

mal records. R. Corbett, L. Creswell, and J. Rice conducted the electrophoresis. C. Cook prepared color photographs. Animals were originally collected with support from NSF grant DEB 7918482. The *Peromyscus* Genetic Stock Center is supported, in part, by NSF grants DIR 9000352 and BIR 9302181. Address reprint requests to Dr. Dawson at the address above.

The Journal of Heredity 1996:87(5)

References

- Dawson WD and Rogers DS, 1993. Genetic linkage map of the deer mouse (*Peromyscus maniculatus*). In: Genetic maps, 6th ed. (O'Brien SJ, ed). Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, 4:232-4:325.
- Dawson WD, Smith MH, and Carmon JL, 1969. A third independent occurrence of the brown mutant in *Peromyscus*. *J Hered* 60:286-288.
- Hance J, 1969. Pseudobrown, a mutant in the oldfield mouse, *Peromyscus polionotus*. *Bull SC Acad Sci* 31:42-43.
- Horner BE and Dawson WD, 1993. Coat color genetics of *Peromyscus*: III. Golden-nugget—a recessive trait in the white-footed mouse, *P. leucopus*. *J Hered* 84:306-311.
- Jackson LJ, 1988. A cDNA encoding tyrosinase-related protein maps to the *brown* locus in mouse. *Proc Natl Acad Sci USA* 85:4392-4396.
- Jackson LJ, Chambers DM, Tsukamoto K, Copeland NG, Gilbert DJ, Jenkins NA, and Hearing V, 1992. A second tyrosinase-related protein, TRP-2, maps to and is mutated at the mouse slaty locus. *EMBO J* 11:527-535.
- Levan G, Levan KL, Hanson C, Szpirer C, Szpirer J, 1992. The rat gene map. *Rat News Lett* 27:10-34.
- Pratt BM and Robbins RJ, 1982. Blonde, a new mutation in *Peromyscus maniculatus* affecting fur, skin and eye pigmentation. *J Hered* 73:69-70.
- Silvers WK, 1979. The coat colors of mice. New York: Springer-Verlag.

Received August 1, 1995
Accepted January 10, 1996

Corresponding Editor: Muriel Davison

Human Microsatellites Applicable for Analysis of Genetic Variation in Apes and Old World Monkeys

T. Coote and M. W. Bruford

In studies of the genetics and social structure of primate populations there is a need to develop highly variable genetic markers for characterizing mating success and the nature of population movement or change through time. Because of their highly polymorphic nature, relatively simple amplification and typing, and the possibility of noninvasive sampling, microsatellites have become the molecular tool of choice in such studies. However, until recently it was assumed that many microsatellite loci, which are primarily situated in noncoding regions of the genome, evolve too rapidly

to be applicable in evolutionarily divergent species. This has often resulted in the time-consuming process of cloning and sequencing microsatellites in new species. Here we describe the application of 11 human microsatellite primer pairs to a large group of primate species. The loci described are informative in all major groups of apes and Old World monkeys, although levels of allelic variability and heterozygosity differ across species. We confirm that with the use of appropriate universally applicable PCR conditions, a subset of human microsatellites are informative genetic markers in a wide range of divergent primate taxa.

Microsatellites, or simple sequences, are short (typically less than 300 bp) tandemly repeated motifs of 2-5 nucleotides that occur at large numbers of loci throughout eukaryotic genomes (Hamada et al. 1984; Tautz and Renz 1984). Their short length makes them amenable to amplification by PCR and subsequent separation on polyacrylamide gels, with the resolution of alleles differing by as little as a single base pair (Litt and Luty 1989; Tautz 1989; Weber and May 1989). Many microsatellites have been found to be highly polymorphic (e.g., Amos et al. 1993), and this variability is derived through variation in the number of repeat units in different alleles. The alleles are inherited in Mendelian fashion and mutation rates can be quite high (Bruford and Wayne 1993; Dallas 1992).

These characteristics have led to the recent utilization of microsatellites as genetic markers in a wide variety of applications. Studies in areas such as kinship (Morin et al. 1994a,b; Queller et al. 1993), population genetic structure (Bruford and Wayne 1993; Di Rienzo et al. 1994; Hughes and Queller 1993; Roy et al. 1994), conservation genetics (Gotelli et al. 1994; Taylor et al. 1994), and forensics (Hagelberg et al. 1991; Jeffreys et al. 1991) are now extensively using this class of genetic marker.

