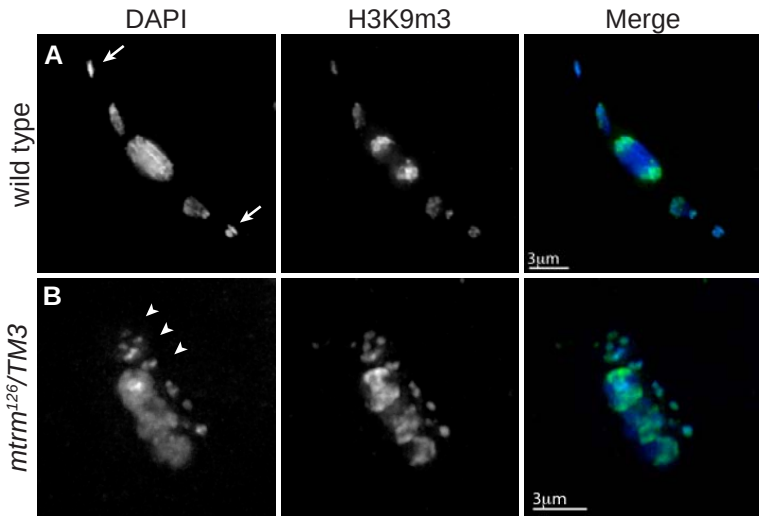

Figure S1 *B* chromosomes are composed of heterochromatin. Fixed oocytes were prepared with antibodies against H3K9m3 (green) and the DNA dye DAPI (blue). (A) Wild-type oocytes displaying the expected pericentric localization of heterochromatin. Arrows indicate the achiasmate 4^{th} chromosomes. (B) $mtrm^{126}/TM3$ oocyte displaying excess staining of the H3K9m3 antibody that co-localizes with the *B* chromosomes (arrowheads).

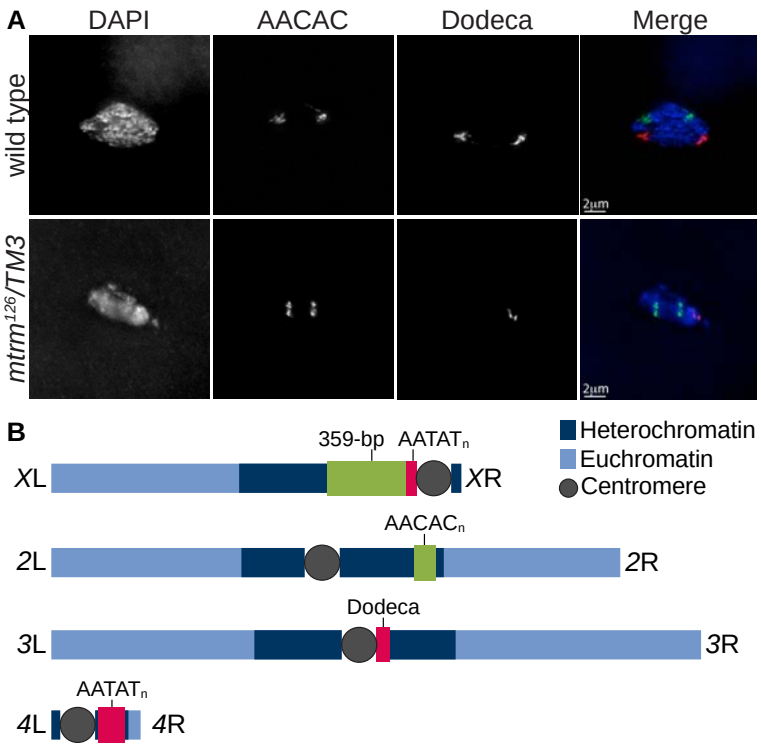

Figure S2 *B* chromosomes do not contain heterochromatic sequences from either the *2nd* or the *3rd* chromosomes. (A) Prometaphase I oocytes labeled with DAPI (blue) and probes to the AACAC heterochromatic repeat on the *2nd* chromosome (green) and to the Dodeca heterochromatic probe of the *3rd* chromosomes (red). Neither wild-type nor *mtrm126/TM3* oocytes show aberrant staining. (B) Schematic showing the location of the 359-bp probe on the *X* chromosome, the $AACAC_n$ probe on *2R*, the Dodeca probe on *3R*, and the $AATAT_n$ probe on the *4th* chromosome.

