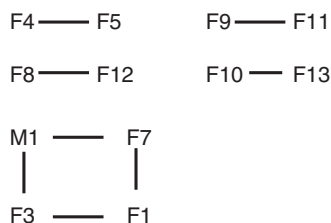


Fig. 1. Graphical representation of individuals representing putative parent-offspring pairs. The lines link individuals that share at least one allele at each microsatellite locus. No representation of the geographical distribution of individuals is intended.

### *Kinship*

Because of the small number of individuals in the data set, we were unable to conduct an extensive analysis of kin relationships. However, one can compare pairs of genotypes in order to identify cases in which two individuals share an allele at every locus and thus represent putative parent-offspring pairs. Conversely, dyads that do not share an allele at every locus cannot be parent-offspring pairs. Four pairs of putative mother-daughter dyads were identified (F4-F5, F8-F12, F9-F11, and F10-F13) (Fig. 1), but the lack of information on the relative ages of the individuals prevented us from determining which individuals were the likely mothers and which were the likely daughters. Four additional individuals, including one male, were found to share alleles at all loci with two other individuals in the data set (Fig. 1). This pattern of allele-sharing is

consistent with a scenario in which M1 and F1 are the parents of the two females (F3 and F7).

#### *Group size*

Excluding one individual (M3) because he was sampled outside of the community range, the Assirik community of chimpanzees numbered at least 15 individuals. Furthermore, we can say that the actual size of the community was probably larger than this minimum number. There was no greater tendency toward duplications of genotypes in the second half of the chronological series (#11-21, excluding #55) than in the first half (#1-10), which suggests that no asymptote of total community size was close to being reached.

#### *Party size and composition*

As is typical of fission-fusion species, the members of a chimpanzee community are rarely (if ever) together at the same time. Instead, they form fluid parties during the day, and nesting parties at night. Researchers have derived nighttime party size by counting nests in a fresh nest group the following morning, and by assuming that the number of nests equals the number of weaned individuals that were present overnight. Comparisons of nest counts to the numbers of habituated chimpanzees have established this as a valid approach, and by genotyping these unhabituated chimpanzees we were able to perform similar tests.

On 6 March 2000, we found a fresh nest site ( $n = 12$  nests) in Lion Valley and collected 12 fecal specimens, eight of which were suitable for DNA analysis. As shown in Table I, these eight samples come from six females and two males, with no duplication. This lack of duplication within a nesting party supports the assumption that for this population, the number of nests found in a morning is a reliable indicator of the number of weaned individuals that nested there the previous night.

#### *Sex ratio*

At 2:13, the sex ratio of 1:6.5 male per females is notably larger than the usual range of sex ratios found in other well-known populations, such as Gombe (1:1.35 [Goodall, 1986]), Tai (1:3 [Boesch & Boesch-Achermann, 2000]), and Mahale (1.36 [Nishida, 1990]). We suspect that the discrepancy between the Assirik sex ratio and the other populations is an artifact of the small sample size; however, only further data will clarify this point.

#### *Ranging*

Fourteen of the 21 fecal specimens came from Lion Valley. However, the three specimens obtained from Stella's Valley are of particular interest. One female (F1) was found in Stella's Valley on 16 February, and in Lion Valley 18 days later. Female 2 was found in Stella's Valley on 20 February, and then in Lion Valley 14 and 41 days later. This is conclusive evidence that the two valleys on opposite sides of Mt. Assirik are used by the same individuals. The closest points between the two valleys are about 7 km apart, in straight-line distance. Female 5 was found in Lion Valley on 6 March, but she had been in Cross Valley 4 days earlier. The straight-line distance between the two valleys is about 3 km. This links the core study area to its periphery.

## DISCUSSION

We compared the amount of genetic variation found in this sample of Assirik chimpanzees with that estimated by the same methods in another chimpanzee population in West Africa [Boesch & Boesch-Achermann, 2000]. The mean expected heterozygosity for the nine markers analyzed in the 15 Assirik chimpanzees was 0.764. A previous study [Bradley et al., 2000] of 45 western chimpanzees from the Tai Forest, Cote d'Ivoire, reported a very similar heterozygosity of 0.790. By revealing similar levels of genetic variability, this comparison implies that the demographic history of the two populations is broadly similar.

In sum, 21 genotyped specimens collected over 47 days yielded new information on five key aspects of chimpanzee socioecology, which substantially enhances earlier data collected over 44 months of field study [McGrew et al., 1981]. Further analysis of the same genetic material could yield even more information (e.g., regarding inbreeding) [Vigilant, 2002]. While such methods are not cheap, they provide useful and predictable information, and thus they are a valuable addition to field primatological techniques.

