

Supplemental figures

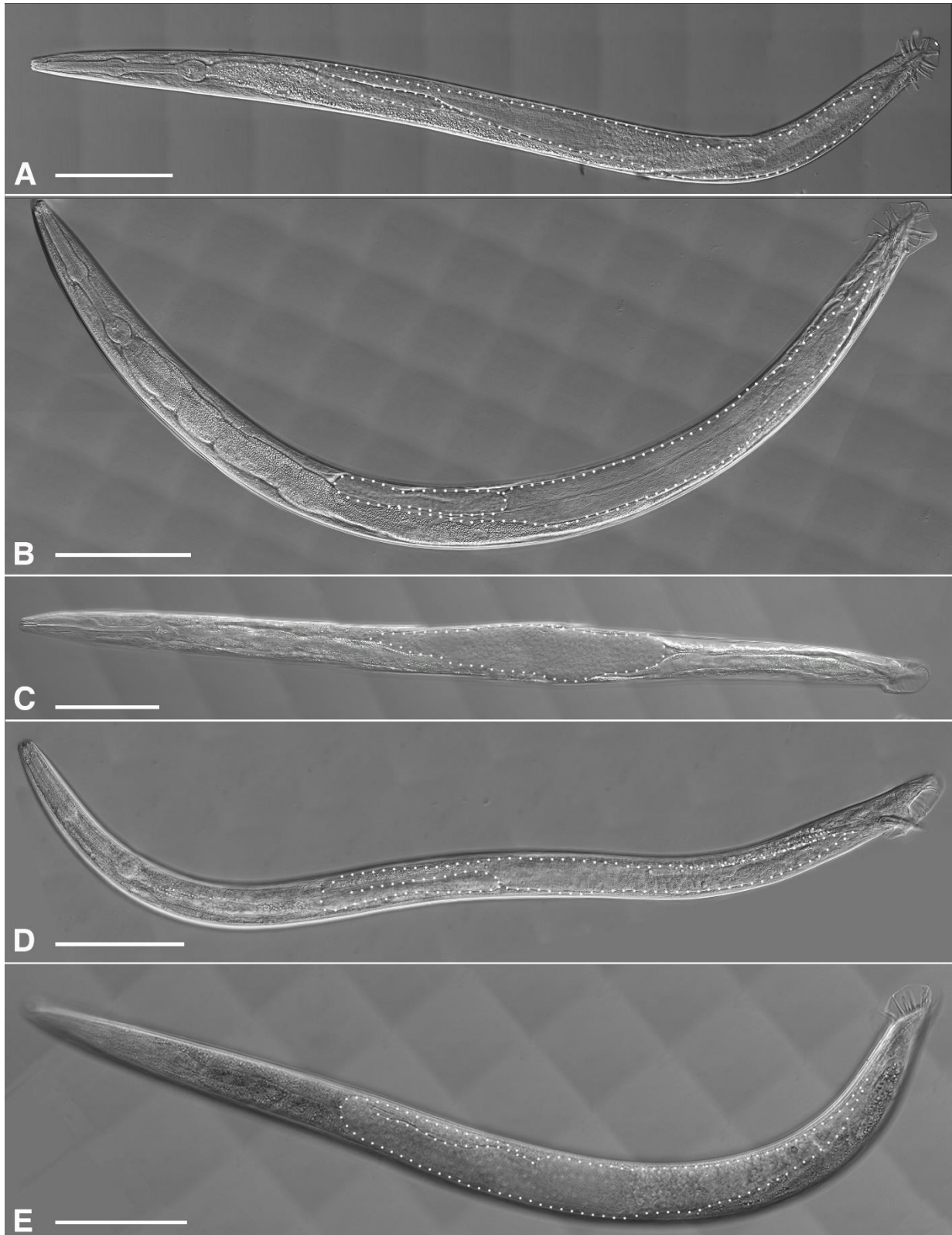


Fig S1. Morphology of males with various genetic background. Shown are micrographs of males from *C. briggsae* (A), *C. nigoni* (B), hybrid F1(b, n) sterile male (C), hybrid F1 fertile F1(b, ir) males carrying an introgression, *zzyIR10330* (D) or *zzyIR10307* (E). Gonads are highlighted in dashed lines. Scale bar: 100µm. Gonads are highlighted with dashed white lines.