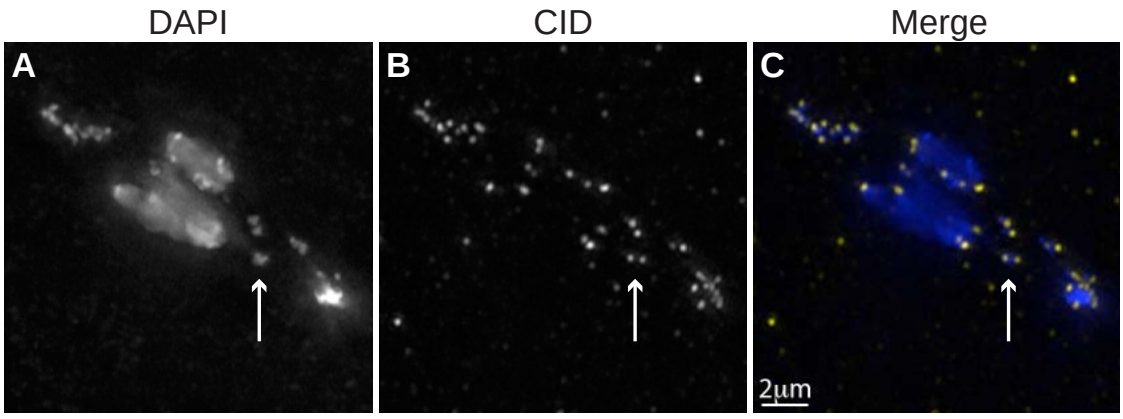

Figure S3 *B* chromosomes appear to contain 2 CID foci in an oocyte from a *mtrm*^{126}/*TM3* female. Arrow points to a *B* chromosome that appears to have CID foci on both ends of the chromosome. DAPI labels the DNA in blue and CID is labeled in yellow.

