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Abstract: The extent and nature of variation in hypervariable regions of DNA have been used in the past as a means to infer the natural histories of populations. We review the interpretation of the extent of genetic diversity for minisatellite DNA in the cheetah to estimate the timing of a population bottleneck in the species and the potential application of a second class of hypervariable DNA, microsatellite DNA, as a molecular tool to examine the natural histories of felid populations. A calibration curve relating the degree of allele fragment sharing in individuals to relatedness in a captive pedigree of cheetahs is presented. This measurement has important applications for management of potential mating in captive management situations.

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Hypervariable genomic variation to reconstruct the natural history of populations: Lessons from the big cats

The extent and nature of variation in hypervariable regions of DNA have been used in the past as a means to infer the natural histories of populations. We review the interpretation of the extent of genetic diversity for minisatellite DNA in the cheetah to estimate the timing of a population bottleneck in the species and the potential application of a second class of hypervariable DNA, microsatellite DNA, as a molecular tool to examine the natural histories of felid populations. A calibration curve relating the degree of allele fragment sharing in individuals to relatedness in a captive pedigree of cheetahs is presented. This measurement has important applications for management of potential matings in captive management situations.

1 Introduction

The extent and nature of variation in minisatellite DNA have been used as a means to examine the natural histories of populations. Minisatellite DNA probes which hybridize to a family of related genomic sequences have been employed to generate multilocus DNA fingerprints. Comparisons of fingerprint patterns within and between individuals in populations provide insight into the dynamics of social structure, mating patterns, and historic inbreeding. Gilbert *et al.* [1] developed feline-specific minisatellite probes to estimate the degree of relatedness in outbred populations of lions as an approach to relate relative kinship to cooperative behavior in lion prides [2]. Lack of variation in multilocus DNA fingerprints in the eusocial naked mole rat led Reeve *et al.* [3] to draw conclusions on the social structure of this species, while the extent of genomic diversity in related populations has been used as an indicator of historic inbreeding [1, 4, 5]. We have characterized the extent of variation for minisatellite DNA [6] in two subspecies of African cheetahs and utilized this as a molecular metric to approximate the timing of a population bottleneck in the species' past.

The cheetah has been revered by man as the world's fastest land mammal. Numbering fewer than 20000 individuals in two regions of sub-Saharan Africa, captive breeding attempts directed at increasing numbers of individuals have met with poor success compared to other felid captive breeding programs [7–9]. As the result of a series of reproductive and molecular genetic analyses of the species directed to address this problem, it became increasingly clear that the cheetah suffered from a lack of genetic diversity for nuclear coding loci. Near monomorphic profiles for allozymes and cell proteins examined by electrophoresis [10, 11], elevated levels of skeletal asymmetry relative to other felid species [12, 13], and lack of variability at the major histocompatibility complex (MHC) locus, as determined by immunological (surgical skin graft) [8] and molecular (restriction fragment length polymorphisms) studies [14], demonstrated that the species exhibited 10–100 times less variation for nuclear coding loci than is normally found in other cat species. Increased spermatozoa abnormalities, decreased fecundity, high infant mortality and increased susceptibility to pathogens were the phenotypic consequences of this species' lack of genetic variability [8, 10–16].

The cheetah's reduced genetic profile was attributed to a postulated historic bottleneck(s), followed by a period of extensive inbreeding [10, 11]. The extent of variation for two rapidly evolving classes of DNA, mitochondrial (mt) and minisatellite, was determined and used to back-calculate the amount of time required to reconstitute variation after a population bottleneck(s) [6]. Moderate levels of variation in mitochondrial RFLP variation among cheetahs were compared to mitochondrial nucleotide divergence between felid species for which fossil dating of a common ancestor had been estimated. Variation in minisatellite DNA as determined with a feline-specific minisatellite probe demonstrated that the cheetah had near comparable levels of diversity to those observed in outbred species of felids including the domestic cat, African leopard, western puma and Serengeti lion ([1, 17], Miththapala *et al.* submitted). These levels were considerably higher than those observed in recently bottlenecked populations (less than 100 years ago) of Asiatic lions or Florida panthers [1, 17]. Both mtDNA and minisatellite DNA variation in cheetahs were interpreted as evidence that cheetahs experienced a bottleneck, not within the last few hundred years, but rather several thousand years ago, likely towards the lower Pleistocene, around 10000 years ago [6].

