UTILITY OF A SATELLITE DNA SEQUENCE AS A GENETIC MARKER IN A HYBRID ZONE OF POCKET GOPHERS (GENUS *GEOMYS*)

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ABSTRACT.— A 4.8 kb *Hae* III restriction fragment length polymorphism (RFLP) distinguishes samples of *Geomys bursarius major* from the majority of individuals of *G. knoxjonesi*. This restriction fragment was isolated from a cosmid library of *G. b. major*. We studied this polymorphism in 124 individuals previously characterized by chromosomal, allozymic (three loci), mitochondrial DNA (mtDNA), ribosomal DNA (rDNA), and DNA content analysis (Baker et al., 1989; Bradley et al., 1991a, 1991b). Against such an established genetic background for each individual, it is possible to better understand how new markers are inherited, and to what extent they resolve relationships within this hybrid zone. Fluorescence *in situ* hybridization indicates this family of satellite DNA is distributed on 66 chromosomes in *G. b. major*, and 60 chromosomes in *G. knoxjonesi*. The chromosomal distribution is interstitial and copy number is highly variable among chromosomes. However, the 4.8 kb *Hae* III RFLP is most likely restricted to a single pair of homologous chromosomes. We conclude that the 4.8 kb band is diagnostic to many individuals, but is not as useful in determining the exact status of hybrid and parental individuals as the allozymes and other DNA markers because it is not fixed between pure parental types.

Key words: pocket gophers, *Geomys bursarius*, *Geomys knoxjonesi*, hybrid zone, *in situ* hybridization, satellite DNA

Even though one of the four well accepted tenets of Darwinian evolution is that there is variation among individuals within a species, studies of the processes of evolution are still limited by the availability of easily detectable, discrete genetic markers that identify individuals, family groups, populations, etc. Such basic evolutionary phenomena as relative fitness, reproductive success, population subdivision, dispersal, gene flow, and effective population size can be better understood if there are sufficient markers to accurately document relationships of individuals within a population. The suite of markers required to address these questions should include not only several non-linked, biparentally inherited characters, but also those that are maternally and paternally inherited. Variations in DNA holds considerable promise for resolving this need. This study is a continuation of our efforts to develop methods to produce such genetic markers for a variety of taxa from natural populations (Simmons et al., 1992; Longmire et al., 1988, 1991, 1992; Baker et al., 1989; Bradley et al., 1991*a*,*b*).

Our design for evaluating evolutionary markers in this study utilizes a hybrid zone between two species of pocket gophers (*Geomys*) where there is a well documented number of individuals representing parental types, F1s, and subsequent crosses (Baker et al., 1989; Bradley et al., 1991*a*,*b*). We studied DNA samples from 124 individuals previously characterized by chromosomal, allozymic (three loci), mitochondrial DNA (mtDNA), and ribosomal DNA (rDNA) differences, as well as differences in DNA content (Baker et al., 1989; Bradley et al., 1991*a*,*b*). Against such an established genetic background for each individual, it is possible to better understand how new markers are inherited and to what extent they resolve relationships within this hybrid zone.

In this study, we examined an RFLP present in a DNA satellite sequence. Although satellite DNA and heterochromatin have received mixed reviews as genetic markers in establishing phylogenetic relationships, there are examples where such sequences are superior to other genetic markers thus far studied (Van Den Bussche et al., 1993; Hamilton et al., 1990, 1992; Simmons et al., 1992). For example, an RFLP in heterochromatic DNA was more diagnostic than allozymes, mtDNA, or rDNA in

