Analytical DNA Fingerprinting in Lions: Parentage, Genetic Diversity, and Kinship

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The application of hypervariable minisatellite genomic families to the reconstruction of population genetic structure holds great promise in describing the demographic history and future prospects of free-ranging populations. This potential has not yet been realized due to unforeseen empirical constraints associated with the use of heterologous species probes, to theoretical limitations on the power of the procedure to track genic heterozygosity and kinship, and to the absence of extensive field studies to test genetic predictions. We combine here the technical development of feline-specific VNTR (variable number tandem repeat) families of genetic loci with the long-term demographic and behavioral observations of lion populations of the Serengeti ecosystem in East Africa. Minisatellite variation was used to quantify the extent of genetic variation in several populations that differed in their natural history and levels of inbreeding. Definitive parentage, both maternal and paternal, was assessed for 78 cubs born in 11 lion prides, permitting the assessment of precise genealogical relationships among some 200 lions. The extent of DNA restriction fragment sharing between lions was empirically calibrated with the coefficient of relatedness, *r*, in two different populations that had distinct demographic histories. The results suggest that reliable estimates of relative genetic diversity, of parentage, and of individual relatedness can be achieved in free-ranging populations, provided the minisatellite family is calibrated in established pedigrees for the species.

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The understanding of the genetic and ecological structure of natural populations has been advanced considerably by the application of molecular measures of genic diversity, particularly allozyme and DNA variation using polymorphic gene probes (Burke 1989; Burke et al. 1989; Gilbert et al. 1990a; Hoelzel and Amos 1988; Jeffreys and Morton 1987; Lewontin 1974; Nevo et al. 1984; Quinn et al. 1987; Reeve et al. 1990; Vassart et al. 1987; Wetton et al. 1987). The discovery of hypervariable minisatellite loci in humans, also called VNTR (variable number tandem repeat) loci, revealed families of related DNA segments dispersed throughout the human and vertebrate genome. Variation at these loci is orders of magnitude more extensive than traditional DNA polymorphisms, presumably because new alleles are generated by both recombinational and mutational mechanisms (Gyllensten et al. 1989; Jarman and Wells 1989; Jeffreys et al. 1988, 1990; Wahls et al. 1990; Wolff et al. 1988, 1989). Such loci consist of short (11–60 bp) tandem repeats that vary in number between alleles and are visualized by Southern analysis after restriction enzyme cleavage of the DNA that flanks the repeat. The composite restriction fragment patterns produced by multilocus VNTR probes provide an individual-specific DNA fingerprint that has been used for assessing paternity, for forensic identification and individualization, for gene mapping, and for antenatal diagnoses (Gilbert et al. 1990b; Jeffreys et al. 1985; Nakamura et al. 1987).

There are at least three potential applications for VNTR analysis in the area of population biology: (1) parentage determination in free-ranging populations for ecological studies of mating systems (Burke 1989; Burke et al. 1989; Quinn et al. 1987; Wetton et al. 1987); (2) estimation of the extent and character of genomic diversity in related populations as an indicator of historic inbreeding or demographic partitioning (Gilbert et al. 1990a; Hoelzel and Amos 1988; Jeffreys and Morton 1987; Kuhnlein et al. 1989; Reeve et al. 1990); and (3) as a measure of kinship among individuals in a natural population (Lynch 1988).

Certain theoretical difficulties with the use of DNA fingerprints in estimating relatedness in free-ranging species have been

raised (Lewin 1989; Lynch 1988) which emphasized that, without the knowledge of locus or allele specificity of the resolved fragments in a DNA fingerprint, it would be difficult to estimate either heterozygosity or relatedness between individuals beyond first-order relatives (Lewin 1989; Lynch 1988, 1990). However, recent empirical results have produced reliable estimates of genetic diversity (Gilbert et al. 1990a,b; Jeffreys and Morton 1987; Reeve et al. 1990) and attempted to correlate DNA fragment sharing with the degree of inbreeding between close relatives (Kuhnlein et al. 1990; Quinn et al. 1987; Reeve et al. 1990). We combine here the accumulated knowledge gained over a 25-year-long study of African lions of the Serengeti ecosystem with feline VNTR probes specifically developed to assess the genetic and kinship structure in felid populations.

Materials and Methods

Lion Populations

Blood and skin biopsy samples were obtained from free-ranging lions that had been chemically immobilized. The Serengeti Ngorongoro Crater and Gir Forest lion populations have been extensively described in previous genetic and behavioral studies (Bertram 1975; Hanby and Bygott 1987; O'Brien et al. 1987a,b; Packer and Pusey 1982, 1983, 1987; Packer et al. 1991a,b; Schaller 1972; Wildt et al. 1987). Domestic cat samples were collected from the NIH cat colony originating from feral cats in Maryland (O'Brien 1980).

Southern Blot Analysis

DNAs were digested with *Pst*I or *Msp*I, separated by electrophoresis, Southern blotting, and hybridization as previously described (Gilbert et al. 1990a,b) with the following modifications: Both molecular weight markers and duplicate samples from the same animal were run on the ends of each gel; ^{32}P-labeled probes were hybridized at a concentration of 1.5×10^6 cpm/ml; final wash conditions were 0.1× SSX, 0.5% SDS at 50°C, done twice for 30 min.

Deriving Felid-Specific VNTR Probes

Genomic DNAs extracted from three unrelated domestic cats were digested with *Eco*RI, ligated into the arms of lambda ZAP (Stratagene), then packaged and plated as described by the manufacturer. Synthetic oligonucleotides based on human core minisatellite sequences (Nakamura et al. 1987) were end-labeled with polynucleotide kinase (BRL). Filters were hybridized in a solution of 5× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate), 50 mM Tris-HCl (pH 7.4), 1× Denhardt's solution (0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll), and 10 μg/ml yeast tRNA with 1×10^5 cpm/ml ^{32}P end-labeled probe for 12–16 h at the temperatures indicated. Washing was done three times in 5× SSC, 0.1% SDS for 5 min. Putative clones were tested for their ability to detect hypervariable sequences by Southern analysis of three domestic cat DNAs. The prehybridization and hybridization solutions contained 7% SDS, 0.5 M sodium phosphate (pH = 7.2), 1 mM EDTA, 7% SDS, and 1×10^6 cpm/ml of ^{32}P-labeled phage DNA or isolated insert. Filters were washed twice in 2× SSC, 0.5% SDS at room temperature and twice in 0.1× SSC, 0.5% SDS at 50°C or 65°C. For genomic clones that showed hybridization to repetitive elements, 100 μg/ml of sheared domestic cat carrier DNA was added to the prehybridization and hybridization buffer. Insert sequences were excised from the lambda ZAP vector by automatic excision (Stratagene) and were contained within the Bluescript KS(+) plasmid (Stratagene). Initially, this entire plasmid was used as a probe, and subsequently smaller fragments containing the hypervariable probe were subcloned into Bluescript KS(+). The nucleotide sequences of the two clones selected for analysis, FCZ8 and FCZ9, were determined: FCZ8 contains a repeat of a 16 bp consensus sequence (AT*GACC*C*TGGG*AGTCCGC) repeated approximately 37 times. For FCZ9, the consensus sequence (CTAATGC*GATC*T*GGGG*TCTGGCGG) was found repeated 65 times. Both of the feline VNTR clones share a common core sequence, *GXXRTGGG*, that is similar to the consensus zeta-globin repeat motif used to select these reagents.

