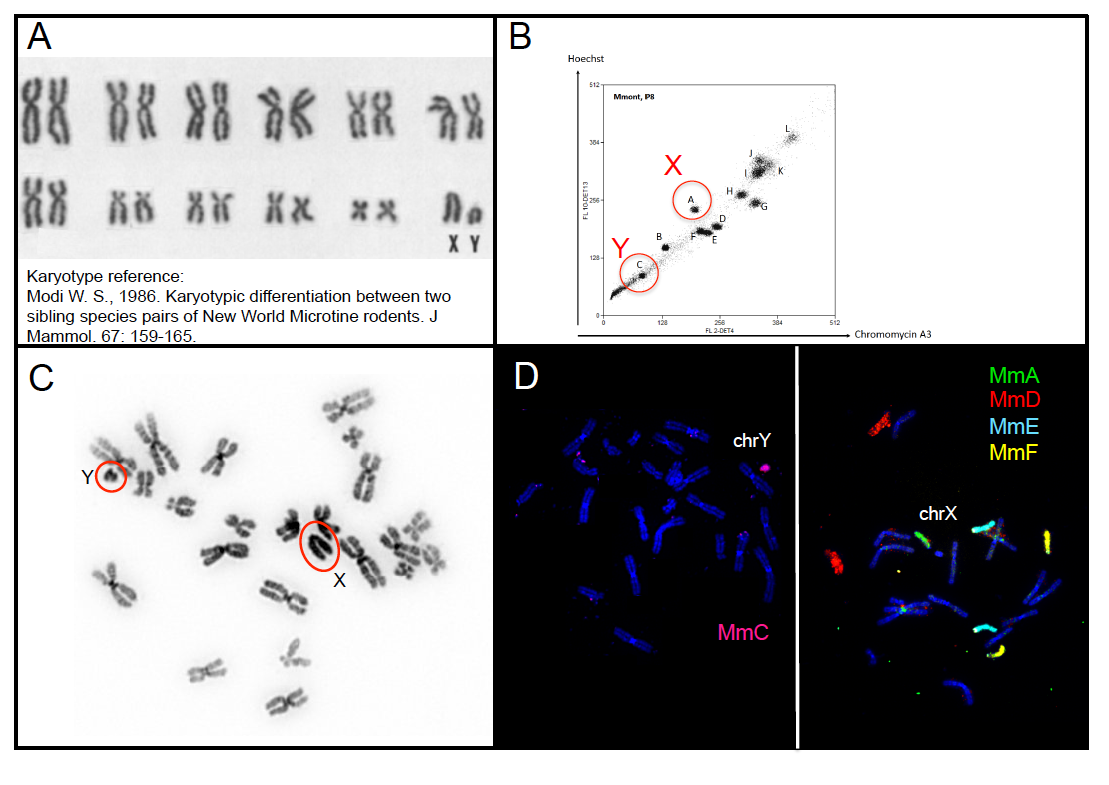
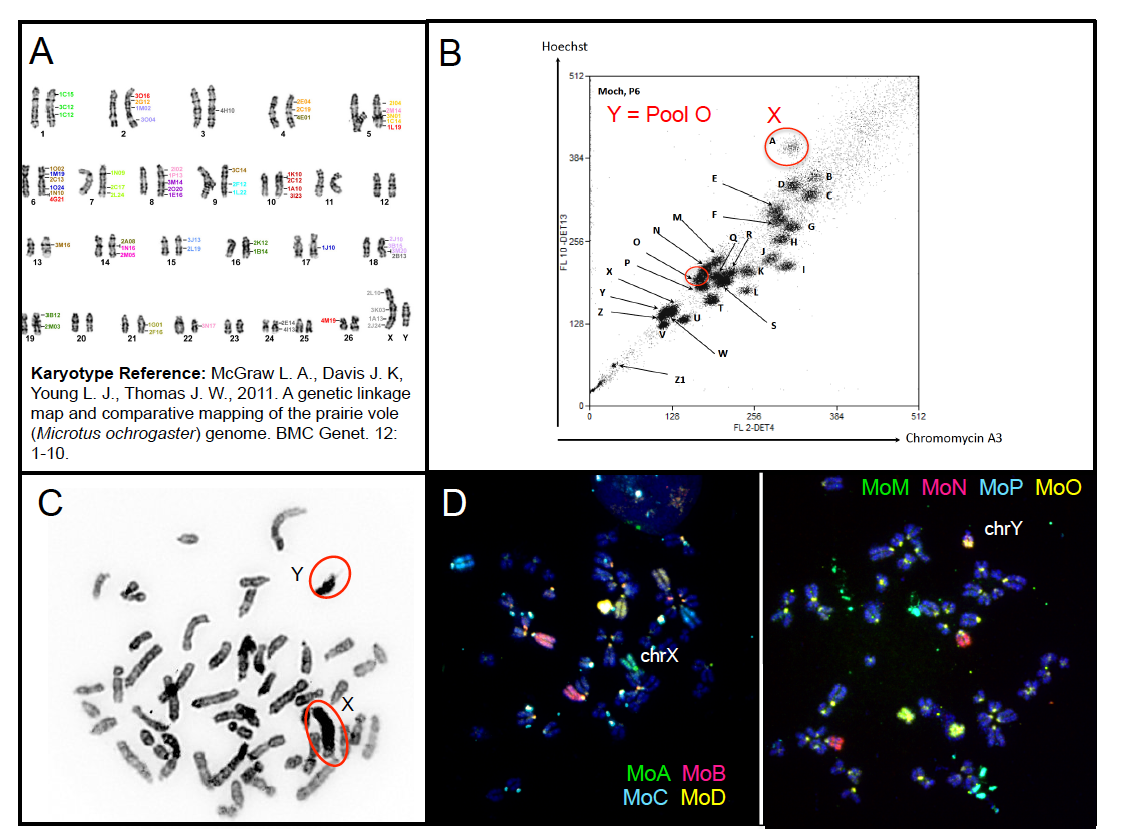
**Figure S1.**