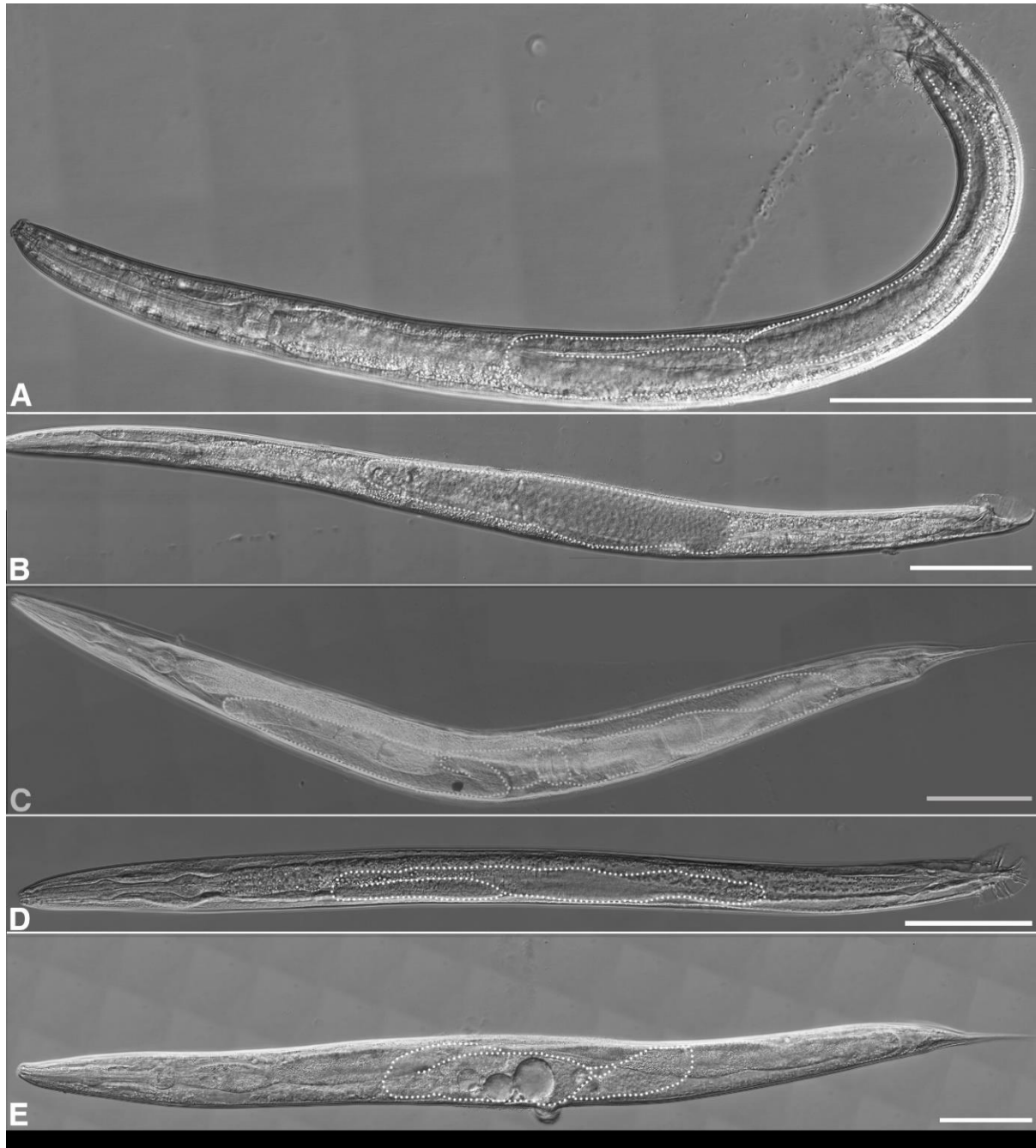


Fig S2. Morphology of hybrid B2(F1 backcross) progeny derived from crossing between the rescued hybrid F1 fertile male with *C. nigoni* female or *C. briggsae* hermaphrodite. Shown are micrographs of progeny from backcrossing the rescued fertile F1 male with maternal *C. nigoni* and *C. briggsae*. (A-C) Hybrid B2 progeny derived from the crossing between the rescued hybrid F1 and *C. nigoni* L4 female. Male and female progeny are shown in (A-B) and (C), respectively. Gonad morphology (highlighted in dash line) is comparable to that of wild-type animal (only one gonadal arm is found) in (A) but not in (B). A fertile B2 female is shown in (C). (D-E) Sterile B2 male (D) and sterile female (E) from the crossing between fertile F1(b,10330) male and sperm-

depleted *C. briggsae* hermaphrodite, respectively. Gonads are highlighted in dash line. Scale bar: 100µm.

