Relationship Between Sequence Homology, Genome Architecture, and Meiotic Behavior of the Sex Chromosomes in North American Voles

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ABSTRACT In most mammals, the X and Y chromosomes synapse and recombine along a conserved region of homology known as the pseudoautosomal region (PAR). These homology-driven interactions are required for meiotic progression and are essential for male fertility. Although the PAR fulfills key meiotic functions in most mammals, several exceptional species lack PAR-mediated sex chromosome associations at meiosis. Here, we leveraged the natural variation in meiotic sex chromosome programs present in North American voles (*Microtus*) to investigate the relationship between meiotic sex chromosome dynamics and X/Y sequence homology. To this end, we developed a novel, reference-blind computational method to analyze sparse sequencing data from flow-sorted X and Y chromosomes isolated from vole species with sex chromosomes that always (*Microtus montanus*), never (*Microtus mogollonensis*), and occasionally synapse (*Microtus ochrogaster*) at meiosis. Unexpectedly, we find more shared X/Y homology in the two vole species with no and sporadic X/Y synapsis compared to the species with obligate synapsis. Sex chromosome homology in the asynaptic and occasionally synaptic species is interspersed along chromosomes and largely restricted to low-complexity sequences, including a striking enrichment for the telomeric repeat sequence, TTAGGG. In contrast, homology is concentrated in high complexity, and presumably euchromatic, sequence on the X and Y chromosomes of the synaptic vole species, *M. montanus*. Taken together, our findings suggest key conditions required to sustain the standard program of X/Y synapsis at meiosis and reveal an intriguing connection between heterochromatic repeat architecture and noncanonical, asynaptic mechanisms of sex chromosome segregation in voles.

KEYWORDS pseudoautosomal region; *Microtus* heterochromatin; telomeric repeats; meiotic synapsis

THE production of haploid sperm and egg via meiosis is dependent on a series of homology-driven events. Chromosomes must first locate their homologous partner within the nucleus and move into spatial proximity. These loose physical associations are formalized by the assembly of the synaptonemal complex (SC), a tripartite protein structure

that tethers homologous chromosomes along their axes and provides a scaffold for the organization of the chromatin loops (Zickler and Kleckner 1999). Concurrent with the initiation of SC assembly, double-strand breaks are programmatically induced across the genome and repaired via homologous recombination (Keeney 2001). The dysregulation or failure of these homology-driven events can lead to aneuploidy (Hassold and Hunt 2001), premature meiotic arrest (Roeder and Bailis 2000), and infertility (Handel and Schimenti 2010). The proper execution of chromosome pairing, synapsis, and recombination is therefore critical for reproduction.

The heterogametic sex chromosomes present a notable exception to this meiotic paradigm. Mammalian X and Y chromosomes evolved from a common set of autosomes

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that experienced divergent evolutionary pressures and reduced recombination after the Y chromosome acquired a sex-determination gene (Graves 1995b; Charlesworth 1996; Lahn and Page 1999). As a result, the X and Y chromosomes lack homology across most of their length. Nonetheless, the heterogametic sex chromosomes must function like a homologous chromosome pair and segregate reductionally at meiosis. To meet this challenge, the X and Y of most mammals retain a small, ~1–5 Mb telomere-adjacent segment of well-preserved sequence homology known as the pseudoautosomal region (PAR) (Mangs and Morris 2007). The critical meiotic activities of pairing, synapsis, and crossing over are concentrated to this narrow interval (Burgoyne 1982), rendering the PAR the most recombinogenic locus in the mammalian genome (Rouyer *et al.* 1986; Page *et al.* 1987; Hinch *et al.* 2014). In most mammals, disruption of sequence homology between X- and Y-linked PAR sequences can trigger meiotic metaphase I arrest and apoptosis (Gabriel-Robez *et al.* 1990; Burgoyne *et al.* 1992; Mohandas *et al.* 1992; Dumont 2017). PAR-spanning mutations may even provide a barrier to gene flow between incipient species (White *et al.* 2012a,b). Importantly, deletions and rearrangements in the PAR have been directly linked to infertility in humans and mice (Burgoyne *et al.* 1992; Jorgez *et al.* 2011).

Although the majority of mammalian species possess a PAR, there are several fascinating, natural exceptions to this rule. Nearly all marsupial species possess degenerate sex chromosomes with no X/Y homology (Graves and Watson 1991). Several nonmurid rodents also appear to lack a PAR (Ashley and Moses 1980; Borodin *et al.* 1995, 2012; de la Fuente *et al.* 2007). In these taxa, physical connections between the heterogametic X and Y chromosomes at meiosis are maintained by proteins rather than homology-driven DNA interactions. In marsupials, polymers of SYCP3, a major protein component of the SC, form a dense plate that anchors the X and Y to a common domain within the cell, thereby counteracting the polarizing tension of the meiotic spindle and ensuring correct X-Y segregation (Page *et al.* 2005). In the Mongolian gerbil, SYCP3 accumulates in a dense mat that paints the Y chromosome and bridges the metaphase plate to coat the distal tip of the X chromosome (de la Fuente *et al.* 2007). These exceptional taxa provide unique insights into the diversity of evolutionary solutions for solving the critical biological challenge of segregating chromosomes with limited or no sequence homology. Extending beyond mammals, there are also numerous examples of asynaptic sex chromosome meiosis in *Coleoptera* (Blackmon *et al.* 2016).

Voles of the genus *Microtus* provide an especially powerful opportunity to investigate the interplay between sex chromosome homology and the emergence of noncanonical mechanisms of meiotic sex chromosome segregation. Across this speciose genus, there is evidence for at least three independent losses of sex chromosome synapsis and recombination at meiosis (Borodin *et al.* 2012). Presumably, the recurrent emergence of the asynaptic condition reflects parallel erosion

of sequence homology between the X and Y along distinct vole lineages. Given that voles radiated from a common ancestor ~2 Mya (Jaarola *et al.* 2004), these observations suggest rapid, dynamic restructuring of sex chromosome architecture in this genus.

Here, we describe a trio of North American voles characterized by distinct meiotic sex chromosome programs, including synaptic, asynaptic, and occasionally synaptic species. We exploit this natural model system to test the relationship between sequence homology, genome architecture, and the meiotic behavior of the sex chromosomes. Our results provide a window onto the genetic processes that govern sex chromosome evolution and offer preliminary insights into the conditions required to sustain the canonical program of X/Y synapsis and recombination at meiosis.

