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Intragenomic movement, sequence amplification and concerted evolution in satellite DNA in harvest mice, *Reithrodontomys*: Evidence from in situ hybridization

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Abstract. Three DNA probes isolated from three species of *Reithrodontomys* (*R. montanus*, *R. megalotis*, *R. fulvescens*) were used to examine within and among species variation in the chromosomal location of satellite DNA and constitutive heterochromatin. These probes hybridized to the centromeric regions on all chromosomes in six species of the subgenus *Reithrodontomys*. Additionally, nearly all extra-centromeric C-band positive regions (with the exception of some heterochromatic material on the X and Y) hybridized to these probes. Within the subgenus *Reithrodontomys* both the chromosomal distribution and organization of satellite DNA has changed throughout evolution. The evolutionary transition has been from a totally centromeric position in *R. fulvescens* to centromeric and non-centromeric regions in other species that have undergone extensive chromosomal rearrangements from the primitive karyotype for peromyscine rodents. In addition, the monomer repeat of the satellite sequence differs between *R. fulvescens* (monomer defined by PstI) and the remaining species in the subgenus *Reithrodontomys* (monomer defined by EcoRI). These results suggest at least two amplification events for this satellite DNA sequence. Models and mechanisms concerned with the homogenization and spread of satellite sequences in complex genomes are evaluated in light of the *Reithrodontomys* data. From a phlyogenetic standpoint, the satellite sequences composing heterochromatic regions were restricted to the subgenus *Reithrodontomys*, which supports morphological differences used to recognize two subgenera, *Reithrodontomys* and *Aporodon*. Probes failed to hybridize to any part of the karyotype of *R. mexicanus* (subgenus *Aporodon*) or to seven species from other closely related genera (*Baiomys*, *Neotoma*, *Nyctomys*, *Ochrotomys*, *Onychomys*, *Peromyscus*, *Xenomys*), some of which are considered as potential sister taxa for *Reithrodontomys*.

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Introduction

Most mammalian chromosomes stain differentially when treated with alkaline or saline solutions producing C-banded (or C^+) regions that define areas of constitutive heterochromatin, and most mammalian species, rodents in particular, demonstrate considerable variation in both the amount and chromosomal distribution of constitutive heterochromatin. In some instances, heterochromatin is restricted to centromeric regions of the chromosomes, whereas in other species heterochromatin can be seen on telomeric regions, interstitial bands, whole chromosomes (including both autosomes and sex chromosomes), and chromosomal arms (Hsu and Arrighi 1971; Pathak et al. 1973; Arnason 1974; Stock 1974; Mascarello and Hsu 1976; Mascarello and Mazrimas 1977; Baker and Barnett 1981). Although no definite function for heterochromatin has been determined (John and Miklos 1979; John 1988), chromosomal variation involving heterochromatin is sometimes coincident with speciation. For instance, species in the rodent genus *Onychomys* can be distinguished by the number of heterochromatic short arms (Baker and Barnett 1981).

Recent molecular studies using restriction endonuclease digestion of total nuclear DNA, cloning and sequencing of specific DNA components, and in situ hybridization of selected DNA sequences to chromosomes have provided new insight into the structure, organization, and evolution of the mammalian genome. By far one of the most actively studied components of the mammalian genome has been highly repetitive DNA. This can exist as either interspersed repeated sequences or as long tandem arrays termed satellites (Singer 1982). Although some satellite DNA sequences can be separated from main band DNA by buoyant density centrifugation, other satellite DNAs cannot and are described as cryptic (Peacock et al. 1977; Skinner 1977). Therefore, the definition of satellite DNA has been broadened to include any DNA that represents a tandem repetition of a unit DNA sequence or repeat (Singer 1982). The

relevance of satellite DNA to mammalian chromosomal architecture can be seen by the fact that the location of most constitutive heterochromatin coincides with the location of satellite sequences along the mammalian chromosome (John 1988). However, considerable heterogeneity exists in the number of satellite DNA families and their chromosomal distribution relative to C-bands.

In this study we examined the genomic organization and chromosomal distribution of satellite DNA sequences and constitutive heterochromatin in the rodent genus *Reithrodontomys* (Mammalia: Rodentia), and related patterns of variation seen in this genus to other species of peromyscine rodents known to demonstrate variation in the chromosomal distribution of heterochromatin. This study was designed to examine the patterns of satellite DNA evolution in order to gain an insight into the structure and organization of the genome, especially as it relates to karyotypic evolution. Our approach was to compare the presence, amount and distribution of satellite DNA and then trace these sequences back through a recognized evolutionary tree (cladogram) and correlate chromosomal location with the evolution of the karyotype. The use of new techniques gave us a new approach for testing or resolving phylogenies erected by classical methodologies (ecology, morphology, behavior, etc.).