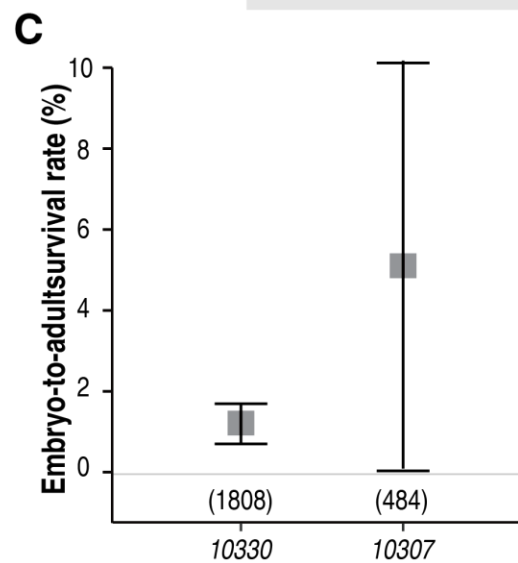
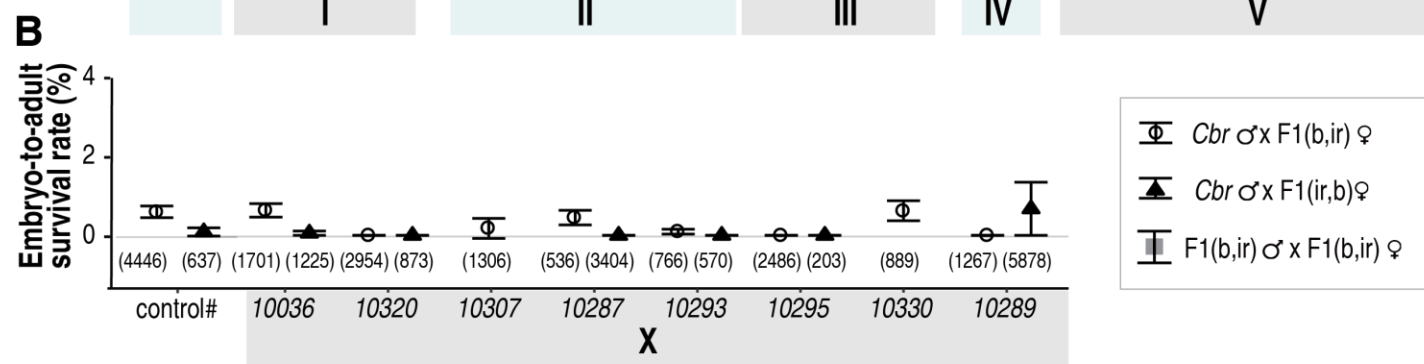
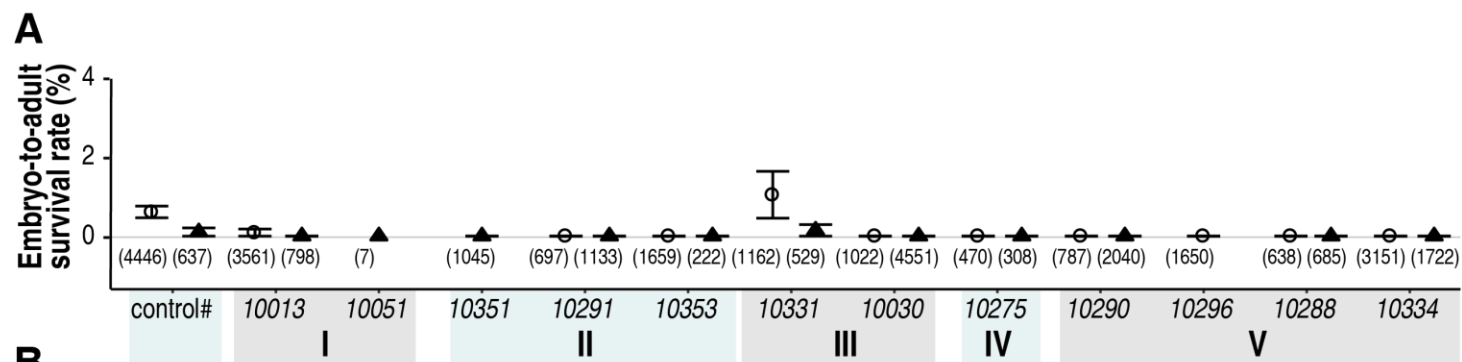