Materials and Methods

Animal husbandry and ethics statement

Adult male Mogollon (*Microtus mogollonensis*; formerly *Microtus mexicanus*) and montane voles (*Microtus montanus*) were live caught in the high-altitude White Mountains of eastern Arizona and temporarily housed at the Biological Sciences Annex at Northern Arizona University (NAU) following protocols approved by the NAU Institutional Animal Care and Use Committee. Wild animals were then transported via courier service to the Yates Mill satellite animal facility operated by North Carolina State University (NCSU) in accordance with protocols approved by the NCSU Institutional Animal Care and Use Committee (approval no. 12-070-O). Prairie vole (*Microtus ochrogaster*) specimens were obtained from a laboratory colony maintained by L.A.M. at NCSU. All animals were killed by CO₂ inhalation.

Spermatocyte cell spreads and immunostaining

Spermatocyte cell spreads were prepared using a standard hypotonic drying down procedure (Peters *et al.* 1997). Cells were immunostained as previously described (Dumont *et al.* 2015) with CREST (anti-human, 1:100 dilution; Antibodies, Inc.), SYCP3 (anti-goat, 1:100 dilution; Santa Cruz Biotechnology), SYCP1 (anti-rabbit, 1:100 dilution; Abcam), and MLH1 (anti-rabbit, 1:100 dilution; BD Biosciences) primary antibodies. AMCA-labeled donkey anti-human, Texas Red-X donkey anti-goat, and FITC donkey anti-rabbit secondary antibodies were used at 1:200 concentration (Jackson ImmunoResearch).

Cell culture and preparation of mitotic metaphase cell spreads

Freshly dissected male kidney tissue was rinsed in 1× Hank's Balanced Salt Solution, minced with a sterile blade, and incubated with collagenase B (2 mg/ml) and primocin (150 µg/ml) for ~3 hr at 37°. Cells were then centrifuged, washed in Hank's Balanced Salt Solution, and cultured in RPMI media supplemented with 10% fetal bovine serum, 1%

GlutaMAX, and primocin at 35° under 5% CO₂. Due to poor growth under these conditions, cultures were transferred to renal cell growth media (Lonza) for subsequent passages.

To harvest metaphase-enriched cell populations, cultures were treated overnight with 50 µg/ml KaryoMAX (Life Technologies). Treated cells were pelleted and gradually resuspended in 75 mM KCl with constant, gentle agitation. The cell suspension was then incubated at 37° for 20 min and promptly pelleted. Cells were slowly resuspended in Carnoy's fixative and left to stand for 20 min. Cells were then pelleted and the fixation step was repeated two additional times. After the third fixation, ~50 µl drops of fixed cell suspension were dropped from a height of ~1 m onto glass slides under humid laboratory conditions. Slides were air dried in a dust-free environment for several days then transferred to -20° for long-term storage.

Whole chromosome sorting and probe synthesis

Stained chromosome suspensions were analyzed on a flow cytometer (Mo-Flo, Beckman Coulter) as described previously (Ng and Carter 2006). Chromosomes from each species were flow sorted on a high-purity sort option into sterile 500 µl Eppendorf tubes containing 33 µl of sterile ultraviolet-treated distilled water. Approximately 1000 copies of each chromosome were isolated. We obtained excellent agreement between the number of flow-sorted clusters and haploid chromosome number in each vole species, suggesting sufficient variation in chromosome size and GC content to uniquely isolate most chromosomes (Supplemental Material, Figures S1–S3).

The flow-sorted chromosome fraction corresponding to the X and Y of a given species was determined via FISH (Figures S1–S3). Based on relative chromosome sizes determined from karyotypes, we first identified candidate X and Y flow-sorted fractions. Approximately 300 chromosomes from each candidate pool were amplified using the GenomiPhi v2 DNA amplification kit according to the manufacturer's protocol with one modification. To enhance yield from the limited quantity of starting material at the potential expense of increased capture bias, the recommended cycling time was extended from 1.5 to 2 hr.

Approximately 1 µg of amplified DNA was digested to ~200–500 bp fragments and fluorescently labeled by nick translation. For each hybridization reaction, ~150–250 ng of labeled probe DNA was precipitated in 100% ethanol at -20° in the presence of 1 µg of mouse Cot-1 DNA (Thermo Fisher) and 0.3 M sodium acetate (pH 5.2). Precipitated probes were washed once in 80% ethanol and resuspended in a hybridization solution (50% deionized formamide, 50% 2× hybridization mix composed of 4× SSC, 0.2% Tween-20, and 20% dextran sulfate). Following resuspension, probes were denatured for 10 min at 70° and then allowed to reanneal at 38° for 30–45 min.

Mitotic metaphase spreads were dehydrated in a 70/90/100% ethanol series for 5 min at each concentration. After air-drying, slides were denatured in 70% formamide/2× SSC

(pH 7.0) at 62–63° for 2 min. Slides were then immediately quenched in ice-cold 70% ethanol, dehydrated in a second ethanol series (70/90/100% ethanol, 5 min each) and air-dried.

Reannealed probes were applied to the denatured, dehydrated slides. The probed area was covered with a 22 × 22 mm coverslip and sealed with rubber cement. Hybridization reactions were incubated overnight in a 37° humid chamber.

Fluorescence microscopy

Slides prepared from renal metaphase and spermatocyte cell spreads were imaged using either a Leica DM5500 B microscope equipped with a Photometrics CoolSNAP HQ² charge-coupled device camera linked to Leica Application Suite (version 2.3.5) software, or an Olympus BX61 epifluorescence microscope with a cooled charge-coupled device camera linked to Smart Capture 3 software. Images were postprocessed and analyzed with the Fiji software package (Schindelin *et al.* 2012).

For immunofluorescence-stained spermatocyte cell spreads, pachytene stage cells were identified by (i) the complete merger of SYCP1 and SYCP3 signals from both homologs for all autosomes, (ii) a full complement of chromosomes, and (iii) minimal background fluorescence. Cells that were damaged during preparation or displayed intensified SYCP3 staining at chromosome termini were not imaged. This later observation signals transition from pachytene into early diplotene.

Single chromosome sequencing and sequencing quality metrics

Amplified genomic DNA from the X and Y flow-sorted chromosomes of *M. ochrogaster*, *M. montanus*, and *M. mogollonensis* was purified by ethanol precipitation in the presence of 0.3 M NaOAc. Paired-end 300 bp libraries (average insert size 764 bp) were prepared from each chromosome pool using Illumina v3 chemistry. The six libraries were uniquely barcoded and sequenced in parallel on a single MiSeq run. Over 6 million paired-end 300 reads were sequenced per chromosome-enriched pool (Table S1).

