Mammalian Genome 6, 521–525 (1995).

Mammalian Genome

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How bats achieve a small C-value: frequency of repetitive DNA in *Macrotus*

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Received: 2 December 1995 / Accepted: 22 April 1995

Abstract. Bats possess a genome approximately 50–87% the size of other eutherian mammals. We document that the events that have achieved or maintained a small genome size in the Mexican leaf-nosed bat *Macrotus waterhousii* have resulted in a lower copy number of interspersed and tandemly repetitive elements. These conclusions are based on examination of 1726 randomly chosen recombinant cosmids, with an average insert size of 35.7 kb and representing 2.6% of the haploid genome of *M. waterhousii.* Probes representative of microsatellites [$(GT)_n$, $(CT)_n$, $(AT)_n$, $(GC)_n$] and a tandem repeat (rDNA) were used to estimate frequency of repetitive elements in the *M. waterhousii* genome. Of the four dinucleotides, $(GT)_n$ was present in 33.5% of the clones, $(CT)_n$ was present in 31.0% of the clones, and $(AT)_n$ and $(GC)_n$ were not represented in any of the clones examined. The 28S rDNA and a repetitive element from *M. californicus* were found in three and four clones, respectively. The dinucleotides $(GT)_n$ and $(CT)_n$ occurred together in the same clone more frequently than expected from chance. Although our data do not allow us to empirically test which mechanisms are maintaining copy number of repetitive DNA in the bat genome, the nonrandom association of these different families of repetitive DNA may provide insight into a mechanism that proportionately reduces diverse families of repetitive DNA that are known to be amplified by very different mechanisms.

Introduction

What are the advantages to being able to control the C-value (Swift 1950) or genome size (Hinegardner 1976) within a cell? It has been hypothesized that to achieve a high metabolic rate, a small genome size is required (Cavallier-Smith, 1985). Among vertebrates, birds and bats have the highest metabolic rate and also have reduced C-values (Bachmann 1972; Szarski 1974; Tiersch and Wachtel 1991). To achieve a reduced C-value it is highly unlikely that single-copy genes can be eliminated, which means that the copy number of repetitive elements must be controlled. In many organisms there is evidence that several types of repetitive elements have been amplified involving a wide variety of mechanisms. The end result of variation in copy number of repetitive elements is the tremendous variation in C-values among closely related taxa (Capanna and Manfredi-Romanini 1971; Bachmann 1972; Flavell et al 1974; Burton et al 1989; Tiersch and Wachtel 1991; Lapitan 1993). Nonetheless, there must also be forces that maintain copy number because there is a limit to cell size and the amount of nonfunctional DNA that an organism can tolerate. For birds and bats, which have reduced C-values, the equilibrium between the forces that amplify repetitive DNA and those that control copy number must be shifted towards reduction of repetitive elements. This study was designed to better understand the characteristics of the low C-value genome of the bat *Macrotus waterhousii.* We have examined repetitive elements that would be amplified by different mechanisms in order to estimate the breadth of the forces that control repetitive DNA copy number in bats.

Although genome size (or C-value) is highly variable among vertebrates, the class Mammalia possesses the least amount of variation. Among the mammalian orders, Chiroptera (bats) has the smallest genome size, ranging from 50% to 87% of that characteristic for more typical mammals (Burton et al. 1989; Capanna and Manfredi-Romanini 1971; Kato et al. 1980). A comparison of the number of chromosomal rDNA sites in rodents and bats suggests that the balance between mechanisms that increase and those that reduce the number of sites in bats is more strongly in favor of reduction of sites than is characteristic of rodents (Baker et al. 1992). Baker and coworkers (1992) further suggest that this containment may be of a general nature, noting that bats also have greatly reduced amounts of C-band material (similar to rDNA, these are discrete blocks of tandemly repeated elements). These authors did not have data to determine whether this mechanism for containment extended to interspersed families of repetitive DNA such as microsatellites, which are hypothesized to increase in number by misalignment. Examination of the frequency of these additional classes of repetitive elements present in the bat genome would provide an estimate of their relative frequency to determine whether interspersed elements are also reduced in copy number per genome. Empirical evidence of the relative frequency of interspersed repetitive elements is critical to understanding the underlying mechanism(s) maintaining the reduced genome size of bats.