Fig S3. Quantification of survival rate of crossing B2 progeny between the *C. briggsae* male and the female of F1 (b, ir) or F1 (ir, b). (A-B) A and B show introgressions on the autosome and X Chromosome, respectively. Control for F1(b, ir) and F1(ir, B) are F1(b, n) and F1(n,b), respectively. Survival rate (%) is shown on the y axis and introgression lines on the x axis. Introgressions are chosen so that they cover the maximum part of individual linkage group shown at the bottom. Note few B2 progeny derived from the crossing can survive. (C) Embryo to adult survival rate for the progeny from reciprocal crosses between the fertile F1(b, ir) males and females.

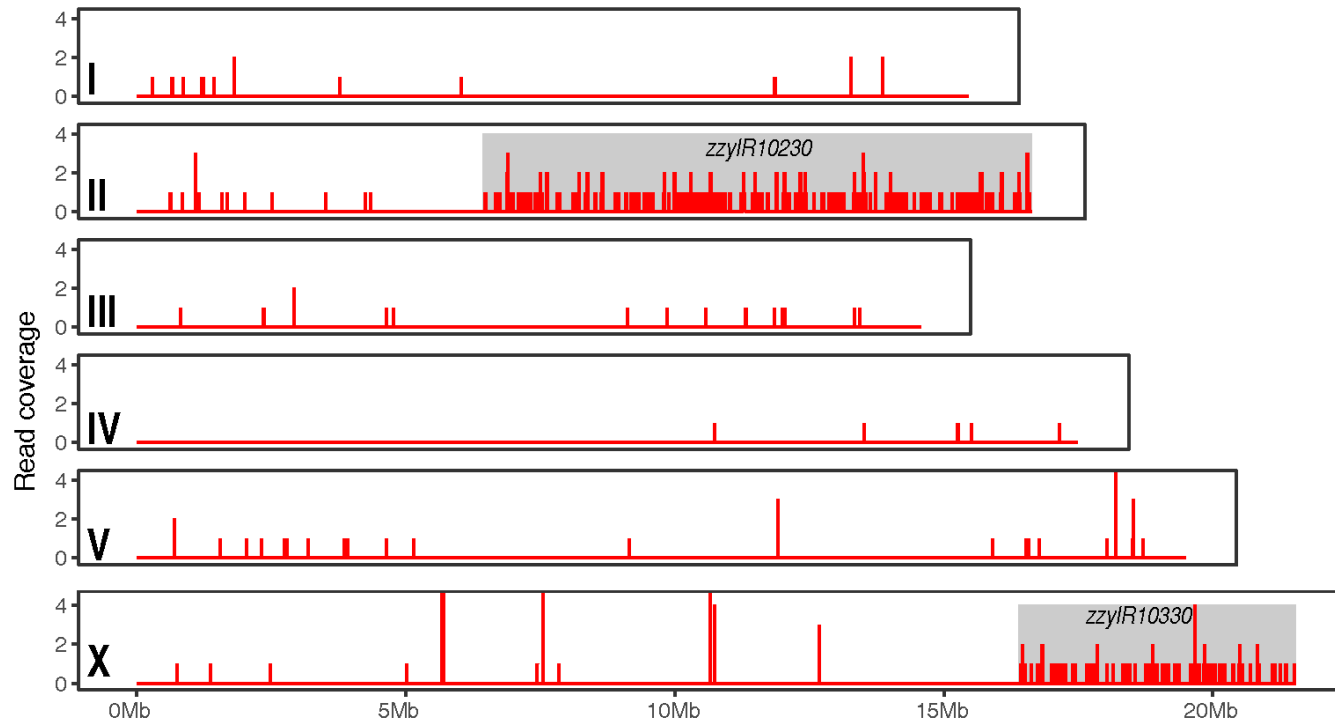


Fig S4. Determining the boundaries of co-segregating fragment that rescues the male sterility produced by zzyIR10330 in *C. nigoni* using NGS. Shown are read coverages (y axis) against the coordinates (x axis) of *C. briggsae* genome. Note that only the read coverages on the right arm of chr-II and chr-X are substantially higher than the rest of the genome, indicating the co-segregating chromosome II arm with introgression zzyIR10330 in *C. nigoni* is essential for its male fertility.