We chose *Reithrodontomys* because the genus is taxonomically diverse, has a well-defined phylogeny, displays extensive variation in terms of the degree of chromosomal evolution, and has taxa which vary in both the amount and chromosomal distribution of constitutive heterochromatin (Hooper 1952; Shellhammer 1967; Carleton and Myers 1979; Robbins and Baker 1980; Engstrom et al. 1981; Hood et al. 1984; Nelson et al. 1984).

We examined seven species of *Reithrodontomys* [*R. fulvescens*, *R. humulis*, *R. megalotis*, *R. montanus*, *R. raviventris*, *R. sumichrasti* (all in the subgenus *Reithrodontomys*), *R. mexicanus* (in the subgenus *Aporodon*)] via in situ hybridization with metaphase chromosomes. In addition, seven closely related genera (*Baiomys*, *Neotoma*, *Nyctomys*, *Ochrotomys*, *Onychomys*, *Peromyscus*, *Xenomys*) were examined.

Materials and methods

Specimens examined. All specimens utilized in this study were collected from natural populations, prepared as voucher specimens and deposited in The Museum, Texas Tech University (identified by a TK number), the Museum of Comparative Zoology, Harvard University (identified by an H number) or the Angelo State University Museum, San Angelo, Texas (identified by an ASK number). These specimens are as follows: (1) *R. creper*, Mexico, Quintanareo, 30 km SE San Miguel (ASK 529, 554). (2) *R. fulvescens*, Oklahoma, Hughes Co., 3.5 km E Raiford (1 male, TK 23472); McIntosh Co., 5 km E Dustin (1 female, TK 23470); Seminole Co., 5.6 km E Seminole (3 males, TK 23409, TK 23411, TK 23445); Texas, Jefferson Co., (1 male, H 135). (3) *R. gracilis*, Mexico, Campechi, 7.5 km W Escarcega (ASK 351); 21.2 km E Ciudad del Carmen (ASK 381). (4) *R. humulis*, Oklahoma, Pottawatomie Co., 4.8 km E Tecumseh (1 female, TK 26412). (5) *R. megalotis*, New Mexico, Luna Co., 36.2 km NE Deming (1 male, H 124); Texas, Castro Co., 8.8 km S, 4 km W Dimmitt (2 males, TK 32283, TK 32309). (6) *R. mexicanus*, Mexico, Veracruz; vicinity of Xomelta, N of Orizaba, approx. 2,440 m (1 female, TK 25749). (7) *R. montanus*, Texas, Castro Co., 8.8 km S, 4 km W Dimmitt (1 female, TK 32290; 2 males, TK 32311, TK 32314); Lamb Co., 23.8 km N Littlefield (1 male, H 126). (8) *R. raviventris*, California, Alameda Co., 4 km W Newark, Newark Slough (1 male, TK 13714). (9) *R. sumichrasti*, Mexico, Oaxaca, 4.8 km N (by road) Suchixtepec (2 females, TK 20995, TK 20997). (10) *Baiomys taylori*, Texas, Garza Co., 22.5 km S, 1.6 km E Post (1 female, TK 32211). (11) *Neotoma floridana*, Oklahoma, Jefferson Co., 11.3 km NE Beyers, Texas (1 female, TK 24199). (12) *Nyctomys sumichrasti*, Mexico, Jalisco, 6 km SE Chamela, Chamela Biological Field Station (1 male, TK 19654). (13) *Ochrotomys nuttalli*, Arkansas, Bradley Co., 12.9 km NW Warren (1 female, TK 26493). (14) *Onychomys leucogaster*, Texas, Garza Co., 25.7 km S, 8 km E Post (1 female, TK 24194; 1 male, TK 24195). (15) *Peromyscus leucopus*, Oklahoma, Kiowa Co., 1.3 km W, 2.4 km S Mountain View (1 female, TK 31585). (16) *Xenomys nelsoni*, Mexico, Jalisco, 6 km SE Chamela, Chamela Biological Field Station (1 female, TK 19647).

Cytological and in situ hybridization procedures. Karyotypes for *N. sumichrasti*, *O. leucogaster*, and *R. raviventris* were prepared from tissue cultures of lung or ear biopsies following the procedures of Baker and Qumsiyeh (1988). All remaining specimens were karyotyped from bone marrow following the yeast-stress method of Lee and Elder (1980). Chromosomes were C-banded as described by Sumner (1972). A minimum of five complete spreads, and in most cases, ten or more spreads were analyzed for each individual listed in Specimens examined.