We have examined three specific classes of repetitive elements in the genome of the New World bat *Macrotus waterhousii* (Chiroptera: Phyllostomidae) and estimated relative copy number and frequency of co-occurrence by examining 1728 independent recombinant cosmids. Hybridization of the genomic cosmid library with genomic DNA provides an estimate for the relative amount of repetitive and unique DNA in the genome (Crampton et al. 1981), whereas hybridization with specific classes of repetitive elements provides insight into the relative contribution of each class to the total amount of repetitive DNA in the genome. The three classes of repetitive DNA we examined included both tandemly and repetitive interspersed sequences: i) dinucleotide microsatellites; ii) the tandemly repeated ribosomal DNA cistron; and iii) a repetitive family of DNA from *M. californicus* that is defined by the restriction endonuclease *Kpn*I. The frequency of microsatellites and rDNA have been well studied in many different mammalian orders and will allow for a comparison of their relative copy number in *M. waterhousii.*

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Materials and methods

Construction of cosmid genomic library. High-molecular-weight DNA was isolated from a male *Macrotus waterhousii* collected from Cuba: Guantanamo Prov., Guantanamo Bay Naval base (TK 32184). We have followed the procedure of Longmire and associates (1991) except that we used previously frozen tissue that was macerated after thawing, rather than blood, as a source for cellular DNA. This method has excellent potential for use in systematically oriented studies because tissues can be collected into lysis buffer and maintained at ambient temperature. Additionally, our studies indicate that the yield is greater in total amount of DNA per unit of tissue and that the molecular weight is higher than we have been able to obtain from other methods with a wide variety of tissues from different organisms.

The cosmid library was constructed as previously described by Longmire and colleagues (1993). After partial digestion with *Sau*3AI and dephosphorylation with calf intestinal alkaline phosphatase, approximately 0.5 μg of *M. waterhousii* genomic DNA was ligated with 1.0 μg of *Bam*HI cloning arms from the cosmid vector sCos-1 (Evans et al. 1989). In vitro packaging was carried out in Giga Pack Gold packaging extracts (Stratagene). Primary infection of *E. coli* host strain DH5αMCR yielded 7.4×10^5 independent recombinants. Average size of inserts in recombinant cosmids was determined by digestion of 20 random primary clones with *Eco*RI followed by electrophoresis within a 0.7% agarose gel. Fragment sizes were determined from photographs of ethidium-stained bands with all bands sized relative to the mobility of bands from two known DNA size standard (bacteriophage-λ DNA digested with *Hin*dIII and a 1-kb ladder).

Molecular characterization of repetitive elements in the genomic library. One thousand seven-hundred and twenty-eight independent clones from the primary library were picked, grown, and archived in 96-well microtiter plates. A replica plater (Sigma Chemical Co.) was used to inoculate nylon membranes (MIS Magna NT 0.45 micron; Biodyne B 0.45 micron) with clones from the microtiter plates. Membranes were incubated at 37°C for 7 h on LB agar containing kanamycin (30 μg/ml) and then transferred to LB agar containing kanamycin and chloramphenicol (170 μg/ml; Sambrook et al. 1989) and grown at 37°C overnight. DNA was fixed onto membranes by placing membranes sequentially on blotting pads soaked in 0.4 M NaOH (5 min), 0.5 M Tris/1.5 M NaCl, pH 7.5 (5 min), and 2 × SSC (5 min), followed by baking at 80°C for 2 h.

Relative abundance and composition of repetitive DNA in the *M. waterhousii* genome was evaluated with several different probes for families of repetitive DNA known to exist in typical mammalian genomes. These probes were hybridized to the *M. waterhousii* library. To evaluate the overall frequency of repetitive DNA, 1 μg of *M. waterhousii* genomic DNA was radioactively labeled by nick translation and hybridized to the library.