Through PCR amplification, microsatellites afford the possibility of genetic analysis using samples obtained noninvasively, such as shed hair (Morin et al. 1994a) and feces (Constable et al. 1995). With such intrinsic advantages, microsatellite loci offer considerable potential for answering a range of questions in molecular ecology and population genetics.

One problem that remains, however, is the sometimes time-consuming process of identifying and sequencing species-specific sequences that flank microsatellite loci, since sequence divergence accumulated

over evolutionary time may be too large to enable annealing of primers, even between quite closely related species. Nevertheless, homologous PCR primer pairs have been successfully used to identify microsatellites conserved in sheep and cattle (Moore et al. 1991), where about 40% of the loci amplified in both species. Also, pilot whale primers from four microsatellite loci produced PCR products in 11 species, representing most of the major cetacean radiations (Schlötterer et al. 1991). The flanking sequences showed a high level of conservation, with average differences of about 3.2% having accumulated over 35-40 million years. Recently, microsatellite primers derived from reed buntings (*Emberiza schoenicus*) have been shown to amplify products in a number of other avian species (Hanotte et al. 1994). A major obstacle to the increased application of such markers to problems in population genetics would be removed if a series of microsatellite markers were to be developed for application in relatively divergent taxonomic groups. Additionally, if such markers were adopted for application in different studies of the same species, and for studies within larger taxonomic groups, data on genetic variation derived from different studies could be compared with greater confidence.

In a related study, we have used human microsatellite primers for parentage analysis and relatedness estimation in savannah baboons, *Papio hamadryas cynocephalus*, (Altmann et al. 1996). In the baboon study we identified human microsatellite primer pairs that reliably amplified polymorphic, highly informative genetic markers. We then applied these markers in other primate species using individuals sampled from all major families of primate. This article describes the results of this experiment, and the implications for future population genetic analysis using microsatellites in primates and other major groups are discussed.

Materials and Methods

Samples

To obtain a full representation of the Primate order for this experiment, we used samples of species throughout the prosimians (lemurs, lorises, and tarsiers), ceboid primates (New World "true" monkeys, marmosets, and tamarins), and anthropoid primates (cercopithecine monkeys and the great and lesser apes, or hominoids). Forty-two individuals from 22 different species, all of different genera, were

Table 1. List of species used for initial screening (based on Martin 1990)

Order Scandentia
Tree shrew (<i>Tupaia tana</i>)
Order Primates
Prosimii (Lemurs, lorises, tarsiers)
Slender loris (<i>Loris tardigradus</i>)
Senegal bushbaby (<i>Galago senegalensis</i>)
Brown lemur (<i>Lemur fulvus</i>)
Mouse lemur (<i>Microcebus murinus</i>)
Anthropoidea
Ceboidea (New World monkeys)
Common marmoset (<i>Callithrix jacchus</i>)
Pygmy marmoset (<i>Cebuella pygmaea</i>)
Goeldi's marmoset (<i>Callimico goeldi</i>)
Douroucouli (night monkey) (<i>Aotus trivirgatus</i>)
Squirrel monkey (<i>Saimiri sciureus</i>)
Brown capuchin (<i>Cebus apella</i>)
Spider monkey (<i>Ateles paniscus</i>)
White-faced saki (<i>Pithecia pithecea</i>)
Cercopithecoidea (Old World monkeys)
De Brazza's monkey (<i>Cercopithecus neglectus</i>)
Langur (<i>Presbytis entellus</i>)
Sulawesi macaque (<i>Macaca nigra</i>)
Savannah baboon (<i>Papio hamadryas cynocephalus</i>)
Guinea baboon (<i>Papio hamadryas hamadryas</i>)
Mandrill (<i>Mandrillus sphinx</i>)
Hominoidea (Greater and lesser apes, humans)
Lar gibbon (<i>Hylobates lar</i>)
Chimpanzee (<i>Pan troglodytes</i>)
Orangutan (<i>Pongo pygmaeus</i>)
Gorilla (<i>Gorilla gorilla</i>)
Human

sampled, as well as one tree shrew (Order: Scandentia), possibly a primitive primate (Table 1).

Unless otherwise acknowledged, samples were selected from the Institute of Zoology blood and tissue bank, and were from captive-bred individuals. DNA was extracted from blood, muscle, or liver using standard phenol/chloroform extraction procedures (Sambrook et al. 1989), followed by precipitation in 100% ethanol, washing in 70% ethanol, and suspension in 1× TE buffer. Genomic DNA extractions were diluted to 1:10 for PCR reactions.