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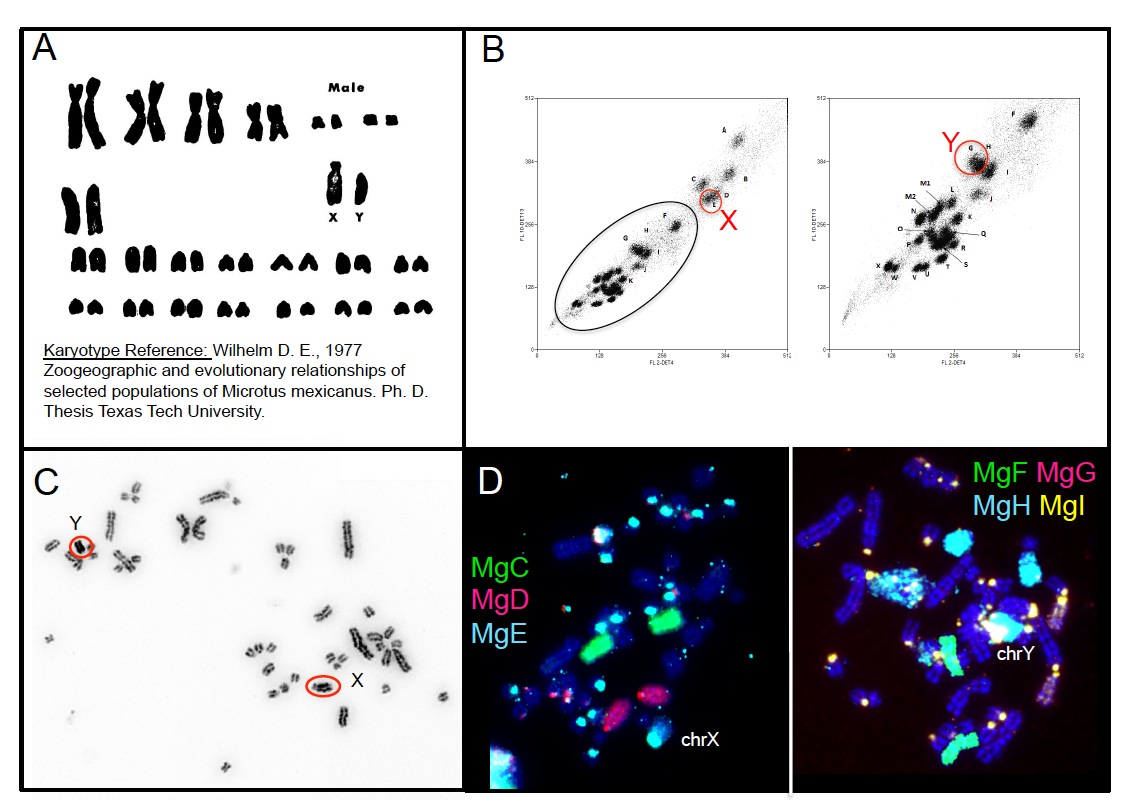
**Figure S1.** (A)Standard*M. montanus* male karyotype. (B) Bivariate male flow karyotype. Two size-matched autosomes appear to cluster together in pool I. (C) Inverted DAPI stained metaphase spread from *M. montanus*. The heterogametic X and Y sex chromosomesare labeled. (D) FISH with probes synthesized from candidate Y and X chromosome flow-sorted pools. Candidate pools were identified based on the size of the X and Y relative to other chromosomes in the *M. montanus* karyotype.

**Figure S2.**



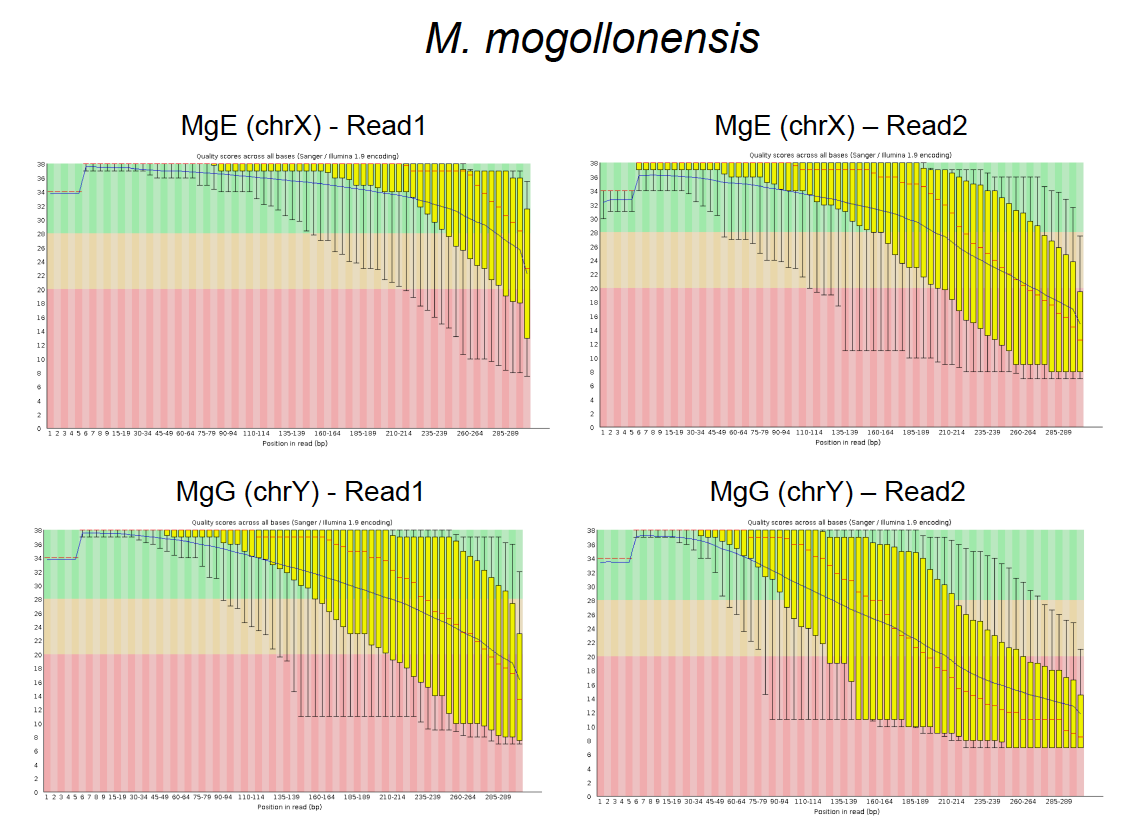
**Figure S2.** (A)Male*M. ochrogaster* G-banded karyotype. (B) Bivariate male flow karyotype. (C) Inverted DAPI stained metaphase spread from *M. ochrogaster*. The heterogametic X and Y sex chromosomesare labeled. (D) FISH with probes synthesized from candidate X and Y chromosome pools. Candidate pools were identified based on the size of the X and Y relative to other chromosomes in the karyotype. The high level of background fluorescence is likely attributable to the ineffectual blocking of repeats with mouse Cot-1 DNA, contamination between poorly resolved flow-sort pools (B), common repeat motifs shared across chromosomes, and cellular debris deposited on the slides.