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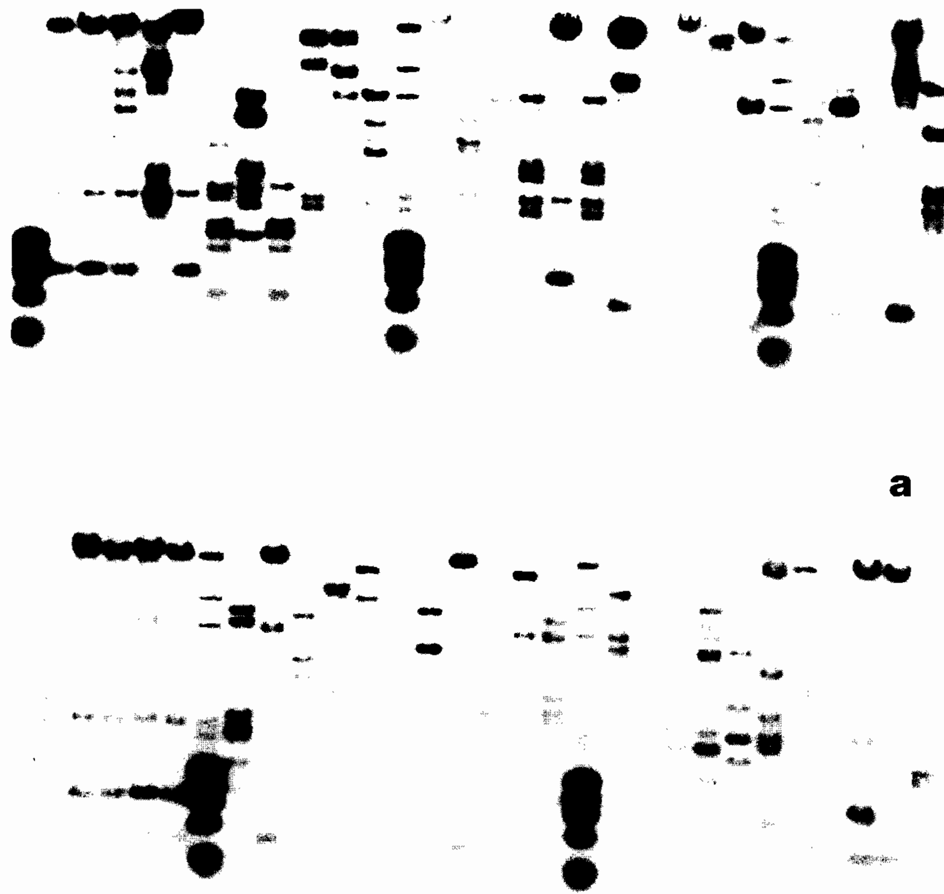


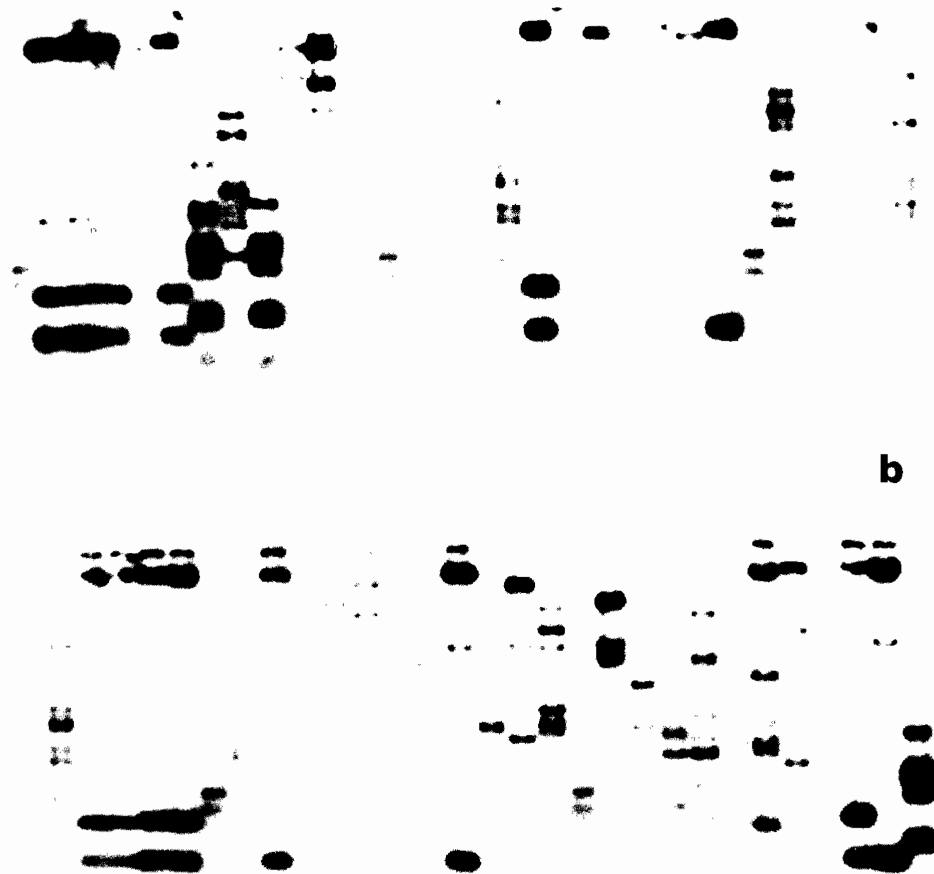
Fig. 1. Hybridization of cosmid blots to (A, above) electroeluted 3.3 kb fragment, and (B, opposite page) nick translated *Geomys* genomic DNA. This technique produces two essentially identical Southern blots for comparing hybridization patterns to different probes. Nearly identical hybridization patterns produced by *Geomys* genomic DNA and the electroeluted 3.3 kb fragment probes indicate the Geo C8 related family of repeats is highly represented in the *Geomys* genome.

distinguishing individuals within a hybrid zone and accurately identifying the chromosomal race of individuals of the white-footed mouse, *Peromyscus leucopus* (Simmons et al., 1992). The potential value of satellite DNA as a genetic marker in populations of *Geomys* has not previously been examined. To explore this potential, we examined a *Hae* III fragment identified by a restriction enzyme screening procedure. This fragment, which was initially visualized from ethidium bromide-stained gels, was present in a subset of the reference samples of *G. b. major* but was absent in a comparable subset of *G. knoxjonesi*.

