Genet. Res., Camb. (1989), **53**, *pp.* 87–93 *With 4 text-figures Printed in Great Britain*

Variable evolutionary stability of *Y* chromosomal repeated sequences in the genus *Mus*

TIMOTHY H. K. PLATT* AND MICHAEL J. DEWEY
Department of Biology, University of South Carolina, Columbia, South Carolina 29208, U.S.A.

(*Received 27 May 1988 and in revised form 14 September 1988*)

Summary

The study reported here is an examination of the organization and evolution of three *Y* chromosomal repeated sequences, designated pBC10-0.6, pBC15-1.1, and pBA33-1.8, in five closely related species of the genus *Mus*. The species distributions of major restriction fragment length polymorphisms produced with a panel of restriction enzymes is used to develop the phylogenetic relationships between the five species studied. However, the apparent degree of relatedness among these species varied a great deal with each of the three probes and was also highly dependent on the particular restriction enzyme used. The usefulness for phylogenetic studies of closely associated sequences varying in evolutionary stability is discussed.

1. Introduction

A variety of repeated sequences have been cloned from mammalian genomes, among them, a number that are specifically and exclusively repeated on the *Y* chromosome. Such sequences have been identified and cloned from species of *Mus* (Eicher *et al.* 1983; Nallaseth *et al.* 1983; Lamar & Palmer, 1984; Bishop *et al.* 1985; Baron *et al.* 1986; Nallaseth & Dewey, 1986; Nishioka & Lamothe, 1986; Platt & Dewey, 1987). The study reported here is an examination of the evolution and organization of three such sequences, designated pBC10-0.6, pBC15-1.1 and pBA33-1.8, in five species of the genus *Mus*, subgenus *Mus* (Marshall, 1986). *In situ* hybridization and Southern analysis show that these sequences are moderately repeated on the *Y* chromosome and in single copy on an autosome or the *X* chromosome of laboratory mice (Nallaseth *et al.* 1983; Nallaseth & Dewey, 1986). The *Y* chromosomal copies are not in the region of the testis determination (*Tdy*), and they appear to be in the region associated with spermatogenesis, sperm motility, and the M720 retroviral sequences (Phillips *et al.* 1982; Platt & Dewey, unpublished). There appear to be approximately 90 copies of pBC10-0.6, 170 of pBC15-1.1, and 130 of pBA33-1.8 on the *Y* chromosome in laboratory mice. These are organized into several repeat families in which the sequences complementary to the probes and parts thereof are interspersed with each other and with other *Y* chromosomal sequences. The three families of repeat sequences represent about 10% of the *Y* chromosomal DNA of laboratory mice.

In a previous study, the evolution of these sequences throughout the genus *Mus* was examined (Maxson, Platt & Dewey, in preparation). In that study, *Eco* RI digested genomic DNA of nine species taken from three subgenera: *Mus*, *Pyromys*, and *Nannomys* (including species which diverged from each other as long ago as 12–15 million years) was probed with each of pBC10-0.6, pBC15-1.1, and pBA33-1.8 at two different hybridization stringencies. The genus was found to divide into two distinct groups, the near and far species, based on such criteria as the minimum hybridization stringency at which signals were detected for the three probes, copy number of sequences homologous to them, and hybridization band pattern similarity across species. The near species, derived as a group from a common phylogenetic branch point 3–6 million years ago (see Fig. 1), show hybridization at higher stringency, have higher copy numbers, and exhibit more band pattern similarity than is found in the more distant species when examined with these probes. This study is a more detailed analysis of the near group as characterized in that study. Hybridization data of a variety of *Y* chromosomal, repeated sequence probes, including AC11 (Nishioka & Lamothe, 1986), ACC2 and ACC3 (Nishioka & Lamothe, 1987) and high-molecular-weight male-specific sequences largely devoid of restriction sites

* Present Address: Department of Genetics, Albert Einstein College of Medicine, Bronx, NY 10461, U.S.A.