To evaluate the composition of clones containing specific repetitive elements, we probed the library with an approximately 2.0-kb repetitive DNA family from the genome of *M. californicus,* which is defined by the restriction endonuclease, *Kpn*I, ribosomal DNA (rDNA), and the four dinucleotide microsatellites. The *Kpn*I fragment was isolated by digesting *M. californicus* genomic DNA with the restriction endonuclease *Kpn*I and purifying the approximately 2.0-kb ethidium staining band from the gel with Prep-A-Gene (BioRad Laboratories). The cloned 28S ribosomal subunit (pI19) was provided by Arnheim (1979). This subunit of the rDNA gene was isolated from the vector, electrophoresed on 0.8% low-melting-point agarose, and purified from the gel with Prep-A-Gene. To insure that all vector DNA was removed from the sample, the purified 28S rDNA was gel purified a second time. Although this probe was isolated from *Mus,* this subunit has been used successfully in hybridization studies on representatives of essentially all genera of phyllostomid bats (Van Den Bussche 1991, 1992) as well as representatives of 13 families of bats, Dermoptera, Primates, and several rodents (Baker et al., 1991). Moreover, this is the same probe used in the chromosomal localization studies that first predicted that bats had a reduced number of rDNA sites as well as reduction in rDNA copy number (Baker et al. 1992).

Abundance of dinucleotide microsatellite repeats were evaluated with approximately 1.2-kb oligonucleotides $(AT)_n \times (TA)_n$, $(GC)_n \times (CG)_n$, $(CT)_n \times (GA)_n$, and $(GT)_n \times (CA)_n$ purchased from Pharmacia LKB. Hereafter, these four microsatellites are referred to as $(AT)_n$, $(GC)_n$, $(CT)_n$, and $(GT)_n$ respectively.

Prior to hybridization, membranes were washed for 1 h at 65°C in 0.1 × SSC, 0.1% SDS. Prehybridization was carried out at 65°C for 1 h in 6 × SSC, 40% formamide (Kodak), 1% SDS, 0.005 M EDTA (pH 8.0), and 0.005 g/ml Carnation evaporated milk. Membranes were hybridized overnight at 42°C in fresh prehybridization solution containing approximately 1×10^6 cpm/ml probe. Probes were labeled with [α-^{32}P]dCTP or [α-^{32}P]dATP by either nick translation or random priming. Nonincorporated label was removed by spin column chromatography (Sambrook et al. 1989). Prior to hybridization, probes were denatured for 10 min at 37°C in 0.1 M NaOH. Following hybridization, membranes were washed once for 15 min in 2 × SSC, 0.1% SDS at room temperature, and twice for 15 min in 0.1 × SSC, 0.1% SDS at 50°C. Washed membranes were autoradiographed at −80°C with Kodak XAR-5 film and two lightning plus intensifying screens. For each probe, all clones were scored on a scale of 0, representing no detectable hybridization, to 3, a completely black spot on an autoradiograph, representing maximum detectable hybridization.

By archiving all recombinant clones in microtiter plates, it is possible to survey the abundance and co-occurrence of various probes in the same clone. Such an examination allows for the detection of nonrandom associations of repetitive elements within the genome (Janecek et al. 1993; Baker et al. 1995). The co-occurrence of two probes in the same cosmid, either more or less frequently than expected, is evaluated by determining the expected frequency of the two probes in a single clone by multiplying the individual frequency of occurrence of the two clones. Standard chi-square statistics using the observed and expected values for occurrence of the two probes were utilized to determine whether the detected association was significantly different from expected on the basis of the frequency of occurrence of the probes taken individually. The apparent interspersion frequency for each of the probes used in this study was calculated by dividing the total size of the haploid genome by the estimated copy number of each probe within the haploid genome.