**Figure S3.**

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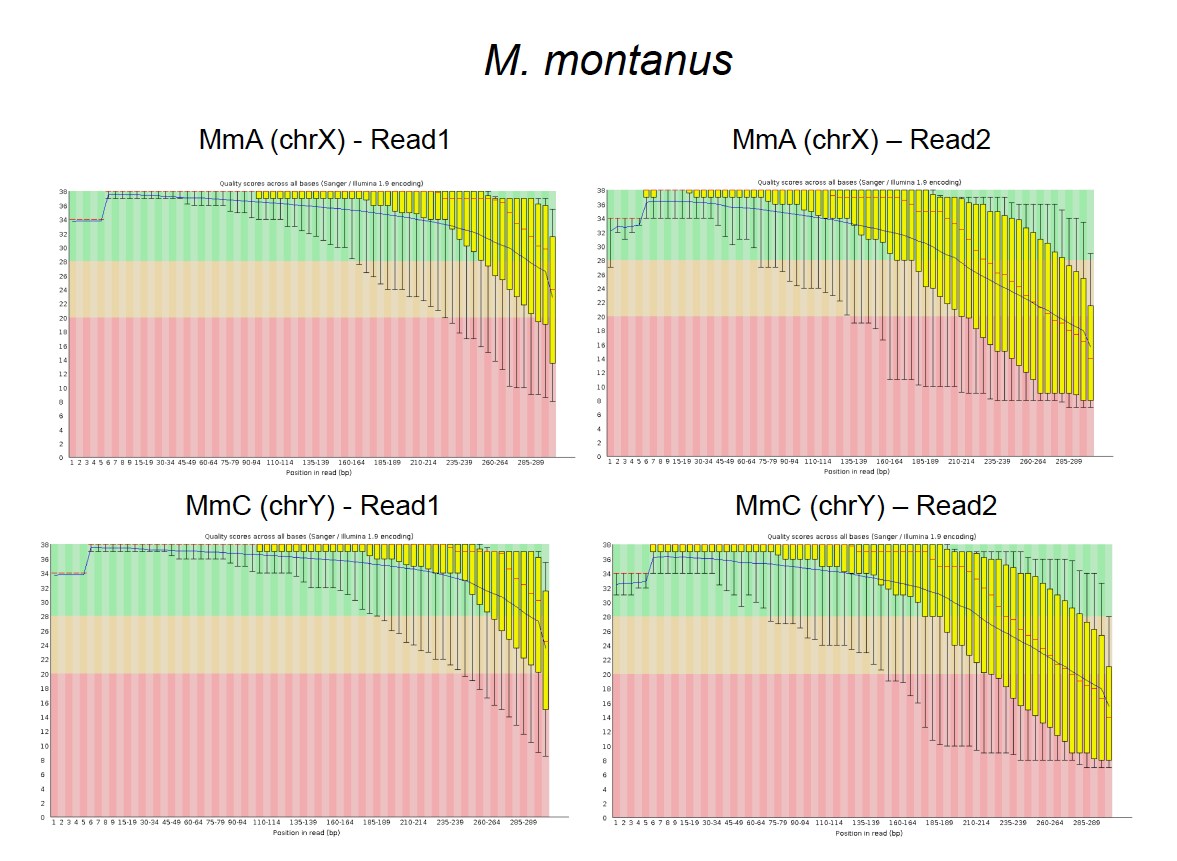
**Figure S3.** (A) Reference*M. mogollonensis* male karyotype. (B) Bivariate flow karyotype with X and Y chromosome flow-sorted pools labeled. The subset of pools separated with higher resolution (right panel) are circled in the left panel. (C) An inverted DAPI karyotype from the wild-caught animal used in this study features an identical chromosome number and fundamental number to the reference specimen in (A). (D) FISH with probes synthesized from candidate X and Y chromosome pools. Candidates were identified on the basis of size inferred from the karyotype. The high level of background fluorescence is likely attributable to the ineffectual blocking of repeats with mouse Cot-1 DNA, poor spreading of chromosomes, contamination between poorly resolved flow-sort pools (see panel B), common repeat motifs shared across chromosomes, and cellular debris deposited on the slides. Gold probes from MgI label 2 autosomes and intensely label the Y; signal from this probe set overwhelms fluorescence from the red MgG probes under the image capture settings used.