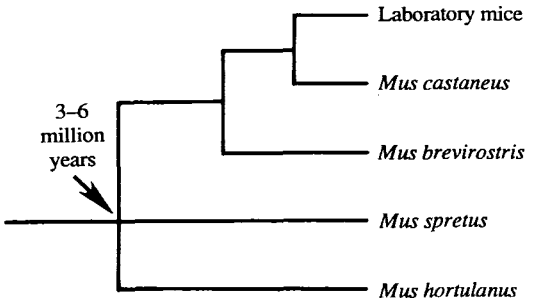

Fig. 1. Phylogenetic relationships of the near species of this study. Many laboratory strains (including C57) are thought to consist of *M. domesticus* autosomes and mitochondrial DNA (Selander *et al.* 1969; Yonekawa *et al.* 1980) and a *M. musculus Y* chromosome (Bishop *et al.* 1985). This phylogeny was derived from comparative studies of protein polymorphism (Bonhomme *et al.* 1984), and divergence of total genomic (Callahan & Todaro, 1978; Brownell, 1983; Martin *et al.* 1985) and mitochondrial DNA (Ferris *et al.* 1983), as well as divergence of *Y* chromosomal repeated sequence, restriction fragment length polymorphisms reported here.

and enriched in the tetranucleotides GATA, GACA, or both (Platt & Dewey, 1987) support the characterization of the near species as a distinct group. Analysis of hybridization of pBC10-0.6, pBC15-1.1, and pBA33-1.8 to genomic DNA of a set of near species permits a more detailed analysis of the group.

This study examines patterns of evolutionary conservation and change in sequences homologous to pBC10-0.6, pBC15-1.1 and pBA33-1.8 in five of the near species, proposing a model for the phylogenetic relationships between C57BL/10Bg (a laboratory strain), *Mus brevirostris* (Peru), *M. castaneus*, *M. hortulanus*, and *M. spretus*. Results indicate the commensal species: *M. musculus* (C57BL/10), *M. castaneus* and *M. brevirostris*, to be phylogenetically closer to each other than to the wild species *M. hortulanus* and *M. spretus*.

There was a great deal of variation in the degree to which individual *Y* chromosomal repeat families appeared evolutionarily conserved, and depended on the particular restriction enzymes used to generate the repeat families. This was especially so in comparisons between the commensal and the wild species, or between the two wild species.

2. Materials and methods

(i) *Animals*

The following strains and species were used in this study: C57BL/10Bg was provided by S. C. Maxson (University of Connecticut, Storrs). *Mus castaneus*, *M. spretus* and *M. hortulanus* were provided by M. Potter (National Institutes of Health) and F. Berger (University of South Carolina). *Mus brevirostris* (Peru) was purchased from the Jackson Laboratories.

(ii) *DNA preparation, electrophoresis, blotting, and hybridization procedures*

Hepatic DNA was isolated by a modified version of the method of Blin & Stafford (1976) from animals that were fasted overnight prior to extraction. DNA preparations were digested overnight in six single restriction enzyme digests for each of the five species. The six enzymes used: *Bam* HI, *Eco* RI, *Hae* III, *Hin* dIII, *Kpn* I and *Pst* I were all obtained from Bethesda Research Laboratories. Digests were carried out using buffer and temperature conditions recommended by the manufacturer, with a three-fold excess of enzyme in 5 mM spermidine. Digests were electrophoresed on 0·8% agarose gels using TAE buffer (40 mM Tris, 20 mM-CH_3COONa, 1 mM-Na_2EDTA, pH adjusted to 8·0 with glacial acetic acid) at 40 V. Approximately 10 μg of DNA was loaded per lane. Gel-separated fragments were Southern transferred to nylon membranes (Amersham International) using the following protocol. Gels were depurinated in one 10 min wash in 0·24 N-HCl, denatured in two 20 min washes in 1·5 M-NaCl, 0·5 M-NaOH, and neutralized in two 45 min washes in 1·5 M-Tris, 3 M-NaCl (pH 7·2). Transfer was effected using 12 × SSPE (1·8 M-NaCl, 120 mM-NaH_2PO_4, 12 mM-Na_4EDTA, pH adjusted to 7·4 with NaOH). Transferred DNA was covalently bound to the nylon membranes with UV irradiation using 5 min exposures on a UV transilluminator (Ultra Violet Products, Inc.).

Ethidium bromide staining showed that there was complete digestion of all genomic DNA preparations for all enzymes used, for all species. It also showed that approximately equal amounts of DNA were usually loaded in each lane across all preparations (data not shown). *Bam* HI digest results are not given for *M. castaneus* in what follows.