Read quality metrics were assessed using FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). We noted a sharp decline in per-base sequence quality as a function of base position within a read (Figures S4–S6). To resolve this issue, we conservatively clipped the last 100 bp from each read using the fastx-trimmer program implemented in the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html).

Reads from the *M. ochrogaster* X chromosome flow sort pool were mapped to the reference genome from this species (MicOch1.0) using the default *bwa mem* settings (Li and Durbin 2010). There is no Y chromosome sequence in this female-derived reference assembly. Currently, there are no available genomic resources for either *M. mogollonensis* or *M. montanus*. We therefore mapped reads from the

X-enriched libraries of these species to the MicOch1.0 reference genome to assess chromosome X coverage. This strategy implicitly assumes that the X chromosome is broadly conserved between species, an assumption that may not be warranted for the rapidly evolving sex chromosomes in this system. Genomic coverage and read depth were calculated using BEDTools (Quinlan and Hall 2010).

To identify intrinsic sequence variables that could drive the amplification biases observed in our *M. ochrogaster* X chromosome-enriched sequence data, we compared the GC content, repetitive element density, and genomic position of the ~15% of the MicOch1.0 chromosome X (chrX) assembly covered by at least one sequenced read and the remaining ~85% of the chrX reference with no mapped reads. The significance of observed differences was assessed by randomly sampling size-matched fragments of the chrX reference to precisely mimic the distribution of contiguous sequenced regions from the *M. ochrogaster* chrX-enriched pool. A total of 1000 synthetic data sets were generated in this manner. The GC percentage, fraction of bases in repeats, and distance (in base pairs) between adjacent sequenced fragments was recorded for each simulated data set.

Validation of the sex chromosome origin of sequenced flow-sorted pools

To confirm that the flow-sorted pools identified by FISH as the X and Y in fact correspond to the heterogametic sex chromosomes, we mapped reads from each vole species to the high-quality human (GRCh38) and mouse (mm10) genome assemblies using *bwa mem* (Li and Durbin 2010). The default mapping settings were relaxed to allow recovery of reads mapping to these divergent assemblies (mapping parameters used: -k 15 -B 3 -O 5). This step enabled us to establish the presence of reads uniquely mapping to the vast majority of conserved genes on the mammalian sex chromosomes (Mueller *et al.* 2013; Bellott *et al.* 2014; Tables S2 and S3).

Given the high level of noise from whole chromosome painting experiments for *M. mogollonensis* and the presence of autosomes size-matched to both the X and Y (Figure S3), we carried out further bioinformatic analyses to confirm the sex chromosome identities of the selected pools from this species. First, we mapped reads from the putative *M. mogollonensis* chrX flow-sort fraction to the MicOch1.0 whole genome assembly using relaxed thresholds (*bwa mem*: -k 15 -B 3 -O 5). 10.4% of reads from the selected flow-sort fraction map to the MicOch1.0 X chromosome (Table S4). This observed percentage is notably greater than the ~1.9% expected in the absence of any enrichment (Table S6). In fact, the X chromosome is more enriched for mapped reads from this sequenced pool than any other assembled chromosome in the MicOch1.0 genome (Table S4). The absence of a reference Y chromosome assembly precludes a comparable analysis with the putative Y chromosome flow-sorted pool.

Cytogenetic observations reveal the heterochromatic status of the *M. mogollonensis* Y chromosome, whereas size-matched autosomes are predominantly euchromatic (Figure

S3). We find that 79.5% of sequenced, mapped reads from the putative *M. mogollonensis* Y chromosome flow-sort fraction span annotated repetitive sequences in the MicOch1.0 reference assembly. We define a read as spanning a repeat if >20% of sequenced bases overlap repeat-masked sequence. This percentage vastly exceeds the 58.9% average repeat content of the reference genome, suggesting that our targeted pool is heavily enriched for Y-derived sequence.

Analysis of shared k-mers

We developed a k-mer-based computational strategy to infer the presence of X/Y homology between the sequenced sex chromosomes of each vole species. For each flow-sorted sex chromosome pool, we first decomposed sequenced reads into their constituent k-mer sequences, where $k = \{30, 33, 35\}$, and reduced this list to a set of nonredundant k-mers. Next, we filtered out k-mers with (i) extreme GC content (<10% or >90%), (ii) containing homopolymer runs of length >10, (iii) mapping to annotated repeat sequences in the MicOch1.0 reference assembly with Hamming Distance ≤ 2 , (iv) corresponding to previously described vole repeat sequences (Table S5), or (v) present in the sequenced data at average coverage >100. We further eliminated k-mers that mapped to autosomal regions defined in the MicOch1.0 reference genome. The remaining set of k-mers should be enriched for sequences derived from the target sex chromosome. The degree of k-mer sharing between the X and Y pools of a given species was then calculated as follows:

$$\frac{N_{\text{shared}}}{N_X + N_Y - N_{\text{shared}}} \times 100\%,$$

where N is the number of unique k-mers.

To validate the utility of this *ad hoc* method, we identified k-mers on the X and Y human (hg38) reference genome and assessed whether the extent and spatial distribution of shared k-mers aligned with the known size and positions of X/Y homologous regions. K-mers that mapped to autosomes, coincided with annotated repeats, exhibited extreme GC content, or contained long homopolymer runs were excluded to mirror the criteria for filtering vole k-mers.

Assessing k-mer sequence complexity

The sequence complexity of a given k-mer can be represented by the number of unique substrings embedded in the sequence. We computed the linguistic complexity of each k-mer as the product of the ratios of the maximum number of words of all lengths $1 \leq i \leq k$ to the theoretical maximum number of words of length i that could be found in the sequence (Trifonov 1990):

$$\text{Sequence complexity} = \prod_{i=1}^k V_i / V_{\text{max}i}$$

V_i is the observed number of different words of length i within the k-mer and $V_{\text{max}i}$ is the maximum number of substrings of

length i in a sequence of length k . For a given alphabet size K , $V_{\max i} = \min(K^i, N - i + 1)$. For a DNA sequence composed of four nucleotides, $K = 4$.