Estimating Genetic Variation with Minisatellite Loci

Genetic variation for each VNTR family was assessed by computation of the average percent difference (APD) in band sharing. The percent difference, PD, = $[F_{ab}/(F_a + F_b)] \times 100$, where F_{ab} is the number of bands that differ between two individuals and F_a and F_b are the total number of bands in individuals a and b, respectively. The APD is the average of PD values in pairwise comparisons of all individuals in a group or population (Gilbert et al. 1990b; Yuhki and O'Brien 1990). APD can be considered as a rough approximator of heterozygosity (with a slightly downward bias), based on genetic models described elsewhere (Gilbert et al. 1990a,b; Lynch 1990). APD is the additive inverse of average percent similarity (APS) or similarity coefficient, "χ" after Jeffreys et al. (1985). We elected to use APD for quantifying population genetic diversity because it should vary in direct proportion to average heterozygosity, the traditional measure of variation. However for estimates of kinship or relatedness we use APS (100 − APD).

The extent of locus heterozygosity was estimated according to Stephens JC, Gilbert DA, Yuhki N, and O'Brien SJ (in preparation) as

$$H = \frac{\sum_{k=1}^{A} s_k}{A - \sum_{k=1}^{A} \sqrt{1 - s_k}} - 1$$

where s_k is the frequency of the kth fragment in the population and A equals the total number of fragments observed overall in polymorphic and monomorphic loci.

Calculating Relatedness between Individual Lions

Coefficients of relatedness, r, were determined directly from pedigree analysis of lion prides according to the convention of Wright (1922) and Hamilton (1964). Thus, unrelated: $r = 0$; full sibs and parent–offspring: $r = 0.5$; grandparents and half-sibs: $r = 0.25$; uncles, aunts: $r = 0.125$; first cousin: $r = 0.0625$.

We have attempted both in experimental design and data analysis to control for the theoretical and practical limits of DNA fingerprinting technology (Burke 1989; Lander 1989; Lewin 1989; Lynch 1988). Since gel-specific differences in banding patterns can occur, we have limited our analysis largely to individuals compared on the same gel. In those cases where the number of animals being compared was larger than the capacity of a single gel, different combinations of individuals were run in pairs of gels to affirm the accuracy of band matching. Initially, only limited demographic data were used to efficiently generate fingerprinting protocols. Therefore, the kinship estimates determined by fingerprint data were done as a blind study reducing the subjectivity in band scoring that might occur if known kin groups were being compared.

Results

Feline-Specific Minisatellite Probes

The human DNA fingerprinting probes 33.15 and 33.6 have been used successfully to describe paternity and genetic

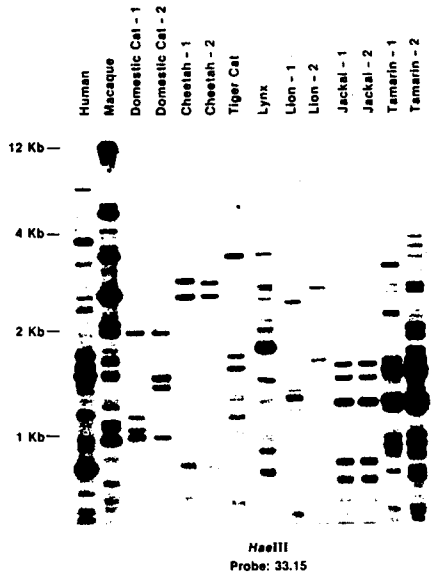

Figure 1. Autoradiogram of genomic DNAs from selected carnivore and primate species, digested with *Hae*III and probed with the human fingerprinting probe 33.15 (Jeffreys et al. 1985), with the final wash conditions at 0.1 × SSC, 0.5% SDS at 50°C. The jackal samples came from the same individual sampled on different occasions. Quantitation of fragment variation in human and cat populations with human 33.15 and 33.6 probes is presented in Table 3.

structure in populations of birds, dogs, and humans (Burke et al. 1989; Gilbert et al. 1990a; Jeffreys et al. 1985; Jeffreys and Morton 1987; Wetton et al. 1987), but they show limited fragment number and variation in domestic cats and other feline species (Jeffreys and Morton 1987). In Figure 1 we present a comparative analysis of three primate, five felid, and one canid species using the human probe 33.15. Both the number of fragments in the resolved range (1–20 Kb) and the extent of fragment variation are considerably lower in cat species than in human, primate, or canid species (Figure 1, Jeffreys and Morton 1987). A panel of more variable marker-probes for felids was developed to avoid the technical limitation of too few restriction fragments, as well as to discover minisatellite families with a broad range of diversity not available with heterologous minisatellite probes. Such reagents were desirable for two reasons. First, because lion prides are composed primarily of related females, parentage is difficult to determine with allozyme, restriction fragment length polymorphism (RFLP) or human DNA fingerprint probe analysis (Jeffreys and Morton 1987; O'Brien et al. 1987b; Packer et al. 1991b; Yuhki and O'Brien 1990). Second, certain populations in three felid species (cheetah, puma, and lion) have reduced genetic variation due to population contractions in their recent history (O'Brien et al. 1983, 1985, 1987b, 1990) and as such would require highly variable minisatellite families for informative assessments of their genetic structure.