Probes were prepared as follows. The three *Y* chromosomally repeated sequences are maintained in pBR325 in the *E. coli* strain LE392. Plasmid DNA was purified from cultures grown out using chloramphenicol to amplify plasmid copy numbers, by a modified version of the method of Godson & Vapnek (1973). Resulting plasmid DNA was digested with *Eco* RI as per genomic DNA above, and electrophoresed on 0·8% agarose gels using TBE buffer (89 mM Tris-borate, 89 mM boric acid, 2 mM-N_2 EDTA). Insert DNA bands were intercepted using DEAE cellulose membrane (NA-45 membrane, Schleicher & Schuell) according to the manufacturer's protocol (application update 364). Purified insert DNA was nick translated to specific activities of approximately 10^9 cpm/μg by a modified version of the method of Maniatis *et al.* (1975) and unincorporated nucleotides were removed with Sephadex G-50 coarse (Pharmacia Inc.) using a spun column technique (Maniatis *et al.* 1982).

Hybridizations were carried out as follows. Each filter was prehybridized at 42 °C in 15 ml of a solution

of 30% formamide, 1% SDS, 50 μg/ml Heparin Na salt, and 1 M-NaCl for a minimum of 6 h. For each filter, 250 ng of nick-translated probe (see above) and 100 μl of 26 μg/μl *Tortula* yeast RNA (Sigma Chemical Co.) were mixed, heated to 100 °C for 10 min, cooled, and added to a hybridization solution of 30% formamide, 10% dextran sulphate, 1% SDS, 0·5 mg/ml Heparin Na salt, and 1 M-NaCl, preheated to 42 °C, and added to replace the prehybridization solution. Hybridizations were carried out overnight at 42 °C.

Post hybridizations were carried out as a series of washes. Filters were washed twice for 20 min in 3 × SSPE at room temperature then once for 10 min in 3 × SSPE at 65 °C, twice for 20 min in 1 × SSPE at 65 °C, and finally, twice for 20 min in 0·1 × SSPE at 65 °C. All autoradiography was done using Kodak X-Omat diagnostic film at −70 °C with intensifier screens.

3. Results

The species used in this study are C57BL/10Bg, *M. castaneus*, *M. brevirostris* (Peru), *M. spretus*, and *M. hortulanus*. C57BL/10 consists of a combination of *Mus musculus Y* chromosomal sequences (Bishop *et al.* 1985) and *M. domesticus X* chromosomes, autosomes and mitochondria (Selander *et al.* 1969; Yonekawa *et al.* 1980) so for purposes of this study, it can be considered to be like *M. musculus*. *M. musculus* (and thus C57BL/10), *M. castaneus*, and *M. brevirostris* are commensal species and *M. spretus* and *M. hortulanus* are wild. All six species trace to a common phylogenetic branch point at three to 6 million years (see Fig. 1). This phylogeny was derived from comparative studies of protein polymorphism (Bonhomme *et al.* 1984), divergence of total genomic (Callahan & Todaro, 1978; Brownell, 1983; Martin *et al.* 1983) and mitochondrial (Ferris *et al.* 1983) DNA sequences, as well as the results presented in this report.

(i) *pBA*33-1.8

A composite of results of hybridizations with pBA33-1.8 is shown in Fig. 2. An examination of the various restriction enzyme digests of the five species shows that the apparent degree of pBA33-1.8 sequence conservation varies significantly depending on which restriction enzyme is used. In *Bam* HI digests a major portion of the hybridization signal is localized to a single 1·0 kbp band that is shared by all five species. Similarly the remaining hybridization signals are localized primarily to three other bands also common to the five species. *Pst* I digests of the near species are similar in presenting most hybridization to a single shared band of 2·0 kbp, and *Hae* III digests of them have virtually identical hybridization patterns so these digests also indicate sequences homologous to pBA33-1.8 to be highly conserved.

In sharp contrast, the banding profiles with the *Hin* dIII and *Eco* RI digests show markedly more phylogenetic divergence for the pBA33-1.8 related sequences. From comparisons of the *Eco* RI restric-

