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DNA Fingerprinting and the Problems of Paternity Determination in an Inbred Captive Population of Guinea Baboons (*Papio hamadryas papio*)

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ABSTRACT. Multilocus DNA fingerprinting was carried out on 65 individuals from a captive colony of guinea baboons (*Papio hamadryas papio*) at Brookfield Zoo, in order to determine the allocation of reproductive success among 7 active males. DNA fingerprinting was found to reveal very low levels of genetic variability in the study population, rendering discrimination of different levels of relatedness, and hence paternity, impossible. A method was therefore developed for emphasizing the region of the fingerprint pattern which revealed the greatest level of band variability, and the effect of this experimental modification on band sharing statistics was tested. Band sharing coefficients among unrelated individuals were significantly lower using the modified system, which was then applied to paternity testing in the whole population. However even when using the modified system, of the 33 offspring analyzed only 4 could be assigned solely to 1 male, 14 offspring were assigned to 1 of 2 males, 7 offspring had 3 potential fathers, and the remainder had 4 or more possible fathers. The implications of the limitations of these data for behavioural studies and genetic management of captive populations are discussed.

Key Words: DNA fingerprinting; Baboons; Inbreeding; Paternity.

INTRODUCTION

Between 1985 and 1988, detailed observations of behavioural affinities and matings were carried out on a captive population of guinea baboons (*Papio hamadryas papio*) housed on 'Baboon Island' at Brookfield Zoo (SAMUELS & ALTMANN, unpubl.). The colony was founded in 1938 with 58 individuals, which were supplemented in 1939 and 1940 with additional females, most of which were guinea baboons, but some were possibly olive baboons (*P. h. anubis*) and hamadryas baboons (*P. h. hamadryas*). Animals have been periodically removed throughout the colony's history, particularly in the 1960's when many males and all individuals which were atypical of the guinea baboon phenotype were removed. Since that time 40–60 baboons have been continuously kept on the island, with about half being adult, and at no time since 1940 have any unrelated individuals been introduced into the colony. It has been presumed, therefore that there is considerable inbreeding in this population (LACY & FOSTER, 1988). Since 1970, maternity records have been kept, but the presence of multiple reproductively active males in the colony has prevented the assignment of paternity to specific individuals (LACY & FOSTER, 1988; TURNER et al., 1992). During the period of this study seven males were actually or potentially reproductively active.

LACY and FOSTER (1988) have attempted to determine paternities in this colony using protein electrophoresis, however this proved impossible as of the 32 loci examined, 31 were invariant. Mean heterozygosity was 1.0%, compared with values from wild baboon populations of 3.9% for *P. h. papio* (LUCOTTE, 1979), 1.8% (LUCOTTE, 1979), 1.7%, and 2.9% (SHOTAKE, 1981) for *P. h. anubis*, 2.8% for *P. h. cynocephalus* (LUCOTTE, 1979), 3.6% and 4.5% (SHOTAKE, 1981), and 5.0% (LUCOTTE, 1979) for *P. h. hamadryas*. These data would indicate that the Baboon Island population suffers depressed levels of genetic variability due probably both to inbreeding and genetic bottlenecks caused by aspects of colony management.

One possible means of uncovering genetic variability in the Baboon Island population, not detected by protein electrophoresis, is to utilize DNA sequences known to be highly variable. To date the most polymorphic DNA loci known comprise a class of hypervariable tandem-repetitive sequences known variously as VNTRs (Variable Number of Tandem Repeats) or minisatellites (WYMAN & WHITE, 1980; JEFFREYS et al., 1985a; NAKAMURA et al., 1987) which mutate rapidly by the gain or loss of different numbers of repeat units. Related sequences of this type are most commonly revealed simultaneously in a multilocus DNA fingerprint (JEFFREYS et al., 1985a, b; see WICKINGS, 1993). Recently, TURNER et al. (1992) carried out multilocus DNA fingerprinting on four randomly chosen individuals from the Baboon Island population, using probes 33.6 and 33.15 (JEFFREYS et al., 1985a, b). Very little variation was apparent in the 33.15 fingerprint pattern, which was not thought to be specific to the individuals analyzed.