To develop hypervariable minisatellite probes useful for these populations, we used five synthetic oligonucleotides homologous to the core sequences of human VNTR loci (Nakamura et al. 1987) to screen three genomic libraries from domestic cats. Positive clones were tested as probes on genomic DNAs from three to five unrelated domestic cats to identify hypervariable molecular clones. A summary of the screening is presented in Table 1. Of 119 clones that showed a positive signal with an oligonucleotide, 25 (21%) revealed VNTR polymorphism. This value is comparable to the yield from a human screen (26%) using a similar cloning strategy (Nakamura et al. 1987). Eight of these putative VNTR clones were characterized further by screening a collection of 16–28 unrelated domestic cat DNAs and 16–18 unrelated lions (Figures 2–4). The results of this survey revealed that two of the probes represented single-locus VNTR variation and six resolved multilocus families under the hybridization conditions selected (Table 2). Examples of the patterns produced in outbred cats, lions, and family pedigrees are illustrated in Figures 2–4.

Genetic variation for each VNTR family was assessed by computation of the average percentage difference (APD) in band sharing. APD with different probes varied from 9.6% to 90.0% in cats and from 49.9% to 93.9% in lions (Table 2).

For paternity determination, we required high APD values; however, high APD and low average fragment frequency would be less useful for estimates of relatedness, as maximum differences would be rapidly approached beyond first-order relatives. Two probes, FCZ8 and FCZ9, were selected for an analysis of genetic profiles of lion populations based on the following criteria. First, on average, the VNTR probe must detect a large number of bands per individual, distributed over a broad molecular weight range. Second, the observed level of polymorphism should be sufficiently high to establish parentage but low enough to detect genetic similarities between distantly related individuals. Third, the standard error of fragment sharing associated with comparisons of restriction fragment profiles among unrelated individuals and individuals of identical degrees of relatedness should be small (Table 2).

A comparison of the genetic diversity resolved by human and feline probes in populations of the same species is presented in Table 3. When the feline FCZ8 and FCZ9 probes were used, the population of domestic cats showed a large average number of fragments per individual (23.9 and 23.0, respectively), but a moderate amount of total variation (APD = 47.3 and 44.5, respectively). By comparison, human probes tested in a group of unrelated human DNAs showed 17.4 fragments per individual with an APD of 76.9%. In cats, the two human probes (33.15 and 33.6) resolved moderate levels of variation (APD = 42.5 and 40.4, respectively), but only a little less than half as many fragments per individual (9.8–11.2) as were seen in human DNAs under standard conditions of hybridization stringency. The increased number of polymorphic fragments revealed by the "FC" (*Felis catus*) probes was the rationale for deriving felid-specific probes.

Table 1. Screen for feline-minisatellite loci using synthetic oligonucleotides of human consensus VNTR families

Human VNTR locus	Consensus oligonucleotide sequence	Temperature (°C)		Positive clones per genome	Tested for VNTR polymorphism	Positive (%)
		Hybridization	Wash			
Zeta-globin	TGGGGCACAGGTTGTGAG	42	48	90	25	6 (24)
Insulin	ACAGGGGTGTGGGG	30	37	64	20	3 (15)
Myoglobin-1	GGAGGTGGGCAGGAAG	37	44	50	36	1 (2.8)
HBV-1	GGAGTTGGGGGAGGAG	37	44	48	20	6 (30)
YNZ22	CTCTGGGTGTCGTGC	37	42	120	18	7 (38)
				372	119	25 (21)

VNTR Population Diversity Reflects Population History

Although genetic variation has been measured in hundreds of populations with allozymes, RFLPs, and mitochondrial DNA (mtDNA) restriction site polymorphism (Nei 1987; Nevo et al. 1984), fewer than 20