**Figure S4.**

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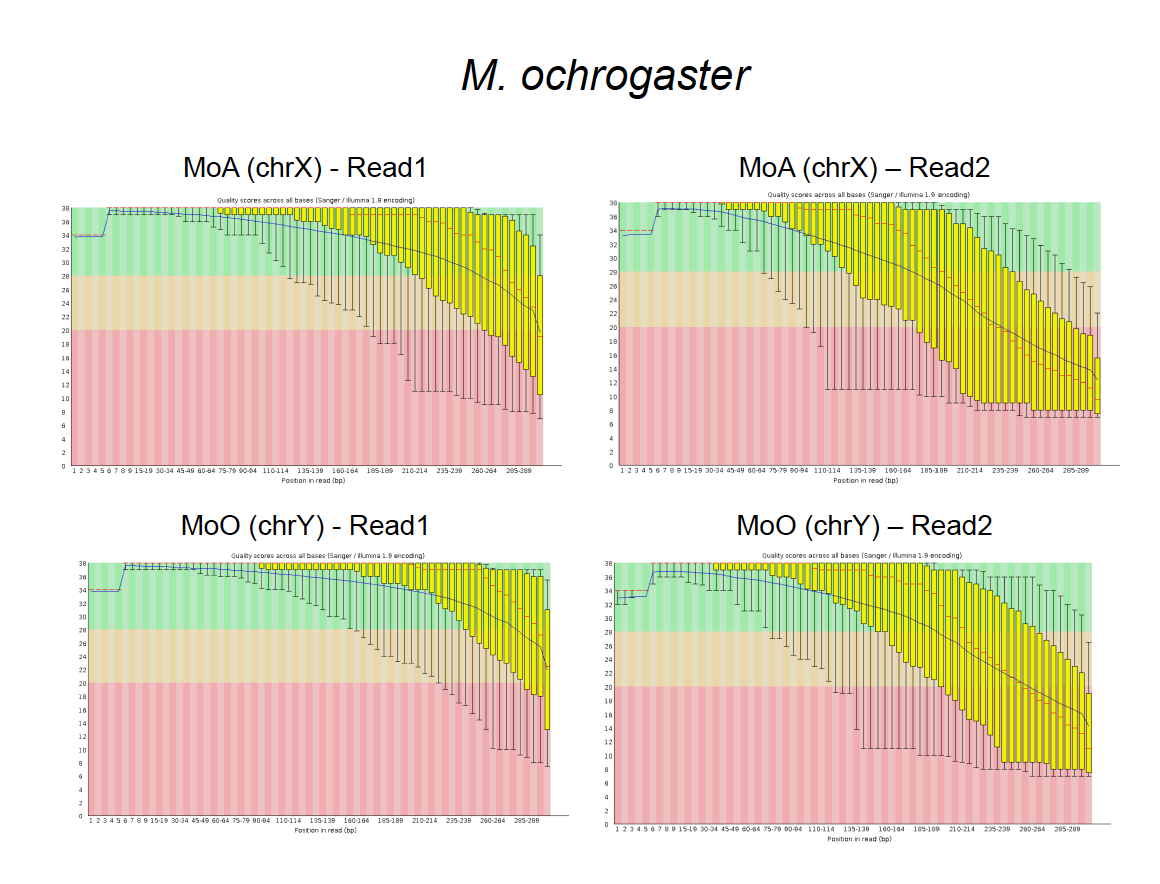
**Figure S4.** Distribution of base quality scores along reads from the *M. mogollonensis* chrX and chrY flow-sorted pools. Figures were generated using FastQC.

**Figure S5.**



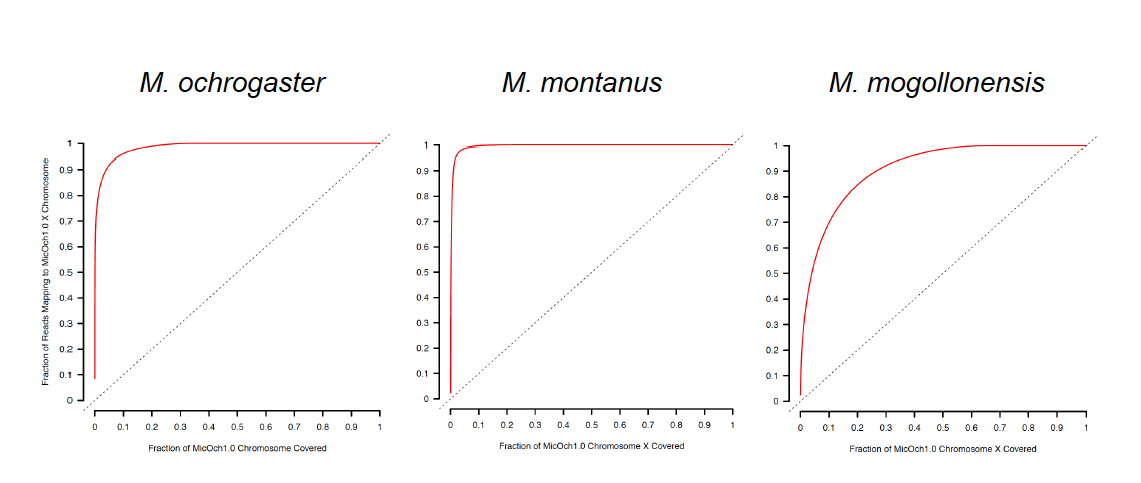
**Figure S5.** Distribution of base quality scores along reads from the *M. montanus* chrX and chrY flow-sorted pools. Figures were generated using FastQC.