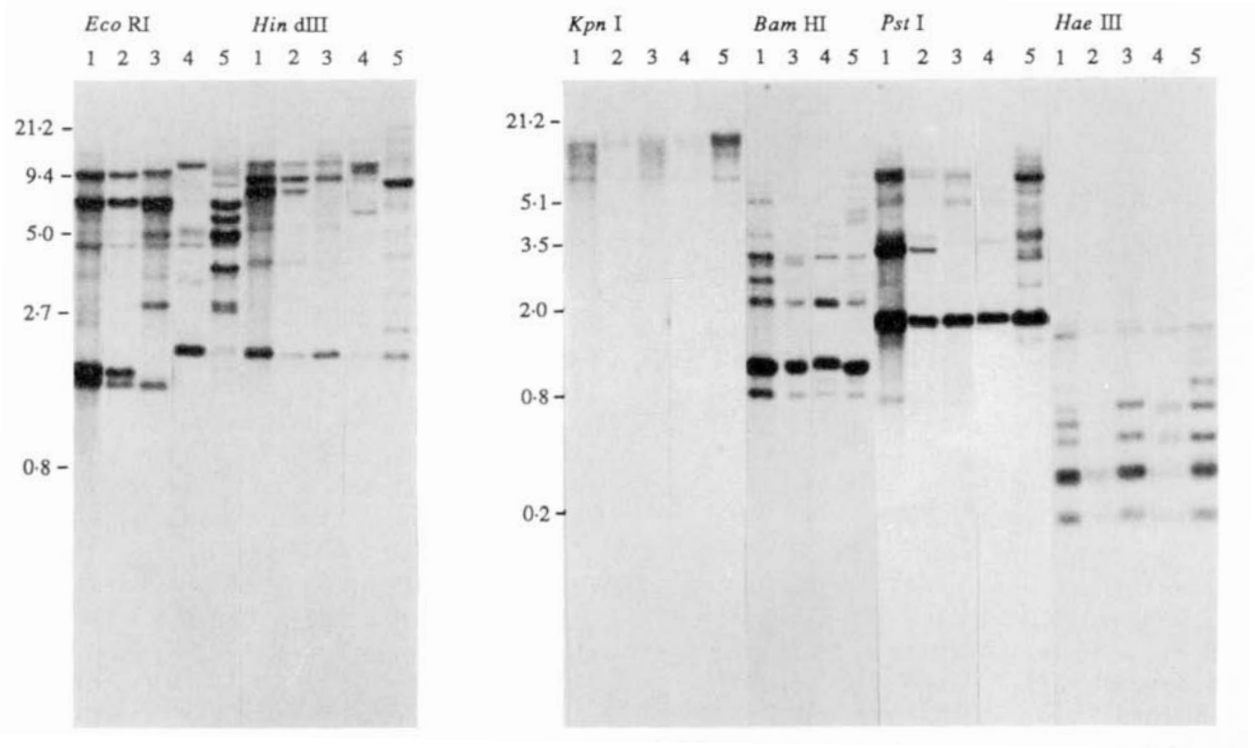

Fig. 2. Hybridization of genomic DNA with pBA33-1.8. Along the side are size marker positions in kbp. Lane 1, *M. musculus* (C57BL/10Bg); lane 2, *M. castaneus*; lane 3, *M. brevirostris* (Peru); lane 4, *M. spretus*; lane 5, *M. hortulanus*.

https://doi.org/10.1017/S0016672300027956 Published online by Cambridge University Press