To determine the genomic location of the satellite sequences of interest, in situ hybridization using metaphase chromosomes was employed. Procedures primarily followed protocols previously described in Moyzis et al. (1987, 1988). Metaphase preparations were denatured for 2 min at 70° C with 70% formamide (Kodak ACS) in 2 × SSC and hybridized for 12–18 h with approximately 1–3 μg/ml biotinylated plasmid DNA (labeled by nick translation as described by Clontech) in 2 × SSC, 500 μg/ml *Escherichia coli* carrier DNA, and 30% formamide. 1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate. Hybridization was maintained at 37° C in moist chambers under sealed coverslips. Following hybridization, the slides were washed for 2 min each in five changes of 2 × SSC, pH 7.0 at 40°–42° C. Hybridization was detected by binding fluorescein-labeled avidin (Vector Laboratories) to biotin-labeled probes. The preparations were amplified once with biotinylated goat anti-avidin antibody (Vector Laboratories) and in some instances two amplifications were required for precise visualization. Slides were counterstained with propidium iodide (Sigma) and 4,6-diamidino-2-phenylindole (DAPI; Sigma). Counterstaining with propidium iodide and viewing with UV light at 436 nm allows the simultaneous observation of the fluorescein-labeled hybridized probe (yellowish green) and total DNA (red), while DAPI viewed at a wavelength of 365 nm allows total DNA to be visualized. Counterstains were applied to slides in an antifade mounting media (Johnson and Araujo 1981) at concentrations of 0.25–0.4 μg/ml (DAPI) and 1.5–2.0 μg/ml (propidium iodide). Photographs were taken with Kodacolor VRG 400 and Extar 1000 film using an Olympus Epi-fluorescent microscope. A total of 5–10 complete spreads were examined for each individual animal and probe combination.

Characterization and cloning of satellite DNA probes. High molecular weight nuclear DNA was purified from either liver or brain using the method of Bingham et al. (1981). Highly repetitive sequences from *Reithrodontomys* were isolated by digestion of total DNA with diagnostic restriction endonucleases that produced a ladder of fragments representing multiples of a monomer repeat, a common feature of satellite DNA sequences (Brutlag 1980; Singer 1982). The digested DNA was electrophoresed on a 0.8% agarose gel, stained with ethidium bromide, and monomer bands were cut from the gel and extracted by electrolution (Weinand et al. 1979; Maniatis et al. 1982). Satellite DNA probes for in situ hybridization were constructed by ligating the isolated DNA con-

Fig. 1 a, b. Restriction site comparisons. **a** DNA blot hybridization of *Reithrodontomys montanus* satellite probe to EcoRI-digested total DNA from: *lanes A–C*, *R. montanus*; *lanes D–H*, *R. megalotis*; *lanes I–J*, *R. raviventris*; *lanes K–L*, *R. fulvescens*. **b** DNA blot hybridization of *R. megalotis* satellite probe to total DNA from *R. fulvescens* digested with PstI (*lane M*) and AccI (*lane N*). *Mr* monomer repeats

taining the monomer repeats to a linear plasmid vector (pUC18) followed by transformation of the *E. coli* host JM 101. The transformed bacteria was plated on 2 x YT plates (Miller 1972) containing ampicillin, 5-bromo-4-chloro-3-indolyl-β-D-glactoside (X-gal) and isopropyl-thiogalactoside (IPTG), and recombinants were selected by color. These recombinants were replated, and plasmid DNA was extracted from them using a mini-plasmid DNA preparation method (Maniatis et al. 1982). The isolated plasmid DNAs were digested with the diagnostic restriction endonuclease used in the cloning experiment, electrophoresed on a 0.8% agarose gel, and transferred to a nylon membrane filter using the DNA blot method of Southern (1975). The filter was hybridized to a probe consisting of the satellite monomer. All recombinants containing the monomer repeat were used for large scale plasmid DNA preparations. The satellite DNA probes used in this study consisted of cloned monomer repeats from *R. megalotis* (pMeg-1; H 124) and *R. montanus* (pMon-1; H 126) defined by EcoRI, and the monomer repeat from *R. fulvescens* (pFul-1; H 135) defined by PstI.

The basic genomic organization of highly repetitive DNA sequences was examined using DNA blot hybridization (Southern 1975). Total DNA (5 μg) was digested with restriction endonucleases and electrophoresed in 0.8% to 2.0% agarose slab gels. After electrophoresis the gel was stained with ethidium bromide, photographed under UV light, and transferred to a Gene Screen Plus nylon hybridization membrane using the alkaline transfer procedure (Chomczynski and Qasba 1984). The dried membrane filter was prehybridized in 50% formamide, 3 x SSC, 5 x Denhardt's solution, 1.0% SDS, and carrier DNA at 42° C and later hybridized in the same solution and at the same temperature to a ^{32}P-labeled probe. Hybridization was for approximately 16–20 h, and the filters were washed at 65° C first with 2 x SSC, 0.1% SDS, and then with 0.1% SSC, 0.1% SDS. Stringency of hybridization was altered for interspecific comparison by varying the temperature of hybridization in 50% formamide (34°–42° C). In addition, the salt concentration (SSC dilution) in washes was altered as well as the temperature in order to increase or decrease stringency. The results were visualized by autoradiography. All probes used in DNA blot experiments were labeled using the random priming method (Feinberg and Vogelstein 1983, 1984).