Patterns of DNA fingerprinting have also been employed to estimate the degree of relatedness or consanguinity in outbred populations with appreciable variation for variable number of tandem repeat (VNTR) loci [1, 18]. We have attempted to compare a sample of cheetahs of known genealogical relationships from the captive populations of cheetahs managed by the Species Survival Plan (SSP) of the American Association of Zoological Parks and Aquaria [8, 9, 19].

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2 Materials and methods

Total genomic DNA was obtained from blood or skin fibroblast cell cultures [20]. Genomic DNA was digested with *Hin*fI and digestion products were electrophoresed in a 1% agarose gel. Southern blot procedures, hybridization and washes followed the procedures of Gilbert *et al.* [1]. A feline-specific minisatellite, FCZ8 [1] was used as the probe. The sequence of the core repeat $(TGGAGAGGTGGGCAGG)_n$ is homologous to the human zeta-globin consensus oligonucleotide used as a screening probe of the feline library. This feline core sequence represents a correction of the previously published sequence, which we now believe was incorrect [1]. Coefficients of relatedness, r, were determined directly from pedigree analysis of animals according to the convention of Wright [21] and Hamilton [22]. For full sibs and parent-offspring: $r = 0.5$; grandparents and half-sibs: $r = 0.25$; uncles, aunts: $r = 0.5$; grandparents and half-sibs: $r = 0.25$; uncles, aunts: $r = 0.125$; first cousins: $r = 0.625$; second cousins: $r = 0.03$; and unrelated individuals: $r = 0$.

3 Results

A pedigree of captive cheetahs including sampled individuals and an autoradiograph showing compared DNA fingerprints of individual cheetah DNAs are presented in Figs. 1 and 2. We measured the percentage of similarity ($PS = 100 - PD$) or band sharing between pairs of individuals in each relatedness class and used the average of these estimates (APS) as the basis for calibration. In Fig. 3, an empirical calibration of the extent of bands shared between cheetahs with the coefficient of relatedness is presented. The results of this calibration show a linear relationship between relatedness and APS as was predicted theoretically [23, 24] and observed elsewhere [1, 18]. The proportionality plus observed transmission data for family groups (unpublished data) affirm the genetic basis of the typed fragments, the independence of fragment alleles' genetic transmission, and the potential for identifying close relatives *vs.* unrelated cheetahs of unknown genealogy. Identifying relative kinship of cheetahs would have direct applications in avoiding further inbreeding among captive mating situations [8, 9, 25].

A second category of hypervariable molecular DNA, microsatellites, has been isolated and characterized in the domestic cat, *Felis catus*, and is being incorporated in a genetic recombination map (Fig. 4) [26]. Microsatellite loci are a class of repetitive genomic DNA with a 1–6 base pair repeat motif. Differences in the number of repeat units within and between individuals result in loci of high polymorphic content [27]. First described for their high heterozygosity in humans [27], microsatellites have been found to be abundant, randomly distributed and highly polymorphic in eukaryotic organisms [28–32]. Recently, the highly polymorphic nature of microsatellites and necessity of only nanogram quantities of even degraded DNA for genotype analysis have led to their successful application to the examination of dynamics of population biology on a molecular genetic level [33–35].