Methodological limitations and strengths. Our experimental design consisted of estimating the level of repetitive DNA in the genome by probing the gridded library with 1 μg of radioactively labeled genomic DNA. The use of cosmid libraries, containing inserts averaging greater than 35 kb, provides an efficient mechanism to rapidly screen a significant portion of the mammalian genome. Because single- and low-copy DNA will be in low abundance in this probe, only recombinant cosmids containing middle to highly repetitive sequences will produce detectable hybridization signal (Crampton et al. 1981). This method probably produces an underestimation of the total amount of repetitive DNA and an overestimation of the truly unique or single copy DNA. For example, some of the recombinant clones that do not produce detectable hybridization under the conditions used may contain divergent members of repetitive DNA families such as LINEs, SINEs, and other transposable elements. Additionally, because our method examines recombinant cosmids with DNA inserts averaging 35 kb, it is possible that any cosmid clone may contain more than a single copy of a given repetitive element. Therefore, the copy number estimates calculated from these data must be considered minimal estimates. However, because the estimates of copy number of repetitive elements in the *M. waterhousii* genome are compared with the abundance and frequency of repetitive elements from a *P. leucopus* cosmid library (Janecek et al. 1993), all estimates should be under the same methodological constraints. Moreover, because a large number of independent cosmids were screened for both studies, it is possible to calculate a 95% confidence interval around the estimates of relative copy number of these various classes of repetitive DNA in each genome (Shaeffer et al. 1990).

Results

Characterization of the M. waterhousii genomic library. Only 35 (2.3%) of the 1728 cosmid clones screened in this study did not hybridize to any of the seven probes examined. Of these 35 cosmids, digestion with the restriction endonuclease *Eco*RI suggested the absence of an insert in two cosmids. These two colonies apparently did not contain a recombinant cosmid or failed to grow to a density that allowed detection of the cosmid DNA by a standard mini prep procedure, bringing the actual number of recombinant cosmids screened for repetitive DNA to 1,726. Mini-prepped DNA from the remaining 33 repeat negative cosmids were visible on an ethidium-stained gel when digested with *Eco*RI (each

clone was verified to have the 6.7-kb DNA fragment characteristic of the sCos-1 vector as well as additional bands totaling approximately 35 kb in size).

The size of the *M. waterhousii* DNA inserted into 20 randomly selected recombinant cosmids ranged from 24.9 to 43.4 kb, with a mean insert size of 35.7 kb. On the basis of this mean insert size, the 1,726 recombinant cosmids represented 6.15×10^7 bp, or 2.6% of the *M. waterhousii* genome, assuming a genome size of 2.4×10^9 bp (based on a DNA content of 5.6 pg/cell; J.W. Bickham, pers. comm.). The total library of 7.4×10^5 primary clones thus is 11-fold representative of the *M. waterhousii* genome.

Characterization of repetitive DNA in the genomic library. Table 1 summarizes the total number of clones from the *M. waterhousii* library that hybridized to each of the seven probes. Hybridization of the *M. waterhousii* library with *M. waterhousii* genomic DNA resulted in some degree of hybridization to 1559 (90.3%) of the recombinant cosmids. Of these positive clones, 471 (30.21%) were scored as maximally hybridizing (score of 3), whereas 751 (48.17%) and 337 (21.62%) were assigned scores of 2 (medium intensity) and 1 (low intensity) respectively.

The *Kpn*I fragment from *M. californicus* produced detectable hybridization in only four recombinant cosmids, whereas the 28S rDNA probe produced detectable hybridization to three recombinant cosmids. The four dinucleotide microsatellites used as probes in this study varied greatly in their representation. Dinucleotides $(GT)_n$ and $(CT)_n$ hybridized to 579 (33.5%) and 536 (31.0%) recombinant cosmids respectively. For $(GT)_n$, 211 of the clones (12.21%) were scored as maximally hybridizing (score = 3), whereas 264 (15.28%) of these clones were scored as 2, and 104 (6.02%) were assigned a score of 1. For $(CT)_n$, 268 of the 536 clones (50.0%) were scored as maximally hybridizing (score = 3), 185 (10.71%) were assigned a score of 2, and 83 clones (4.80%) were scored as 1. No hybridization was detected with either dinucleotide probe $(GC)_n$ or $(AT)_n$.

For all pairwise comparisons of the eight probes used in this study, the co-occurrence of clones hybridizing was as expected from their individual representation in the library. However, pairwise comparisons between $(GT)_n$ and $(CT)_n$ showed a significantly higher frequency of co-occurrence than expected from their individual representation in the *M. waterhousii* library ($P < 0.001$).