Microsatellite Primers

We selected 85 microsatellite primer pairs for potential use in paternity determination in baboons. The selection process was carried out mainly using the published list of microsatellite loci isolated during the human genome mapping project (Gyapay et al. 1994; Weissenbach et al. 1992) and which are available from Research Genetics Inc. as Human MapPairs[®]. The loci selected were, wherever possible, situated on different human chromosomes to minimize the possibility of linkage effects, and each possessed a mean heterozygosity value in humans of >0.8. In addition, we included several other human microsatellites, some of which

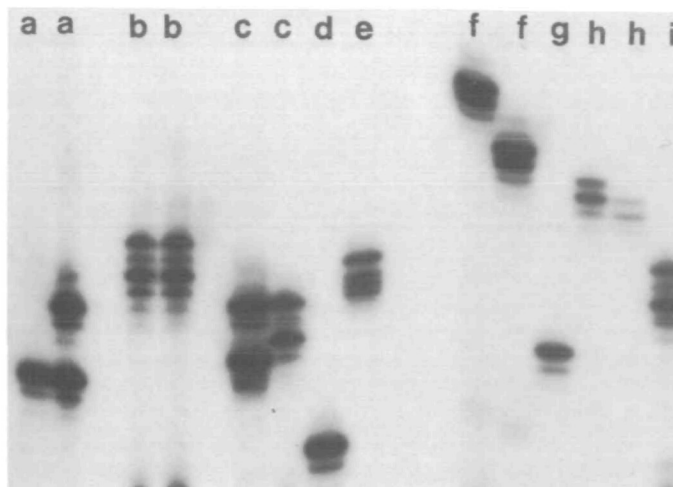


Figure 1. Primate variability assay using locus *D7S503*, showing amplification in Old World monkeys and apes: (a) gorilla; (b) orangutan; (c) chimpanzee; (d) Lar gibbon; (e) mandrill; (f) savannah baboon; (g) Sulawesi macaque; (h) langur; (i) De Brazza's monkey. There was no amplification in the tree shrew, in prosimians, or in New World monkeys.

have been previously identified for application in chimpanzees (Morin et al. 1994b).

A rapid screening, involving the amplification of each of the 85 loci in five unrelated baboons showed that 15 of these primer pairs had potential for use as polymorphic markers in the savannah baboon (*P. hamadryas cynocephalus*). Primers for 11 loci that produced the strongest, most unambiguous signals were used for this experiment: *D1S207*, *D2S141*, *D4S431*, *D6S271*, *D6S311*, *D7S503*, *D11S925*, *D13S159*, *D16S420*, *D17S791*, and *D6S287*.

Amplification Procedure

The protocol used for amplifying the microsatellites is described below. A Hybaid Omnigene thermal cyclor was used.

The forward (5') primer of each pair was end-labeled with 32p-γATP using T4-poly-nucleotide kinase (New England Biolabs) and manufacturer's buffer (70 mM Tris-HCl (pH 7.6)). All PCR reactions were carried out in a total of 10 μl containing the following: genomic DNA diluted 1 in 10, 140 μM dNTPs, 10% DMSO, 1 mM MgCl₂, and 0.45 units Taq DNA polymerase with NH₄ buffer [160 mM (NH₄)₂SO₄, 670 mM Tris-HCl (pH 8.8 at 25°C), 0.1% Tween-20], and the volume made up with dH₂O.

PCR amplifications were then carried out as follows: one 3 min denaturation at 95°C followed by seven cycles of 45 s at 95°C, 1 min at 50°C annealing temperature and 90 s at 72°C. This was followed by a further 30 cycles with the annealing temperature adjusted to 54°C and a 10 min final extension step at 72°C. This reaction protocol was derived to be applicable to all the primers and was used in all the spe-

cies studied. After amplification, 4 μl of product was loaded onto a 6% denaturing polyacrylamide gel. For a molecular weight marker we used the Multi-Pol DNA Sequencing System (Clontech Laboratories, USA) and pooled the A and T reactions of the polycloning region of single-stranded M13 mp18. Gels were exposed to autoradiographic film for between 5 h and 5 days.