Methods and Materials

Individuals for this study were collected along a naturally-occurring hybrid zone in eastern New Mexico (Baker et al., 1989; Bradley et al., 1991*a*; Pembleton and Baker, 1978). High molecular weight DNA was isolated following the protocol of Longmire et al. (1991) with the exception that finely diced frozen liver or muscle tissue (0.5 g) was substituted for blood as a DNA source. Approximately 5 ug of DNA from each individual was digested with *Hae* III and electrophoresed on 0.8% agarose gels. Gels were transferred to Boehringer Mannheim positively charged nylon membranes under alkaline conditions (0.4 M NaOH).

A cosmid library was constructed from genomic DNA isolated from an individual with the genetic characteristics of a pure parental type of *G. b. major* (Baker et al., 1989). This library was obtained by partially digesting genomic DNA with *Sau*3A I, followed by dephosphorylation and ligation to *Bam*H I prepared sCos-1 cloning arms (Evans et al., 1989). The ligated DNA was packaged using Gigapack II Gold extracts (Stratagene) and infected into DH5aMCR bacteria. Cloning a total of



1.0 ug of partially digested *G. b. major* DNA yielded 394,460 independent recombinants for a 5.3-fold representation of the *G. b. major* genome

A 1X representation of the library (75,000 clones) was plated and screened by hybridization to the electroeluted *Hae* III fragment. Eighty-five positive clones were selected and recombinant cosmids were recovered using conventional miniprep methods (Stallings et al., 1990). The clones were digested with *Eco*R I and electrophoresed on 0.8% agarose gels to obtain restriction patterns of the respective cosmids. The gels were bidirectionally blotted. One membrane was hybridized to the electroeluted 3.3 kb *Hae* III fragment, whereas the other membrane was hybridized to genomic *G. b. major* DNA under the following conditions. Membranes were prehybridized for 2 h at 42° C in 35% formamide, 6X SSC, 0.005 M EDTA (pH 8.0), and 0.25% w/v powdered milk (Vassart et al., 1987). Hybridization was performed overnight at 42° C in the same solution containing 1X 10^6 cpm/ml of probe. Following hybridization, membranes were washed two times for 15 min at 23° C in 2X SSC, 0.1% SDS, and two times for 15 min at 50°C in 0.1X SSC and 0.1% SDS. Washed membranes were autoradiographed at -70° C in cassettes containing intensifying screens. The electroeluted fragment and cosmid clones were nick translated to specific activities greater than 10^8 cpm/ug and used to screen Southern blots containing *Hae* III digested *G. b. major* DNA using the same hybridization conditions listed above.

Linearized insert DNA from clone Geo C8 (500 ng) was predigested for 15 min into fragments ranging from 100-500 bp using 60 units (U) DNA polymerase I/DNase I at 37° C in the absence of dNTPs, followed by biotinylation for 90 min using Enzo Diagnostics Bio-11-dUTP and an additional 35 U DNA polymerase I at 15° C. Cells containing metaphase chromosomes were extracted from the bone marrow of four animals following the yeast-stress method of Lee and Elder (1980). Fixed cell suspensions were washed in fresh methanol:acetic acid solution (3:1) immediately prior to use. Cell suspensions were dropped and flame dried onto slides. To determine the chromosomal location of clone Geo C8, a fluorescence *in situ* hybridization procedure modified from Moyzis et al. (1987; 1988) was employed as fol-