In this paper we describe a further study in this population using DNA fingerprinting, designed both to quantify any residual genetic variation occurring among the colony members, and to test whether a combination of different multilocus minisatellite probes could be used to unequivocally identify fathers in this inbred population.

MATERIALS AND METHODS

DNA fingerprinting was carried out using the general protocols described in BRUFORD et al. (1992), with the following modifications specific for guinea baboon fingerprints. DNA was extracted from 3 ml of frozen baboon blood with: (1) an equal volume of phenol; (2) an equal volume of phenol/chloroform; and (3) a similar volume of chloroform:isoamyl alcohol (JEFFREYS & MORTON, 1987). The DNA was subsequently precipitated in two volumes of 100% ethanol, and dissolved in TE (10 mM Tris, 1 mM EDTA pH8.0).

PROBE/ENZYME TEST GEL

Five μg samples of high molecular weight DNA were taken from four individuals belonging to different family groups (based on matrilines traced back 15 years) and were digested separately with *Hinf* I, *Hae* III, *Alu* I, and *Mbo* I. The digested DNA was electrophoresed at 1.5 v/cm through a 25 cm, 1% agarose gel until 2 kb fragments had migrated 200 mm, and was blotted onto a nylon membrane under vacuum. The membrane was probed with 33.6 and 33.15 (JEFFREYS et al., 1985a) in phosphate hybridization buffer (WESTNEAT et al., 1988; BRUFORD et al., 1992) and washed at low stringency (0.26 M Na_2HPO_4, 1% SDS for 15 min., 2 × SSC, 0.1% SDS for 15 min., both at 60°C). Autoradiographic film was exposed for between two and five days before being developed.

Population Fingerprint Gels

Five μg samples were digested to completion with *Hinf* I and electrophoresed at 1.5 v/cm through a 25 or 30 cm, 1% agarose gel until all 2 kb fragments had migrated approximately 200 mm. A modified system was then developed, where gels were electrophoresed at 2 v/cm through a 25 cm, 1% gel for an extended period until 4 kb fragments had migrated at least 200 mm. All other conditions were carried out as described previously except hybridization and washing, which was carried out at 58°C. Gels were loaded with mother/offspring pairs in adjacent lanes, and all seven possible fathers loaded at the end of each gel; inter-gel comparisons were not attempted.

DNA fingerprint patterns were analyzed as described in Bruford et al. (1992). Pairwise comparisons were made between mothers and offspring, and each possible father and all offspring. Band sharing coefficients were calculated and used as a measure of genetic similarity among individuals. Unique, diagnostic fingerprint bands were also used, where present, to assign paternity. Bands were scored as being shared where the difference in their migration distances was no greater than 0.5 mm from the origin, and where there was no more than a two-fold intensity difference among them.

RESULTS

Probe/enzyme Test Gel

Fingerprint bands were scored, and band sharing coefficients were calculated for each pair-wise comparison of adjacently loaded samples for all eight probe/restriction enzyme

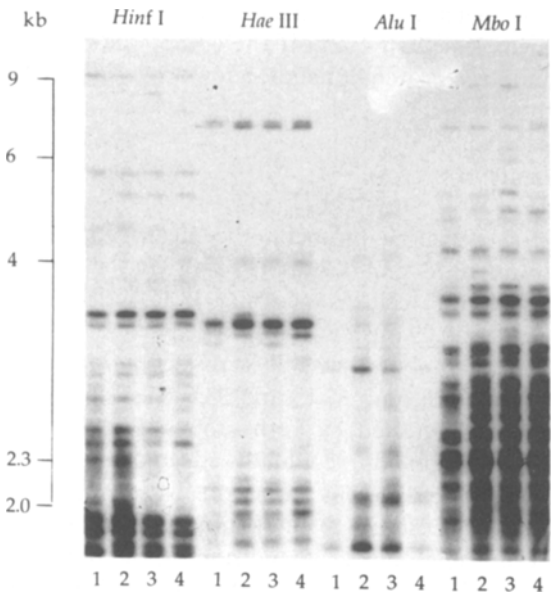

Fig. 1. DNA fingerprint of four randomly chosen baboons digested separately with *Hinf* I, *Hae* III, *Alu* I, and *Mbo* I, and probed with 33.15.