Results

Screening of all Primate Species

The 11 primer pairs selected were those that were the most informative of 85 human primer pairs tested during the screening of five unrelated baboons for an allied study (Altmann et al. 1996). On initial screening, strong signals were obtained for locus *D7S503* for each species of Old World monkey and ape, and allelic variability could be seen within and among species (Figure 1). However, no obvious microsatellites were detected in any of the New World monkeys, prosimians, or in the tree shrew. A similar pattern, where amplification occurred only in Old World monkeys and apes, was found in a further five loci tested (*D13S159*, *D6S311*, *D1S207*, *D6S271*, and *D2S141*).

Subsequently, a second experiment was carried out, using larger samples of apes and Old World monkey species, but excluding New World species and prosimians. Microsatellites were amplified from a number of individuals from selected species to attempt to gain a crude measure of allelic diversity and heterozygosity. Five further loci (*D4S431*, *D11S925*, *D16S420*,

Table 2. Primate allele size and allelic diversity (bp)

	<i>D13S159</i>	<i>D7S503</i>	<i>D2S141</i>	<i>D1S207</i>	<i>D6S311</i>	<i>D6S271</i>	<i>D4S431</i>	<i>D11S925</i>	<i>D17S791</i>	<i>D16S420</i>	<i>D6S287</i>
De Brazza's monkey	158	143-147	128	137-143	240-242	210	216-220	182	148-158	177	146-148
Diana monkey	156	139-145	128	147-155	238	182-194	234-252	178-184	—	181-215	146
White colobus	158	139-153	128	155	238-244	168	306-314	182-190	156-158	175	128
Langur ^a	136-140 (4)	151-167 (5)	128 (1)	131-153 (2)	234-238 (2)	172-178 (3)	226 (1)	182-212 (3)	146-168 (5)	195-201 (4)	136-140 (3)
Sulawesi macaque	138-144	137-139	128	141-153	242-262	182-184	242	228-230	160-182	—	138-140
Savannah baboon	168-172	155-161	128-130	135-139	226-232	168-196	214-220	192-194	172-176	197	148-154
Guinea baboon ^a	170-174 (3)	155-167 (6)	128-130 (2)	139-149 (4)	232-234 (2)	186-196 (3)	214-218 (3)	190-194 (2)	160-172 (2)	191-199 (2)	142-152 (3)
Mandrill	218	147-153	150	139-147	238-244	168-194	240	190-192	160-168	189	138-154
Orangutan ^a	216-218 (2)	141-153 (5)	140-152 (2)	125-127 (2)	208 (1)	166-194 (1)	238-256 (4)	188-210 (3)	162-172 (6)	187-197 (3)	162 (3)
Gorilla	160-168	135-145	162-164	151-175	230-238	184-206	264	174-194	156-164	155-175	154
Chimpanzee ^a	160-188 (9)	137-145 (4)	140-152 (5)	135-167 (11)	210-234 (7)	176-194 (10)	244-268 (8)	170 (1)	156-178 (11)	177-197 (6)	160-176 (5)
Human ^a	170-198	149-171 (7)	144-158 (7)	133-149 (8)	232-266 (12)	144-202 (13)	244-256	174-198 (8)	162-186 (11)	175-197 (8)	158-174

^a Number of alleles where sample size exceeds three. Langur, *n* = 4; Guinea baboon, *n* = 6; orangutan, *n* = 7; chimpanzee, *n* = 10; human, *n* = 12.

D17S791, *D6S287*) that had given positive results in the baboon population were also selected.

Despite the small sample sizes available, the 11 loci were found to be polymorphic in nearly all of the species studied, though the level and nature of the polymorphisms were found to differ among species. As expected, certain loci were found to be more informative in some species than in others. For example, only two alleles of 128 and 130 bp were typed for *D2S141* for all the Old World monkeys (these were the only alleles found at this locus in 90 individuals in our savannah baboon paternity analysis). However, there were five alleles in eight orangutan individuals. Allelic diversity and range of allele size detected in all species for the different loci are shown in Table 2.

Although each locus used for this study demonstrated a large amount of variability, putative alleles were detected in some species which did not show the characteristic slippage products normally seen within the same and other species. In the highly variable locus *D11S925*, just one 170 bp allele was found in all 10 chimpanzees, whereas 8 different alleles were found in 12 humans with a heterozygosity of 0.9. All orangutans were homozygous for one 208 bp allele at locus *D6S311*, and all individuals possessed an identical 166 bp allele at locus *D6S271* and one 162bp allele at locus *D6S287*. It is possible that major insertions or deletions in the flanking sequences or within the repeat elements of this apparent lack of polymorphism. However, in each case the locus was highly variable in other species. Apart from these very few exceptions, all the loci used were highly polymorphic and heterozygous in our panel of humans and informative genetic markers for the species tested (Figure 2).

