Supplementary Material

Figure S1. Picture of the plastic bottle device.

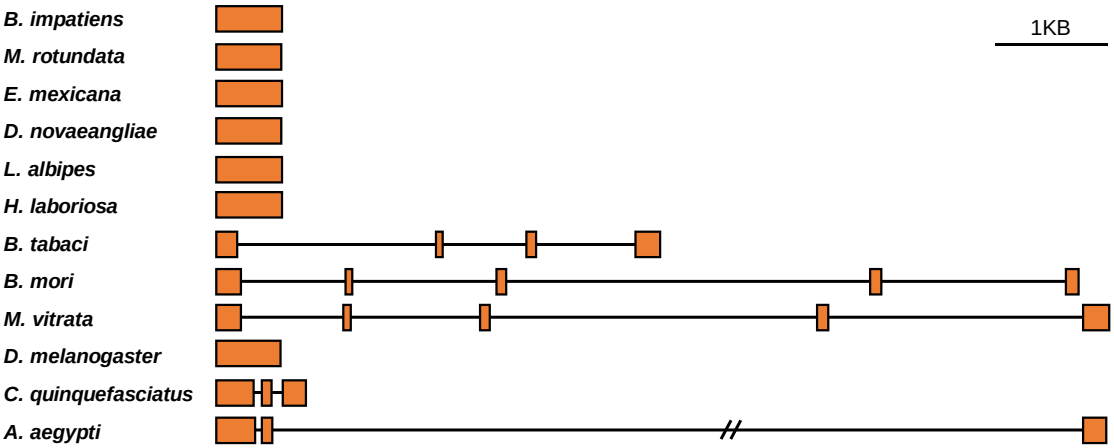

Figure S2. Genomic structure of the *ix* gene for several insects.

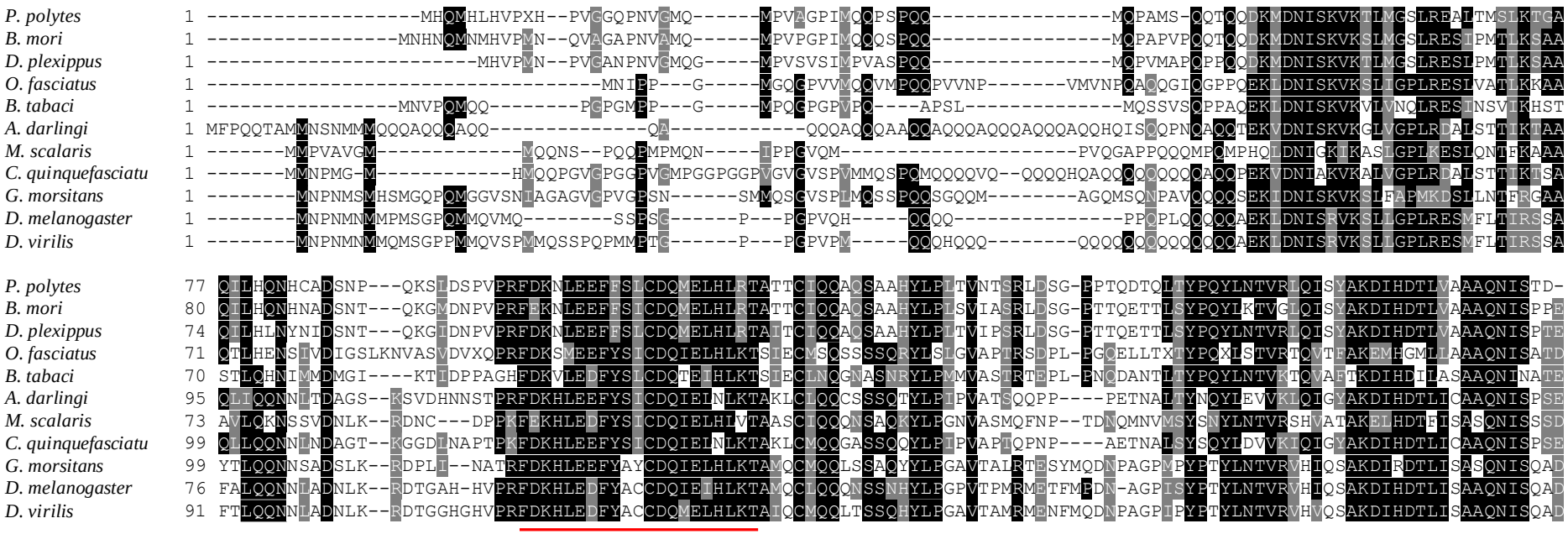

Figure S3. Amino acid alignment of intersex IX homologues. Conserved and similar amino acid residues are indicated as white letters on a black background and white letters on a dark grey background, respectively. Non-conserved residues are indicated as black letters on a white background. The DSX^F binding region is underlined.

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BtIX^C1  MNVPQMQQPGPGMPPGMPQGPGPVPQAPSLMQSSVSQPPAQEKLDNISKVKVLVNQLRESI
BtIX^F   MNVPQ.....APSLMQSSVSQPPAQEKLDNISKVKVLVNQLRESI
BtIX^C2  ..................................................................
BtIX^M   MNVPQMQQPGPGMPPGMPQGPGPVPQAPSLMQSSVSQPPAQ.....
                                                   Med29
BtIX^C1  NSVIKHSTSTLQHNIMMDMGIKTIDPPAGHFDKVLEDFYSLCDQTEIHLKTSIECLNQGN
BtIX^F   NSVIKHSTSTLQHNIMMDMGIKTIDPPAGHFDKVLEDFYSLCDQTEIHLKTSIECLNQGN
BtIX^C2  ..............MMDMGIKTIDPPAGHFDKVLEDFYSLCDQTEIHLKTSIECLNQGN
BtIX^M   ..................................................................