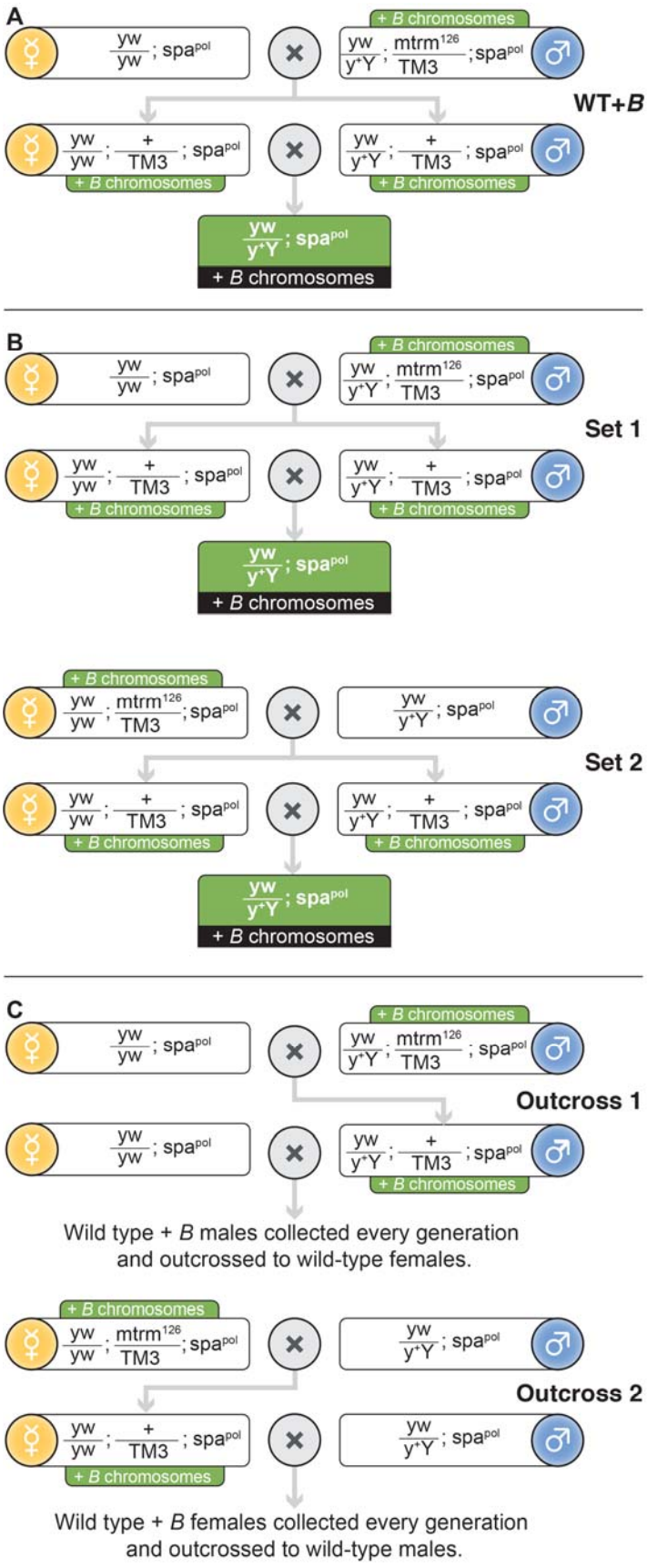
A
yw/yw ; spa^pol
+ B chromosomes
yw/y+Y ; mtrm126/TM3 ; spa^pol
WT+B
yw/yw ; +/TM3 ; spa^pol
+ B chromosomes
yw/y+Y ; +/TM3 ; spa^pol
+ B chromosomes
yw/y+Y ; spa^pol
+ B chromosomes
B
yw/yw ; spa^pol
+ B chromosomes
yw/y+Y ; mtrm126/TM3 ; spa^pol
Set 1
yw/yw ; +/TM3 ; spa^pol
+ B chromosomes
yw/y+Y ; +/TM3 ; spa^pol
+ B chromosomes
yw/y+Y ; spa^pol
+ B chromosomes
+ B chromosomes
yw/yw ; mtrm126/TM3 ; spa^pol
yw/y+Y ; spa^pol
Set 2
yw/yw ; +/TM3 ; spa^pol
+ B chromosomes
yw/y+Y ; +/TM3 ; spa^pol
+ B chromosomes
yw/y+Y ; spa^pol
+ B chromosomes
C
yw/yw ; spa^pol
+ B chromosomes
yw/y+Y ; mtrm126/TM3 ; spa^pol
Outcross 1
yw/yw ; spa^pol
yw/y+Y ; +/TM3 ; spa^pol
+ B chromosomes
Wild type + B males collected every generation
and outcrossed to wild-type females.
+ B chromosomes
yw/yw ; mtrm126/TM3 ; spa^pol
yw/y+Y ; spa^pol
Outcross 2
yw/yw ; +/TM3 ; spa^pol
+ B chromosomes
yw/y+Y ; spa^pol
Wild type + B females collected every generation
and outcrossed to wild-type males.