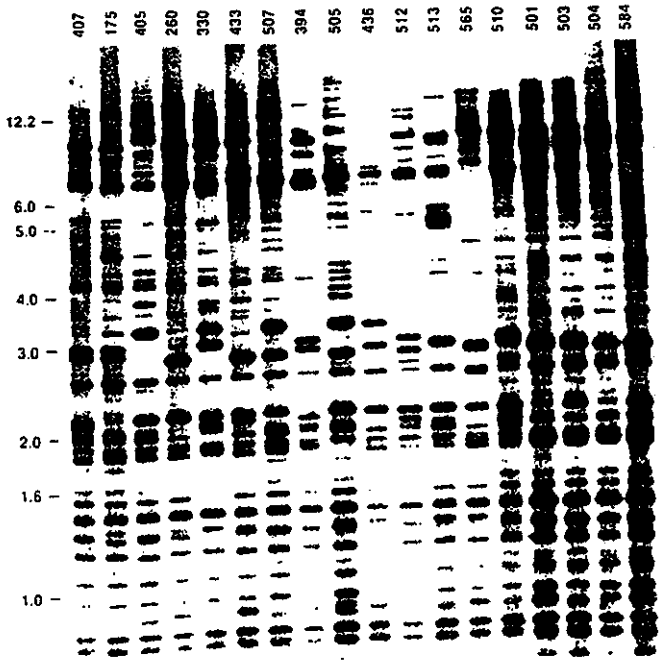

Figure 2. DNA fingerprint patterns observed in animals of known relatedness. Genomic DNA was digested with *Hin*fI and probed with a feline-specific minisatellite, FCZ8 [1]. Individual cheetah studbook numbers relate to pedigrees in Fig. 1.

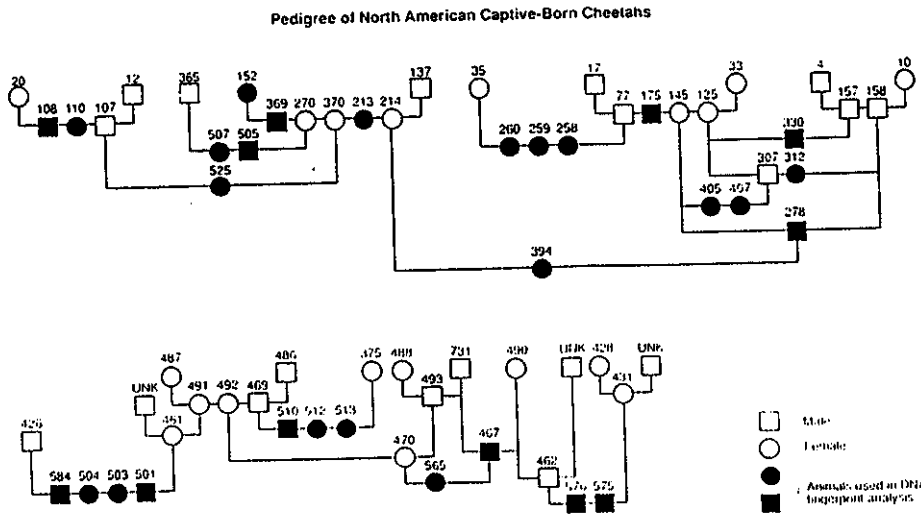

Figure 1. Pedigree of North American captive-born cheetahs. Darkened figures indicate animals used in the calibration curve [7, 19]. Numbers are International Cheetah Studbook numbers [19].

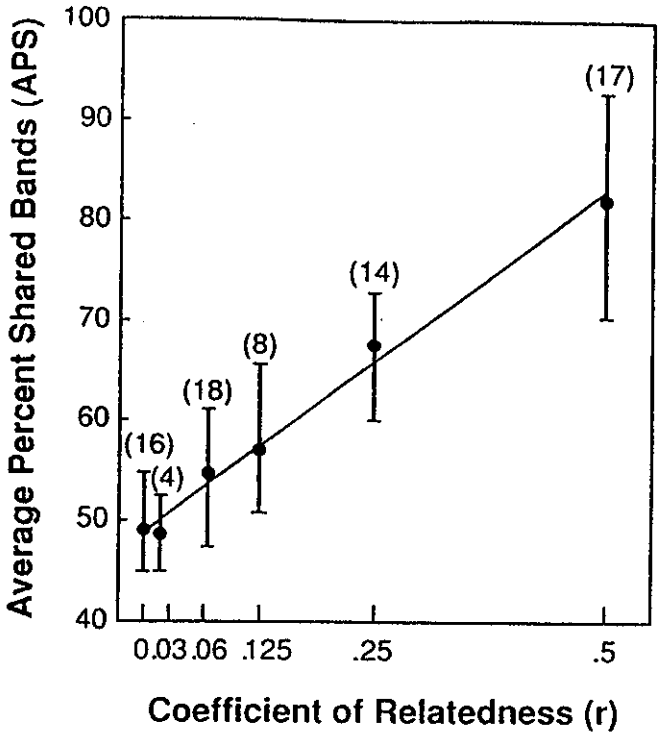

Figure 3. Calibration curve of the average percentage of similarity (APS) of shared bands in DNA fingerprints versus the coefficient of relatedness, *r*, in the cheetah. Numbers in parentheses indicate the number of pairwise comparisons used in the analysis for a particular *r* value. Error bars represent one standard deviation.

