



Figure S2 Results from the analysis of RAD-sequencing data for *G. affinis* and *G. holbrooki*.

(A) Tile plot showing the number of markers (color intensity) found in any number of males (horizontal axis) and females (vertical axis) for *G. affinis*. Tiles for which association with sex is significant ($p < 0.05$, Chi-squared test with Bonferroni correction) are highlighted with a red border. Among the 433 markers significantly associated with sex, 397 (92%) were present in all females and absent from all males (top left corner). (B) Tile plot showing the number of markers (color intensity) found in any number of males (horizontal axis) and females (vertical axis) for *G. holbrooki*. Tiles for which association with sex is significant ($p < 0.05$, Chi-squared test with Bonferroni correction) are highlighted with a red border. Among the 309 markers significantly associated with sex, 195 (63%) were present in all males and absent from all females (bottom right corner). (C) Manhattan plot showing $-\log_{10}(p_{\text{association with sex}})$ from a Chi-squared test on the number of males and number of females in which a marker is present for *G. affinis* markers aligned to the genome of *X. maculatus*, with odd linkage groups (LGs) colored in yellow with light background and even LGs colored in blue with dark background. The dashed line indicated a p -value for association with sex of 0.05 after Bonferroni correction. Most markers significantly associated with sex (78%) were aligned to LG1 of *X.*

maculatus. (D) Manhattan plot showing $-\log_{10}(p_{\text{association with sex}})$ from a Chi-squared test on the number of males and number of females in which a marker is present for *G. holbrooki* markers aligned to the genome of *X. maculatus*, with odd linkage groups (LGs) colored in yellow with light background and even LGs colored in blue with dark background. The dashed line indicated a p-value for association with sex of 0.05 after Bonferroni correction. Most markers significantly associated with sex (78%) were aligned to LG16 of *X. maculatus*.