Discussion

One of the challenges faced by geneticists working on primate ecology, behavior, and population genetics is to identify highly variable loci that have informative homologues in other primate species. This is particularly important in the case of microsatellites as they have largely replaced multi- and single-locus minisatellite probes in population genetic research, especially where specific genotype information is required to track genes across generations and where samples need to be collected noninvasively. Comparisons of multilocus DNA fingerprint data across populations and among species have proved problematic in the past, since it is rarely possible to prove that homologous VNTR sequences are being compared within the patterns, and single-locus minisatellites are often inapplicable, as their characteristics have been shown to vary, sometimes greatly, across relatively narrow taxonomic ranges (Gray and Jeffreys 1992; Martin et al. 1992). This experiment was carried out with the aim of providing a set of informative PCR-based VNTR markers that could potentially be applied across many species. The experiments we carried out were of two kinds: the first addressed the question of the applicability of the markers throughout the entire primate order, and the second addressed the more detailed characteristics of the markers in the apes and Old World monkeys.

In the first experiment, all six pairs of primers tested amplified microsatellites in all species of apes and Old World monkeys. However, in contrast, there was a complete absence of cross-amplification between the human primers and the New World monkeys, prosimians, and the tree shrew. The fact that these microsatellite sequences appear to be present in all apes and Old World monkeys is interesting giv-

en that paleontological evidence places the common evolutionary origin of the Hominoidea (apes) and Cercopithecoidea (Old World monkeys) at around 30 million years bp (see Martin 1990), and a striking feature of these results is the high level of heterozygosity and polymorphism found within the ape and Old World monkey samples. We found that polymorphisms were detected within species even where only two individuals were represented, with up to the maximum possible four alleles. In addition, the nature of each species' allele size-range was almost always different from any other, while the alleles retained the characteristic slippage products usually diagnostic of a variable microsatellite locus, suggesting a species- or group-specific allelic distribution at these microsatellite loci.

In the second experiment, carried out to examine the characteristics of these markers in more detail in apes and Old World monkeys, all 11 loci showed considerable levels of heterozygosity and allelic diversity throughout the majority of the species studied. For example, locus *D2S141* seems likely to be most informative in ape species, becoming less variable in species more phylogenetically distinct from humans; *D16S420* detected only three alleles in eight Guinea and savannah baboon individuals and all were homozygotes; only two alleles were detected at locus *D13S159* in seven homozygous orangutan individuals; and *D11S925* is unlikely to be informative for chimpanzees.

Although many of the populations studied here were likely to show decreased levels of polymorphism due to the generally low genetic variability encountered in captive primate populations, primarily due to founder effect, genetic drift, and inbreeding (e.g., Bruford and Altmann 1993; Morin and Ryder 1992), high levels of vari-