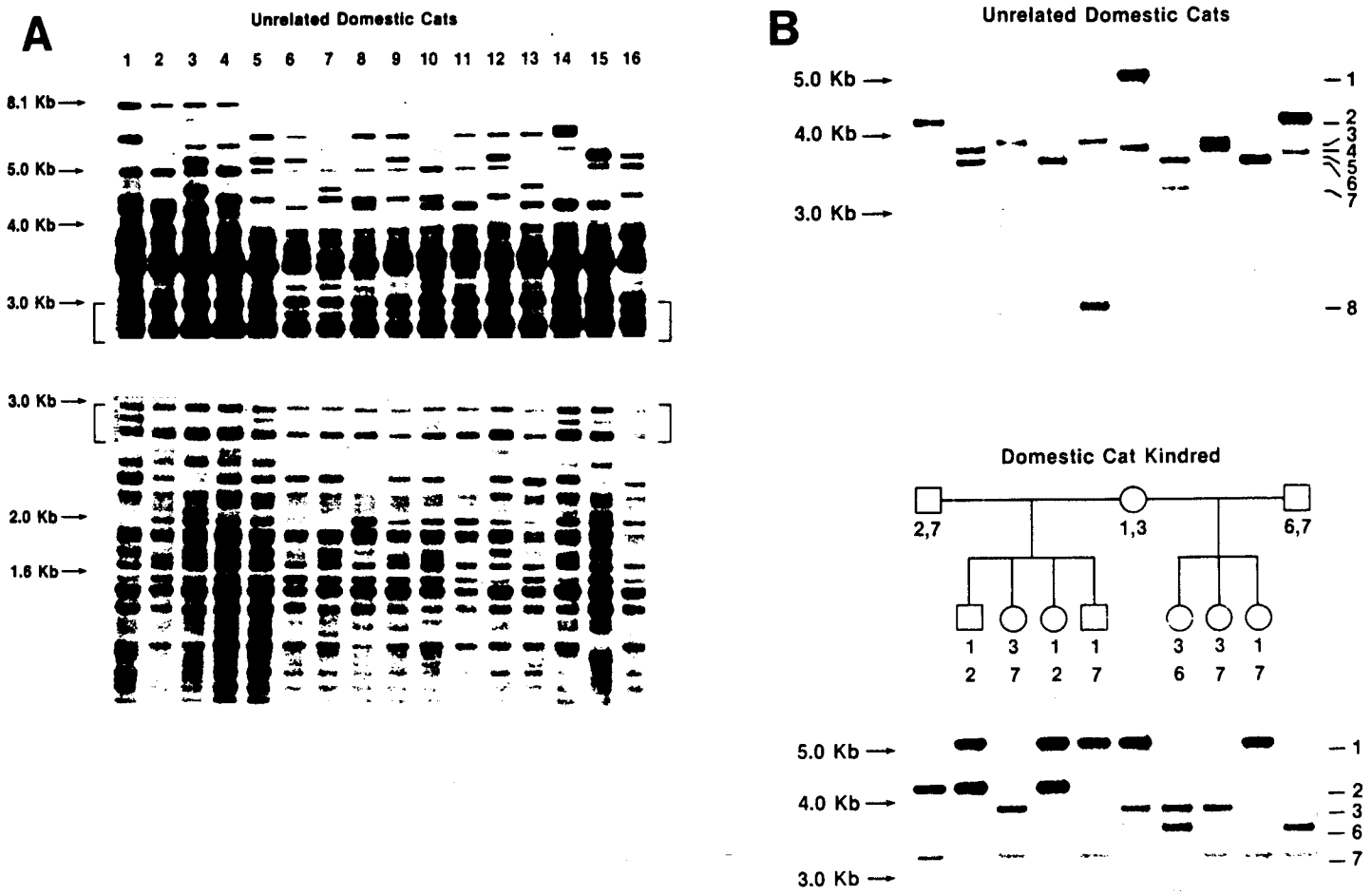

Figure 2. **(A)** DNAs from unrelated domestic cats digested with *MspI* and hybridized with probe FCZ8. Brackets indicate area of overlap between a lighter exposure (**bottom**) and darker exposure (**top**) of the same autoradiogram. **(B)** Unrelated cat and cat pedigree DNA digested with *PstI* and hybridized with probe FCI11. Numbers are allele designations.

species have been studied using DNA fingerprinting. Because minisatellite alleles change at a rate 100–1,000 times faster than conventional allele variation, their population variation, compared to control populations, should reflect more recent historic events (e.g., founder effect, bottlenecks, assortative mating) (Gilbert et al. 1990a; Reeve et al. 1990). We present in Table 3 an estimate of minisatellite family diversity using both APD and a new measure of heterozygosity at hypervariable minisatellite loci (Stephens JC, Gilbert DA, Yuhki N, and O'Brien SJ, in preparation) in an outbred population of domestic cats and in three different lion populations that differ in their genetic structure and recent demographic history (O'Brien et al. 1987a,b; Packer et al. 1991b; Wildt et al. 1987).

The Serengeti is a large outbred population of about 3,000 lions that live in the Serengeti Plains in Tanzania (Packer et al. 1988; Pusey and Packer 1987; Schaller 1972). The Ngorongoro Crater population consists of about 100 lions living adjacent to but geographically isolated from the larger Serengeti population. The Crater lions are known to have descended from 10 survivors of an epizootic of biting flies in 1962. Eight of these animals plus seven others that immigrated into the Crater between 1959 and 1964 were the founders of the present population (Packer et al. 1991b). The third samples were from the relict population of Asiatic lions, *Panthera leo persica*, which resides in the Gir Forest Sanctuary in the Gujarat State in western India. The present Gir Forest population numbers 250 individuals, but these are descended from a population that dropped to less than 20 animals at the turn of the last century (O'Brien et al. 1987a). The Serengeti population shows appreciable allozyme and major histocompatibility complex (MHC) genetic variability (comparable to domestic cat, mouse and man) and healthy reproductive characteristics (O'Brien et al. 1987b; Wildt et al. 1987; Yuhki and O'Brien 1990). The Gir lions exhibit no allozyme or MHC variation whatsoever, as well as elevated levels of developmental sperm abnormalities and diminished testosterone levels (O'Brien et al. 1987b; Wildt et al. 1987; Yuhki and O'Brien 1990). The moderately bottlenecked Ngorongoro lion population fell between these two extremes in each measurement.

The feline minisatellites confirmed the genetic impoverishment of the Gir lion sample detected by other methods (Figure 3 and Table 3). The Ngorongoro and Serengeti populations had similar APD and heterozygosities, although the Ngorongoro estimate had a threefold greater standard deviation than did the Serengeti. The Serengeti lion population revealed 80 distinct VNTR fragments, 45 of which were polymorphic. The Crater lions resolved 75 VNTR bands, 37 of which were polymorphic. There were no genetically fixed fragment differences between the Serengeti and Crater populations, although each had three to five polymorphic alleles that were

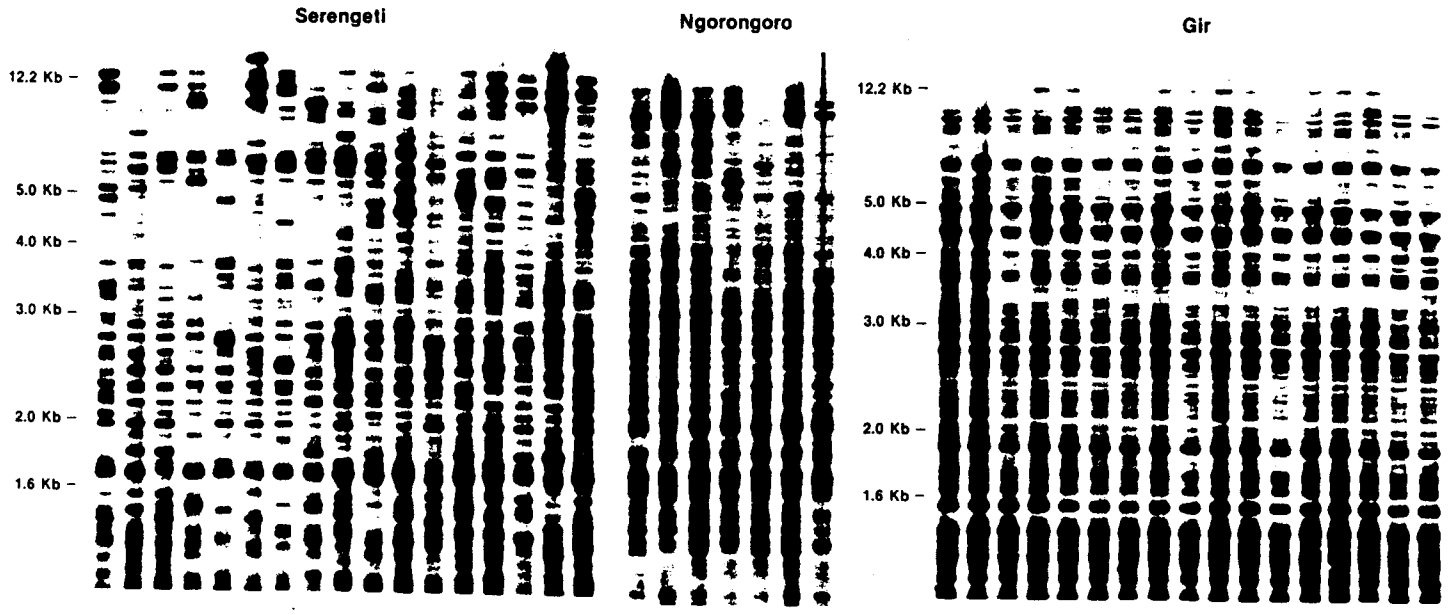

Figure 3. Unrelated African lions from indicated populations (see text) digested with *MspI* and hybridized with probe FCZ8.