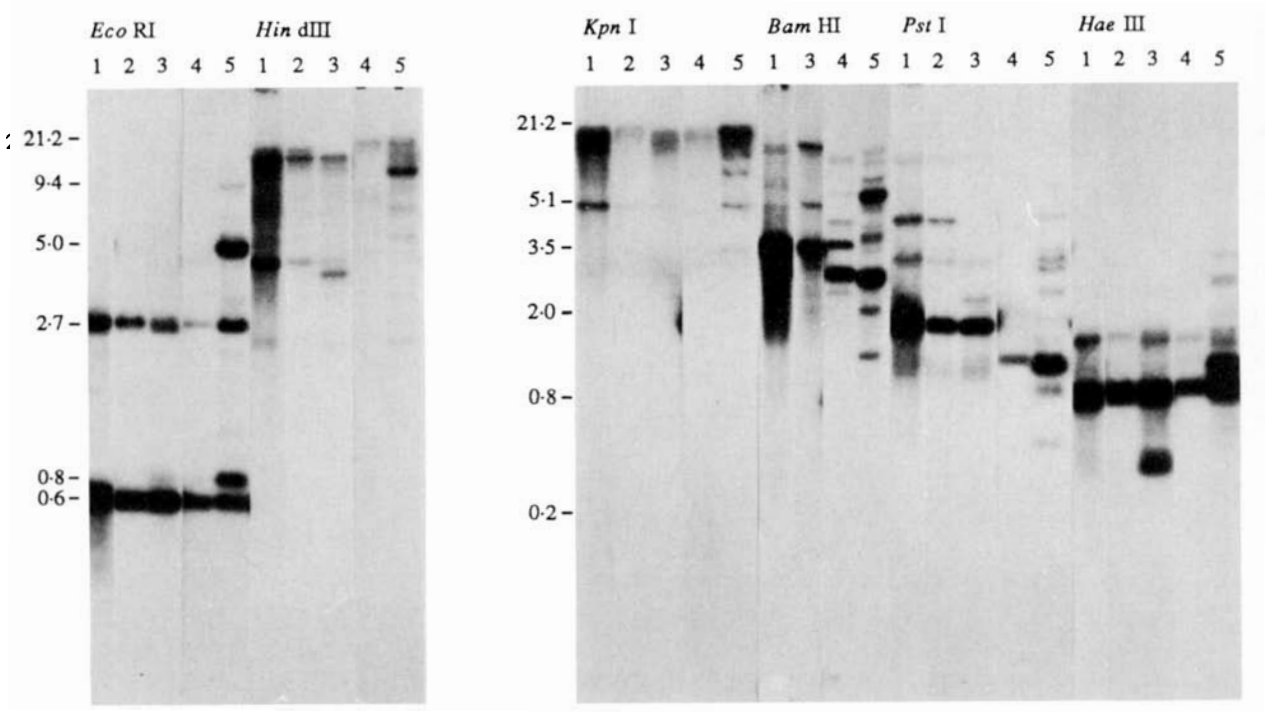

Fig. 3. Hybridization of genomic DNA with pBC10-0.6. Along the side are size marker positions in kbp. Lane 1, *Mus musculus* (C57BL/10Bg); lane 2, *M. castaneus*; lane 3, *M. brevirostris* (Peru); lane 4, *M. spretus*; lane 5, *M. hortulanus*.

tion profiles of *M. spretus*, *M. hortulanus*, and any of the commensal species there appeared to be very little similarity among the three. From one species to the next, practically all of the restriction families in one species is absent in the other species and replaced by an entirely new array of families.

Although the three commensal species are very similar, *M. brevirostris* appears to be diverging from *M. musculus* and *M. castaneus*. *Eco* RI digests of *M. musculus* and *M. castaneus* share a doublet at approximately 1·4 kbp where *M. brevirostris* has just the smaller of the two bands. These are major hybridization bands representing a significant percentage of the copies of sequences homologous to pBA33-1.8 in these species. *M. brevirostris* in turn has prominent hybridization bands at 2·7 and 5·0 kbp not found in the other two species. *M. brevirostris* also differs from the other two commensal species for *Pst* I digests. *M. musculus* and *M. castaneus* have a major shared band at 3·5 kbp which *M. brevirostris* lacks.

(ii) *pBC*10-0.6

A composite of hybridization results with pBC10-0.6 is shown in Fig. 3. As with pBA33-1.8, different types of restriction enzymes appear to show different degrees of divergence in the near species. *Eco* RI digests show sequence conservation with most hybridization in a 620 bp band that is shared by the five species. *Bam* HI and *Pst* I digests show interspecific differences for major hybridization bands. As in pBA33-1.8 hybridizations *Hae* III digests show most hybridization to be in a conserved band and restriction enzyme digests which show divergence show more of it between the commensal and wild species than within the commensals. They also show more divergence within the wild species than within the commensals. *M. brevirostris* appears to be farther from *M. musculus* than is *M. castaneus* in *Hin* dIII, and *Pst* I digests where it lacks hybridization bands that they have, and *Hind* dIII, *Pst* I and *Hae* III digests where it has one or more bands that they lack.

(iii) *pBC*15-1.1

A composite of hybridization results with pBC15-1.1 insert DNA is shown in Fig. 4. As in hybridizations with pBA33-1.8, different types of restriction enzyme digests appear to show varying degrees of divergence within the near species but this observation is complicated by the band pattern distributions observed in *M. spretus*. It seems to have diverged more from the other near species for this probe than for the other two. Thus in *Eco* RI digests probed with pBC15-1.1, most hybridization is observed at 3·7 and 4·3 kbp for all of the near species except *M. spretus*. Similarly, *M. spretus* shows much reduced hybridization in *Hin* dIII digests at 1·8 kbp. The four other species show prominent hybridization there. High stringency hybridizations show the five species to share a major *Hae* III band at approximately 400 bp.