**Figure S6.**

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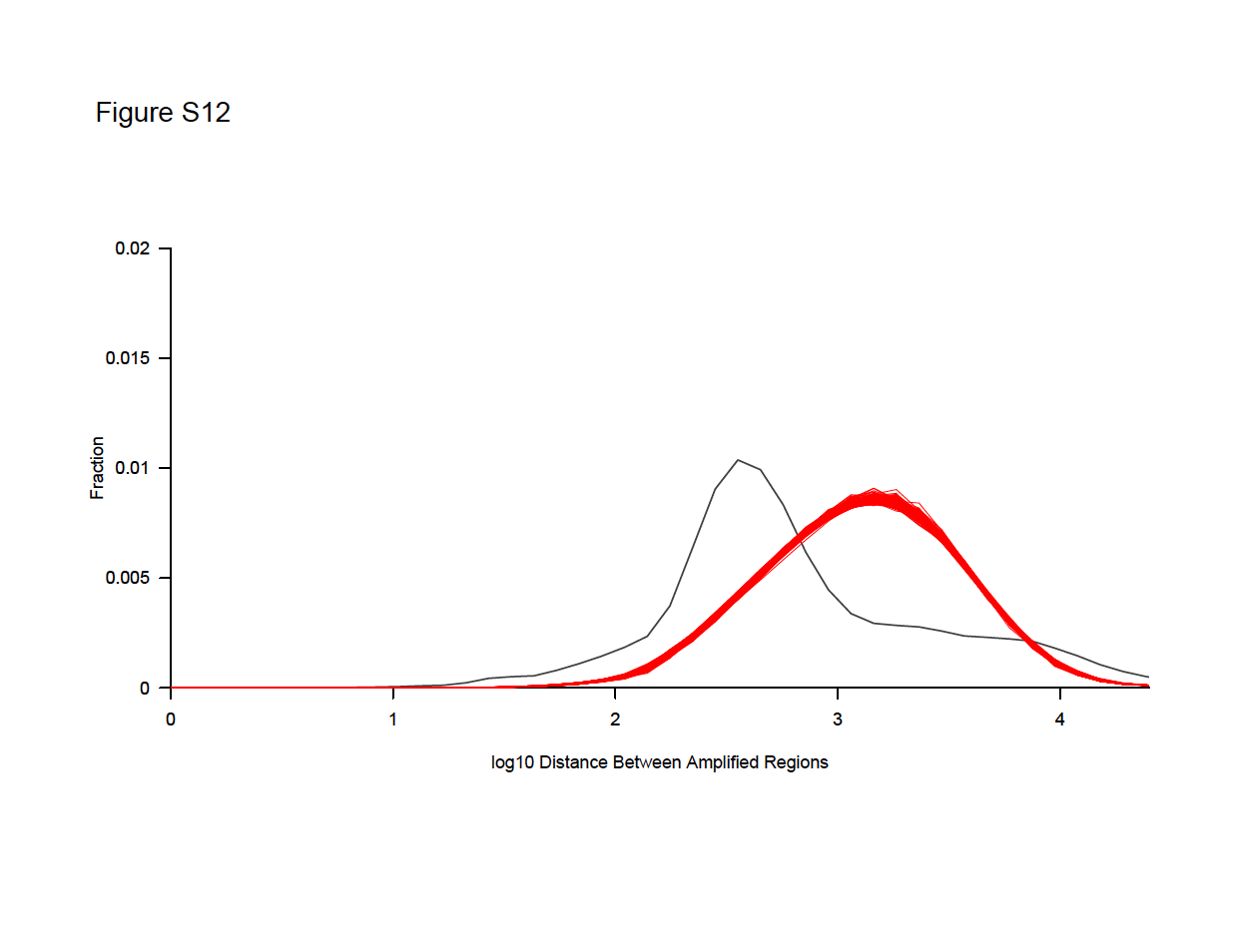
**Figure S6.** Distribution of base quality scores along reads from the *M. ochrogaster* chrX and chrY flow-sorted pools. Figures were generated using FastQC.

**Figure S7.**



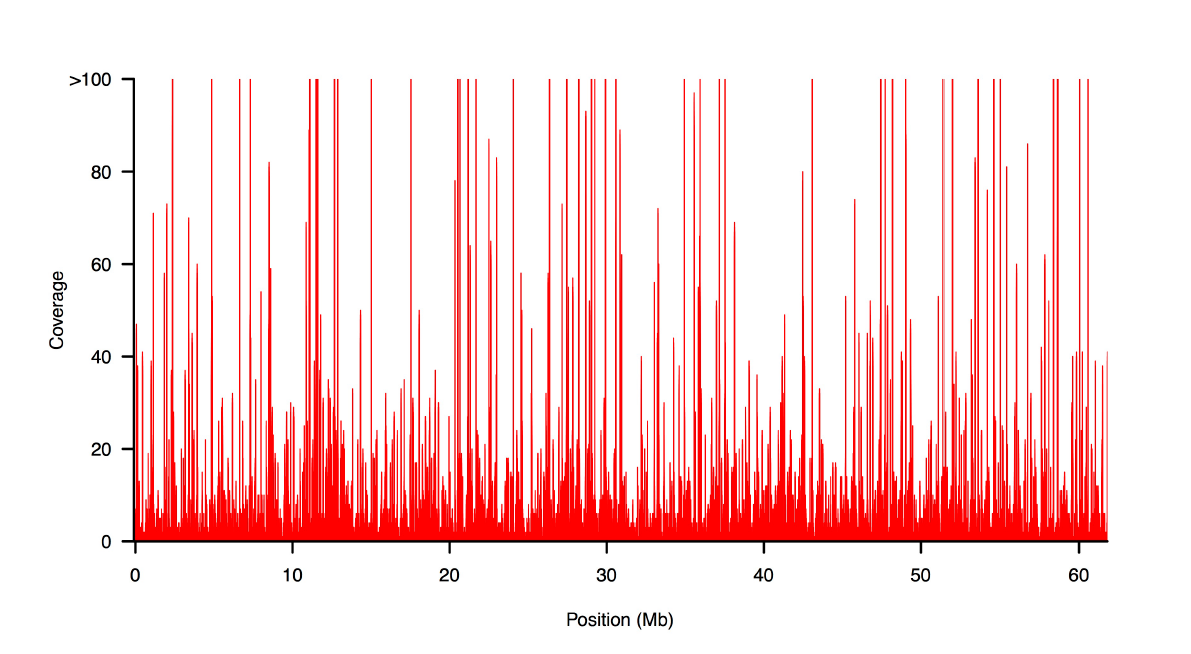
**Figure S7.** The fraction of reads from the *M. ochrogaster*, *M. montanus*, and *M. mogollonensis* chrX flow-sorted pools that map to the MicOch1.0 chrX reference assembly. For each species, the cumulative fraction of reads mapping to the X is plotted against the fraction of the chrX reference covered by at least one sequenced read.