## ACKNOWLEDGMENTS

We thank J. Arno for field assistance, B. Bradley for laboratory assistance, D. Lukas for analytical assistance, D. Deaton for word processing, and the Direction des Parcs Nationaux du Sénégal for permission to do the field research.

## REFERENCES

- Baldwin PJ, McGrew WC, Tutin CEG. 1982. Wide-ranging chimpanzees at Mt. Assirik, Senegal. *Int J Primatol* 3:367–385.
- Boesch C, Boesch-Achermann H. 2000. *The chimpanzees of the Tai Forest*. Oxford: Oxford University Press. 316p.
- Bradley BJ, Boesch C, Vigilant L. 2000. Identification and redesign of human microsatellite markers for genotyping wild chimpanzee (*Pan troglodytes verus*) and gorilla (*Gorilla gorilla gorilla*) DNA from faeces. *Conserv Genet* 1:289–292.
- Bradley BJ, Chambers KE, Vigilant L. 2001. Accurate DNA-based sex identification of apes using non-invasive samples. *Conserv Genet* 2:179–181.
- Constable JL, Ashley MV, Goodall J, Pusey AE. 2001. Noninvasive paternity assignment in Gombe chimpanzees. *Mol Ecol* 10:1279–1300.
- Goodall J. 1986. *The chimpanzees of Gombe*. Cambridge, MA: Harvard University Press. 673p.
- Goosens B, Setchell JM, Vidal C, Dilambaka E, Jamart A. 2003. Successful reproduction in wild-released orphan chimpanzees (*Pan troglodytes troglodytes*). *Primates* 44:67–69.
- Marchant LF, Ensminger A, Pruettz J, McGrew WC. 2000. Highly successful, non-invasive collection of DNA from wild chimpanzees. *Pan Africa News* 7:20–21.
- McGrew WC, Baldwin PJ, Tutin CEG. 1981. Chimpanzees in a hot, dry and open habitat: Mt. Assirik, Senegal, West Africa. *J Hum Evol* 10:227–244.
- Moore J. 1992. "Savanna" chimpanzees. In: Nishida T, editor. *Topics in primatology*. Vol. I. Human origins. Tokyo: University of Tokyo Press. p 99–118.
- Morin PA, Moore JJ, Chakraborty R, Jin L, Goodall J, Woodruff DS. 1994. Kin selection, social structure, gene flow, and the evolution of chimpanzees. *Science* 265:1193–1201.
- Morin PA, Chambers KE, Boesch C, Vigilant L. 2001. Quantitative PCR analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes verus*). *Mol Ecol* 10:1835–1844.
- Nishida T, editor. 1990. *The chimpanzees of the Mahale mountains*. Tokyo: University of Tokyo Press. 328p.
- Pruettz JD, Marchant LF, Arno J, McGrew WC. 2002. Survey of savanna chimpanzees (*Pan troglodytes verus*) in southeastern Senegal. *Am J Primatol* 58:35–43.
- Sullivan KM, Mannucci A, Kimpton CP, Gill P. 1993. A rapid and quantitative DNA sex test—fluorescence-based PCR analysis of X-Y homologous gene amelogenin. *Biotechniques* 15:636–641.
- Tutin CEG, McGrew WC, Baldwin PJ. 1983. Social organization of savanna-dwelling chimpanzees, *Pan troglodytes verus*, at Mt. Assirik, Senegal. *Primates* 24:154–173.

- Vigilant L, Hofreiter M, Siedel C, Boesch C. 2001. Paternity and relatedness in wild chimpanzee communities. *Proc Natl Acad Sci USA* 98:12890–12895.
- Vigilant L. 2002. Technical challenges in the microsatellite genotyping of a wild chimpanzee population using feces. *Evol Anthropol* 11(Suppl 1):162–165.
- Waits L, Luikart G, Taberlet P. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol Ecol* 10:249–256.
- Whiten A, Goodall J, McGrew WC, Nishida T, Reynolds V, Sugiyama Y, Tutin CEG, Wrangham RW, Boesch C. 1999. Cultures in chimpanzees. *Nature* 399:682–685.
- Williamson EA, Feistner ATC. 2003. Habituating primates: processes, techniques, variables and ethics. In: Setchell JM, editor. *Field and laboratory methods in primatology: a practical guide*. Cambridge: Cambridge University Press. p 25–39.
- Woodruff D. 1993. Non-invasive genotyping of primates. *Primates* 34:333–346.