Results

Genome Organization

Upon digestion with the restriction endonuclease EcoRI, a satellite DNA ladder with a periodicity representing a 350 bp monomer repeat was found in species of the subgenus *Reithrodontomys* including *R. megalotis*, *R. montanus*, *R. sumichrasti*, *R. raviventris*, and *R. zacatecae* (Fig. 1a). Alternatively, *R. fulvescens* (subgenus *Reithrodontomys*) had a satellite DNA component homologous to the component found in the above species, but possessed a 340 bp monomer repeat defined by PstI (Fig. 1b).

Cloned satellite DNA monomers from *R. megalotis* and *R. montanus* were used in conjunction with DNA blot hybridization to examine the phylogenetic distribution of this satellite DNA sequence in other taxa of *Reithrodontomys* and peromyscine rodents. Even though DNA blot hybridizations were conducted at several different stringencies (see Materials and methods), no homologous satellite DNA sequence was seen in species of *Reithrodontomys* from the subgenus *Aporodon* represented by *R. gracilis*, *R. creper*, and *R. mexicanus* in this study. In addition, DNA blot hybridizations to individuals of other peromyscine rodent genera including *Onychomys*, *Neotoma*, and *Peromyscus* did not reveal any satellite sequences homologous to those found in species of *Reithrodontomys*.

Chromosomal distribution

The variation in amount and distribution of C-band positive material in *Reithrodontomys* is illustrated in Fig. 2a–d and Table 1. The variation in genomic location of the satellite DNA sequences is illustrated in Fig. 3 and Table 1. The satellite sequence probes (pMeg-1, pMon-1, pFul-1) hybridized to all centromeres on all chromosomes of six of the seven *Reithrodontomys* species examined. Additionally, the non-centromeric in situ hybridization of the probes to the autosomes of these species exactly matches the C-band positive regions of the karyotypes (Table 1). In situ hybridization of the X chromosomes generally matches the C-band distribution of heterochromatin except in *R. fulvescens*, *R. montanus* and *R. raviventris*. The only Y chromosome observed to hybridize beyond the centromeric region was found in a specimen of *R. fulvescens* which showed hybridization on the proximal one-third of the chromosome (Fig. 3). No in situ hybridization was detected on the chromosomes of the representative of *Reithrodontomys* from the subgenus *Aporodon* (*R. mexicanus*) or to any chromosomes from representatives of *B. taylori*, *N. floridana*, *N. sumichrasti*, *O. nuttalli*, *O. leucogaster*, *P. leucopus* and *X. nelsoni* (Table 1).