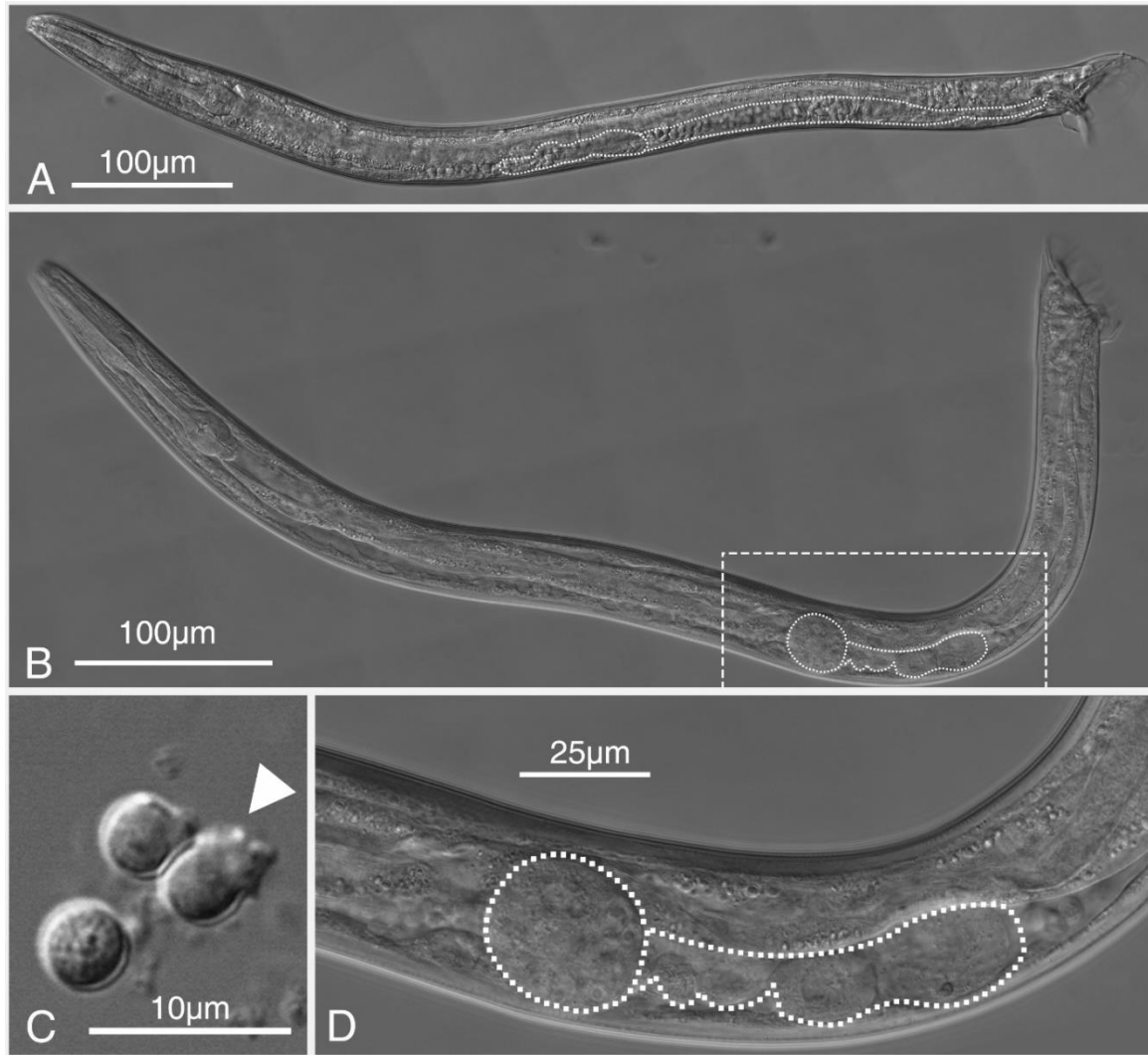


Fig S5. Morphology of hybrid F1 sterile males from crossing between introgression-bearing *C. nigoni* male and *C. briggsae* hermaphrodite. Note that F1 (n, b) males are inviable. (A) Sterile F1(10353, b) male. (B) Sterile F1(10275, b) male. (C) Spontaneous sperm activation in F1(10353, b) male (indicated with white triangle). (D) A magnified view of the malformed gonad in F1(10275, b) male.