BtIX^C1  ASNRYLPMMVASTRTEPLPNQDANTLTYPQYLNTVKTQVAFTKDIHDILASAAQNINATE
BtIX^F   ASNRYLPMMVASTRTEPLPNQDANTLTYPQYLNTVKTQVAFTKDIHDILASAAQNINATE
BtIX^C2  ASNRYLPMMVASTRTEPLPNQDANTLTYPQYLNTVKTQVAFTKDIHDILASAAQNINATE
BtIX^M   ..........................................KTFMTSLRLLHKILMLLSEP
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Figure S4. Amino acid alignments of predicted proteins coded by four *ix* transcripts. The conserved domain (Med29) of IX is highlighted in yellow.

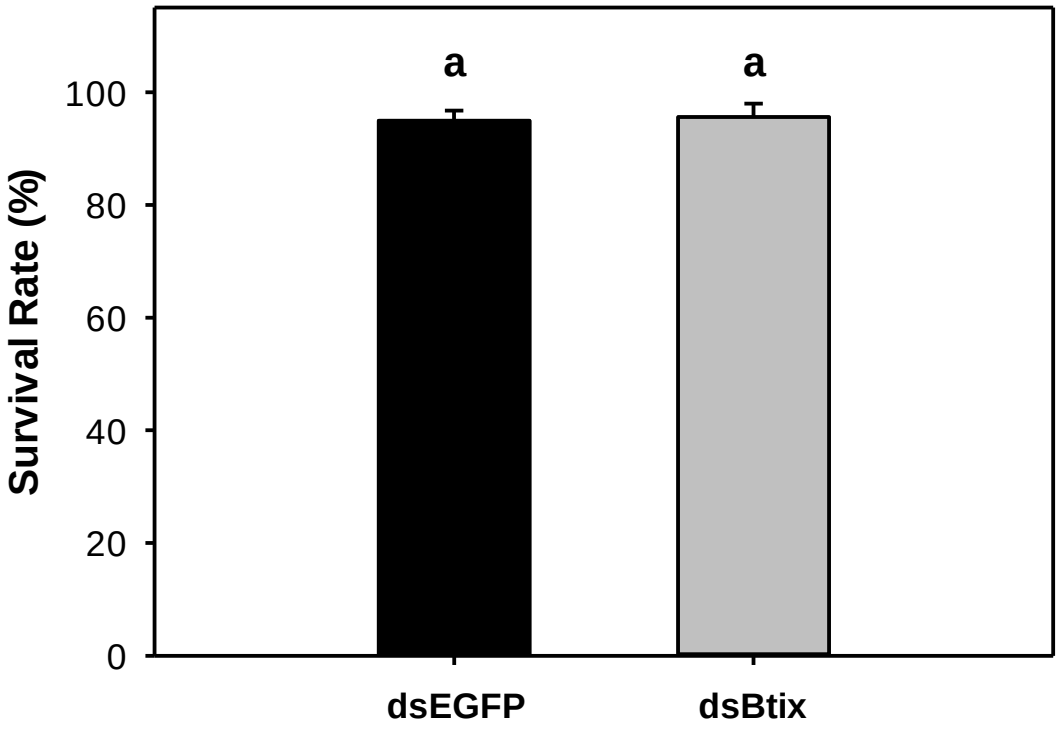

Figure S5. The effect of RNAi of *Btix* on the survival rate of newly emerged female adults. Bars with the same letter are not significantly different from each other at P<0.05 based on Tukey's test. Each point represents the mean ± SE from three independent experiments.

Figure S6. RNAi phenotypes of *Btix*. D0-D3, 0-3 days after RNAi, respectively.

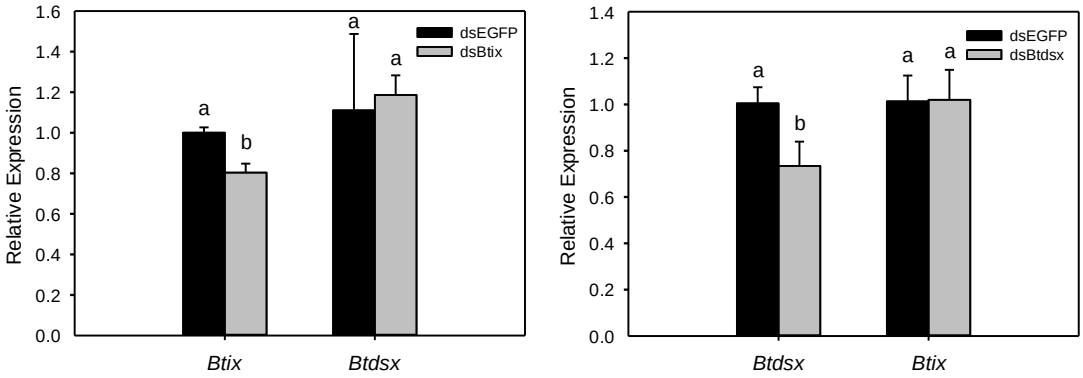

Figure S7. Effect of RNAi *Btix* or *Btdsx* on the expression of *Btix* and *Btdsx*. Bars with the same letter are not significantly different from each other at P<0.05 based on Tukey's test. Each point represents the mean ± SE from three independent experiments.