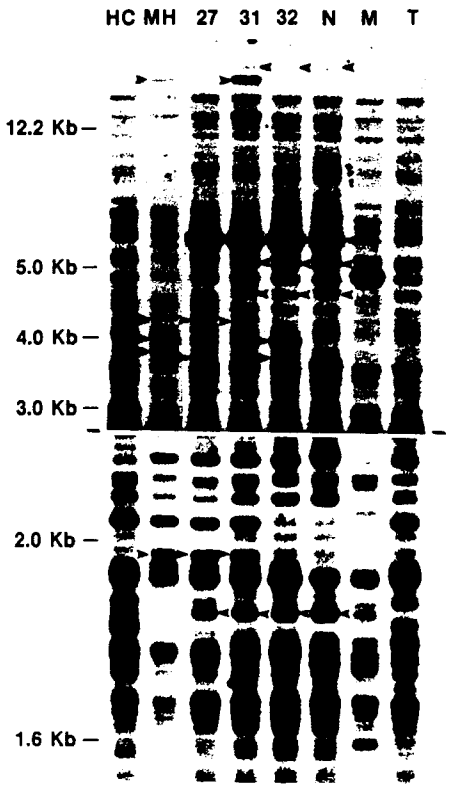

Figure 4. Paternity determination in the Campsite lion pride using *PstI* and probe FCZ8. HC and MH are resident males; letter designations are the females, and numbers are the offspring. Rightward arrows indicate diagnostic fragments for paternity; leftward arrows indicate diagnostic fragments for maternity. Dashes at 2.8 Kb indicate splice between a lighter exposure (**bottom**) and darker exposure (**top**) of the same autoradiogram. Although each set of putative mothers and each set of candidate fathers tend to be close relatives, an average of four (range one–seven) diagnostic unique maternal and five (range two–nine) unique paternal bands could be detected in each cub. In most cases two or more different restriction enzymes were necessary to implicate maternity and paternity.

not found in the other population. These results are not inconsistent with the history of the Crater population having descended from 15 founder lions, including Serengeti immigrants in the 1960s (Packer et al., 1991b). Nonetheless, it is notable that the allozyme and MHC data suggest greater genetic depletion from the founder event than the VNTR variation reveals. It does seem that the founder event that preceded the Crater population was less extreme than the events that led to genetic uniformity in the present Gir Forest lion population.

Parentage and Genealogy of African Lion Prides

Lions in the Serengeti National Park and in Ngorongoro Crater, Tanzania, have been the subject of intense behavioral and ecological studies for nearly 25 years (Bertram 1975; Bygott et al. 1979; Hanby and Bygott 1987; Packer and Pusey 1982, 1983; Packer et al. 1988; Pusey and Packer 1987). Lions live in social groups called "prides" that typically consist of two–nine adult females, their dependent young, and a coalition of two–six adult males. Female pride members are usually close relatives, as are most male coalition partners, but the resident males are usually unrelated to the females (Pusey and Packer 1987). Although individual lions can be identified (Packer et al. 1991b; Pennycuick and Rudnai 1970), paternity cannot be reliably determined for cubs because female lions typically mate with several males during each estrus period (Packer and Pusey 1987; Schaller 1972). Further, maternity of cubs is sometimes uncertain because of the synchronous breeding and communal rearing strategies of female lions (Bertram 1975, 1976; Packer et al. 1988).

In order to establish parentage precise-

Table 2. Genetic aspects of feline-specific hypervariable minisatellite genome families

Species	Probe	Restriction enzyme	APD[a] for unrelated individuals ±SD (%)	Average no. bands per individual	Total fragments scored in population	Average fragment frequency (range)
Domestic cats	FCZ8	*MspI*	47.3 ± 3.6	24	77	0.31 (0.06–1.0)
	FCZ9	*MspI*	44.5 ± 3.7	23	67	0.34 (0.07–1.0)
	FCI11	*MspI*	90.0 ± 5.4	2	13	0.14 (0.10–0.3)
	FCM14	*PstI*	75.4 ± 8.7	15	93	0.17 (0.06–0.7)
	FCM3	*HinfI*	61.1 ± 8.5	2	5	0.40 (0.20–0.6)
	FCH5	*PstI*	74.3 ± 3.7	11	61	0.18 (0.08–1.0)
	FCH18	*HinfI*	24.6 ± 3.8	19	36	0.54 (0.06–1.0)
	FCY4	*HinfI*	9.6 ± 5.3	20	25	0.80 (0.08–1.0)
Lions	FCZ8	*MspI*	53.0 ± 3.1	18	48	0.35 (0.05–1.0)
	FCZ9	*MspI*	49.9 ± 3.1	23	75	0.30 (0.06–1.0)
	FCI11	*PstI*	93.9 ± 4.1	2	15	0.12 (0.08–0.2)

[a] APD is average percentage difference in band sharing; see text.