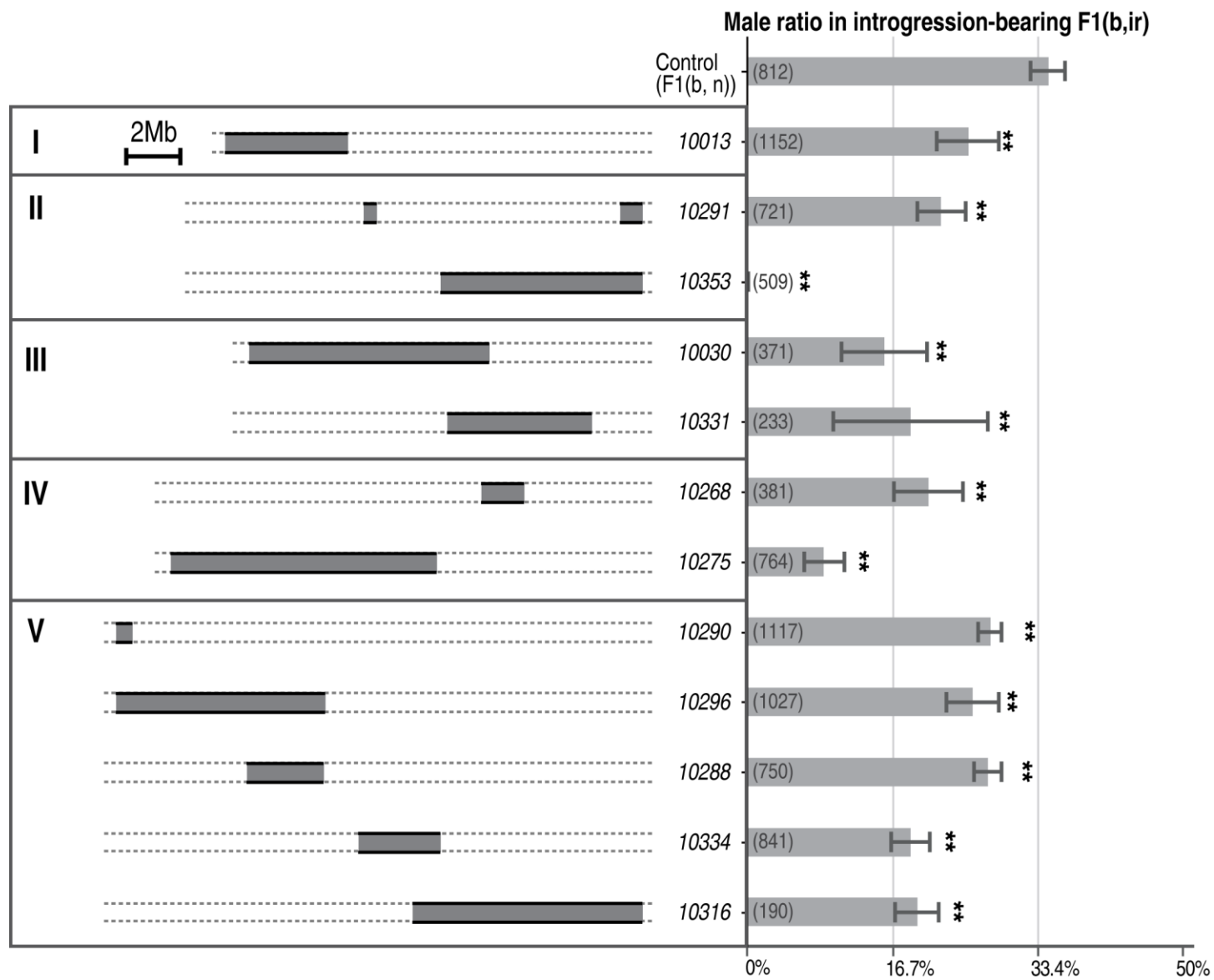


Fig S6. Male ratio in introgression-bearing F1(b, ir) hybrids. Shown are the percentages of males carrying an autosome-linked introgression out of all F1(b, ir) progeny carrying the same introgression. Those with a significant deviation from the control, i.e., F1 (b, n), are indicated with ** ($p < 0.01$, student's t test). Introgressions are shown on the left with name and linkage group indicated as in Fig. 2A. The introgression that kills all hybrid F1(b, ir) progeny (Fig. 5A) is not scored for the male ratio. Note that all autosome-linked introgressions show a significant decrease in male ratio compared with control group, suggesting a detrimental effect of homozygosity of autosomal introgression on hybrid F1 male viability.