Data availability

All data necessary for confirming the conclusions of the article are present within the main text and the supplemental materials. File S1 contains all supplemental figures and associated figure legends and is available for download through Figshare. Tables S1–S12 are supplied in a single Excel spreadsheet on Figshare. Sequence data are available as fastq files through GenBank under BioProject accession no. PRJNA415618. Supplemental material available at Figshare: <https://doi.org/10.25386/genetics.6359510>.

Results

Diversity of early meiotic sex chromosome programs in North American voles

The SC is a structural protein complex that provides a scaffold for the organization of the chromatin loops at meiosis (Zickler and Kleckner 1999). The sequential assembly of the SC during early meiosis can be tracked using expression patterns of SYCP3 and SYCP1, key protein components of the axial/lateral and transverse elements of the SC. At the onset of meiosis, SYCP3 begins to accumulate in isolated patches along the condensing chromosome axes. As meiosis progresses, SYCP3 signals coalesce into continuous strands that extend along the full axis of each chromosome. At the completion of synapsis, the transverse element protein SYCP1 is incorporated into the SC and links SYCP3 signals from homologous autosomes along their lengths (Dobson *et al.* 1994). On the heterologous X and Y chromosomes, this final stage of synapsis—marked by the colocalization of both SYCP1 and SYCP3 along the chromosome axis—is restricted the PAR (Page *et al.* 2006; Figure 1).

Male voles of the genus *Microtus* exhibit variable levels of X/Y association at meiosis, with evidence for multiple, independent losses of X/Y synapsis along the vole phylogeny (Borodin *et al.* 2012). Here, we confirmed one of these transitions in a trio of North American voles: *M. mogollonensis*, *M. montanus*, and *M. ochrogaster* (Figure 1 and Table 1). In *M. montanus*, the short arm of the Y chromosome is almost always synapsed with the terminal end of the X chromosome long arm at pachytene. In contrast, in *M. mogollonensis*, the X and Y chromosome axes never synapse, although sex chromosomes were always in close spatial proximity in the cell ($n = 121$ spermatocytes; Table 1). *M. ochrogaster* appears to be in the midst of an evolutionary transition, with approximately half of spermatocytes from this species possessing synapsed sex chromosomes and the other half exhibiting the asynapsed condition (Figure 1 and Table 1). Although X/Y synapsis is a rapidly evolving trait between species, the agreement between our findings and earlier results (Borodin *et al.* 2012) suggests that this phenotype may be fixed within species.

Figure 1 Representative pachytene stage spermatocytes from (A) *M. montanus*, (B) *M. mogollonensis* and (C and D) *M. ochrogaster*. Cells were immunostained for SYCP3 (red) to label the meiotic chromosome axes, SYCP1 (green) to mark regions of complete synapsis, and CREST (blue) to visualize centromeres. The heterogametic sex chromosomes are circled and enlarged in each panel. In A and D, sex chromosomes are synapsed at a terminal PAR, indicated by the colocalization of SYCP1 and SYCP3 signals. In B and C, sex chromosomes remain unsynapsed, with no SYCP1 signal detected along the sex chromosome axes.

Single chromosome sequencing of flow-sorted pools

We hypothesized that variation in meiotic sex chromosome programs among these three closely related vole species reflects distinct levels of X/Y homology in these taxa. To directly test this prediction, we aimed to sequence flow-sorted X and Y chromosomes from *M. montanus*, *M. ochrogaster*, and

Table 1 Fraction of cells with synapsed sex chromosomes from three vole species

Species	2N	No. of pachytene spermatocytes	% X/Y synapsis
<i>M. mogollonensis</i> (n = 2)	44	121	0
<i>M. montanus</i> (n = 1)	24	116	95
<i>M. ochrogaster</i> (n = 1)	54	118	40

M. mogollonensis (see *Materials and Methods*; Figures S1–S3 and Table S1).

Approximately 300 X and Y chromosomes from each vole species were amplified by isothermal strand displacement (Walker *et al.* 1992). Use of a limited amount of starting material can result in strong amplification bias (Chen *et al.* 2014; de Bourcy *et al.* 2014). To first assess the degree of bias introduced in our sequencing libraries, we mapped sequenced reads from the amplified *M. ochrogaster* X chromosome pool against the reference MicOch1.0 assembly. Approximately 6.3% of sequenced reads map to the reference X chromosome (Table S6). This represents a notable enrichment over the ~1.9% of reads expected from this chromosome in the absence of any enrichment (chrX length 61.8 Mb, assembled diploid genome size 3.3 Gb), but this percentage remains surprisingly low. We attribute this unexpected result to two related considerations. First, the MicOch1.0 reference genome is a draft quality assembly based on short paired-end reads. Sequences derived from repeat-rich regions on the X chromosome have likely been collapsed to single loci. Consistent with this explanation, the assembled length of the X chromosome is only about half its estimated size based on visual inspection of the karyotype and the length of comparably sized chromosomes (Figure S2). Second, the presence of shared heterochromatic repeats between the X chromosome and autosomes could result in spurious mapping of X chromosome derived reads to the autosomal genome fraction. Indeed, we uncover evidence for shared repetitive sequences between the X and multiple autosomal centromeres (see below; Figure 2C).

In addition to the modest enrichment for chrX reads, reads that do map to the chrX reference sequence provide coverage over just 14.6% of the chromosome. More than 90% of sequenced reads originate from <5% of the X chromosome sequence (Figure S7). By cross-species mapping to the MicOch1.0 reference assembly, we infer comparable levels of amplification bias for flow-sorted X chromosome sequences of *M. montanus* and *M. mogollonensis* (Table S7). In all cases, the overwhelming majority of reads map to a small fraction of X chromosome sequence, with the bulk of the X chromosome covered by no reads (Figure S7).

We next sought to rule out the possibility that intrinsic sequence properties drive this amplification bias, resulting in disproportional representation of genomic regions defined by specific sequence features. On average, regions of the MicOch1.0 chrX assembly covered by at least one sequenced read from the *M. ochrogaster* chrX-enriched library have

lower GC content and higher repetitive element density than regions with no sequence coverage ($P < 0.001$ by simulation; Table S8). However, observed differences in GC and repetitive element content between these fractions are very small, calling into question their biological significance. Moreover, we anticipate an enrichment of reads in repetitive sequences if repeats are frequently collapsed on the reference assembly. Amplified regions also tend to be more tightly clustered than expected by chance ($P < 0.001$ by simulation; Figure S8), although a visual inspection of coverage does not reveal an overt departure from uniformity (Figure S9). This spatial trend may be driven, in part, by the poor quality and the large number of gaps in the reference assembly. Taken together, these bioinformatic analyses uncover no obvious sequence feature that can, in isolation, account for the marked amplification bias we observe.