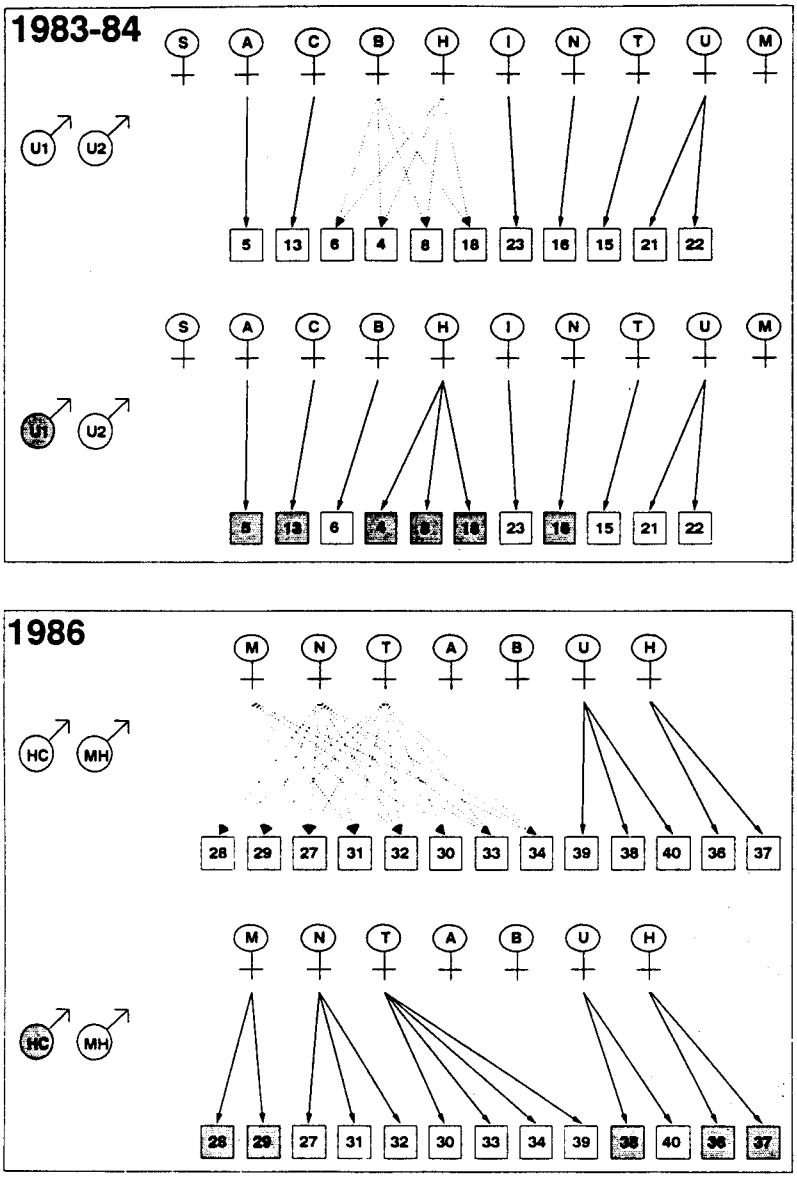

Figure 5. Pedigree of Campsite pride covering the tenure of two different male coalitions. The upper box shows the offspring born in 1984 and 1985, while the males U1 and U2 were residents. These males were replaced by HC and MH, and their offspring born in 1986 are represented in the lower box. Single letters represent the adult females, and numbers represent offspring. For each box, the upper pedigree represents the kinship structure as estimated by behavioral observation. Dashed arrows represent maternity that could not be accurately assigned from behavioral observation, as all cubs were born at approximately the same time. The bottom pedigree is the actual parentage determined by DNA fingerprinting. In the lower half, solid arrows indicate maternity; shaded males were fathers of shaded offspring, and unshaded males were fathers of unshaded offspring. The "1986" box shows the only case in the entire analysis where behavioral data suggested that an adult female ("U") was the only possible mother, and DNA fingerprinting assigned this cub ("39") to another female ("T"). In every pride analyzed, all adult females were included whether or not they could be excluded based on behavioral data. Cubs 38 and 40 represent the only case of mixed paternity in a litter that was observed in this analysis.

ly, blood and skin biopsy samples were collected from 193 individually recognized lions from the Serengeti population between 1985 and 1989 (Table 4). Leukocyte and/or fibroblast DNAs extracted from pride mothers, coalition males, and cubs from 44 litters were examined using FCZ8 to establish maternity and paternity for 78 cubs born during the sampling period. All adult residents of a pride were included as potential parents in each paternity gel. The results of parentage analysis for cubs born in two prides, each with only two putative fathers, is illustrated in Figures 4 and 5.

For each of 78 cubs parentage determination was unequivocal and in each paternal implication the father was a resident male for the pride. In all cases but one, the mother identified by DNA fingerprinting was one of several pride females implicated by behavioral observations. (The exceptional cub was born within the same pride to another female.) In 23 of 24 litters a single male was the father for the entire litter despite the fact that females often accept multiple copulatory partners during estrus. In general, DNA fingerprinting verified maternal associations and mating success of resident males and also appears to provide a robust measure of parentage even when the candidate mothers or fathers are closely related.

Calibration of Kinship and VNTR Band Sharing

The family structure of the African lion prides listed in Table 4 was determined based upon behavioral observation and confirmed by the paternity analysis as illustrated in Figure 5. Knowledge of the prides' genealogical structures allowed for an empirical calibration of the extent of band sharing with the coefficient of relatedness, r, defined as the expected fraction of genes in two individuals that are identical by descent from a common ancestor (Hamilton 1964; Wright 1922) between related lions. Thus for first-order relatives (parent–offspring, full siblings) $r = .5$; for half siblings, $r = .25$; for first cousins, $r = .125$, and so on. We then measured the percentage of *similarity* (PS = 100 − PD) of band sharing between pairs of individuals in each relatedness class and used the average of these estimates (APS) as the basis for calibration.

The results of this calibration for Serengeti lions are presented in Figure 6A. In the analysis 76 lions born in different parts of the Serengeti with no known kinship links were assumed to be "unrelated." Unrelated lions shared 49.0% ± 3.3% (range = 40%–56%) of their fragments, while first-order relatives ($r = .5$) shared 79.6% ± 2.2% (range = 76%–86%). The frequency distributions of these two classes do not overlap, thus establishing a lower limit (unrelated: 49.0%) and an upper limit (first-order kin: 79.6%) of band sharing.

Beyond first-order relatives the extent of band sharing decreases gradually as a curvilinear function of r. Although the variance in APS is small within the various relatedness categories, the distributions for individuals related by $r = .125, .25$, and .5 overlap sufficiently that it would be impossible to classify a pair of individuals as siblings vs. cousins based solely on band sharing data. However, most individuals can be assigned to one of three discrete groups of relatedness: first, animals with a level of band sharing (APS) indicative of nonrelatives; second, individuals that