As with the other probes, the commensals appear closer to each other than to the wild species and closer to each other than the wild species are to each other. Thus *Eco* RI digests of the commensals share a band

https://doi.org/10.1017/S0016672300027956 Published online by Cambridge University Press

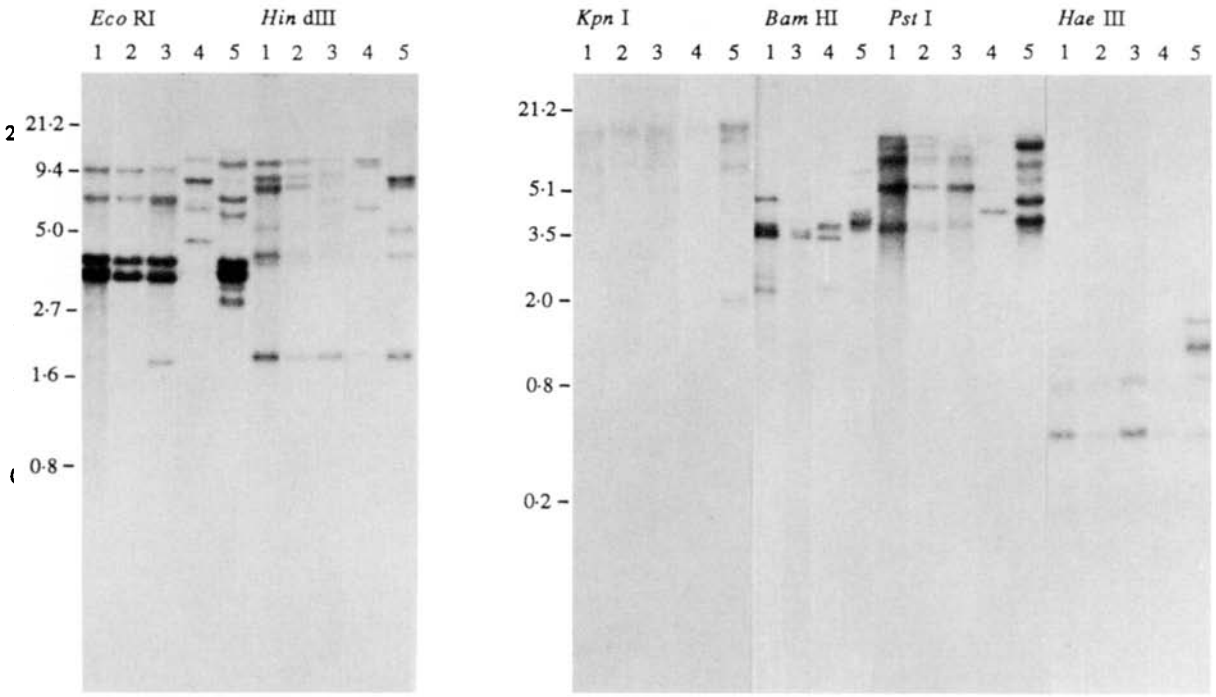

Fig. 4. Hybridization of genomic DNA with pBC15-1.1. Along the side are size marker positions in kbp. Lane 1, *M. musculus* (C57BL/10Bg); lane 2, *M. castaneus*; lane 3, *M. brevirostris* (Peru); lane 4, *M. spretus*; lane 5, *M. hortulanus*.

at 9·4 kbp and *Pst* I digests of them have a prominent band at 7·8 kbp not found in the wild species.

(iv) *Kpn I digests*

When genomic DNA of the five near species is digested with *Kpn*I and probed with pBA33-1.8, pBC10-0.6, or pBC15-1.1, sequences homologous to them are found to be largely devoid of restriction sites. This observation holds for all five species tested for all three probes, with most pBA33-1.8 *Kpn* I fragments over 20 kbp, most pBC10-0.6 fragments at approximately 20 kbp, and most pBC15-1.1 fragments approximately 15 kbp with some minor banding, down to 2·0 kbp (in pBC15-1.1 hybridizations) that is more readily observed at lower stringency (data not shown).

4. Discussion

The study reported here is an analysis of the organization and evolution of three *Y* chromosomal repeated sequences: pBA33-1.8, pBC10-0.6, and pBC15-1.1 in five species of the genus *Mus*. There was a clear distinction between the commensal and wild species of this study. When DNA of these species was probed with pBC10-0.6, pBC15-1.1, or pBA33-1.8, there was much more apparent similarity within the commensal mice (C57BL/10, *M. castaneus* and *M. brevirostris*), than either between them and the two wild species, *M. spretus* and *M. hortulanus*, or between the two wild species. This was reflected in the number of shared hybridization bands when various digests of genomic DNA of the five species were probed with the *Y* chromosomal sequences. The data also indicated that *M. musculus* is closer phylogenetically to *M. castaneus* than to *M. brevirostris*. This relationship is similarly supported by the distribution of a variety of protein polymorphism markers (Bonhomme *et al.* 1984).