**Figure S8.**

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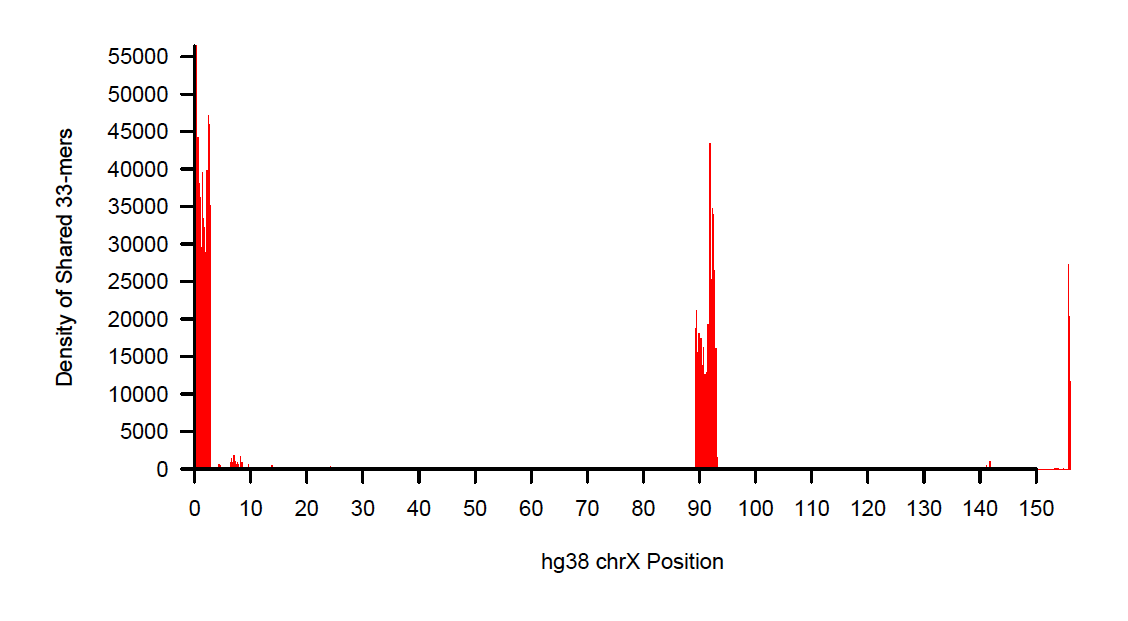
**Figure S8**. The distribution of distances between adjacent sequenced regions in the *M. ochrogaster* chromosome X flow-sorted amplified pool is shown in black. The expected distribution of distances based on 1000 random simulations is shown in red. Data were smoothed using a Loess smoothing parameter 0.1 for ease of visualization.

**Figure S9.**

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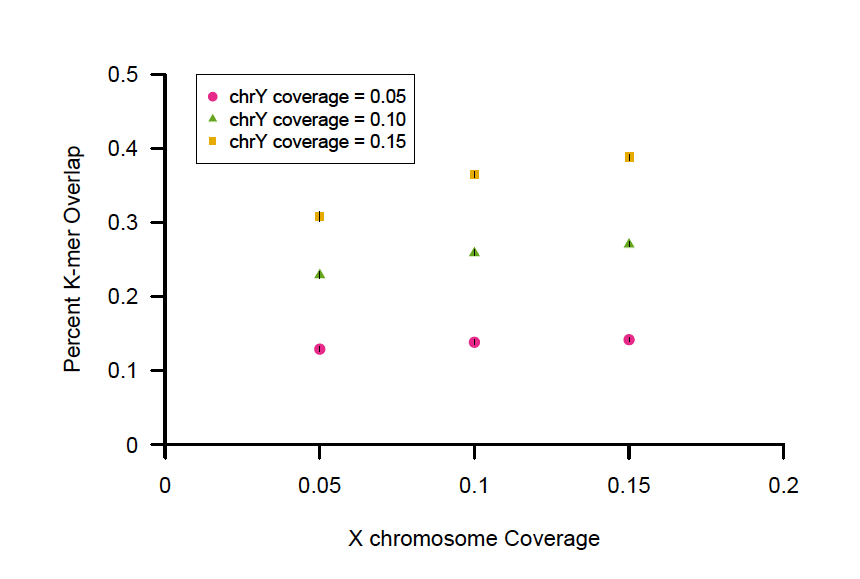
**Figure S9.** Distribution of read coverage from the *M. ochrogaster* chrX flow-sorted pool along the MicOch1.0 chrX assembly.

**Figure S10.**



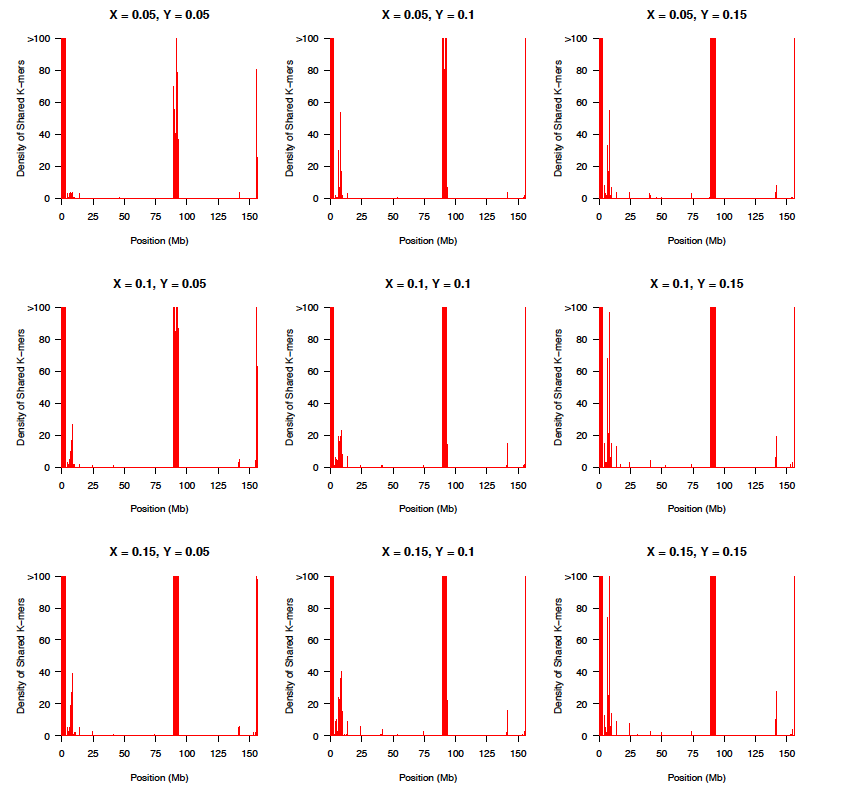
**Figure S10.** Shared X and Y chromosome 33-mers in the human reference assembly localize to the two distal human PARs and the interstitial X-transposed region.