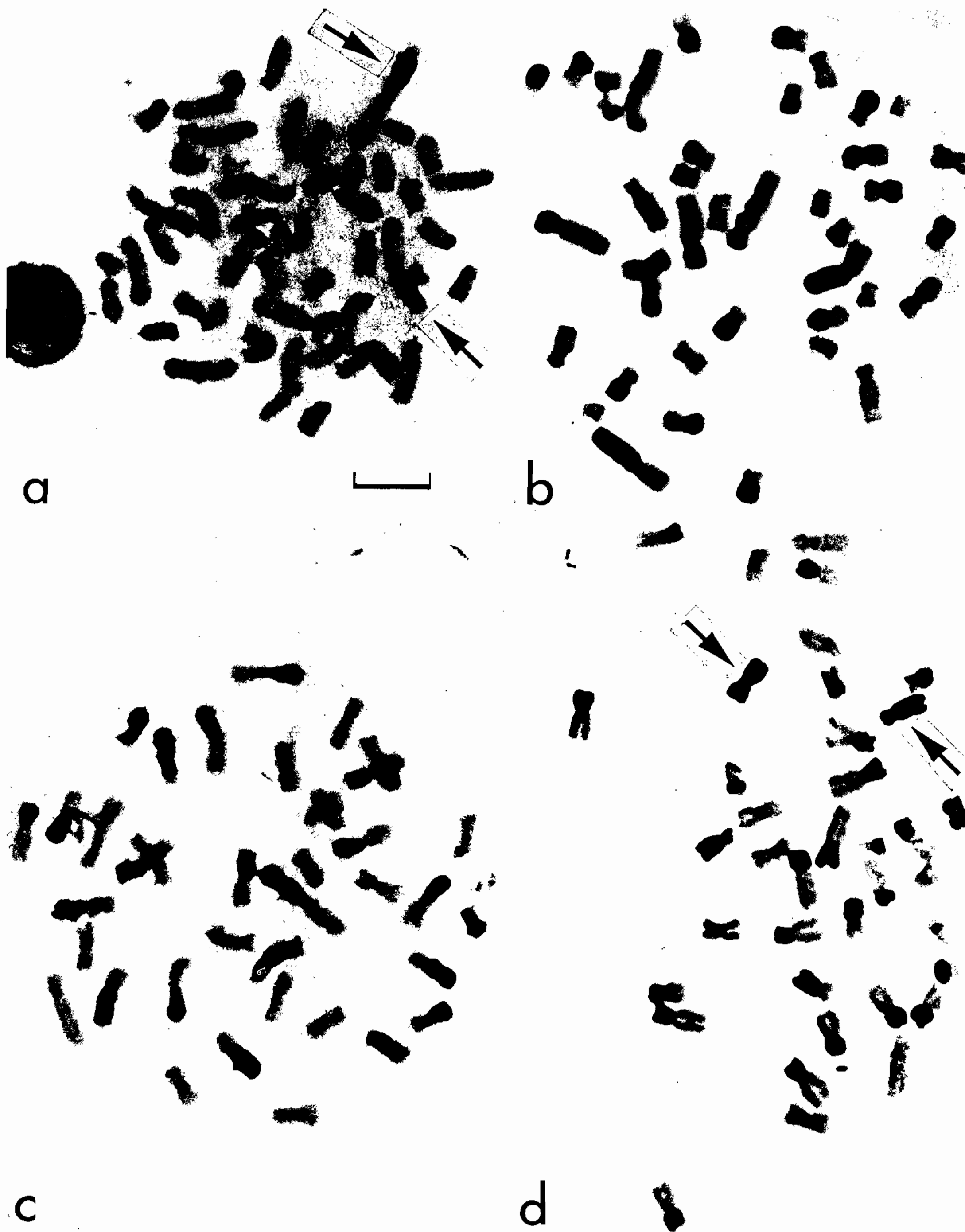

Fig. 2a–d. C-banded chromosomes of **a** *Reithrodontomys fulvescens*, **b** *R. montanus*, **c** *R. sumichrasti* and **d** *R. raviventris*. Note that C-band positive material is restricted to the centromeric regions of *R. fulvescens* except for the telomeric regions of the short arms of the X chromosomes (*arrows*). *R. montanus* exhibits numerous heterochromatic additions on both long and short arms of chromosomes. *R. sumichrasti* exhibits numerous heterochromatic additions, primarily appearing as short arm blocks. *R. raviventris* has the most extensive heterochromatic additions of the seven species of *Reithrodontomys* examined in this study. Two chromosomes (*arrows*) appear to be entirely heterochromatic. Bar represents 10 μm

Discussion

Phylogenetic distribution of satellite sequences

Reithrodontomys offers an opportunity to examine the patterns and processes of chromosomal evolution via modifications in the distribution of heterochromatin. There is a well-documented phylogeny for *Reithrodontomys* and related genera, and extensive empirical data exist for the patterns of chromosomal evolution seen in these rodents. For example, *R. fulvescens* shares an identical G-band pattern in most, if not all, chromo-