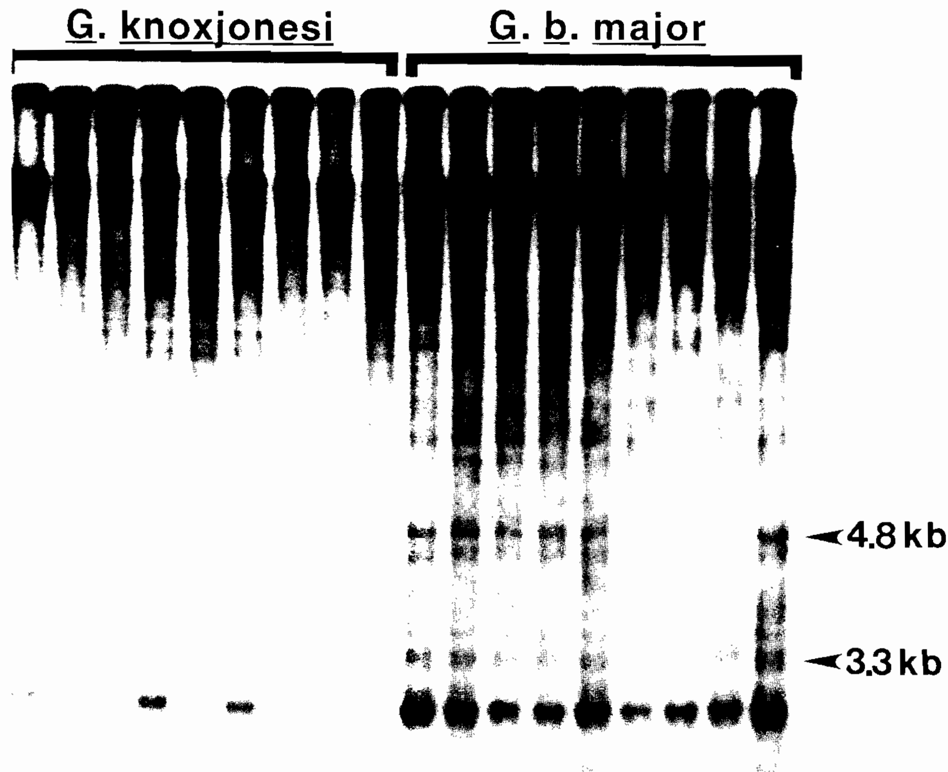


Fig. 2. *Hae* III digested DNAs form *G. b. major* and *G. knoxjonesi* probed with clone Geo C8. Arrows indicate locations of 3.3 kb fragment that was used to screen the cosmid library, and the 4.8 kb marker fragment that was diagnostic for *G. b. major*. Note the presence of several additional marker fragments for both *G. knoxjonesi* and *G. b. major*.

lows. Slides were treated with 200 µl of RNase A (100 ug/ml in 2X SSC, pH 7.0) for 60 min in a humidified incubator at 37° C, and then washed in 4 changes of 2X SSC followed by 4 washes in 70, 80, 95, and 100% EtOH. Slides were denatured for 2 min in 70% formamide in 2X SSC at 70° C, and subsequently chilled for 2 min in 70% EtOH at 4° C and dehydrated in an ethanol series (80, 95, and 100%). The biotinylated probe was denatured for 5 min in 70% formamide in 2X SSC at 70° C and placed on ice for 2 min. Slides were hybridized overnight in a humidified chamber at 37° C with 95 ng of denatured, biotinylated probe DNA in 50% formamide in 2X SSC, 100 µg *E. coli* carrier DNA. Following hybridization, slides were washed in 4 changes of 50% formamide in 2X SSC and 4 changes of 2X SSC at 40° C. All wash times were for 2 min. Slides were placed in BT buffer (0.30M sodium chloride, 0.10M sodium bicarbonate, 0.005% Tween-20) for 2 min at room temperature and then blocked for 5 min with 5% BSA in BT buffer at room temperature. A 50 µl aliquot of fluorescein isothiocyanate (FITC)-labeled avidin (1:700 dilution in 5% BSA/BT buffer) was added, and the slides incubated at 37° C for 45 min. Following incubation, slides were washed in 4 changes of BT buffer at 40° C. Chromosomes were counterstained with propidium iodide (125 µl/ml) in antifade (p-phenylenediamine dihydrochloride). Chromosomes were visualized using a Zeiss Axioplan microscope and recorded using Ektar 1000 and Kodacolor 400 film.

Results

After *Hae* III digestion and electrophoresis of *Geomys* genomic DNA, a 3.3 kb fragment of repetitive

DNA was visible in reference samples of *G. b. major* but not in reference samples of *G. knoxjonesi*. This *Hae* III fragment was then electroeluted and used to screen the *Geomys* cosmid library.

From the initial 85 clones, 73 positive clones were chosen for further study. These clones were digested with *Eco*R I and bidirectionally blotted to nylon membranes. These 73 clones were separated into 41 groups based on similarity of restriction patterns. The largest of these groups contained 15 clones that displayed identical restriction patterns, and there were a total of 12 groups that contained two or more clones, whereas 28 groups represented unique clones.