Most interestingly, the apparent degree of relatedness among these species varies a great deal with each of the three *Y* chromosomal probes and also depends on the particular restriction enzyme used. When the five species of this study were probed with pBA33-1.8, pBC10-0.6, and pBC15-1.1, different restriction enzymes showed very different pictures as to the evolutionary stability of each probe. Thus, in some instances we observed that over relatively short evolutionary time periods, some *Y* chromosome-specific repetitive elements are subject to rapid and profound changes that frequently involve all or most of the copies of each repeat family. Such is the case, for example, with male genomic DNA digested with *Eco* RI and probed with pBA33-1.8 or *Pst* I digests probed with pBC10-0.6. From one species to the next, practically all of the restriction families in one species is absent in the other species and replaced by an entirely new array of families. Conversely, other combinations of probe and restriction enzyme yield hybridization patterns that are largely conserved among the five species, as was the case for DNA digested with *Pst* I and probed with pBA33-1.8, or *Eco* RI digests probed with pBC10-0.6.

Two basic mechanisms may be responsible for altering restriction profiles of specific DNA sequences.

https://doi.org/10.1017/S0016672300027956 Published online by Cambridge University Press

One is the simple accumulation of point mutations at particular restriction sites along with the spread of the mutations throughout the rest of the copies of the repeat family. The other involves the generation and spread of rearrangements such as insertions, deletions, or inversions. Extensive structural characterization will be required to allow an evaluation of the types of molecular changes responsible for such instability. Whatever the mechanism, our data would indicate that the susceptibility to such changes is not uniformly distributed among the *Y* chromosomal repetitive elements.

The results in this and other recent studies (Platt & Dewey, 1987; Maxson, Platt & Dewey, in preparation) serve to underscore both the usefulness and limitations of repetitive elements as phylogenetic markers. Mouse *Y* chromosomal repetitive elements evolve rapidly and are usually detectable only in species of *Mus* and not beyond (Lamar & Palmer, 1984; Nallaseth & Dewey, 1986; Nishioka & Lamothe, 1986; Platt & Dewey, 1987). However, they can be most useful for making phylogenetic distinctions among the members of the *Mus* species, and their applicability depends on the particular combination of probe and restriction enzyme. With one combination we see evidence for an ancestor common to the commensal species, *M. spretus*, and *M. hortulanus*. For example, in Fig. 2 all species displayed essentially the same *Bam* HI or *Hin* dIII banding profiles. With other combinations of probe and restriction enzyme phylogenetic distinctions are possible within the closely related commensal mice. With this approach the results are not easy to quantify and require the exercise of caution in their interpretation. The most clearly interpretable data are those in which there are single band differences among species whose overall banding profiles are otherwise quite similar. Examples include the 1·8 kbp *Eco* RI and the 3·0 *Pst* I restriction fragments in Fig. 2 present in *M. musculus* and *M. castaneus* but absent from *M. brevirostris*. Single band similarities between species whose banding profiles are otherwise very dissimilar (such as the 3·0 kbp *Eco* RI fragment in the *M. brevirostris* and *M. hortulanus* lanes 3 and 5 in Fig. 2) are less likely to reflect common ancestry.

The authors thank Debra Williams for assistance in preparation of this manuscript. Support for this work came from Public Health Service Grant HD 17523 from the National Institute of Child Health and Human Development.

References

Baron, B., Metezeau, P., Hatat, D., Roberts, C., Goldberg, M. E. & Bishop, C. (1986). Cloning of DNA libraries from mouse Y chromosomes purified by flow cytometry. *Somatic Cell and Molecular Genetics* **12**, 289–295.

Bishop, C. E., Bousot, P., Baron, B., Bonhomme, F. & Hatat, D. (1985). Most classical *Mus musculus domesticus* laboratory mouse strains carry a *Mus musculus musculus* Y chromosome. *Nature* (*Lond.*) **315**, 70–72.

Blin, N. & Stafford, P. W. (1976). A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Research* **3**, 2302–2308.