Discussion

Genomic organization and characterization. Ninety percent of the 35-kb cloned *M. waterhousii* fragments contain highly or middle repetitive DNA. The frequency of such elements by identical methods indicated that 99% of the cloned fragments of *Peromyscus leucopus* genomic DNA contained highly or middle repetitive DNA (Janecek et al. 1993). The percentage of the clones containing repetitive DNA in the *M. waterhousii* genomic library is reduced by 9.2% when compared with the repetitive DNA present in the *P. leucopus* library (Janecek et al. 1993). These data are interpreted as indicating that *M. waterhousii* has a reduction in the total amount of repetitive DNA relative to those organisms characterized by more typical C-values (humans and *P. leucopus*).

Macrotus californicus (the California leaf-nosed bat), the sister-taxon to *M. waterhousii,* possesses in its genome a repetitive family of DNA defined by the restriction endonuclease *Kpn*I that can be visualized by fluorescence with ethidium bromide. With identical methods, digestion of *M. waterhousii* DNA with *Kpn*I does not reveal this repetitive family. However, probing the *M. waterhousii* cosmid library for this *Kpn*I family resulted in hybridization to four of the 1726 clones, with three of these clones hybridizing with maximal intensity (score of 3). These results indicate that the DNA sequence making up the repetitive *Kpn*I family in *M. californicus* is also present in *M. waterhousii,* but this family of DNA has been differentially amplified or deleted in the respective genomes since their divergence from a common ancestor.

Most mammals possess the rDNA cistron in approximately 300–500 copies. However, Baker and associates (1992) have shown that bats have a reduced number of rDNA sites compared with rodents. We found only three 28S rDNA clones in the *M. waterhousii* library. If our estimate of 2.6% of the genome being represented by this gridded sublibrary is correct, and assuming that each insert contains a single copy of the rDNA cistron, which is approximately 38 kb in *M. waterhousii* (Van Den Bussche 1991), this would translate to approximately 115 copies of the rDNA cistron in the *M. waterhousii* genome. These data are interpreted as compatible with the hypothesis that bats have a reduced copy number of the rDNA cistron (Baker et al. 1992).

Microsatellites are relatively short runs of tandemly repeated DNA with repeat lengths of 6 bp or less (Stallings 1992). A characterization of the relative abundance of microsatellites in the *M. waterhousii* genome is important for understanding whether repetitive families other than the tandemly repeated rDNA cistron are also less abundant in the bat genome. From a computer search of human and rat (*Rattus norvegicus*) microsatellites in the major data bases, Beckman and Weber (1992) found that among the dinucleotides, $(GT)_n$ was the most abundant, followed by $(CT)_n$, whereas $(AT)_n$ and $(GC)_n$ were only infrequently detected. In our study, the four dinucleotide microsatellites varied greatly in their representation among the 1726 recombinant cosmids examined and followed the same general trend detected by Beckman and Weber (1992). The oligonucleotide probe for the dinucleotide microsatellite $(GT)_n$ hybridized to 33.5% of the *M. waterhousii* recombinant cosmid clones (Table 1). Examining a *P. leucopus* cosmid library with comparable insert size, Janecek et al. (1993) found that approximately 88% of the clones (2013 clones examined) contained $(GT)_n$ sequences (Table 2).

Copy numbers of $(GT)_n$ sequences vary considerably among mammalian genomes. There are approximately 100,000 copies of $(GT)_n$ within the *Mus* genome (Hamada et al. 1982), 50,000 copies in human (Stallings et al. 1991), and 75,000 copies in *P. leucopus* (Janecek et al. 1993). Assuming that 2.6% of the genome was examined, we estimate that the *M. waterhousii* genome contains at least 22,000 $(GT)_n$ repetitive sequences. Copy number of $(GT)_n$ sequences in *M. waterhousii* indicates a reduction relative to other mammals. The dinucleotide $(CT)_n$ was the second most abundant microsatellite repeat present in the *M. waterhousii* library. We estimated $(CT)_n$ sequences to be present in at least 20,000 copies in the genome of *M. waterhousii.* Therefore, the frequency of $(CT)_n$ sequences in *M. waterhousii* is reduced relative to the 50,000 copies in the *P. leucopus* genome (Table 2).