The bidirectional blots were hybridized to the electroeluted 3.3 kb fragment and to *Geomys* genomic DNA (Fig. 1). Essentially all of the clones hybridized to the *Hae* III fragment and to genomic DNA of *G. b. major* in an identical manner, indicating that sequences sharing a high level of identity to the electroeluted band are highly represented in the *G. b. major* genome.

Cosmid clones were used to screen Southern blots containing *Hae* III digested genomic DNA to determine which clones best distinguished *G. knoxjonesi* from *G. b. major*. Clone Geo C8 (a member of the group of 15 identical clones) generated a complex banding pattern that included a strongly hybridizing fragment at 4.8 kb that differentiated the majority of *G. b. major* individuals and hybrids from *G. knoxjonesi* individuals (Fig. 2). The results of screening DNA samples from 124 individuals representative of both reference samples and individuals from the hybrid zone for the presence or absence of the 4.8 kb fragment is summarized in Table 1.

Fluorescence *in situ* hybridization of clone Geo C8 to two individuals of *G. b. major* revealed 66 chromosomes containing these sequences (Fig. 3*a*). Such hybridization occurred interstitially, but in nearly all examples the interstitial bands were nearer to the centromeric regions rather than the telomeric. In nearly all chromosomes, hybridization did not occur at the centromere or telomere regions. No visible hybridization is apparent in four large and two small acrocentric elements. The amount of hybridization varied greatly among chromosomes. *In situ* hybridization of clone Geo C8 to *G. knoxjonesi* karyotypes revealed 60 chromosomes with hybridization patterns similar to those of *G. b. major*, and 10 acrocentric elements (four large and six small) with no visible hybridization (Fig. 3*b*).

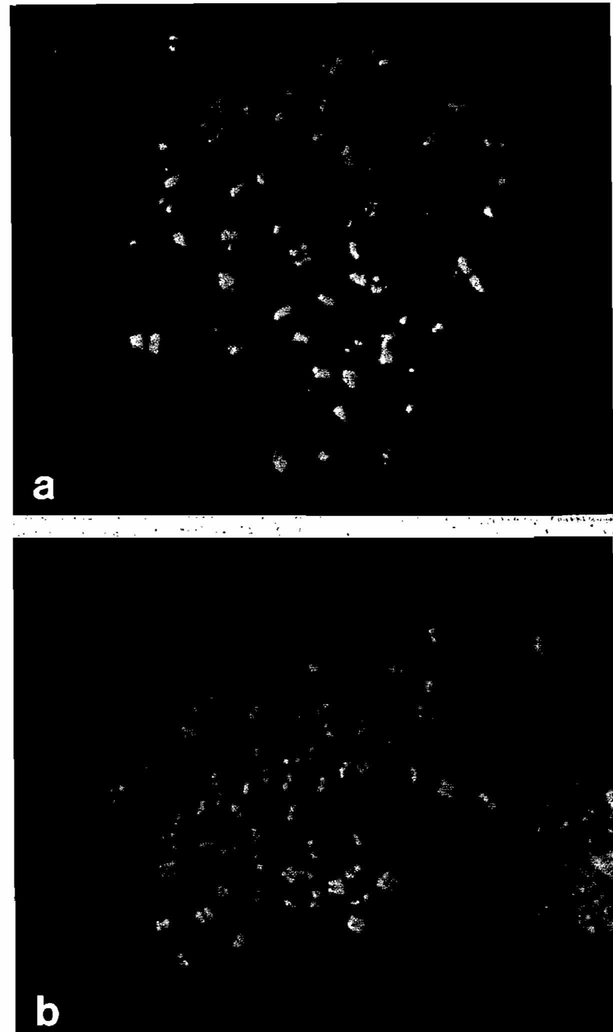


Fig. 3. Fluorescent *in situ* hybridization of clone Geo C8 to reference samples of metaphase chromosomes of, (A) *G. b. major*, and (B) *G. knoxjonesi*. Yellow signal indicates hybridization of probe. The chromosomes are counterstained orange with propidium iodide.

Table 1. Summary of examination of 124 individuals of Geomys *for the presence of a 4.8 kb restriction fragment length polymorphism revealed by probing genomic DNA with the cosmid clone Geo C8. MR = reference sample of* G. b. major; *KR = reference sample of* G. knoxjonesi. *Percent* major *was derived from allozymes and diploid number as described in Baker et al.(1989). These 124 individuals are the same as were reported in Baker et al. (1989), and Bradley et al. (1991*a*).*

percent *major*—>	MR	8/8	7/8	6/8	5/8	4/8	3/8	2/8	1/8	0/8	KR
band present	17	23	7	2	5	12	3	1	3	1	2
not present	0	0	0	0	0	2	0	0	3	22	21