Table 1. DNA fingerprint data for probe/enzyme test gel.

Enzyme	Mean number of bands (±s.d., $n=4$)		Mean band sharing coefficient (±s.d., $n=3$ comparisons)	
	33.15	33.6	33.15	33.6
Hinf I	23.50±2.6	20.25±1.8	0.854±0.04	0.832±0.06
Hae III	17.75±2.5	22.50±2.4	0.831±0.03	0.802±0.03
Alu I	12.25±3.3	13.75±2.5	0.750±0.05	0.786±0.04
Mbo I	31.25±3.4	26.20±4.2	0.801±0.04	0.775±0.04

comparisons (n=three comparisons for each probe/restriction enzyme combination). Figure 1 shows the 33.15 autoradiogram, and Table 1 summarizes the band sharing results for both probes. The number of bands scored (N) ranged from 12.25 for *Alu* I digested DNA probed with 33.15 to 31.25 for *Mbo* I digested DNA probed with 33.15. Mean band sharing coefficients (x) were uniformly extremely high, ranging between 0.75 and 0.85 with different probe/restriction enzyme combinations ($x=[(N_{ab}/N_a)+(N_{ab}/N_b)]/2$, JEFFREYS et al., 1985b; BURKE & BRUFORD, 1987). *Hinf* I was the restriction enzyme selected for future analyses as it gave the best compromise between high band resolution and the number of bands that could be unambiguously scored (see Fig. 1, BRUFORD et al., 1992), although *Mbo* I also gave a highly informative pattern. A feature of all enzymes was the relative low intensity of high molecular weight bands compared with low molecular weight bands.

A prominent feature of the most informative enzyme systems in this population (*Hinf* I and *Mbo* I) was that fingerprint bands below 4 kb were almost invariant. In another experiment, DNA from 20 individuals, chosen randomly from the population, was digested with *Hinf* I and probed with 33.15 and 33.6, and had a mean band sharing coefficient of 0.836. The mean number of bands scored was 29.38, however of these, 15 bands were totally invariant (data not shown). Therefore for further analyses, the protocol was modified to electrophorese all fragments less than 4 kb off the end of the gel. To compensate for the low intensity of the high molecular weight bands, a lower hybridization stringency was used (see Materials and Methods).

POPULATION DNA FINGERPRINTS

Using the modified fingerprinting protocol, the mean number of bands scored per individual using 33.15 was 16.73±3.17 (s.d.) (n=54), and using 33.6 was 10.44±2.47 (n=48). The mean band sharing coefficient using 33.15 was 0.608±0.086 (s.d.) (n=45 comparisons), and was 0.614±0.094 using 33.6 (band sharing coefficients were calculated for adjacent lane comparisons only). Unusually, cross comparison of autoradiograms of the same membranes probed with both 33.15 and 33.6 revealed a considerable degree (up to 50%) of overlap of bands detected by both probes. For this reason, only the 33.15 bands and non-overlapping 33.6 bands were analyzed, to avoid non-independence among sets of bands detected by the two different probes (JEFFREYS, BROOKFIELD, & SEMEONOFF, 1985; JEFFREYS et al., 1985a, b; BURKE & BRUFORD, 1987). The mean band sharing coefficient for the population using only 33.15 and non-overlapping 33.6 bands was 0.610± 0.074 (s.d.) and the corresponding mean number of bands analyzed was 20.93±3.64. Using these data, the probability of identity $p=3.2\times10^{-5}$ ($p=x^n$, where x=the mean band sharing coefficient and n=the mean number of bands per individual, JEFFREYS et al.,

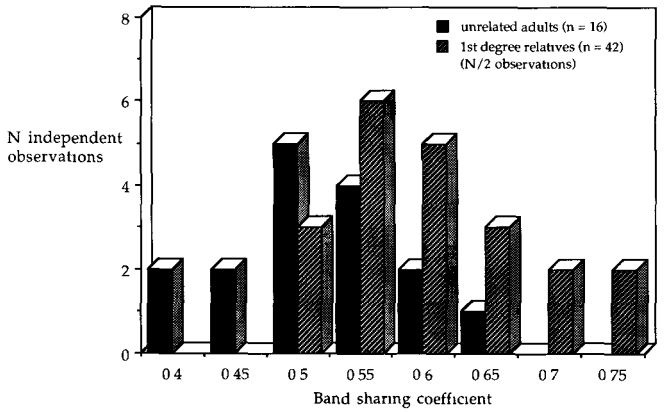

Fig. 2. Band sharing coefficient frequency distributions for first degree relatives and unrelated individuals.