**Figure S11.**

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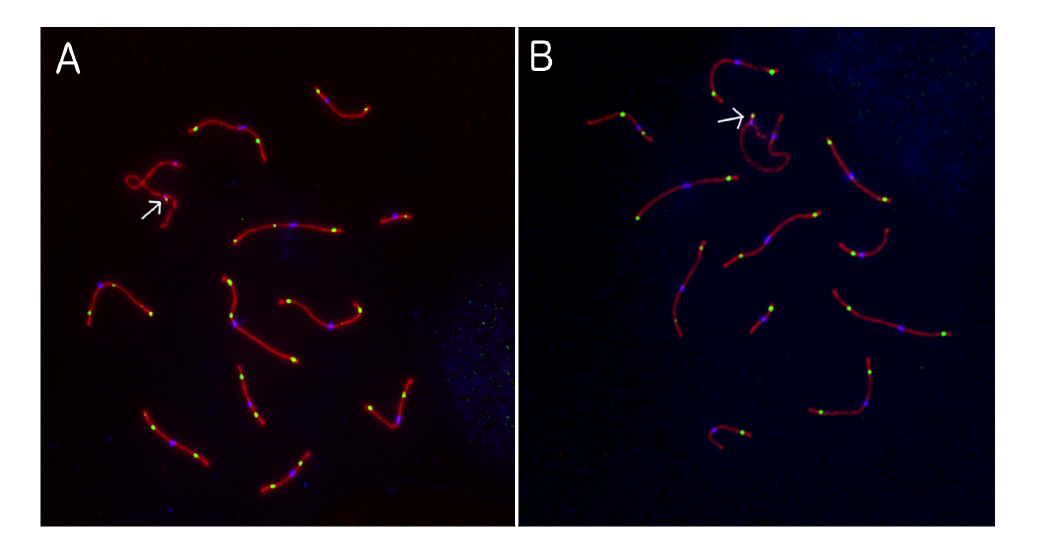
**Figure S11.** Down-sampling the number of X and Y chromosome 33-mers in humans yields levels of k-mer sharing that are proportional to the extent of sequence coverage. For example, randomly sampling 15% of X chromosomes 33-mers and 15% of Y chromosome 33-mers produces highest percent k-mer sharing. In contrast, randomly sampling 5% of X chromosomes 33-mers and 5% of Y chromosome 33-mers produces the lowest percent k-mer sharing, as expected.

**Figure S12.**

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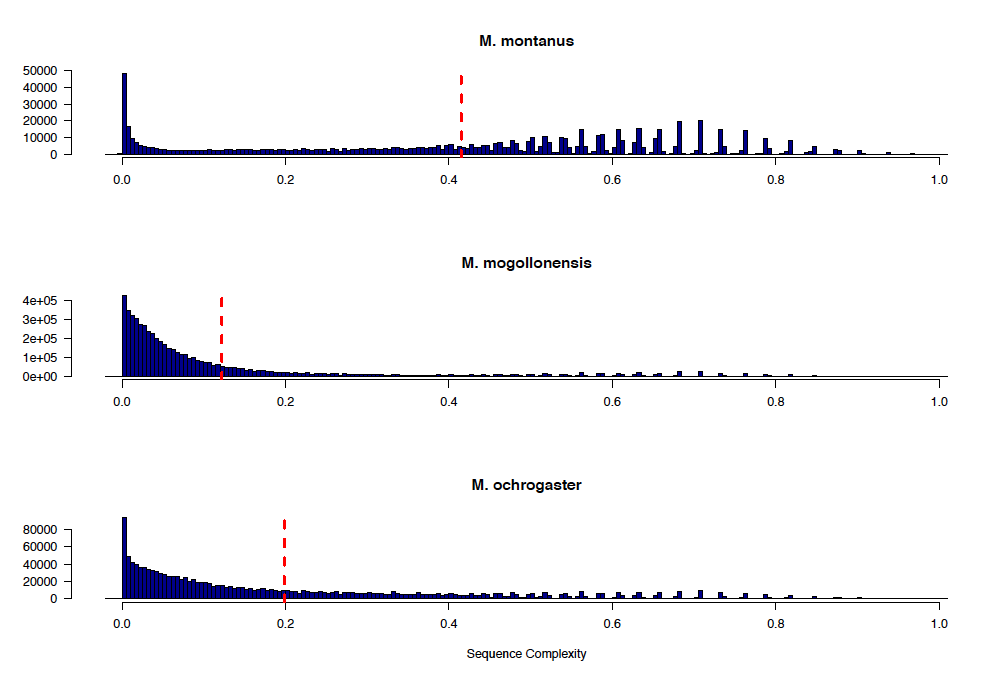
**Figure S12.** Randomly down-sampling X and Y 33-mers in humans by the specified fractions retains the expected distribution of shared k-mers across the human chrX.

**Figure S13.**



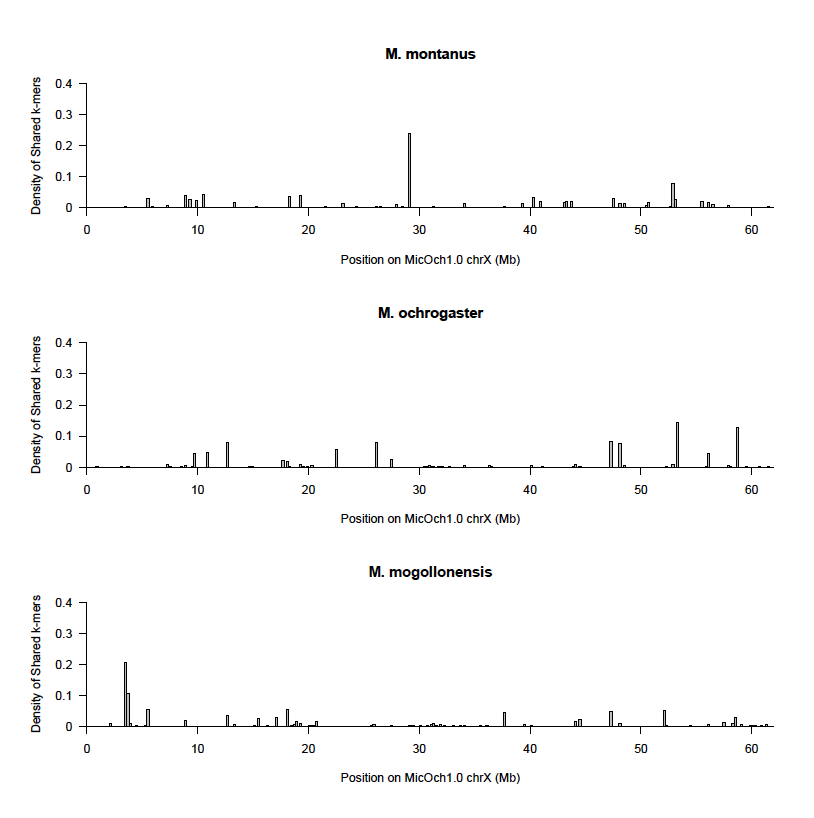
**Figure S13**. Crossing over on the X/Y synapsed region in *M. montanus*. Pachytene spermatocytes were immunostained with antibodies against SYCP3 (red), CREST (blue), and MLH1 (green), a mismatch repair protein that localizes to sites of crossing over on the mature meiotic axis. White arrows designate crossovers on the synapsed PAR.

