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Abstract: Short tandem repeat polymorphismus (STRP), or microsatellites, are widespread among vertebrate genomes and are useful in gene mapping and population studies due to their high level of length polymorphism. The authors describe the isolation, characterization, and PCR amplification of 10 microsatellite loci from the domestic cat, Felis catus. The flanking primer sequences were conserved among other Felidae species, and amplification products demonstrated abundant polymorphism in puma, lion, cheetah, and domestic cat. The cheetah sample exhibited the lowest level of polymorphism for these loci among felid species.
Evolutionary Conservation of Ten Microsatellite Loci in Four Species of Felidae

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Short tandem repeat polymorphisms (STRP), or microsatellites, are widespread among vertebrate genomes and are useful in gene mapping and population studies due to a high level of length polymorphism. We describe here the isolation, amplification, and characterization of 10 microsatellite loci from the domestic cat, Felis catus. The flanking primer sequences were conserved among other Felidae species, and amplification products demonstrated abundant polymorphism in lion, cheetah and domestic cat. The cheetah sample exhibited the lowest level of polymorphism for these loci among felid species.

Microsatellite loci are short repetitive elements that exhibit a tandem repeat of a 1–6 base pair (bp) motif. Variations in the number of repeat units result in loci of high polymorphic information content. Since the heterozygosity of these loci was first described in humans (Weber and May 1989), they have been found to be abundant, randomly distributed, and highly polymorphic in all eukaryotic organisms examined to date. Their short length, generally less than 100 bp, leads to facilitated genotyping by PCR (polymerase chain reaction) technology. These loci have rapidly become the polymorphic marker of choice in genetic recombination maps and in the fine-scale mapping desirable in positional cloning. Genetic recombination maps are published or in progress in human, mouse, cow, pig, sheep, cat, dog, and numerous other animal and plant species (Bishop et al. 1994; Dietrich et al. 1992; Gyapay et al. 1994).

Recently, the highly polymorphic nature of microsatellites and the need for 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 (Bruford and Wayne 1990). Microsatellite loci have been used in recent examination of the social structure of pilot whales (Amos et al. 1995), genetic diversity in the bottlenosed wombat and endangered Ethiopian wolf (Gottelli et al. 1994; Taylor et al. 1994) and in assessing paternity in chimpanzees and ant colonies (Evans, in press; Morin et al. 1993).

We report on the isolation and characterization of 10 highly polymorphic (dC-dG)/(dA-dT), dinucleotide repeat loci in the domestic cat genome. We demonstrate that the 10 cat microsatellite primer pairs amplify products of predicted size in lion, cheetah, puma, Asian leopard cat, and Geoffroy's cat, suggesting their evolutionary conservation across all Felidae. Individual loci exhibit ample heterozygosity even in the genetically impoverished cheetah to serve as useful molecular genetic markers.

Materials and Methods

Genomic DNA

We extracted genomic DNA from leukocyte or tissue specimens (Sambrook et al. 1989) from the following species: domestic cat—10 unrelated individuals were used; cheetah—five unrelated captive individuals of the southern subspecies Acinonyx jubatus jubatus, collected from Kruger Park, Transvaal, or Namibia and five free-ranging individuals of the east African subspecies A. j. raineyi, collected in Tanzania and Kenya; puma—10 unrelated individuals from throughout the geographical range of the species from North America to South America; lion—two individuals from each of four populations, (1) Serengeti National Park, Tanzania, (2) Ngornogorgor Crater, Tanzania, (3) Kruger Park, South Africa, and (4) Namibia.

Construction of a genomic library, screening with radiolabeled oligonucleotide and wash conditions were as in Die- trich et al. (1992). We monitored counts of radiolabeled hybridization filters of the library screen after each wash. When the average number of counts per filter approximated 1,000–2,000 cpm on a handheld monitor, we blotted filters and exposed them to X-Omat AR film overnight. Following a secondary screen, we prepared single-stranded DNA from recombinants using a Qiagen M13 mini kit and sequenced them using a Prism Ready Reaction Dye Primer Cycle Sequencing Kit (Applied Biosystems) and an Applied Biosystems 373A DNA Sequencer. Primer pairs were designed in unique sequence flanking the microsatellite using a sequence analysis program (Primer; vers. 0.5; Lin- coln, Daly and Lander, Whitehead Institute for Biomedical Research, Cambridge, MA). All primer pairs were designed for uniform amplification conditions and a 'Tm of 60'°. Amplification products for individual loci

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were initially examined in 4% agarose gels to ensure product fidelity.