Discussion

When hybridized to Southern blots containing *Hae* III digested genomic DNA samples, the Geo C8 clone revealed a 4.8 kb fragment in all pure *G. b. major*. This fragment was present in parentals found in the hybrid zone, and in the reference sample from a geographically distant population. It was also present in 33 out of 38 hybrid individuals examined (Table 1 and Appendix). Of the 15 individuals that had a predominance of genetic markers (chromosomes, allozymes, mtDNA, and rDNA) from the *G. b. major* genome, all were identified by the presence of the 4.8 kb fragment revealed by clone Geo C8. Baker et al. (1989) identified 17 hybrid individuals as potential F_1s. These 17 individuals were further divided into subsets that appeared to be F_1s in all markers (six individuals) and a group that was F_1-like (11 individuals), based on rDNA. Baker et al. (1989) concluded that these 11 individuals were probably the result of some cross other than between parental types. The 4.8 kb marker of clone Geo C8 was present in all individuals that qualified as F_1s using the above criteria (Baker et al., 1989). This fragment was also present in all but two of the F_1-like individuals. Of the nine individuals whose genetic markers were predominately identified as *G. knoxjonesi* by Baker et al. (1989), six possessed the 4.8 kb marker. One of 23 individuals identified as pure *G. knoxjonesi* from the hybrid zone also had the 4.8 kb marker present. Although this may indicate that this individual has a hybrid ancestry, there is some data that suggests that the 4.8 kb fragment is polymorphic in parental populations of *G. knoxjonesi*. Evidence that the 4.8 kb fragment is not absent from pure *G. knoxjonesi* is provided by the observation that 2 of 24 individuals from the reference sample of *G. knoxjonesi* possessed this genetic marker.

Two questions that would aid in understanding the significance of this variation in satellite DNA are: 1) what is the chromosomal distribution of this satellite family of tandemly repeated sequences in *G. knoxjonesi* and *G. b. major;* and 2) what is the chromosomal distribution of the subset of this tandemly repeated element that contains the specific restriction site that produces the 4.8 kb marker distinguishing *G. b. major* from *G. knoxjonesi*.

Concerning the first question, the results of fluorescence *in situ* hybridization studies (Fig. 3) revealed that this family of repetitive elements distinguishes *G. b. major* (present on 66 chromosomes) from *G. knoxjonesi* (present on 60 chromosomes). Although Bradley et al. (1991*c*) did not find that fluorescence *in situ* hybridization with seven other repetitive sequences was useful in distinguishing *G. b. major* from *G. knoxjonesi*, clone Geo C8 can certainly be added to the numerous other characters that distinguish most individuals of these two species. One other feature of the chromosomal distribution of this particular family of satellite DNA is unique from that described for heterochromatin in other rodents. Heterochromatin in *Peromyscus*, *Reithrodontomys*, *Onychomys*, *Mus*, *Thomomys*, and *Dipodomys* is localized within centromeric or telomeric regions, but not interstitially as seen in Figure 3 (Bradley et al., 1991*c*; Hamilton et al., 1990, 1992; Joseph et al., 1989; Van Den Bussche et al., 1992). Additionally, the heterochromatin in these other species of rodents is C-band positive. In *Geomys*, however, autosomal C-band material is generally restricted to the centromeric regions and does not reflect the pattern seen in Figure 3 (Smolen, 1992; Qumsiyeh et al., 1988). Thus, the family of repeats detected by clone Geo C8 is not localized within centromeric heterochromatin and is not C-band positive..

Concerning the chromosomal distribution of the 4.8 kb RFLP, the following observations are interesting. If this RFLP was distributed on all of the chromsomes that *in situ* hybridized to clone Geo C8, then it would be expected that all hybrid individuals would have this marker, although the intensity of hybridization would be expected to decrease as the proportion of the *G. b. major* genome decreased. However, two observations suggest that this particular RFLP is localized on a single homologous pair of chromosomes as has been proposed for an RFLP family in fish (Haaf et al., 1993). First, F_1-like individuals are found that do not contain the 4.8 kb marker. If this marker was distributed on multiple chromosomes, then by basic independent assortment, one would expect the presence of this fragment in all (or nearly all) hybrid individuals that contained equal representations of the genomes of *G. b. major* and *G. knoxjonesi*. Baker et al. (1989) hypothesized that some of their F_1-like individuals were the result of crosses between individuals that were 1/8 and 8/8 *G. b. major* and individuals that were 7/8 and 0/8 *G. b. major*. The F_1-like individuals that do not have this marker were two of the individuals that Baker et al. (1989) hypothesized were not F_1s. If this diagnostic RFLP is present in a single block on one pair of chromosomes, then up to 25% of the offspring of these types of crosses might not possess this marker. A two

character distribution is almost as likely—if a one homologue distribution is true then 1/4 of the 3/8's and 1/2 of the 2/8's should lack the band.