Table 3. DNA variation in VNTR loci in cat, lion, and human populations

Species	Population	DNA fingerprint: No. individuals	Probe	Restriction enzyme	No. fragments/ individual	APD ± SD (%)[b]	Heterozygosity (%)[a]: DNA fingerprint	MHC RFLP[c]	Allozymes[d]
Domestic cat	Eastern U.S.	17	FCZ8	*Mspl*	23.9 ± 3.3	47.3 ± 3.6	46.0	28.9	8.2
		15	FCZ9	*Mspl*	23.0 ± 2.6	44.5 ± 3.6	41.8		
		18	33.15	*Hinfl*	11.2 ± 2.3	42.5 ± 2.7	56.7		
		16	33.6	*Hinfl*	9.8 ± 1.9	40.4 ± 3.8	35.1		
Human	ATCC cell lines	33	33.6[e]	*HaeIII*	17.4 ± 1.8	76.9 ± 9.4	86.0	17.4	6.7
Lion	Serengeti	76	FCZ8	*Mspl*	18.0 ± 3.5	49.0 ± 3.3	48.1	21.8	3.8
		17	FCZ9	*Mspl*	23.2 ± 3.3	44.5 ± 3.0	50.0		
	Ngorongoro Crater	6	FCZ8	*Mspl*	21.6 ± 3.8	51.5 ± 10.5	43.5	8.0	1.5
	Gir Forest	16	FCZ8	*Mspl*	23.2 ± 0.8	3.8 ± 0.2	2.8	0.0	0.0
		16	FCZ8	*Rsal*	22.9 ± 0.2	2.6 ± 0.7	2.9		

Heterozygosity was estimated using the method of Stephens JC, Gilbert DA, Yuhki N, and O'Brien SJ (in preparation), in which DNA fragments are presumed to represent randomly distributed alleles of chromosomally dispersed VNTR loci. Twenty-two sampled individuals of known relatedness were used (Packer et al., 1991b).

For APD estimates of Ngorongoro Crater, the six most distantly related individuals were compared (Packer et al., 1991b).

MHC data are from Yuhki and O'Brien (1990).

Allozyme data are from Harris and Hopkinson (1972); O'Brien (1980); and O'Brien et al. (1987b).

Human probe data are from Gilbert et al. (1990b).

are distantly related but not close relatives (r = .03–.125); and, third, lions that are genetically as similar as close kin, either siblings or cousins.

The calibration curve for the 23 lions sampled from the Ngorongoro Crater is presented in Figure 6B. Even with the Crater population's different genealogical structure (due to common descent from a limited number of founding individuals) and our small sample size, it is still possible to specify levels of relatedness. First-order relatives (r = .5) had an APS = 71.7% ± 11.1% (range = 51%–84.6%), whereas nonrelatives, based on comparison to the Serengeti lions, had an APS of 36.9% ± 4.2% (range = 31.6%–41.8%). As in the Serengeti population, the APS for individual comparisons of intermediate relatedness (r = .125–.5) had only slight overlap with "unrelated," displayed a direct proportionality to r, and were generally lower than the APS for first-order relatives (Figure 6B).

Once the correlation between band sharing and kinship was established, the genetic relationship between individuals for which no demographic data exist could be estimated. We tested the calibration by determining the APS between pairs of male lions traveling together in Serengeti coalitions, many of whose natal origins were unknown. The results of 15 coalition pairs show that each comparison fell into one of two nonoverlapping classes (Figure 7). The first group shared 75%–85% of their bands, similar to APS values for cousins and siblings. The second group shared 45%–53% of their bands, values similar to those found among known, unrelated lions. These latter male groups are likely composed of animals that originate from different prides, with no kinship links, while the former group are likely brothers, cousins, or close kin that came from the same natal pride.

Table 4. Composition of studied lion prides

Pride	Observed since	Total no. animals observed	Mean pride size	No. animals sampled 1985–1989[a]: Candidate mothers	Candidate fathers[b]	Offspring	Total
Serengeti							
Masai	1966	286	20	7	4*	15	26
Mukoma	1966	34	5	2	4*	1	7
Shambles	1966	68	7	2	4*	2	8
SRI	1966	51	8	2	3**	3	8
Sangere	1974	41	9	3	3**	10	16
Loliondo	1974	127	18	10	3	7	23
Campsite	1974	148	24	10,7	2,2***	11,13	38
Campsite 2	1984	11	5	1	2***	1	6
Simba	1974	115	19	7	2****	9	19
Sympatica	1974	16	4	1	2****	1	4
Transect	1986	33	12	3	2	5	10
Other Serengeti prides (N = 10)	1966/74	800	8	—	—	—	43
Ngorongoro prides (N = 7)	1963	558	10	—	—	—	23
Total no. of different lions		2,050		48	18	78	216

A total of 21 Serengeti prides and seven Ngorongoro Crater prides were sampled between 1985 and 1989. Eleven of these prides were the subject of parentage determination of 78 cubs (see text).

Four male coalitions were at various times resident in multiple prides.

* Coalition of four males resident in three prides.

** Coalition of three males resident in two prides.

*** Coalition of two males resident in two prides.

**** Another coalition of two different males resident in two prides; campsite pride was sampled twice during the tenure of two different male coalitions.

Discussion

The development and characterization of VNTR families specifically selected for cats provides some important advances in our understanding of wildlife populations. As is the case for human minisatellite families (Nakamura et al. 1987), feline VNTR families differed appreciably in the extent of differences between individuals, the number of monomorphic fragments in a population, the number of polymorphic alleles, and the average and variance of fragment frequency. The wide variation in polymorphism characteristics of the cat probes allowed us to overcome technical limitations posed by the use of human