Figure S4 Crosses to introduce *B* chromosomes into a wild-type stock. (A) To analyze the *B* chromosomes in a wild-type genetic background by CID foci in ovaries, *mtrm*^{126}*/TM3* flies were crossed to either females or males from a wild-type stock. From this cross, their *TM3 Sb, Ser* progeny were collected and crossed to each other. The *TM3* balancer ensured the *mtrm*^{126} mutation was no longer present, and the balancer was lost from the stock after a few generations. From this cross a WT+*B* stock was created. (B) For the larval neuroblast experiments, male and female *mtrm*^{126}*/TM3* flies from the *B* chromosome-containing stock were crossed to either females or males from a wild-type stock. From this cross, their *TM3 Sb, Ser* progeny were collected and crossed to each other as described in (A). From these crosses, two stocks were created: a wild-type stock with paternally-derived *B* chromosomes (Set 1) and a wild-type stock with maternally-derived *B* chromosomes (Set 2). Each set of crosses for the neuroblast experiment was started twice. (C) Crosses were initiated as in (B) but then non-*mtrm*^{126} progeny were again crossed to wild-type flies and progeny were subsequently outcrossed to wild type for four additional generations. For each outcross set, the crosses were initiated four times and the data pooled.

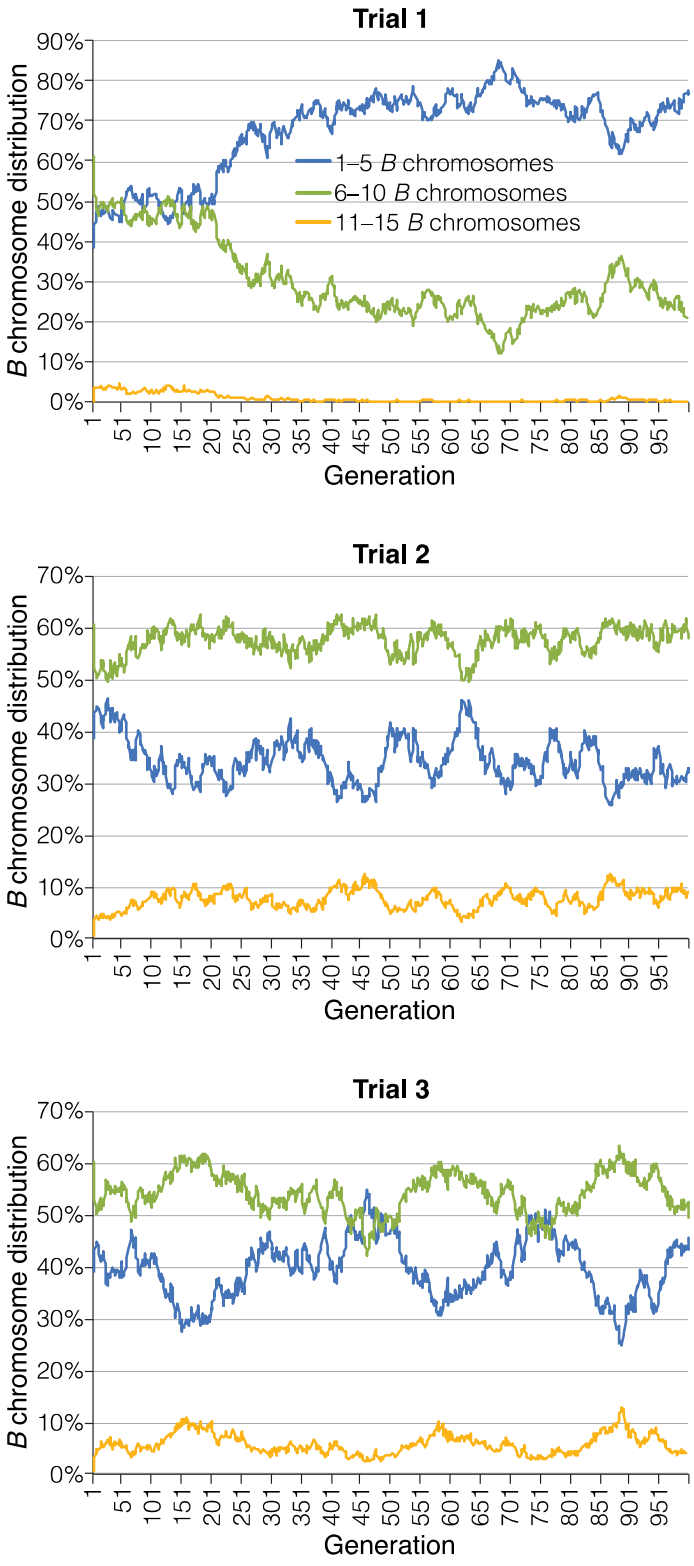

Figure S5 Simulation of 1,000 generations of sibling crosses. Three trials were performed beginning with 6 *B* chromosomes per parent and the number of *B* chromosomes at each generation was binned into groups of 1–5, 6–10, or 11–15 *B* chromosomes per progeny. 1,000 male and 1,000 female progeny were randomly selected for the next generation. These trials show that *B* chromosomes will persist in sibling crosses with random segregation over 1,000 generations.

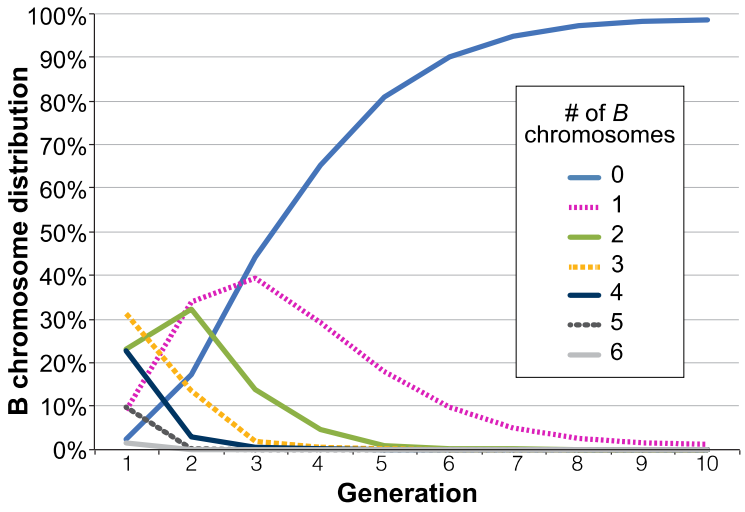

Figure S6 Simulation of 10 generations of outcrossing. Assuming 1,000 flies per generation, the number of *B* chromosomes quickly drops to zero for the majority of flies in the outcross model and *B* chromosomes are almost completely absent from the stock by generation 10.

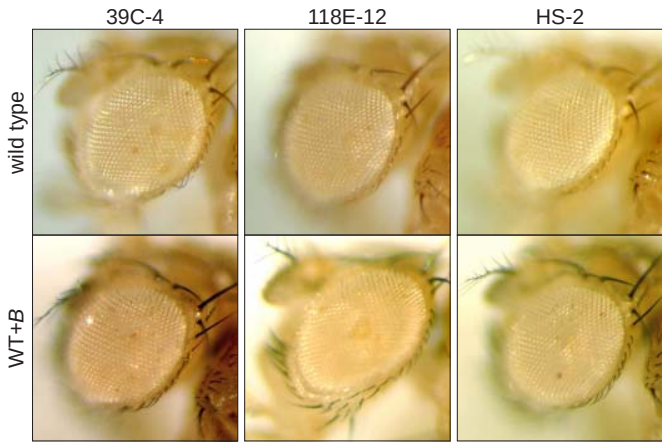

Figure S7 Some PEV reporters were unaffected by the presence of *B* chromosomes. Three insertion reporter lines with the w^+ gene inserted in the pericentric region of chromosomes *2L* (39C-4), *3R* (118E-12), and *3L* (HS-2) were not affected by the presence of *B* chromosomes. Females carrying the reporters were crossed to males of the indicated genotypes.