A critical assumption in our study is that all conditions for hybridization in our study are identical to those of Janecek and colleagues (1993) so that any reduction in the amount of repetitive DNA that we detect in the bat genome is a characteristic of the

Table 1. Representation of seven probes in 1726 independent clones from a cosmid library constructed from *Macrotus waterhousii* genomic DNA. Percentages are shown in parentheses; some values do not total 100 owing to rounding error.

Probe	Negative clones	Positive clones
$(AT)_n$	1726 (100.00)	0 (0.00)
$(GC)_n$	1726 (100.00)	0 (0.00)
$(CT)_n$	1190 (68.95)	536 (31.05)
$(GT)_n$	1147 (66.45)	579 (33.55)
rDNA	1723 (99.82)	3 (0.17)
KpnI	1722 (99.77)	4 (0.23)
M. waterhousii genomic DNA	167 (9.68)	1559 (90.32)

Table 2. Comparison of the copy number (with 95% confidence intervals) and apparent interspersion frequency for the dinucleotide microsatellites $(GT)_n$ and $(CT)_n$ from hybridization of three genomic libraries. The estimated genome size for each organism is also presented.

	$(GT)_n$		$(CT)_n$		
	Copy number	Interspersion frequency	Copy number	Interspersion frequency	Genome size (bp)
P. leucoopus[a]	75,049 ± 1,210	1/40 kb	50,832 ± 1,851	1/59 kb	3.0×10^9
M. waterhousii	22,239 ± 1,488	1/106 kb	20,646 ± 1,460	1/115 kb	2.4×10^9
G. hirsutum[b]	485 ± 228	1/3,370 kb	1,768 ± 432	1/922 kb	1.8×10^9

[a] Janecek et al. (1993).
[b] Baker et al. (1995).

genome and not an artifact of methodology. If conditions for hybridization between the two studies were different, this would affect the clones that were scored as 1 or 0. To test the repeatability of the two studies, several randomly chosen clones from each taxon scoring 1 and 0 for the microsatellites $(GT)_n$ and $(CT)_n$ from the *Macrotus* and *Peromyscus* libraries were archived in the same microtiter plate, stamped on the same membrane, grown, and hybridized under the conditions outlined earlier. The autoradiograms were developed for a longer period of time than in the original studies so that we would be able to more clearly determine whether any hybridization was occurring in the clones scored as 0. These hybridizations produced results identical to those reported by Janecek and associates (1993) and our study. A small subset of the *Peromyscus* and *Macrotus* clones that scored either 1 or 0 for the microsatellite $(GT)_n$ is shown in Fig. 1.

One possible explanation for the reduced copy number of repetitive elements in the bat genome is that this simply follows arithmetically the reduction in total genome size seen in bats. Recently these same dinucleotide repeats have been examined in the Upland cotton (*Gossypium hirsutum*) genome, an organism that shares with bats the characteristic of having a reduced genome size (Baker et al. 1995). Because probes and conditions for hybridization experiments were identical between the present study and that for *G. hirsutum* and *P. leucopus*, we can directly compare the interspersion frequency of the two most abundant dinucleotide microsatellites in these genomes. Calculating the apparent interspersion frequency takes into account the different genome sizes and will allow us to evaluate whether the reduction in copy number

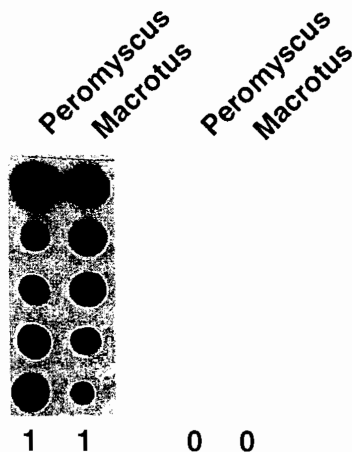

Fig. 1. Autoradiogram of a subset of $(GT)_n$ microsatellite containing cosmids from the *Peromyscus* and *Macrotus* libraries that were originally scored as 1 or 0. These cosmids were grown on the same membrane and hybridized under the conditions described in the Materials and methods. The autoradiogram was overdeveloped to show that those cosmids scored as 0 in both studies produced no detectable hybridization. Similar results are obtained with cosmids containing the microsatellite $(CT)_n$.