Inference of X-Y homology from k-mer sharing

Given that the chromosome-wide distribution of amplified regions is largely random, we reasoned that X/Y homology could be inferred from shared sequences present in the amplified X and Y chromosome pools from a given species. We developed a k-mer based computational strategy to define the set of sequences present in each X and Y flow-sorted pool (see *Materials and Methods*). This approach is agnostic to the availability of a reference genome, a key consideration given the absence of high-quality sex chromosome assemblies for voles. A subset of the k-mers in a given pool may be derived from size-matched autosomes that co-sort with the sex chromosome of interest (Figures S1–S3). Importantly, we do not expect contamination from the same autosomes in the X and Y chromosome fractions due to their pronounced size differences (Figures S1–S3). Thus, the intersection of k-mer sets from the X and Y chromosome-enriched flow-sorted pools of a given species should reflect the degree of sex chromosome homology. This homology will include the PAR, as well as regions of conserved ancestral identity between the X and Y.

To validate this strategy, we first asked how many k-mers are shared between the high-quality human X and Y chromosome assemblies. Although both sex chromosomes have been sequenced for several other mammalian species, including mouse (Mouse Genome Sequencing Consortium *et al.* 2002; Soh *et al.* 2014) and chimpanzee (Chimpanzee Sequencing and Analysis Consortium 2005; Hughes *et al.* 2010), the PAR is embedded within gaps or altogether missing from these assemblies, precluding their use as benchmarks in this analysis. We fractionated the human X and Y chromosome sequences (hg38) into their constituent $k = 33$ bp sequences and quantified the extent of 33-mer sharing between the sex chromosomes. Approximately 2.7% of 33-mers are shared between the X and Y chromosomes (Table S9). These shared k-mers are spatially concentrated in three clusters corresponding to the two terminal human PARs and the X-transposed region at Xq21.31 (Figure S10). To mimic the sparse coverage of the vole sequence data, we then randomly selected 5–15% of

Figure 2 Whole chromosome painting on metaphase cell spreads in (A) *M. mogollonensis*, (B) *M. montanus*, and (C) *M. ochrogaster*. X chromosome probes were cross-hybridized to the Y chromosome (and vice versa) to test for sequence homology between the sex chromosomes. (A') *M. mogollonensis* Y chromosome probes hybridized to the *M. mogollonensis* X chromosome and (A'') *M. mogollonensis* X chromosome probes hybridized to the *M. mogollonensis* Y chromosome. (B') *M. montanus* Y chromosome paints hybridized to the *M. montanus* X chromosome and (B'') X chromosome probes hybridized to the Y chromosome. (C') *M. ochrogaster* X chromosome FISHed with *M. ochrogaster* Y chromosome paints and (C'') *M. ochrogaster* X chromosome probes hybridized to the Y chromosome of this species.

human k-mers on each sex chromosome and computed the degree of k-mer sharing between these downsampled data sets. In all cases, the extent of k-mer sharing was proportional to the known, annotated size of the human PAR and the extent of downsampling (Figure S11). Furthermore, we still observe highly specific localization of shared k-mers to known regions of X/Y homology (Figure S12). We conclude that our k-mer approach provides qualitative and spatial information about the identity and location of the PAR.

We applied this k-mer strategy to assess whether each of the three North American vole species possesses an X/Y homologous region (Table 2). We focus on sequences of length $k = 33$, but note that our qualitative findings are replicated for $k = 30$ and $k = 35$ (Table S10). *M. mogollonensis* harbors the highest percentage of chromosome X 33-mers shared with the Y. We observe a 1.6- and 3.2-fold reduction in 33-mer sharing in *M. ochrogaster* and *M. montanus*, respectively (Table 2). Overall, levels of k-mer sharing oppose naïve predictions based on the frequency of meiotic sex chromosome synapsis in these taxa.

Broad-scale features of sex chromosome homology validated by whole chromosome painting

We next sought to validate patterns of X/Y k-mer sharing in a series of chromosome painting experiments. For each of the three vole species, we developed chromosome-specific probes from X and Y chromosome flow sorted pools and hybridized both sex chromosome probe sets to metaphase preparations from the same species (see *Materials and Methods*). If k-mer based trends mirror true levels of X/Y homology, we reasoned that X and Y chromosome probes should cohybridize over a larger region in *M. mogollonensis* and *M. ochrogaster* than *M. montanus*.

Consistent with predictions based on k-mer sharing, we find clear signals of probe colocalization across the short arm of the X chromosome and the distal end of the Y in *M. mogollonensis* (Figure 2B). Fluorescent probe signals are strongly suppressed on the X chromosome long arm and over much of

the Y, likely reflecting the dense heterochromatic content of these regions (Figure S3). X chromosome probes display promiscuity for the centromeres of acrocentric chromosomes in this species and we detect faint fluorescent signals on one autosome pair. This latter signal is likely attributable to contamination of the X chromosome flow-sorted fraction with that of a size-matched autosome (Figure S3).

M. ochrogaster, the species with intermediate levels of X/Y synapsis at meiosis, also possesses shared X-Y homology detected via chromosome painting (Figure 2C). Although X chromosome paints hybridize to much of the Y chromosome, the reciprocal signal (Y chromosome paint on the X) is not detected. The distal region of the chrX long arm and the centromere-proximal region on chromosome Y remain unpainted, consistent with their heterochromatic status (Figure S2). Curiously, these regions participate in the synaptic associations observed in a subset of spermatocytes from this species (Figure 1B).

Although the *M. montanus* X and Y almost always synapse at meiosis, cohybridization of both sex chromosome paints to loci on the X or Y was never observed (Figure 2A). We conclude that sex chromosome synapsis in this species must be mediated by heterochromatic repeat sequences over which probe binding is suppressed or restricted to a region too small to be visualized using this technique. A third interpretation, that X/Y synapsis is driven by nonhomologous sequence interactions, is unlikely given the presence of crossover events within the synapsed region (Figure S13).

In summary, sex chromosomes from the asynaptic species *M. mogollonensis* and the partially asynaptic species *M. ochrogaster* possess clear regions of homology as assessed by both k-mer sharing and whole chromosome painting. However, we observe limited k-mer sharing and no reciprocal probe hybridization between the synaptic X and Y chromosomes of *M. montanus*. Thus, the presence of X/Y homology alone is not sufficient to drive sex chromosome synapsis at meiosis, paralleling earlier observations in European voles (Acosta *et al.* 2011).