1985b; BRUFORD et al., 1992), and the mean allele frequency per locus $q=0.376$ (where q is related to the mean band sharing coefficient x by $x=2q-q^2$, JEFFREYS et al., 1985b). The mean number of alleles per locus, which is the reciprocal of the mean allele frequency was 2.65.

To investigate the likelihood of distinguishing first degree relatives (i.e. mother/offspring and sib/sib pairs) from "unrelated" individuals (i.e. from different matrilines) band sharing frequency distributions were derived for these two subsets of the population. The distributions are shown on the histogram in Figure 2. A large degree of overlap was observed between the two relatedness classes, and only the tails of the two distributions were mutually exclusive. Mean band sharing coefficients were calculated for unrelated individuals [0.537 ± 0.094 (s.d.)] and first degree relatives [0.649 ± 0.093 (s.d.)]. The *expected* mean band sharing coefficient for first degree relatives can be predicted, based on the mean allele frequency in unrelated individuals q_{ur} which was 0.319. The expected mean band sharing coefficient for first-order relatives was 0.724 [where $q_{1st}=(1+q-q^2)/(2\text{-}q)$, BRUFORD et al., 1992] considerably higher than the observed mean value (though within one standard deviation).

PATERNITY ANALYSIS

Due to the unreliability of assigning first-order relatives (and therefore father-offspring pairs) in the population, the seven potential fathers and known mothers were compared with all offspring for the presence of unique diagnostic bands which would unequivocally assign paternity. Figure 3 shows an example autoradiogram of a population fingerprint

Table 2. Paternity inclusion data in the Baboon Island offspring, on the basis of the possession of one or two unique paternal alleles.

	Number of possible fathers			
	1 father	2 fathers	3 fathers	4+ fathers
Number of offspring (n/33)	4*	14@	7§	8

*Two different males positively assigned, paternity divided 2:2; @ four different males implicated, including those positively assigned; § the same four males implicated.

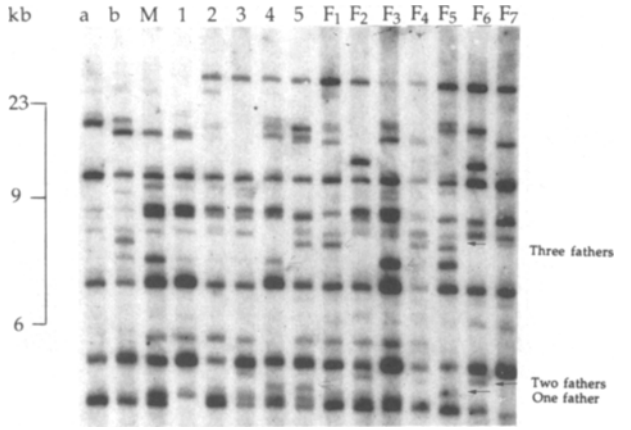

Fig. 3. DNA fingerprint of two siblings (a and b), a mother (M), four offspring (1, 2, 3, and 4), a grandchild (5), and the seven potential fathers (F_1, F_2, F_3, F_4, F_5, F_6, and F_7).

probed with 33.6. Examples of bands present in one, two, and three potential fathers, (but not the mother) and their segregation in several offspring are arrowed. Using this approach, offspring were classed into four categories dependent on the number of possible fathers that could be assigned on the fingerprints. Only 4 offspring out of a total of 33 could be positively assigned to fathers on the basis of the transmission of one or two bands unique to one male parent (see Table 2). Of these offspring paternity was assigned in one individual due to the presence of two unique bands, whereas only one diagnostic band was present in the other three offspring.