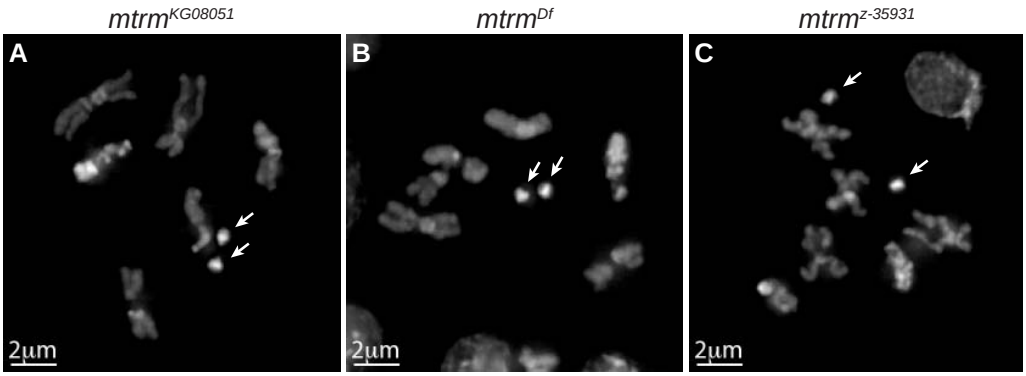

Figure S8 Other *mtrm* mutant stocks, including the progenitor of the *mtrm*^{126} stock, do not contain *B* chromosomes. (A) Larval neuroblast from the *mtrm*^{126} progenitor stock, (*y*^{1}***; PSUPor-Pexo70***^{KG08051} ***mtrm***^{KG08051} ***ry***^{506}***/TM3, Sb***^{1} ***Ser***^{1}). (B) Larval neuroblast of the y *w/y+; Df(3L)66CT2-T10/TM3, Sb, Ser; spa*^{pol} deficiency stock uncovering *mtrm.* (C) Larval neuroblast of a *mtrm* mutation discovered in the Zuker collection (*mtrm*$^{z-35931}$*/TM6B*).

Files S1-S2

Available for download as AVI files at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.160556/-/DC1.

File S1 Multiple *B* chromosomes are observed in a living oocyte from a *mtrm^{126}/TM3* mother. In a living prometaphase I oocyte from a *mtrm^{126}/TM3* mother, multiple B chromosomes can be observed moving dynamically on the meiotic spindle in a fashion similar to the *4th* chromosomes. DNA is labeled in green and α-tubulin is labeled in red. Movie is displayed at 5 frames per second.

File S2 Multiple *B* chromosomes are observed in a living oocyte from a *mtrm^{126}/TM3* mother. This is the same movie as File S1 but with only the DNA channel shown in black and white for easier viewing of the *B* chromosomes. Movie is displayed at 5 frames per second.

Files S3-S4

Available for download as Word files at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.160556/-/DC1.

File S3 Script modeling the *B* chromosome sibling cross experiment. This Perl script begins with 6 *B* chromosomes each for 1,000 males and 1,000 females. These flies are mated to one another, producing between 0 and 20 progeny. The progeny are created by randomly moving *B* chromosomes to one of two poles of a gamete and then randomly selecting a pole to continue to the next generation. More than 1,000 males and 1,000 females are created in each generation, but only 1,000 of each are randomly selected to survive and reproduce in the next generation.

File S4 Script modeling the *B* chromosome outcross experiment. This Perl script begins with 1,000 females with 6 *B* chromosomes each. These females are crossed to non-*B* chromosome-carrying males, producing an oocyte with a random number of *B* chromosomes between 0 and 6. The progeny then continue to the next generation and produce offspring containing some random number of *B* chromosomes between 0 and the number contributed in the previous generation. This continues for 10 generations.

Table S1 *X* and *4th* chromosome nondisjunction in females with achiasmate *X*s

	Wild type	**WT+*B***
Total progeny	1002	909
Adjusted total	**1025**	**1640**
% diplo-*X*	0.4%	2.4%
% nullo-*X*	0.4%	2.2%
Total % *X* NDJ	**0.8%**	**4.6%**
% diplo-*4*	0.9%	21.8%
% nullo-*4*	0.8%	7.8%
Total % *4th* NDJ	**1.7%**	**29.6%**

FM7w/y w; spa^{pol} females with and without *B* chromosomes were tested for *X* and *4th* chromosome NDJ.