Table 2 Sex chromosome 33-mer sharing in North American voles

Species	No. of X 33-mers	No. of Y 33-mers	No. of shared 33-mers	% Shared k-mers
<i>M. mogollonensis</i>	384,757,254	320,347,290	9,472,308	1.362
<i>M. montanus</i>	142,382,487	147,777,835	1,224,327	0.424
<i>M. ochrogaster</i>	244,978,285	182,802,467	3,666,169	0.864

Reduced *k*-mer sequence complexity in species with asynaptic sex chromosomes

One interpretation for the unexpected relationship between sex chromosome synapsis and homology is that X/Y homology in *M. mogollonensis* is limited to repetitive sequences. Indeed, cytogenetic observations indicate that both sex chromosomes from this asynaptic species are dominated by heterochromatin (Figure 2B and Figure S3). Bioinformatic filters targeting *k*-mers in known, annotated repeats resulted in the removal of 12.82% of all X chromosome 33-mers in *M. mogollonensis*, but only 5.78% of X chromosome 33-mers in the synaptic species, *M. montanus* (Table S10). Thus, the *M. mogollonensis* sex chromosomes appear to harbor an elevated load of annotated repeat sequences relative to those of *M. montanus*.

Motivated by these observations, we sought to characterize the sequence composition of *k*-mers from each species. To this end, we calculated the linguistic complexity of X and Y *k*-mers in each vole species as a function of the observed number of unique “words” embedded within each *k*-mer (see *Materials and Methods*). *k*-mers derived from repeat-rich sequences should exhibit reduced complexity scores compared to *k*-mers from unique single copy sequences.

Consistent with our expectations, 33-mers from *M. mogollonensis* have reduced sequence complexity relative to *M. montanus* (Mann–Whitney *U*-test: $P < 10^{-15}$ for both sex chromosome comparisons; Figure 3 and Figure S14). *M. ochrogaster* exhibits an intermediate distribution of sequence complexity scores (Mann–Whitney *U*-test: $P < 10^{-15}$ for all comparisons). After eliminating all *k*-mers with a complexity score < 0.4 , X/Y 33-mer sharing is decreased fivefold for *M. mogollonensis* and twofold for *M. ochrogaster*. In contrast, removing low-complexity *k*-mers has minimal effect on the degree of 33-mer sharing in *M. montanus* (Table S10 and Table 3). We conclude that X/Y homology in *M. mogollonensis* and *M. ochrogaster* is largely restricted to heterochromatic repeat-rich sequences.

Based on our benchmark analyses of the human sex chromosomes, we expected shared, high-complexity X/Y 33-mers to cluster in a specific region or regions across the X chromosome, corresponding to the PAR(s). Instead, the distribution of shared X/Y 33-mers in *M. ochrogaster* is surprisingly uniform (Figure S15). A cluster of *M. montanus* X/Y shared *k*-mers localizes to MicOch1.0 chrX:29.0–29.1 Mb. This region is homologous to the intergenic interval between *Cldn34d* and *Mir669m-2* on mouse chrX:76.86–77.01 Mb, but shows no clear homology to the human genome by a BLAST-like alignment tool search (Kent 2002). If this region

indeed corresponds to the *M. montanus* PAR, it is not homologous to the mouse or human PAR and would provide an additional example of the poor conservation of this locus throughout mammalian evolution (Toder *et al.* 1997; Graves *et al.* 1998; Toder and Graves 1998; White *et al.* 2012b).

Low-complexity *k*-mers from asynaptic species are enriched for subtelomeric repeats

Prior cytogenetic analyses in voles have identified individual repeat families that have undergone lineage-specific expansion, yielding diverse heterochromatin sequence environments across species (Marchal *et al.* 2004a, 2006; Acosta *et al.* 2008, 2009). We next asked whether the low-complexity *k*-mer fractions from *M. mogollonensis*, *M. ochrogaster*, and *M. montanus* are enriched for identical focal repeat units. We began by partitioning low-complexity 33-mers (complexity scores < 0.4) into their constituent 2–8 bp motifs and tallying observed motif counts for each pool. In *M. montanus*, low-complexity sequences from the X and Y are replete with AG/CT dinucleotide repeats (Table S11). This finding concords with the high abundance of (AG)_n and (CT)_n sequences in mammalian genomes (Tóth *et al.* 2000). For both the X and Y chromosome pools from *M. ochrogaster* and *M. mogollonensis*, the most frequently observed motifs within low-complexity *k*-mers are derivatives of the TTAGGG subtelomeric repeat unit (Figure 4 and Table S11). This finding is unlikely an artifact of the preferential amplification of subtelomeric regions, as we observe no local enrichment of *M. ochrogaster* X chromosome reads mapping to distal regions on the reference assembly (Figure S9).

To elucidate whether the apparent enrichment of telomere repeats is attributable to blocks of tandemly arrayed TTAGGG repeats or interspersed instances of this motif, we examined the composition of sequenced reads containing this hexamer sequence. A majority of TTAGGG-harboring, sequenced reads possessed multiple contiguous repeat units in both *M. mogollonensis* and *M. ochrogaster* (Table S12). However, in *M. montanus*, the majority of instances of the TTAGGG motif occur in isolation (Figure S16 and Table S12). Thus, the tandem-repeat architecture of TTAGGG sequences in the two species with asynaptic sex chromosome segregation is distinct from that in *M. montanus*.

TTAGGG motifs are clustered in two discrete, interstitial regions on the vole X chromosome

There are three potential explanations for the preponderance of (TTAGGG)_n sequences within the low-complexity X and Y

Figure 3 Histograms of sequence complexity scores for 33-mers in the amplified flow-sorted sequence pools from (A and B) *M. mogollonensis*, (C and D) *M. ochrogaster*, and (E and F) *M. montanus*.

chromosome k-mer fraction of *M. mogollonensis* and *M. ochrogaster*. A historical chromosome fusion event(s) may have transferred the subtelomeric repeat array into a novel interstitial context. Subsequent runaway amplification could have led TTAGGG repeats to dominate the heterochromatic content of the sex chromosomes from these species, as observed in several mammalian taxa (Meyne *et al.* 1990). Alternatively, the TTAGGG repeat sequence may constitute a core component of pericentromeric α -satellite DNA (Meyne *et al.* 1990; Ventura *et al.* 2006). Finally, interstitial TTAGGG sequences could arise from telomerase activity during the repair of double-strand breaks (Nergadze *et al.* 2007; Ruiz-Herrera *et al.* 2008).