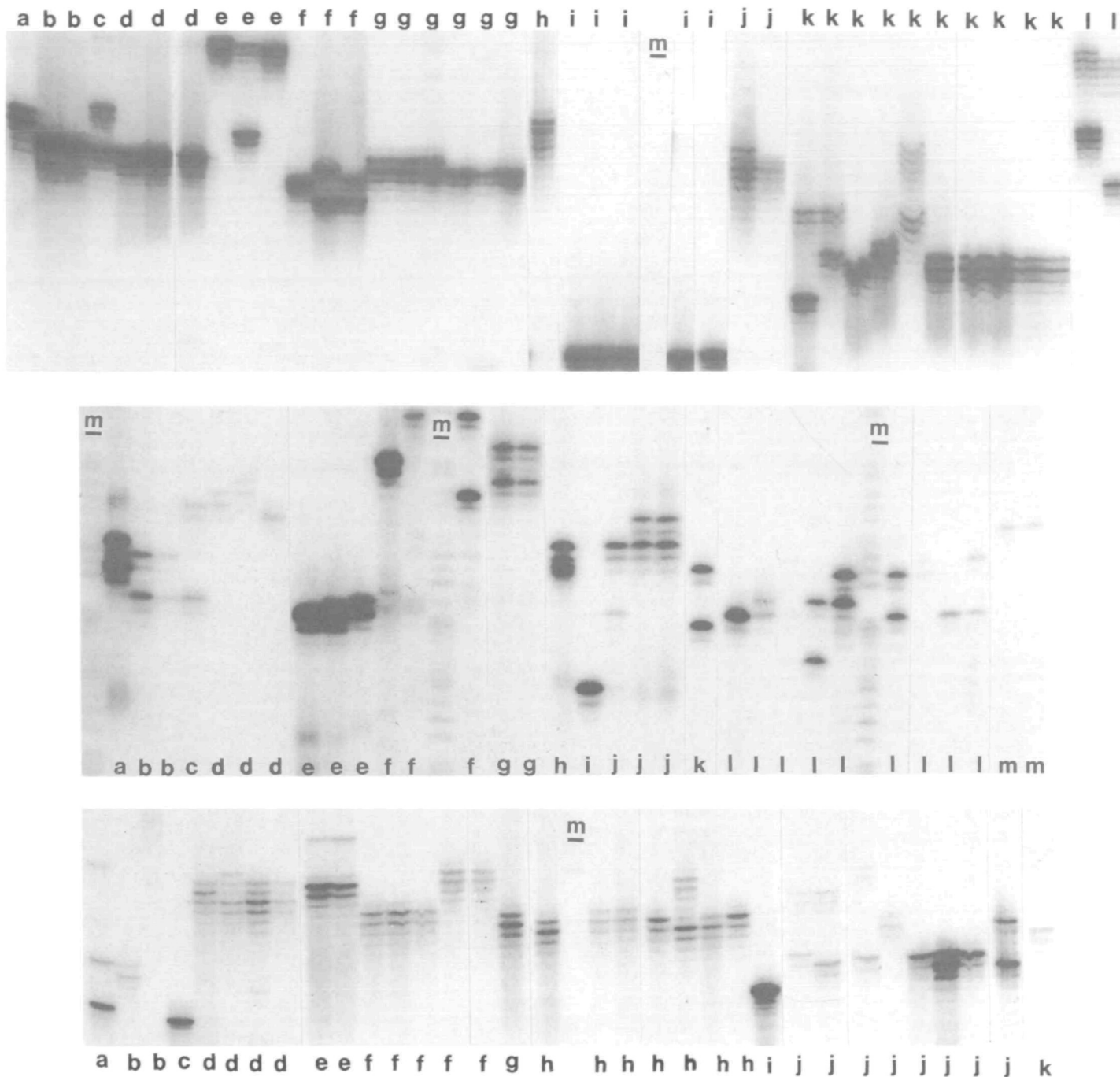


Figure 2. (Top) Locus *D6S311*: (a) De Brazza's monkey; (b) Diana monkey; (c) white colobus; (d) langur; (e) Sulawesi macaque; (f) savannah baboon; (g) Guinea baboon; (h) mandrill; (i) orangutan; (j) gorilla; (k) chimpanzee; (l) human (*m* = molecular weight marker). (Center) Locus *D7S503*: (a) De Brazza's monkey; (b) Diana monkey; (c) white colobus; (d) langur; (e) Sulawesi macaque; (f) savannah baboon; (g) Guinea baboon; (h) mandrill; (i) Lar gibbon; (j) orangutan; (k) gorilla; (l) chimpanzee; (m) human (*m* = molecular weight marker). (Bottom) Locus *D16S420*: (a) De Brazza's monkey; (b) Diana monkey; (c) white colobus; (d) langur; (e) savannah baboon; (f) Guinea baboon; (g) mandrill; (h) orangutan; (i) gorilla; (j) chimpanzee; (i) human (*m* = molecular weight marker).

ability were generally found. This makes the monomorphism found at *D11S925* in chimpanzees and at *D6S311*, *D6S271*, and *D6S287* in orangutans somewhat unexpected. The possibility that these results are PCR artifacts cannot be ruled out, but is unlikely because of the consistent nature of the results at the other loci studied and the fact that the experiment was repeated several times with the same result.

Previous studies of primates have shown that the conservation of flanking sequences has only been able to detect polymorphisms in closely related species. Inoue and Takenaka (1993) found that the primers designed for Japanese macaques detected size differences in other macaques as well as three different species of Cercopithecoïd monkeys. Primers designed specifically for chimpanzees (Takenaka et

al. 1993) were uninformative and unsuitable for individual discrimination among Japanese macaques because the banding pattern was monomorphic.