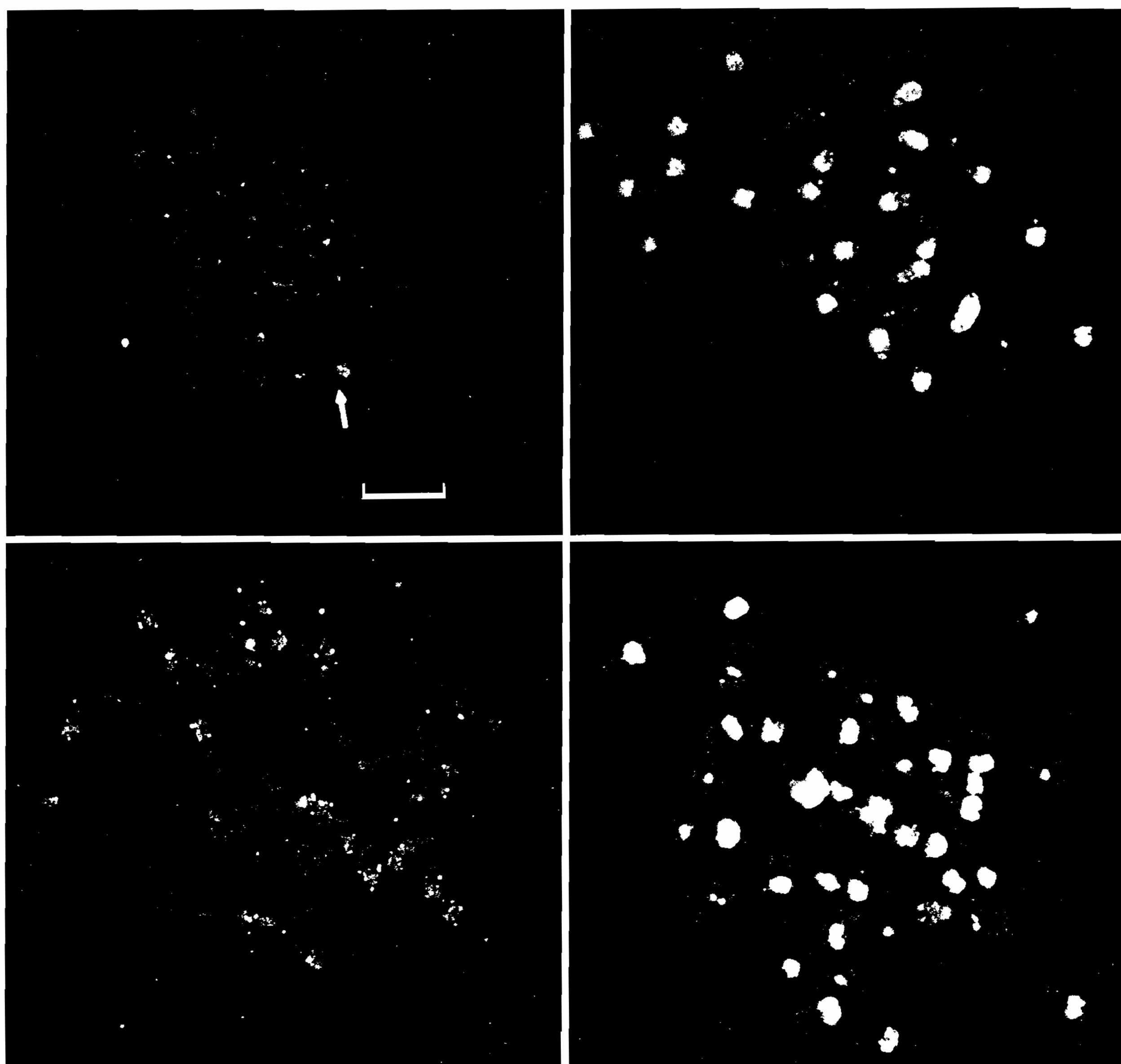

Fig. 3. In situ hybridization of biotin-labeled sequences isolated from *Reithrodontomys* to metaphase chromosomes. *R. fulvescens* chromosomes (*upper left*) are hybridized with the *R. megalotis* probe. *R. montanus* (*upper right*), *R. sumichrasti* (*lower left*) and *R. raviventris* (*lower right*) are hybridized with the *R. montanus* satellite sequence. *R. fulvescens* has a karyotype like the proposed primitive karyotype for the genus. Hybridization occurs only on the centromeric regions of all chromosomes except for approximately 30% of the Y chromosome which has hybridized to the satellite sequence (*arrow*). The remaining three species have hybridization patterns which are highly correlated with the amount and distribution of C-band positive material shown in Fig. 1. Bar represents 10 μm

somes with the karyotype proposed as primitive for peromyscine rodents (Koop et al. 1984). In addition, the G-band patterns for many chromosomes in *R. fulvescens* are shared with European *Apodemus*, African *Aethomys* and Australian *Melomys* (Koop et al. 1984; Baker et al. 1988). In contrast to *R. fulvescens*, *R. megalotis* and *R. montanus* have karyotypes that are unique to one or two taxa and are thought to have undergone karyotypic megaevolution (Baker and Bickham 1980, 1984). In the proposed primitive karyotype of *R. fulvescens* satellite sequences were restricted to centromeric regions and this probably reflects the primitive condition for this family of satellite DNA. In the species with highly derived karyotypes such as *R. megalotis* and *R. montanus* the satellite DNA is also found on all centromeric regions of all chromosomes, plus many non-centromeric regions including, telomeric regions, short arm blocks, interstitial bands, and long arm blocks (Fig. 3; Table 1).

From a phylogenetic standpoint, the satellite sequences composing heterochromatic regions were restricted to the subgenus *Reithrodontomys* (Table 1), which supports morphological differences used to recognize two subgenera, *Reithrodontomys* and *Aporodon* (Howell 1914; Hooper 1952). The lack of detectable hy-

Table 1. Chromosomal variation of the C-band heterochromatin and satellite DNA for *Reithrodontomys* and closely related taxa