The second observation is that when this band is present in populations of pure parental *G. knoxjonesi*, it is easily visualized and does not appear in any of the individuals as a faint band. Within hybrid individuals, there is some variation in intensity (perhaps due to variation in the homozygous and heterozygous condition) but there is no difficulty in determining presence or absence of this band. We can not rule out at this time that some of the variation observed among individuals is due to variation in copy number of this specific repeat within the single block.

In summary, this band is diagnostic to many individuals but it certainly is not as useful in determining the exact status of hybrid and parental individuals as the allozyme and other DNA markers (Baker et al., 1989; Bradley et al., 1991*a*,*b*). The reason for this lower level of resolution is most likely that the marker is not fixed between pure parental types.

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Appendix

Genotypes of individuals are as follows: MR = reference sample of G. b. major; *KR = reference sample of* G. knoxjonesi ; *M* = G. b. major *from zone; H = heterozygote; K* = G. knoxjonesi *from zone. rDNA values expressed as percentage of* G. b. major. *TK 28191-30750 from Baker et al. (1989), TK 30751-30796 from Bradley et al. (1991a).*

TK#	SEX	2N	mtDNA	rDNA	Adh	Ldh	Pep major	percent presence	4.8 kb marker
28191	M	72	M	100	M	M	M	M 8/8	+
28192	F	70	K	0	K	K	K	K 0/8	-
28194	F	70	K	0	K	K	K	K 0/8	-
28195	F	70	K	0	K	K	K	K 0/8	-
28196	F	70	M	0	H	K	K	H 1/8	-
28197	F	70	K	0	K	K	K	K 0/8	-
30505	F	71	K	100	H	H	H	H 4/8	+
30509	F	72	M	100	M	M	M	M 8/8	+
30512	F	72	M	100	M	M	M	M 8/8	+
30646	M	72	M	100	M	M	M	M 8/8	+
30647	M	71	K	47.1	H	H	H	H 4/8	+
30648	M	70	K	5.6	K	K	K	H 0/8	-
30649	F	70	K	0	K	K	K	K 0/8	-
30650	F	71	K	38.7	H	H	K	H 3/8	+
30651	F	70	K	0	K	K	K	K 0/8	-
30652	M	72	M	100	M	M	M	M 8/8	+
30653	M	72	K	97.5	M	M	H	H 7/8	+
30654	M	71	K	81.2	H	H	M	H 5/8	+
30655	F	70	K	13.2	K	K	K	H 0/8	-
30659	M	71	K	65.0	H	H	H	H 4/8	+
30661	M	71	K	35.9	H	H	H	H 4/8	+
30662	F	71	K	77.9	H	H	H	H 4/8	+
30663	F	72	M	100	M	M	M	M 8/8	+
30664	M	71	K	70.1	H	M	H	H 5/8	+
30666	M	72	M	100	M	M	M	M 8/8	+
30670	F	72	M	100	M	M	M	M 8/8	+
30671	M	70	K	0	K	K	K	K 0/8	-
30672	M	70	K	0	K	K	K	K 0/8	-
30675	F	71	K	59.2	H	H	H	H 4/8	+
30676	F	72	M	100	M	M	M	M 8/8	+
30682	M	72	M	100	M	M	H	H 7/8	+
30683	F	72	K	62.7	H	H	H	H 5/8	+