DISCUSSION

The results of this study illustrate the problems of measuring relatedness within inbred populations. The Baboon Island colony clearly has an extremely low level of genetic variability, a fact already apparent from previous studies (LACY & FOSTER, 1988; TURNER et al., 1992). Such low levels of genetic variability revealed by DNA fingerprinting are extremely rare in natural populations, except where the underlying social system produces highly inbred individuals (e.g. FAULKES et al., 1990; REEVE et al., 1990) or founder effect, genetic drift, and population bottlenecks have contributed (GILBERT et al., 1990; WAYNE et al., 1991; PACKER et al., 1991), and are normally confined to deliberately inbred lines of model species (JEFFREYS et al., 1987; KUHNLEIN et al., 1989, 1990). The occurrence of low genetic variability in captive populations of wild species is now becoming apparent (e.g. HANOTTE et al., 1991; MORIN & RYDER, 1991; BRUFORD et al., 1991) and is almost certainly due to a lack of outbreeding genetic management by the institutions in question, either by the over-representation of successful captive breeders or because of the lack of intervention to prevent inbreeding. A combination of both of these effects has led to the Baboon Island colony being highly inbred. An alternative explanation for the low level of genetic variability observed is that it represents a species-wide phenomenon, i.e. that all populations show depressed levels of genetic polymorphism. However, to date, no data are available on band sharing coefficients in wild guinea baboon populations for comparison with Baboon Island.

The mean band sharing coefficient of 0.836 for bands greater than 2 kb precluded any form of relatedness analysis due to the low numbers of individuals in the population combined with the very small expected differences in band sharing coefficient between relatedness classes. However, in common with many other species, the variability of bands within the fingerprint pattern appeared to be length-dependent (BURKE et al., 1991), with the more variable loci being between 4 kb and 18 kb. In this case, bands below 4 kb long were unusually totally invariant.

The removal of the invariant bands and further electrophoretic separation of the higher molecular weight variable bands, improving the resolution of subtle molecular weight differences, had the combined effect of reducing the mean population band sharing coefficient from 0.836 to 0.610, allowing the possibility of the discrimination of relatedness in certain cases. However, the combined effects of the still elevated band sharing value and of conducting the analysis across generations, rendered the unequivocal distinction between relatedness classes impossible, with only the band sharing coefficients at the tails of the distribution (less than 0.45 for unrelated individuals and greater than 0.65 for first-order relatives) proving diagnostic. In addition, the frequencies of the first-order relative class were not normally distributed, as would be expected in a random sample of individuals taken from an outbred population.

Because of the poor resolution of relatedness using band sharing coefficients in the population, the presence of rare or unique paternal alleles was investigated as a means of assigning offspring to fathers. The mean allele frequency in the population fingerprint was 0.376, and the mean number of alleles expected per locus, its reciprocal, was 2.66. Assuming that alleles have equal frequency in the population, the mean allele frequency required to produce a unique paternal specific allele at a given locus is 0.125 (to distinguish it from the six other potential fathers and the mother), considerably less than the observed value. As it was impossible to assign specific bands to loci in the baboon fingerprint, the exact number of loci present in the fingerprint was not known, and thus it was not possible to estimate the probability of an offspring inheriting a diagnostic allele in the fingerprint as a whole.

Of the 33 offspring analyzed, only 4 possessed one or more paternal specific alleles. Three of those offspring were assigned on the basis of one band only. The use of one band for a paternity inclusion would not normally be considered strong evidence, as the probability of false inclusion of an unrelated non-father is 0.537 in this population [$I' = x^m$, where x is the mean band sharing coefficient among unrelated individuals and m is the number of paternal specific bands in the offspring — in three cases 1, (BROOKFIELD, 1989; BRUFORD et al., 1992), in the one case where two diagnostic bands are present, this probability diminishes to 0.289]. However, all the potential fathers were sampled and the probability of a mutation occurring which would create a band of the exact molecular weight to produce a false inclusion is extremely low.

The limitations of this approach were, however, illustrated by the fact that out of 33, 29 of the offspring remain unassigned (see Table 2).

With the addition of data from using other multilocus VNTR probes which detect different loci and with single locus analysis, it may perhaps be possible to assign a large proportion of the offspring to one of the seven potential fathers. Additionally, these data, by excluding at least three of the seven males in nearly all cases, and in combination with observations of behavioural affinities, may eventually prove adequate to solve paternity in all offspring, and test whether a correlation exists between behavioural and genetic paternity in this population.

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