

**Supplementary Fig. S3.** Comparisons between males and female which include greater numbers of individuals help to refine sets of candidate sex-linked loci. Each point represents the number of putative sex-specific loci based on presence and absence patterns across random draws of individuals for different numbers of each sex. Females and males are color-coded in white and black, respectively. When comparing only a few individuals of each sex, many loci appear to be present uniquely in one sex and absent in the other sex, and this pattern holds for both males and females. But as greater numbers of each sex are compared, the numbers of putatively sex-specific loci drop precipitously. After comparing all nine female and eleven male hellbenders, we retained a set of 12 loci present in all males and absent in all females (putatively Y-linked) and a set of 100 loci present in all females and absent in all males (putatively W-linked).