Taxon	C-band heterochromatin distribution						References for C-bands	Highly repeated DNA distribution					
	Cen-tro-meric	Telo-meric	Inter-stitial	Long arm block	Short arm block	En-tire		Cen-tro-meric	Telo-meric	Inter-stitial	Long arm block	Short arm block	En-tire
R. fulvescens	25	X	–	–	–	Y	This study	25	–	–	–	–	–
R. humulis	25+1 single	–	5	–	6+X	Y	This study	25+1 single	–	ND	–	ND	–
R. megalotis	21	2 (SA)	1 (LA)	–	9+X	Y	This study	21	2 (SA)	1+single	–	8+single	–
R. mexicanus	26	–	–	–	–	–	Hood et al. (1984)	–	–	–	–	–	–
R. montanus	19	–	–	4+X	7	Y	Hood et al. (1984)	19	–	–	4	6+X+single	–
R. raviventris	19	–	–	2+X, Y	10	1	Hood et al. (1984)	19	–	–	2+Y	10+X?	1
R. sumichrasti	21	2	–	X	10	Y	Hood et al. (1984)	21	2	–	X	10	–
Baiomys taylori	24	–	–	–	–	–	Yates et al. (1979)	–	–	–	–	–	–
Neotoma floridana	26	–	–	–	1+X	–	This study	–	–	–	–	–	–
Nyctomys sumichrasti	26	–	–	–	–	–	Haiduk et al. (1988)	–	–	–	–	–	–
Ochrotomys nuttalli	26	–	1 (LA)	–	1+X	Y	Engstrom and Bickham (1982)	–	–	–	–	–	–
Onychomys leucogaster	24	–	5 (LA)	–	17+X	Y	Baker et al. (1979)	–	–	–	–	–	–
Peromyscus leucopus	24	5 (SA)	4 (LA)	–	–	–	Arrighi et al. (1976); Robbins and Baker (1981)						
Xenomys nelsoni	24	–	–	–	–	–	Haiduk et al. (1988)	–	–	–	–	–	–

All numbers listed indicate the number of pairs (unless stated otherwise) for each condition. The terms telomeric and centromeric indicate the presence of heterochromatin or hybridization near or at these chromosomal structures. ND, not determined; LA, long arm; SA, short arm; –, not present

bridization for any species outside the subgenus *Reithrodontomys* is interesting in light of the fact that all additional species examined are considered to be peromyscine rodents and such genera as *Baiomys*, *Peromyscus*, and *Onychomys* are considered potential sister taxa for *Reithrodontomys*. Also, at least some species in these genera contain large amounts of C-band positive material in their karyotypes (Baker et al. 1979; Yates et al. 1979; Robbins and Baker 1981).

Origin and dispersal of satellite sequences

The origin of repeated DNA sequences within the genome undoubtedly involves some form of amplification (Britten and Kohne 1966, 1968; Smith 1974, 1976, 1978); however, the actual processes involved in the amplification of satellite or repeated sequences, the dispersal of these sequences, and their maintenance in the genome are less clear.

To explain the presence of these satellite sequences in all six species of the subgenus *Reithrodontomys*, as well as their presence in the centromeric regions of all chromosomes within each species, requires some mechanism which not only produced sequence similarity in the common ancestor of the subgenus, but also produced sequence similarity in the centromeric regions of nonhomologous chromosomes (homogenization). This mechanism must also account for the absence of these sequences in the subgenus *Aporodon*, as well as other closely related genera which may have shared a recent common ancestor with *Reithrodontomys*.

Macgregor and Sessions (1986) have offered a possible explanation for what is observed in *Reithrodontomys*. Their model suggests that highly repeated DNA sequences originate and grow at specific chromosomal sites such as telomeres or centromeres, or wherever they are tolerated, and then disperse from these sites throughout the genome by subsequent chromosomal rearrangements. Their predictions are that recently evolved sequences should be homogeneous in structure, localized in discrete groups (at telomeres and centromeres), and taxonomically restricted in distribution. These predictions are supported by evidence from Urodeles (Macgregor and Sherwood 1979; Batistoni et al. 1986; Cremisi et al. 1988) and plants (Flavell 1986).

As mentioned above, in species of *Reithrodontomys* that have the more primitive karyotype, such as *R. fulvescens*, these satellite sequences are restricted to the centromeric region. In those species that have undergone moderate to extensive chromosomal evolution, such as *R. megalotis*, *R. sumichrasti*, *R. montanus*, *R. humulis*, and *R. raviventris*, the amount of satellite sequence and distribution (non-centromeric) throughout the genome is more extensive.

A model proposed by Saumweber (1987) and Schweizer et al. (1987) described how associations of chromosomes in intermitotic nuclei (Rabl polarization; Rabl 1885) may provide an opportunity for various chromosomal regions either to contact or to come into close proximity with each other. Essentially, Rabl polar-

ization creates a unique or specific ordering of the chromosomes within the haploid genome. The arrangement is one in which nonhomologous arms of most similar length are associated with one another. This allows centromeres to associate with one another and, less frequently, it permits telomeres to come into close contact with other telomeres on arms of similar length and equidistant interstitial regions. Rabl orientation has been described in various animal and plant cell nuclei (Comings 1980).