In an effort to discriminate between these alternatives, we examined the chromosome-wide distribution of TTAGGG-containing sequenced reads on the MicOch1.0 chrX reference

assembly. We reasoned that if repeats are relics of historical chromosome rearrangements or core components of heterochromatic blocks, they should spatially cluster at a few loci in the genome. If, instead, repeats arise from spontaneous DNA repair events, they may be distributed among a larger number of historical repair sites and are unlikely to be conserved between species. In agreement with the first pattern, reads from the *M. ochrogaster* chrX pool that contain the TTAGGG hexamer predominantly localize to two intervals on the chrX reference assembly (chrX:11.5 and chrX:30.5 Mb; Figure S17). TTAGGG-containing reads from the *M. mogollonensis* X chromosome pool are also enriched at these two loci, albeit with reduced intensity likely owing to divergence from the reference. Although *M. montanus* reads containing subtelomeric repeats map to the chrX:11.5 interval, we find no signal at the chrX:30.5

Table 3 Percentage of high-complexity, nonrepetitive k-mers shared between the X and Y chromosomes

Species	No. of X 33-mers	No. of Y 33-mers	No. of shared 33-mers	% Shared 33-mers
<i>M. mogollonensis</i>	221,111,605	38,137,825	534,336	0.207
<i>M. montanus</i>	77,348,266	89,937,043	416,032	0.249
<i>M. ochrogaster</i>	59,896,135	74,726,628	247,897	0.184

locus. We speculate that the 30.5 Mb region corresponds to the site of a historical chromosome fusion event that occurred in the common ancestor of *M. mogollonensis* and *M. ochrogaster*, giving rise to the bi-armed architecture of the X chromosome in these species. The poor quality of the vole reference genome, the sparse coverage of our single chromosome sequence data, and the low resolution of whole chromosome painting limit further insights into the origin and identity of this putatively translocated sequence.

In summary, low-complexity sequences in *M. ochrogaster* and *M. mogollonensis* are dominated by subtelomeric repeats that map to two interstitial regions of the X chromosome. In contrast, the *M. montanus* sex chromosomes are enriched for (AG)_n/(CT)_n microsatellites, revealing sharp differences in the repeat composition of the sex chromosomes between these closely related species.

Discussion

We investigated patterns of sex chromosome homology and architecture in three North American vole species characterized by distinct meiotic X/Y segregation programs. Using a combination of DNA sequence analyses and cytogenetic approaches, we demonstrated a greater degree of X/Y sequence homology in voles with sex chromosomes that never (*M. mogollonensis*) and occasionally (*M. ochrogaster*) synapse at meiosis compared to the species with obligate X/Y synapsis (*M. montanus*). These findings provide several key insights into the evolution and meiotic function of the PAR.

First, our results indicate that sex chromosomes harboring significant homology can nonetheless fail to synapsis and recombine at meiosis. We uncover the highest fraction of X/Y k-mer sharing in the asynaptic vole *M. mogollonensis*, but much of this signal is driven by heterochromatic sequences. Notably, *M. mogollonensis* exhibits a marked enrichment of low-complexity shared k-mers relative to the synaptic species *M. montanus* (Figure 4 and Figure S14), and removal of these putative repetitive sequences causes a pronounced drop in the extent of inferred X/Y homology based on k-mer sharing (Table 2 and Table 3). Together, these observations suggest that sex chromosome synapsis is dependent on factors other than the mere presence of sequence homology. The epigenetic landscape, the density and genetic architecture of heterochromatic regions across the sex chromosomes, and the availability of sufficient X/Y sequence homology across a contiguous expanse of

unique sequence are likely defining features of a functional PAR (Acosta *et al.* 2011; Kauppi *et al.* 2011; Powers *et al.* 2016).

Second, our findings suggest that even short regions of X/Y homology are sufficient to drive synapsis. The obligate pattern of meiotic synapsis between the *M. montanus* sex chromosomes (Figure 1A and Table 1) strongly implies the presence of a PAR, but multiple lines of evidence indicate that this homologous region is likely quite short, on the order of <1 Mb. For one, the putative PAR is evidently small enough to escape detection via chromosome painting on metaphase chromosomes. Additionally, shared X/Y k-mers in this species localize to a short ~200 kb region on the MicOch1.0 reference (Figure S15) and crossover events in the *M. montanus* PAR are only observed at the extreme distal end of the synapsed interval (Figure S13). We acknowledge that the conclusion of a very short *M. montanus* PAR is seemingly at odds with the observation that ~1/10 of the Y chromosome is synapsed with the X at meiosis (Figure 1A). X/Y synapsis may be initiated at a small region of homology, and ultimately extended into nonhomologous regions, as reported in other mammals (Tres 1977; Solari 1988; de la Fuente *et al.* 2012). Alternatively, the size of the chromatin loops organized on the SC could differ between the PAR and nonsynapsed regions, yielding variable relationships between the SC length and physical sequence length over distinct genomic compartments. This phenomenon has been previously described in house mice (Kauppi *et al.* 2011).

Third, our data add to mounting evidence establishing a general link between the accumulation of heterochromatin and the emergence of asynaptic mechanisms for sex chromosome segregation in voles (Jiménez *et al.* 1991; Marchal *et al.* 2004b; Acosta *et al.* 2011). Indeed, several vole species with asynaptic X/Y meiosis possess giant sex chromosomes characterized by runaway amplification of repetitive sequences and massive heterochromatic blocks (Megias-Nogales *et al.* 2003; Marchal *et al.* 2004b). Despite these hints at an apparent association, it remains unclear whether asynaptic sex chromosome segregation arose as a cause or consequence of changes in the heterochromatic composition of the sex chromosomes. On the one hand, the emergence of asynaptic meiosis and the associated loss of the meiotic function of the PAR may have resulted in relaxed selection on the X and Y and, consequently, the rapid accumulation of sex chromosome heterochromatin. On the other hand, the expansion of heterochromatic regions on the sex chromosomes may have imposed strong selective pressures for the emergence

Figure 4 The relative frequency of all 4096 possible hexamer motifs present in 33-mers from each sequenced sex chromosome pool in (A and B) *M. mogollonensis*, (C and D) *M. ochrogaster*, and (E and F) *M. montanus*. For each pool, hexamer counts were standardized to the observed count of the most frequent hexamer. Motifs are plotted in alphabetical order along the x-axis. Motifs related to the TTAGGG subtelomeric repeat sequence are indicated in red. The identity of motifs with a relative frequency >0.75 is provided (black dots).

of an alternative mechanism as a safeguard against deleterious rearrangements borne from nonallelic homologous recombination. Analyses of the heterochromatin composition of sex chromosomes from additional *Microtus* taxa as well as

species ancestral to *Microtus* may help tease apart the direction of causality. Whether heterochromatin plays a direct role in asynaptic/achiasmate sex chromosome segregation in voles, as observed in *Drosophila* (Dernburg *et al.* 1996), or

whether this relationship is simply correlative also remains an open question.