All the primers were selected from the human genome database for use in savannah baboons, and those that showed informative variation within that species also hybridized with DNA from all the other Old World monkey and ape families.

The possibility remains open that with appropriate PCR conditions, microsatellite primers designed for certain species may be informative in more divergent taxa than previously believed or demonstrated. With the recent increase in the use of microsatellite techniques for analyzing population genetic structure and evolution there is a need for further studies of this type. Certainly, from the thousands of microsatellites so far isolated from the human genome, a significant number will be applicable for use in a range of other primates, though these experiments demonstrate a variability too high for direct comparisons between species in phylogenetic analysis.

From the Conservation Genetics Group, Institute of Zoology, Regents Park, London NW1 4RY, UK. This work was supported by NSF grant IBN-9223335 to J. Altmann, University of Chicago, and by the Institute of Zoology. We are grateful to Research Genetics Inc. for permission to publish results using these primers and for help and information about them. Helen Stanley provided information on other primer sequences. We would like to acknowledge the help of Jeanne Altmann and Robert Wayne for helpful discussions and for critically reviewing earlier versions of this manuscript. All samples were from the Institute of Zoology blood and tissue bank, except the savannah baboon samples which were taken from a wild population in Amboseli National Park, Kenya. Valuable technical assistance was provided by Susan Haines and Harriet Green, and photography was carried out by Terry Dennett at the Institute of Zoology. Lastly, we would like to thank those people who gave blood for human analysis: Sheila Boddy, Tamsin Burland, Steve Casey, Dave Cheesman, Simon Francis, Miranda Kadwell, Yasmin Mohammed, Tim Robson, and Ilik Saccheri.

The Journal of Heredity 1996:87(5)