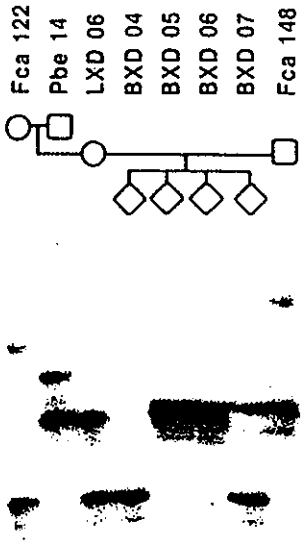

Figure 4. Autoradiograph of PCR-amplified microsatellite alleles for locus *Fca 23* in a three-generation interspecific backcross pedigree of domestic cat × Asian leopard cat. This cross is being constructed as an aid for constructing a gene map of the domestic cat including both coding loci (Type I) and highly polymorphic microsatellite loci (Type II) [39]. Radiolabeled PCR amplification products were electrophoresed in a 6% polyacrylamide denaturing gel. Segregation of alleles clearly shows Mendelian inheritance. *Fca*, domestic cat; Pbe, Asian leopard cat; LXD, F1 generation (leopard cat × domestic); BXD, backcross individuals.

Table 1. Characterization of ten microsatellite loci in four species of felidae

Locus	Heterozygosity/(# of alleles) Domestic cat	Cheetah	Puma	Lion
Fca 8	0.89 (7)	0.84 (8)	0.49 (4)	0.73 (4)
Fca 23	0.75 (7)	0.43 (4)	0.50 (4)	0.51 (3)
Fca 35	0.60 (4)	0.60 (3)	0.50 (4)	0.79 (5)
Fca 43	0.70 (7)	0.00 (1)	0.85 (8)	0.40 (2)
Fca 45	0.86 (8)	0.43 (3)	0.10 (2)	0.23 (2)
Fca 77	0.63 (5)	0.00 (1)	0.62 (3)	0.76 (4)
Fca 78	0.76 (6)	0.27 (2)	0.71 (6)	0.85 (6)
Fca 90	0.85 (8)	0.00 (1)	0.83 (7)	0.77 (5)
Fca 96	0.85 (6)	0.74 (7)	0.82 (7)	0.74 (5)
Fca 126	0.77 (5)	0.64 (4)	0.66 (4)	0.85 (7)
Average =	0.77	0.39	0.61	0.66

The evolutionary conservation of a subset of these loci isolated from a genomic library of *Felis catus* has been examined as a source of polymorphism across Felidae species [26]. Ten primer pairs amplified across the evolutionary range of Felidae [36] by polymerase chain reaction (PCR) generated products in the domestic cat, puma, lion, cheetah, Asian leopard cat and Geoffrey's cat, of predicted size, with the classic "stutter" band artifact of PCR amplification of microsatellite loci. A broad range of heterozygosity was observed both between species for a single locus and among loci within a single species (Table 1). Differences in the rate of mutation for individual microsatellite loci is suggested by the wide range of heterozygosity levels (0–0.842) observed in the cheetah. If microsatellite loci were reduced to homozygosity in the cheetah by the proposed lower Pleistocene bottleneck [6], then rapidly mutating loci would have reconstituted variation while other less rapidly mutating loci would not.

The polymorphism of microsatellite loci across felids offers much potential as molecular markers for (i) species or subspecies identification (44% of alleles scored in the cheetah were subspecies specific); (ii) a sensitive measure of population genetic divergence; (iii) parentage assessment in natural populations; and (iv) forensic matching of individuals in cases of human attacks and other situations. Each of these applications offers the prospect of valuable insight into the past, present and future status of free-ranging wildlife species [37, 38].

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