Several trends are predicted by the Rabl model (Schweizer et al. 1987): (1) metacentric chromosomes should have similar or symmetric patterns of C-bands in both arms; (2) karyotypes composed of chromosomes with approximately similar arm lengths and ratios should have telomeric rather than interstitial C-bands; (3) karyotypes composed of chromosomes of different sizes and arm ratios should have interstitial C-bands in addition to telomeric bands; and (4) in nonhomologous chromosomes with arms of similar length, there should be concerted evolution of C-bands. These predictions have held true in some plants (Schweizer and Ehrendorfer 1983), mammals (rodents of the genus *Peromyscus*; Hazen et al. 1977), and insects (John et al. 1985). If these predictions are true and C-band patterns are determined by both chromosomal architecture and arm length, then similar C-band patterns (not sequence composition) might occur in distant or related species with similar karyomorphology as a result of convergence or parallelism, not common ancestry.

The prediction that in nonhomologous chromosomes with arms of similar length, there would be concerted evolution of C-bands appears to be true for *Reithrodontomys*. The Rabl model also predicts that interstitial bands would occur in conjunction with telomeric bands and be the same distance from the centromeres as are the telomeres from the centromeres. Our observations in *R. humulis* (Table 1) which has interstitial, as well as telomeric areas of hybridization do not follow this prediction (in situ hybridized karyotype not shown). An alternative model has been reviewed by Walsh (1987) which involves replication by rolling circles of DNA. One appealing aspect of this model is that DNA can be easily moved between nonhomologous chromosomes even though nuclear orientation does not allow them to come into contact. However, with the data presented here it is difficult to test the rolling circle model.

Evidence for concerted evolution and intragenomic movement among nonhomologous chromosomes

The concept of concerted evolution describes processes by which a sequence can be amplified, homogenized throughout the genome, and distributed among both homologous and nonhomologous chromosomes (Kyrstal et al. 1978; Arnheim et al. 1980; Dover 1982). Several mechanisms including unequal crossing over, gene conversion, sequence transposition and rolling circles have been observed or hypothesized to be active in certain genomes (Jackson and Fink 1981; Klein and Petes 1981; Dover 1982; Walsh 1987), and these mechanisms may act together or individually during the process of concerted evolution.

There is definite evidence for concerted evolution of satellite DNA in the subgenus *Reithrodontomys*. For instance, it is known from G-band data that linkage groups in mammals are highly conserved (O'Brien et al. 1985; Baker et al. 1987). If chromosome 1 is compared in a series of rodents such as *Neotoma*, *Rattus*, *Oryzomys*, and *Peromyscus* the G-banding patterns are almost identical. The same holds for the respective chromosomes 2 and 3. However, if chromosome 1 is compared with chromosome 2 and then each with chromosome 3 in *Peromyscus*, the G-band patterns are very different. This is what is expected if nonhomologous chromosomes are evolving independently. This divergence in nonhomologous elements is not observed when the intragenomic satellite sequences are compared among nonhomologous chromosomes in the subgenus *Reithrodontomys*. The satellite DNA sequences appear to be more similar on nonhomologous chromosomes within a species, whereas satellite sequences on the centromeric flanking regions of homeologous chromosomes in closely related genera (*Peromyscus* and *Onychomys*) are totally different even though G-band patterns in euchromatic arms remain indistinguishable.

There are two alternative hypothesis for explaining the patterns of distribution observed in *Reithrodontomys*. The ability to discriminate between the two would assist in our understanding of the dynamic nature of the genome. One possibility would be that the event which established this repeat in the ancestor of the subgenus *Reithrodontomys* occurred only once. The alternative explanation would be a continual form of amplification with subsequent purging of the old repeat and replacement with the recently amplified repeat.

It would appear that the amplification, removal of old satellite DNA and subsequent spread of the new sequence to centromeric regions of nonhomologous chromosomes, has occurred at least twice in the evolution of the subgenus *Reithrodontomys*. This supposition is based on the following observations. First, it is assumed that either the EcoRI site (*R. megalotis* and *R. montanus*) or the PstI site (*R. fulvescens*) defining the monomer repeat unit of the satellite sequence was primitive when the initial amplification and dispersal of the amplified sequence took place. If this is true, then it would appear that the derived site (EcoRI if PstI is primitive or alternatively, the PstI site if EcoRI is primitive) has totally replaced the primitive site in some species.

It is impossible to determine with these data which of the cases is true. Also it is impossible to determine if this amplification of large blocks occurred for some chromosomes in a common ancestor for several species, or if this amplification is a unique event for each chromosomal pair for each species. However, the broad array of position and amount of the repeated sequence that appears throughout the subgenus *Reithrodontomys* suggests that the process has been active throughout the evolution of the subgenus. The original amplification of this repeated sequence appears to be a form of con-

certed DNA evolution. This would account for the presence of similar, if not identical, sequences on nonhomologous chromosomes.

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