of microsatellite clusters is due to the reduced genome size in bats. If the reduced copy number of microsatellites were due to a straight arithmetic reduction in the bat genome, then we would expect the interspersion frequency of these clusters to be essentially the same in the *P. leucopus, G. hirsutum,* and *Macrotus* genomes. However, if there is some mechanism(s) reducing the microsatellite clusters, then we would expect to see microsatellite clusters distributed at greater distances from each other throughout the genome of *M. waterhousii* and *G. hirsutum* compared with that in *P. leucopus.*

As with the estimates of relative abundance, considerable variation exists for interspersion frequencies of $(GT)_n$ and $(CT)_n$. Interspersion frequencies of $(GT)_n$ have been estimated as one $(GT)_n$ cluster every 54 kb in humans (Moyzis et al. 1989), 40 kb in *Peromyscus* (Janecek et al. 1993), 21 kb in *Rattus* (Stallings et al. 1991), 18 kb in *Mus* (Stallings 1992), and 3370 kb in *G. hirsutum* (Baker et al. 1995). Based on our sampling of the *M. waterhousii* genome, $(GT)_n$ microsatellite clusters are distributed over greater distances in the bat genome compared with other mammals, yet are more frequent than detected in the Upland cotton genome in that we calculate on $(GT)_n$ cluster every 106 kb (Table 2). Although fewer genomes have been examined for the dinucleotide repeat $(CT)_n$, a pattern similar to that seen with $(GT)_n$ is detected. For example, that apparent interspersion frequency of $(CT)_n$ is one repeat cluster every 59 kb in *P. leucopus* (Janecek et al. 1993) and 922 kb in *G. hirsutum,* whereas it is one cluster every 115 kb in *M. waterhousii.*

When the relative abundance and interspersion frequency data from *M. waterhousii* are interpreted in light of similar data from *P. leucopus* and other mammals characterized by larger genome size as well as *G. hirsutum,* which has a reduced genome size, it appears that the *M. waterhousii* and Upland cotton genomes have greatly reduced the number of these repeats and that this reduction is not due to just having a smaller sized genome.

When the co-occurrence of probes occurring in the same clone is examined, one significant association is detected. The dinucleotide microsatellites $(GT)_n$ and $(CT)_n$ co-occurred more frequently than expected from their individual representation in the genome. Examination of the frequency of co-occurrence of these same families of repetitive DNA in *P. leucopus* and *G. hirsutum* suggested that these families of DNA were randomly distributed (Janecek et al. 1993; Baker et al. in press). Because there was a significant nonrandom association of the dinucleotides $(GT)_n$ and $(CT)_n$, it is possible that this may provide one mechanism for riding all of these families of repetitive DNA proportionally. If there is movement of repetitive DNA among nonhomologous chromosomes, as has been shown in other studies (Hamilton et al. 1990, 1992), then this may provide insight into the mechanism that is reducing the amount of repetitive DNA in the bat genome.

The bat genome can now be characterized as having a reduced copy number (relative to other mammals thus far studied) of rDNA, heterochromatin, microsatellites, and a nonrandom association of the dinucleotide $(GT)_n$ and $(CT)_n$. The repetitive elements mentioned above are hypothesized to be amplified by a wide va-

riety of mechanisms including rolling circle replication, slippage-repair, and unequal crossing-over. The implications are that the genome size in bats is controlled by broad-based regulatory forces that maintain low copy numbers of repetitive DNA families. Whether this regulation of repetitive DNA involves a single or several different copy number containment mechanisms cannot be determined with our data. However, the nonrandom association of two families of microsatellites in the bat genome may provide insight into a mechanism that can reduce these families of repetitive DNA proportionately.

Acknowledgments. Specimens for this study were donated from the frozen tissue collection at The Museum, Texas Tech University. We thank Holly A. Wichman, Cheryl A. Schmidt, and James C. Cathey for comments on an earlier version of this manuscript. This study was supported by National Science Foundation grant BSR-91-07-143 to R.J. Baker.

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