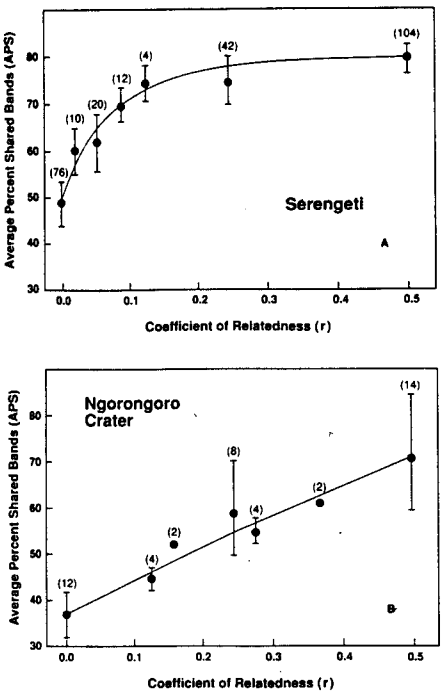

Figure 6. Calibration curve of the average percentage of similarity (APS) versus the coefficient of relatedness, *r*, in (**A**) the Serengeti lion population and (**B**) the Ngorongoro Crater lion population. Points are the mean of APS for two restriction enzymes, *Msp*I and *Rsa*I, between the indicated number of lions with standard deviation. Numbers above the points are the actual number of animals compared to derive APS. Each individual in a relatedness class was compared at random to only one other individual in order to assure independent and equivalent weight to each individual's phenotype (Lynch 1990). For example, for first-order relatives ($r = .5$), we examined 104 lions, or 52 comparisons; for $r = .25$, we compared 42 lions, or 21 comparisons, and so on. In the Serengeti, unrelated lions were animals from different locations in the park with no known kinship links. In Ngorongoro Crater, because all the animals were related due to their descent from a few Serengeti founder lions (O'Brien et al. 1987b; Packer et al. 1991b), unrelated animals indicate a comparison of Ngorongoro lions to Serengeti lions. Both sexes were used equivalently in calibration since no sex bias was discovered when they were treated separately. For relatedness classes .125–.5, the *r* values were known exactly from behavioral observations plus pedigree reconstruction. In certain distant lion relationships, only a range for *r* was known, namely: Serengeti, $.016 < r < .031$, $.031 < r < .062$, $.062 < r < .125$; Ngorongoro, $.062 < r < .25$, $.16 < r < .38$, $.25 < r < .5$. In these cases the midpoint value for *r* is plotted on the abscissa. The homogeneity of variance for each curve was investigated using Bartlett's test for groups of different sample sizes. For the Ngorongoro, $\chi^2 = 3.953$, $p = .139$; for the Serengeti, $\chi^2 = 7.645$, $p = .022$, where the data at $r = 0$ are more heterogeneous than the other *r* values. The Ngorongoro data can be approximated by a linear function, although this same function (and other linear, quadratic, or cubic models) cannot be fitted to the Serengeti curve. The Serengeti curve is then significantly different from the Ngorongoro relationship and can only be described by higher order statistical analysis involving a piecemeal linear model.

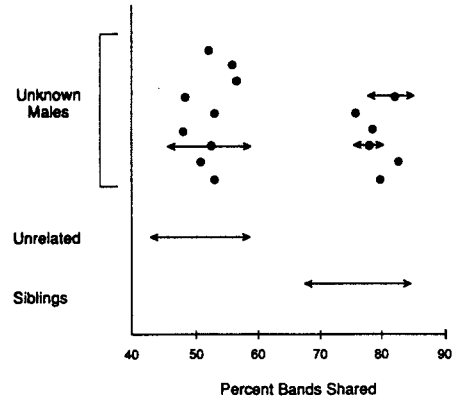

Figure 7. Summary of band sharing estimates for male coalition partners for which no demographic or historic data exists. For coalitions that contained only two males, dots represent the percentage of similarity (PS) estimates for both restriction enzymes used; in cases where coalition size was greater than two, the arrows represent the ranges of individual PS values and dots represent the APS between coalition members. The arrows for "siblings" and "unrelated" show the range of PS values for animals known either to be siblings (full siblings or half siblings) and those known to originate from different parts of the Serengeti, respectively.

probes in cats, as well as the theoretical concerns that had been raised about the efficacy of DNA fingerprinting in estimating diversity and relatedness in wildlife species (Lewin 1989; Lynch 1988). The cat probes were effective in establishing parentage in a "worst case" situation among lion prides where parentage was uncertain and the candidate parents were often closely related (and thereby impossible to discriminate by allozyme or human minisatellite methods). Further, the molecular reagents revealed genetic diminution in a population of Asiatic lions that had been shown to be compromised in allozyme and MHC-RFLP variation as well. Finally, a calibration curve that related the extent of DNA fragment sharing to the coefficient of relatedness (r) was derived for two free-ranging lion populations from East Africa.

The differences in the shape of the calibration curves are significant and intriguing (Figure 6A vs. 6B). The Ngorongoro curve can be fitted to a linear model, as would be predicted by theoretical expectations (Lynch 1988), whereas the Serengeti curve is more curvilinear or "piecemeal" linear. The reason for the difference is not obvious, but it could be the result of differences in population histories. At issue here is why should offspring of unrelated fathers ($r = .25$) have equivalent levels of band sharing (APS $\cong$ 80%) as do full siblings or parent-offspring comparisons ($r = .25$; see Figure 6A)? The long term demographic observations over many generations, do not give any support to the explanation that the Serengeti population is consanguinous or subdivided; all observations support panmixia of a large outbred population. Other factors that might influence the shape of calibration curves would include the gene family used, restriction enzyme, mutation rates, fraction of invariant loci, allele frequency distribution, and variance of APS estimates. Unanticipated nonlinearity of calibration curves similar to the Serengeti pattern has also been suggested by studies of certain avian species (Jones et al., in press; Kuhnlein et al. 1990).

Our results and those of others (Kuhnlein et al. 1990; Quinn et al. 1987; Reeve et al. 1990) suggest that three criteria should be considered before using DNA fingerprinting for genetic estimates in natural populations. First, the probe and enzyme system chosen must be based on matching the polymorphism characteristics of the gene family and the information sought. Fingerprinting probes with a lower proportion of shared bands between unrelated individuals than the probes used here might satisfactorily distinguish between siblings and cousins, but, due to alignment difficulties, they might not differentiate between moderately related and unrelated animals. Second, the population should be prescreened, and probe and enzyme combinations that show a low variance associated with band sharing estimates should be identified. In the Serengeti, DNA fingerprinting clearly distinguishes between close relatives, distant relatives, and nonrelatives. But the high variance associated with band sharing values in the Ngorongoro Crater makes kinship estimation in this population less reliable. Although on theoretical grounds the variance in any relatedness class should depend solely on APS (Lynch 1988), the technical limitation of fragment resolution of different gene-enzyme systems does affect the variance appreciably; so variance must be determined empirically. Finally, whenever possible, calibration of band sharing estimates with animals of known pedigrees or genealogies should be attempted so that band sharing estimates between individuals of unknown background can be correctly interpreted.

The ability to determine relative kinship among individuals would have several potential applications in wildlife biology. First, because of the rapid rate of new allele production, the method would be more sensitive than previous procedures (mtDNA, allozymes, nuclear RFLP) to recent genetic partitioning of populations, particularly within historic times (Gilbert et al. 1990a; Reeve et al. 1990). Second, the procedure would have direct application

to captive breeding programs in which avoidance of inbreeding and outbreeding effects might be anticipated a priori (Soule et al. 1986). Third, a number of intriguing evolutionary hypotheses about ecological and behavioral strategies depend on mating and cooperative behavior between relatives and/or nonrelatives (Bertram 1975; Bygott et al. 1979; Hanby and Bygott 1987; Packer et al. 1988; Packer and Pusey 1982, 1983; Pusey and Packer 1987; Schaller 1972). The last application is the subject of an extension of this study (Packer et al. 1991a).

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