**Genotyping of Unrelated Individuals**

One primer of each pair was 5' end-labeled in a 15-μl reaction that included 75 μCi of [γ-33P]ATP at 6,000 Ci/mmol (New England Nuclear), 10× reaction buffer (Sambrook et al. 1989) and primer concentration of 1.5 μM by T4 polynucleotide kinase for 30 min at 37°C. We found that due to instability of the isolate to freeze-thaw, it was best to incorporate isothenase into a PCR product on the day of isothenase delivery. Radiolabeled PCR products could be visualized after electrophoresis in polyacrylamide gels up to 2-3 weeks later. Amplification of microsatellite loci proceeded in a 10-μl reaction, including the 10× buffer and dNTP concentration recommended by Perkin Elmer Cetus, 1.2 units of Taq DNA polymerase (Boehringer Mannheim), 0.1 Unit of Perfect Match (Stratagene), and 50 ng of DNA with primer concentrations of 200 nM for each unlabeled primer and 1.3 nm of end-labeled primer. Reactions were amplified using MJ Research Programmable Thermal Cycler (MJ Research) as follows: denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 3 min, with a final cycle of 10 min. We denatured products by an equal volume of formamide and electrophoresed them in an automated gel. 6% denaturing polyacrylamide (National Diagnostics) in 0.5× TBE buffer for 2-3 h. Gels were then exposed to Whatman 3MM blotted paper and then exposed to XAR film for 6-24 h.

**Results and Discussion**

Ten microsatellite clones were sequenced from a domestic cat, Felis catus, puma, Puma concolor, and the cheetah, Acinonyx jubatus. Among the six species, Primers were designed based on the following sequence. The 10 primer pairs used to amplify microsatellites from a domestic cat, Felis catus, puma, Puma concolor, the cheetah, Acinonyx jubatus, and three additional cheetahs (O'Brien 1986) were amplified for each of the six species and exhibited a high polymorphism (Table 1). The primer pairs represented the oldest and most recent evolutionary events, respectively, of the Felidae spanning approximately 13 million years (O'Brien 1986).

There is a broad range of heterozygosity observed among loci within a single species, ranging from 0% to 36% in all species. This suggests a large number of mutation events for each individual. The most isozyme analyses of isozyme and soluble proteins have shown abundant genetic variation in large populations throughout all species, cat, lion, and puma, with estimated average heterozygosity of 7.0%, 3.7%, and 1.8%-6.7%, respectively (Newman and O'Brien 1986).
The amplification of satellite DNA in the thermal cycler was followed by PCR, for a final cycle at 70°C for 7 min. The PCR products were analyzed in a 1% agarose gel and stained with ethidium bromide (0.5 μg/mL) before gelting and exposure to a UV lamp.

On the other hand, for the basic library, based on primer pairs with a high degree of informativeness in other analyses, the bands of PCR products were separated using agarose gel electrophoresis (1%). Each lane contained a standard DNA size control as well as a range of PCR product sizes. The bands of interest were excised from the gel, eluted, and purified using the QIAquick Gel Extraction Kit. DNA was quantified using a spectrophotometer.

Figure 2. Autoradiographs of PCR-amplified microsatellite alleles for loci Fca 270 in a three-generation intraspecific backcross population of domestic cat × Asian leopard cat. This cross is being constructed as an aid to constructing a gene map of the domestic cat, including both coding loci (Type I) and highly polymorphic microsatellite loci (Type B). Lanes: 1 = Fca 270 cDNA, 2 = Fca 270 PCR, 3 = Fca 270 PCR + Fca 270 cDNA, 4 = Fca 270 cDNA, 5 = Fca 270 PCR + Fca 270 cDNA.

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References


The amplification of microsatellites across a broad species range has previously been demonstrated by Moore et al. (1993), who observed successful amplification of 27 of 48 ovine primer pairs in bovine DNA. 62% of which exhibited polymorphism, and by Bowcock et al. (1994), who observed amplified ovine primer pairs in chimpanzee, gorilla, and organutus but found that allele frequencies could not be used to generate genetic distances. The success of amplification of microsatellites across species boundaries depends on the conservation of primer sequences. Caution needs to be taken in interpreting results in that null alleles (alleles for which there is no discernible product due to the lack of conservation of primer sequence) could result in the detection of heterozygous individuals, skewing the data toward a higher frequency of homozygous individuals. To test for the presence of null alleles, it is necessary to examine the number of homozygous individuals in the population, which significantly greater than that expected under Hardy-Weinberg equilibrium.

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Developmental genetics is the new frontier of a genetic revolution. The new level of understanding developmental genetics is a result of this book's title: An Introduction to Developmental Genetics. The book has been written by Dr. Michael R. Waters, an expert in the field of developmental genetics. The book has been written for students and researchers who are interested in understanding how genetic information is used to create the complex structures of the body. The book is divided into three parts: 1) an introduction to the concepts of developmental genetics, 2) an introduction to the basic genetic principles, and 3) an introduction to the developmental processes. The book is filled with clear and concise explanations of the latest research in the field of developmental genetics. The book is an excellent resource for anyone who is interested in understanding the complex processes that govern the development of the body. The book is highly recommended for anyone who is interested in the field of developmental genetics.