Bonhomme, F., Catalan, J., Britton-Davidson, J., Chapman, V. M. Moriwaki, K., Nevo, E. & Thaler, L. (1984). Biochemical diversity and evolution in the genus *Mus*. *Biochemical Genetics* **22**, 275–303.

Brownell, E. (1983). DNA/DNA hybridization studies of muroid rodents. *Evolution* **37**, 1034–1051.

Callahan, R. & Todaro, G. J. (1978). Four major endogenous retrovirus classes each genetically transmitted in various species of *Mus*. In: *Origins of Inbred Mice*. (ed. H. C. Morse), pp. 689–713. New York: Academic Press.

Eicher, E. M., Phillips, S. J. & Washburn, L. L. (1983). The use of molecular probes and chromosomal rearrangements to partition the mouse Y chromosome into functional regions. In: *Recombinant DNA and Medical Genetics* (eds. A. Messer and I. H. Porter), pp. 57–71. New York: Academic Press.

Ferris, S. D., Sage, D. R., Prager, E. M., Ritte, U. & Wilson, A. C. (1983). Mitochondrial DNA evolution in mice. *Genetics* **105**, 681–721.

Godson, G. N. & Vapnek, D. (1973). A simple method of preparing large amounts of $\phi\chi$174 RFI supercoiled DNA. *Biochemical Biophysica Acta* **299**, 516.

Lamar, E. E. & Palmer, E. (1984). *Y*-encoded, species-specific DNA in mice: evidence that the *Y* chromosome exists in two polymorphic forms in inbred strains. *Cell* **37**, 171–177.

Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

Maniatis, T., Jeffrey, A. & Kleid, D. G. (1975). Nucleotide sequence of the rightward operator of phage λ. *Proceedings of the National Academy of Sciences* (*USA*) **72**, 1184–1192.

Marshall, T. (1986). Systematics of the genus *Mus*. *Current Topics in Microbiology and Immunology* **127**, 12–18.

Martin, S. L., Voliva, C. F., Hardies, S. C., Edgell, M. H. & Hutchinson, C. A. III. (1985). Tempo and mode of concerted evolution in the L1 repeat family of mice. *Molecular Biology and Evolution* **2**, 127–140.

Nallaseth, F. S. & Dewey, M. J. (1986). Moderately repeated mouse *Y* chromosomal sequence families present distinct types of organization and evolutionary change. *Nucleic Acids Research* **14**, 5295–5307.

Nallaseth, F. S., Lawther, R. P., Stallcup, M. R. & Dewey, M. J. (1983). Isolation of recombinant bacteriophage containing male specific mouse DNA. *Molecular and General Genetics* **190**, 80–84.

Nishioka, Y. & Lamothe, E. (1986). Isolation and characterization of a mouse *Y* chromosomal repetitive sequence. *Genetics* **133**, 417–432.

Nishioka, Y. & Lamothe, E. (1987). Evolution of a mouse *Y* chromosome sequence flanked by highly repetitive elements. *Genome* **29**, 380–383.

Phillips, S. J., Birkenmeier, E. H., Callahan, R. & Eicher, E. M. (1982). Male and female DNAs can be discriminated using retroviral probes. *Nature* **297**, 241–243.

Platt, T. H. K. & Dewey, M. J. (1987). Multiple forms of male-specific simple repetitive sequences in the genus *Mus*. *Journal of Molecular Evolution* **25**, 201–206.

Selander, R. K., Hunt, W. G. & Yang, S. Y. (1969). Protein polymorphism and genetic heterozygosity in two European subspecies of the house mouse. *Evolution* **23**, 379–390.

Singh, L., Purdom, I. F. & Jones, K. W. (1980). Sex chromosome-associated satellite DNA: evolution and conservation. *Chromosoma* **79**, 137–157.

Wensink, P. C., Tabata, S. & Puchl, C. (1979). The clustered and scrambled arrangement of moderately repetitive elements in *Drosophila* DNA. *Cell* **18**, 1231–1246.

Yonekawa, H., Moriwaki, K., Gotsh, O., Watanabe, J., Hayashi, J.-C., Miyashita, N., Petras, M. L. & Tagashira, Y. (1980). Relationship between laboratory mice and the subspecies *Mus musculus domesticus* based on restriction endonuclease cleavage patterns of mitochondrial DNA. *Japan Journal of Genetics* **55**, 289–296.

https://doi.org/10.1017/S0016672300027956 Published online by Cambridge University Press