Appendix. Continued

TK#	SEX	2N	mtDNA	rDNA	Adh	Ldh	Pep major	percent presence	4.8 kb marker
30684	M	71	K	65.3	H	H	H	H 4/8	-
30685	M	72	M	100	M	M	M	M 8/8	+
30686	F	70	K	0	K	K	H	H 1/8	-
30687	M	71	K	51.7	H	H	H	H 4/8	+
30688	F	72	M	100	M	M	M	M 8/8	+
30689	F	72	K	100	M	H	H	H 6/8	+
30691	M	70	K	0	K	K	K	K 0/8	-
30692	M	71	K	0	H	H	H	H 4/8	+
30693	M	72	M	88.0	M	H	M	H 7/8	·
30694	M	72	M	100	M	M	M	M 8/8	+
30696	M	72	K	100	M	M	M	H 8/8	+
30697	M	72	M	100	M	M	M	M 8/8	+
30700	F	71	M	74.0	H	H	H	H 4/8	-
30701	M	72	M	100	H	M	M	H 7/8	-
30702	M	70	K	0	H	K	K	H 1/8	+
30703	M	72	M	100	M	M	M	M 8/8	+
30704	M	70		0	H	K	K	H 1/8	+
30705	M	72	M	100	M	M	M	M 8/8	+
30706	M	72	M	100	M	M	K	H 6/8	+
30707	F	70	K	0	K	K	K	K 0/8	-
30709	F	70	K	0	K	K	K	KR	-
30710	F	70	K	0	K	K	K	KR	-
30711	M	70	K	0	K	K	K	KR	-
30712	M	70	K	0	K	K	K	KR	-
30713	M	70	K	0	K	K	K	KR	-
30714	M	70	K	0	K	K	K	KR	-
30715	M	70	K	0	K	K	K	KR	-
30716	F	70	K	0	K	K	K	KR	-
30717	F	70	K	0	K	K	K	KR	-
30718	M	70	K	0	K	K	K	KR	-
30720	M	70	K	0	K	K	K	KR	+
30722	M	72	M	100	M	M	M	MR	+
30723	M	72	M	100	M	M	M	MR	+
30725	M	72	M	100	M	M	M	MR	+
30726	M	72	M	100	M	M	M	MR	+
30727	M	72	M	100	M	M	M	MR	+
30728	M	72	M	100	M	M	M	MR	+
30729	M	72	M	100	M	M	M	MR	+
30733	F	71	M	100	M	M	M	MR	+
30734	F	72	M	100	M	M	M	MR	+
30735	F	71	M	100	M	M	M	MR	+
30736	F	72	M	100	M	M	M	MR	+
30737	M	71	M	100	M	M	M	MR	+
30738	F	72	M	100	M	M	M	MR	+
30739	F	72	M	100	M	M	M	MR	+
30740	M	71	M	100	M	M	M	MR	+
30741	F	70	M	100	M	M	M	MR	+
30742	M	71	M	100	M	M	M	MR	+
30743	M	70	K	0	K	K	K	KR	-
30744	F	70	K	0	K	K	K	KR	-

Appendix. Continued

TK#	SEX	2N	mtDNA	rDNA	Adh	Ldh	Pep major	percent presence	4.8 kb marker
30746	F	70	K	0	K	K	K	KR	-
30747	F	70	K	0	K	K	K	KR	-
30748	M	70	K	0	K	K	K	KR	-
30751	M	70	K	0	K	K	K	K 0/8	-
30752	F	70	K	0	K	H	K	H 1/8	-
30753	F	70	K	0	K	K	K	K 0/8	-
30754	F	71	K	90.6	H	H	H	H 4/8	+
30755	F	71	K	26.1	H	H	H	H 4/8	+
30756	M	71	M	22.9	H	H	M	H 5/8	+
30757	M	70	K	0	K	K	K	K 0/8	-
30759	M	70	K	0	K	K	K	K 0/8	+
30760	F	70	K	0	K	K	K	K 0/8	-
30761	F	72	M	100	M	M	M	M 8/8	+
30763	M	72	M	100	M	M	M	M 8/8	+
30766	F	70	K	0	K	K	K	K 0/8	-
30770	F	71	K	0	K	K	K	H 1/8	+
30771	F	70	K	0	K	K	K	KR	+
30772	F	70	K	0	K	K	K	KR	-
30773	M	70	K	0	K	K	K	KR	-
30774	F	70	K	0	K	K	K	KR	-
30775	F	70	K	0	K	K	K	KR	-
30776	M	70	K	0	K	K	K	KR	-
30777	M	71	M	100	H	-	M	H 4/6	-
30778	F	70	K	0	K	K	K	K 0/8	-
30779	F	72	K	100	K	H	K	H 3/8	+
30780	M	70	K	0	K	K	K	K 0/8	-
30781	F	71	M	79.2	H	H	H	H 4/8	+
30782	F	71	K	92.2	H	H	H	H 4/8	+
30783	F	70	K	0	K	K	K	K 0/8	-
30784	F	72	M	97.1	M	M	H	H 7/8	+
30785	M	72	M	100	M	M	H	H 7/8	+
30786	F	72	M	100	M	M	M	M 8/8	+
30788	M	72	M	100	M	M	M	M 8/8	+
30789	M	70	K	0	K	K	K	K 0/8	-
30790	M	71	M	100	H	H	M	H 5/8	+
30791	F	70	K	0	K	K	K	K 0/8	-
30792	F	70	K	93.8	H	H	H	H 3/8	+
30793	F	72	M	100	M	M	M	M 8/8	+
30794	F	72	M	100	M	M	M	M 8/8	+
30795	F	72	M	100	H	M	M	H 7/8	+
30796	F	72	M	100	M	M	M	M 8/8	+