The lack of X/Y synapsis in *M. mogollonensis* raises the question of how proper segregation of the heterologous sex chromosomes is ensured in this species. Cytogenetic studies in diverse mammals with asynaptic sex chromosomes underscore a key role for the axial/lateral element protein SYCP3. In marsupials, SYCP3 polymerizes into a dense plate that links both the X and Y until their segregation at metaphase I (Page *et al.* 2002, 2005). In Mongolian gerbils, SYCP3 paints the Y chromosome and distal tip of the X, forming a protein bridge between the two sex chromosomes (de la Fuente *et al.* 2007). In the European vole (*Microtus duodecimcostatus*), the X and Y chromosomes are tethered by a SYCP3 filament that persists through meiosis I (de la Fuente *et al.* 2012). A similar mechanism is observed in the water vole (*Arvicola terrestris*), suggesting that SYCP3 assumed a specialized role in sex chromosome segregation in the common ancestor of *Microtus* (de la Fuente *et al.* 2012). Thus, whereas the fusion or translocation of autosomal sequence onto the sex chromosomes provides one evolutionary solution to counteract the erosion of sequence homology between the X and Y (Graves 1995a; Toder and Graves 1998; Blackmon and Demuth 2015), many mammals, including some vole taxa, have pursued an alternative evolutionary strategy to surmount this biological challenge: the development of a SYCP3-dependent meiotic sex chromosome segregation mechanism.

Although the unique modifications of the axial/lateral element protein SYCP3 set asynaptic mammalian species apart from their synaptic counterparts, sex chromosome meiosis in these exceptional taxa exhibits some shared features with other mammals. Most notably, at the onset of meiosis in all mammals, the X and Y chromosomes undergo a series of orchestrated movements that culminate in telomere binding to the nuclear envelope and bouquet formation. Telomere repeats (and the proteins that bind them) are critical for initiating and stabilizing these nuclear envelope attachments (Cooper *et al.* 1998; Chikashige *et al.* 2006; Ding *et al.* 2007). Indeed, shortened or absent telomeres compromise chromosome binding to the nuclear envelope, leading to impaired synapsis, recombination, and apoptosis (Liu *et al.* 2004).

Our analysis of sequenced, flow-sorted X and Y chromosomes revealed a striking enrichment for the telomeric (TTAGGG)_n repeats on the sex chromosomes of species with no or occasional synapsis, *M. mogollonensis* and *M. ochrogaster*. Prior studies of asynaptic sex chromosome segregation have posited that telomeric repeats can provide terminal connections between nonhomologous X and Y chromosomes (Solari and Ashley 1977; Ashley and Moses 1980). However, the (TTAGGG)_n repeats in voles cluster into two large interstitial blocks (based on the MicOch1.0 reference assembly), and cannot mediate telosynaptic X/Y associations. Instead, this repeat architecture raises the possibility that interstitial (TTAGGG)_n repeats are, like telomeres, anchored to the nuclear envelope in early meiosis (Meyne *et al.* 1990).

At points of telomere attachment, the axial elements are splayed out along the nuclear envelope and manifest as cone-shaped anchors (Page *et al.* 2002, 2006; de la Fuente *et al.* 2007). These telomere-associated modifications suggest that SYCP3 protein possesses a unique binding affinity for (TTAGGG)_n repeats. Indeed, pull-down experiments consistently uncover an enrichment of SYCP3 binding in repetitive DNA (Pearlman *et al.* 1992; Hernández-Hernández *et al.* 2008; Johnson *et al.* 2013), although direct evidence of preferential binding to (TTAGGG)_n repeats is currently lacking. These modified axial elements may seed the formation of atypical SYCP3 structures that link the sex chromosomes through the first meiotic division, as previously proposed (Page *et al.* 2006; de la Fuente *et al.* 2007, 2012). Although it is unclear what triggers these modifications, the ability of SYCP3 to self-polymerize could directly aid this dynamic restructuring of the chromosome axes at points of telomere attachment to the nuclear envelope (Yuan *et al.* 1998). If these axial element modifications are driven by primary DNA sequence—and not features specific to telomeres—interstitial (TTAGGG)_n repeat blocks could also possess a modified SC architecture capable of producing SYCP3 links between the asynaptic X and Y.

Despite the plausibility of this model, we observed no overt modification of the sex chromosome axes in early meiotic surface-spread spermatocytes from any of the three vole taxa examined in this investigation. However, we did not analyze cells from later meiotic stages, and the two-dimensional compression of cells in our spermatocyte spread assays obscured the three-dimensional expression of SYCP3. Future cytogenetic investigations that jointly analyze the meiotic dynamics of telomeric repeats and the axial elements in squashed specimens will be required to ascertain a possible relationship between (TTAGGG)_n repeats and SC modifications associated with achiasmate sex chromosome segregation.

Although further details of the mechanism of sex chromosome segregation in North American voles remain to be elucidated, the emergence of a novel X/Y meiotic program appears to have shaped key aspects of chromosome architecture and evolution in North American voles. Our combined genomic and cytogenetic analysis suggests erosion of X/Y sequence homology at unique sequences in species with asynaptic sex chromosome meiosis, and the conservation of sequence identity across a narrow PAR in a vole species with synaptic sex chromosome meiosis. In addition, the loss of sex chromosome synapsis is associated with increased accumulation of low-complexity sequences on the X and Y, presumably reflecting the loss of evolutionary constraint to maintain sequence homology. Taken together, our findings provide an initial case study into how the meiotic function of the PAR shapes sex chromosome evolution and underscore the critical need for evolutionary models that explicitly account for this important feature of sex chromosome biology (Blackmon and Brandvain 2017).

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