**Figure S14.**

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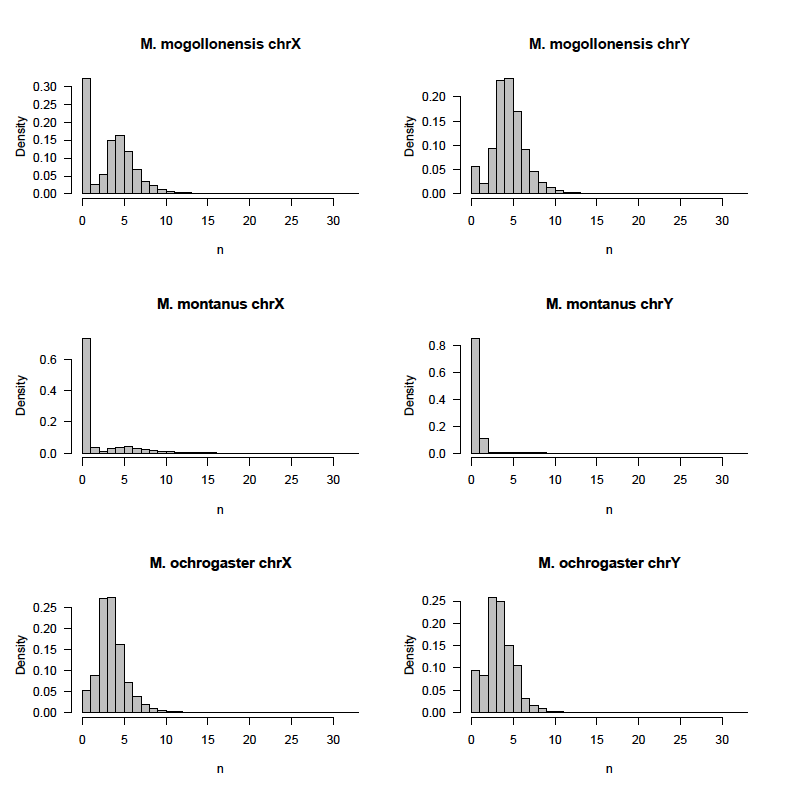
**Figure S14.** Histograms of sequence complexity scores for shared chrX and chrY 33-mers in *M. montanus*, *M. mogollonensis*, and *M. ochrogaster*. The mean of each distribution is denoted by a red dashed vertical line.

**Figure S15.**

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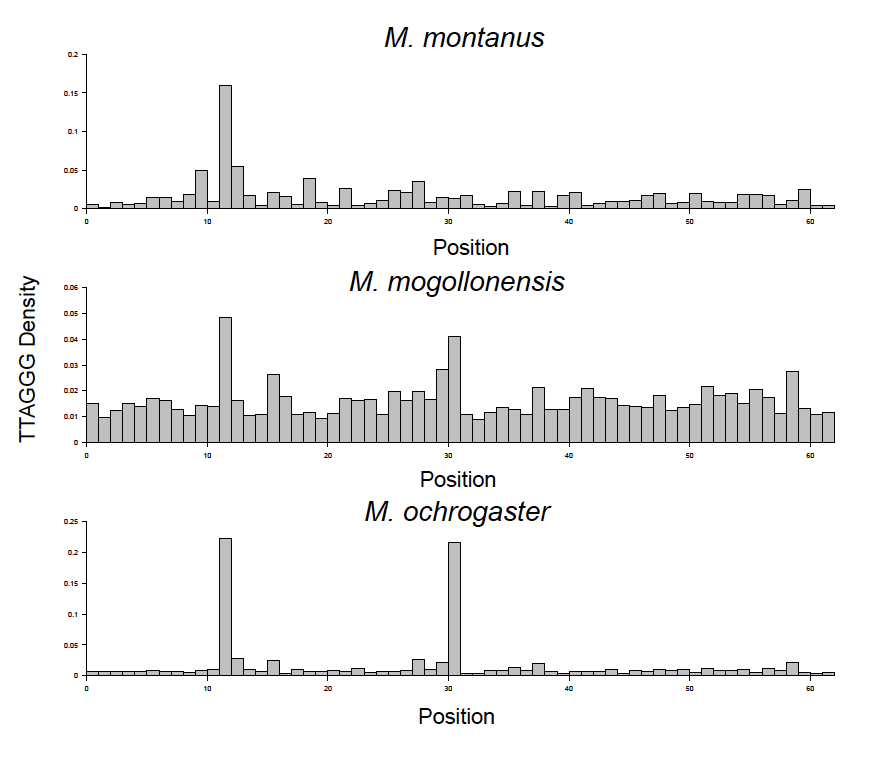
**Figure S15.** The density of shared X/Y 33-mers in 200kb windows is plotted along the MicOch1.0 chrX reference assembly. The observed number of shared k-mers in each bin was scaled by the total number of shared chrX and chrY k-mers. Unlike the comparable distribution of shared k-mers between the human X and Y chromosomes (Figures S14 and S16), shared k-mers are distributed along the full length of the chromosome, with no clear evidence for a PAR.

**Figure S16.**



**Figure S16.** Histograms depicting the number of consecutive TTAGGG hexamers in sequenced reads harboring at least one occurrence of the repeat. On both sex chromosomes from *M. montanus*, most instances of this motif are sporadic. In contrast, TTAGGG sequences are commonly arrayed in tandem on the *M. ochrogaster* sex chromosomes and the *M. mogollonensis* Y.

**Figure S17.**



**Figure S17.** The fraction of mapped reads containing at least one instance of the TTAGGG sequence motif is plotted in 20kb windows along the MicOch1.0 chrX reference assembly.