References

- Altmann J, Alberts SC, Haines SA, Dubach J, Muruthi P, Cooté T, Geffen E, Cheesman DJ, Mututua RS, Saiyalel SN, Wayne RK, Lacy RL, and Bruford MW, 1996. Behaviour predicts genetic structure in a wild primate group. *Proc Natl Acad Sci USA* 93:5797-5801.
- Amos W, Schlötterer C, and Tautz D, 1993. Social structure of pilot whales revealed by analytical DNA profiling. *Science* 260:670-672.
- Bruford MW and Altmann J, 1993. DNA fingerprinting and the problems of paternity determination in an inbred captive population of Guinea baboons (*Papio hamadryas papio*). *Primates* 34(3):403-411.
- Bruford MW and Wayne RK, 1993. Microsatellites and their application to population genetic studies. *Curr Opin Genet Devel* 3:939-943.
- Constable JJ, Packer C, Collins DA, and Pusey AE, 1995. Nuclear DNA from primate dung. *Nature* 373:393.
- Dallas JF, 1992. Estimation of microsatellite mutation rates in recombinant inbred strains of mice. *Mammal Genome* 5:32-38.
- Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, and Freimer NB, 1994. Mutational processes of simple-sequence repeat loci in human populations. *Proc Natl Acad Sci USA* 91:3166-3170.
- Gotelli D, Sillero-Zubiri C, Applebaum GD, Roy MS, Gorman DJ, Garcia-Moreno J, Ostrander EA, and Wayne RK, 1994. Molecular genetics of the most endangered canid: the Ethiopian wolf *Canis simensis*. *Mol Ecol* 3:301-312.
- Gray IC and Jeffreys AJ, 1992. Evolutionary transience of hypervariable minisatellites in man and the primates. *Proc R Soc Lond B* 243:241-253.
- Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, Bernardi G, Lathrop M, and Weissenbach J, 1994. The 1993-94 Genethon human linkage map. *Nat Genet* 7:246-339.
- Hagelberg E, Gray IC, and Jefferys AJ, 1991. Identification of the skeletal remains of a murder victim by DNA analysis. *Nature* 352:427-429.
- Hamada H, Seidman M, Howard BH, and Gorman CM, 1984. Enhanced gene expression by the poly (dT-dG) poly (dC-dA) sequence. *Mol Cell Biol* 4:2622-2630.
- Hanotte O, Zanon C, Pugh A, Greig C, Dixon A, and Burke T, 1994. Isolation and characterization of microsatellite loci in a passerine bird: the reed bunting *Emberiza schoenicus*. *Mol Ecol* 3:529-530.
- Hughes CR and Queller DC, 1993. Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. *Mol Ecol* 2:131-137.
- Inoue M and Takenaka O, 1993. Japanese macaque microsatellite PCR primers for paternity testing. *Primates* 34(1):37-45.
- Jeffreys AJ, Allen AJ, Hagelberg E, and Sonnberg A, 1991. Identification of the skeletal remains of Josef Mengele by DNA analysis. *Nature* 352:427-429.
- Litt M and Luty JA, 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am J Hum Genet* 44:397-401.
- Martin RD, 1990. Primate origins and evolution: a phylogenetic reconstruction. London: Chapman and Hall.
- Martin RD, Dixon AF, and Wickings EJ (eds), 1992. Paternity in primates: genetic tests and theories. Implications of human DNA fingerprinting. Basel: Karger.
- Moore SS, Sargeant LL, King TJ, Mattick JS, Georges M, and Hetzel DJS, 1991. The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. *Genomics* 10:654-660.
- Morin PA and Ryder O, 1992. Founder contribution and pedigree inference in a captive breeding colony of lion-tailed macaques, using mitochondrial DNA and DNA fingerprint analyses. *Zoo Biol* 10(4):341-352.
- Morin PA, Moore JJ, Chakraborty R, Jin L, Goodall J, and Woodruff DS, 1994a. Kin selection, social structure, gene flow, and the evolution of chimpanzees. *Science* 265:1193-1201.
- Morin PA, Wallis J, Moore JJ, and Woodruff DS, 1994b. Paternity exclusion in a community of wild chimpanzees using hypervariable simple sequence repeats. *Mol Ecol* 3:469-478.
- Queller DC, Strassman JE, and Hughes CR, 1993. Microsatellites and kinship. *Trends Ecol Evol* 8(8):285-288.
- Roy MS, Geffen E, Smith D, Ostrander ES, and Wayne RK, 1994. Patterns of differentiation and hybridization in North American canids, revealed by analysis of microsatellite loci. *Mol Biol Evol* 11(4):553-570.
- Sambrook J, Fritsch EF, and Maniatis T, 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
- Schlötterer C, Amos B, and Tautz D, 1991. Conservation of polymorphic simple sequence loci in cetacean species. *Nature* 354:63-65.
- Takenaka O, Takasaki H, Kawamoto S, Arakawa M, and Takenaka A, 1993. Polymorphic microsatellite DNA amplification customized for chimpanzee paternity testing. *Primates* 34(1):27-35.
- Tautz D, 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res* 17:6463-6471.
- Tautz D and Renz M, 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Res* 12:4127-4138.
- Taylor AC, Sherwin WB, and Wayne RK, 1994. Genetic variation of microsatellite loci in a bottlenecked species: the northern hairy-nosed wombat *Lasiorninus kreftii*. *Mol Ecol* 3:277-290.
- Weber JL and May PE, 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am J Hum Genet* 44:388-396.
- Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vaysseix G, and Lathrop M, 1992. A second-generation linkage map of the human genome. *Nature* 359:794-801.

Received June 20, 1995

Accepted January 26, 1996

Corresponding Editor: Oliver A. Ryder

Estimating and Testing Hypotheses About the Number of Genes Using Inbred-Backcross Data

K. M. Eskridge and D. P. Coyne

We describe an approach to estimate and test hypotheses about the number of genes that differ between the donor parent and the recurrent parent when using the inbred-backcross breeding procedure. The method is used to estimate the number of genes determining resistance of common beans (*Phaseolus vulgaris* L.) to common bacterial blight incited by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye. beans.

The backcross breeding procedure has long been used to successfully transfer a major gene(s) controlling a desired trait from a donor or nonrecurrent parent to a superior cultivar or line (recurrent parent). Genes are transferred by repeated backcrossing to the recurrent parent and selecting in each generation for the desired trait (Briggs 1935; Briggs and Knowles 1967). This breeding procedure has generally been used to transfer major genes controlling such traits as disease resistance to superior cultivars of self-pollinated crops, inbred lines of cross-pollinated crops, or cultivars of cross-pollinated crops. Generally, two or more backcrosses are made to the recurrent parent in order to reconstitute most of the genotype of the desired parent along with the designated genes from the donor parent, followed by several generations of selfing to attain a homozygous line. It is useful to plant breeders using the inbred-backcross procedure to know the approximate number of genes (k) that differ between the recurrent and nonrecurrent parent controlling a desired quantitative trait. In ad-