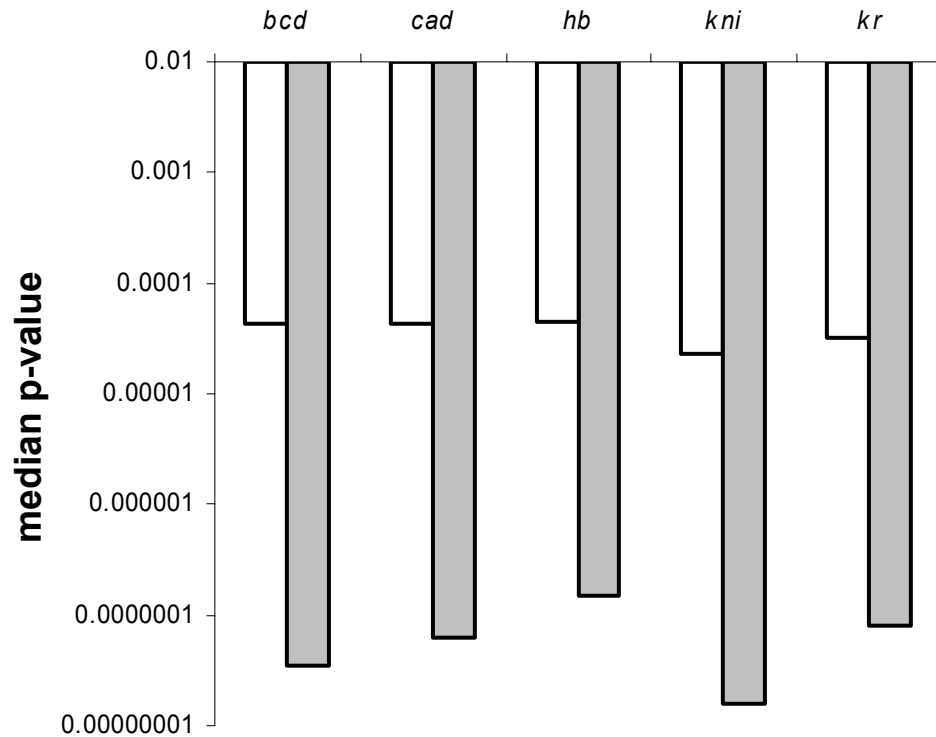
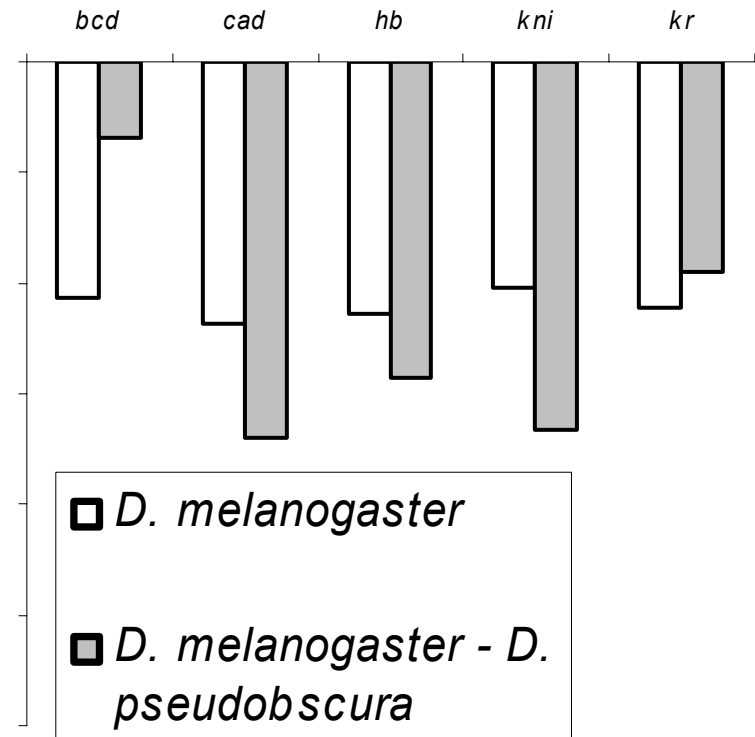


Many characterized binding sites do not seem to be evolving under constraint. We defined a binding site as not conserved if its p-value in the four way alignment of *S. cerevisiae*, *S. paradoxus*, *S. mikatae*, and *S. bayanus* was higher (less significant) than in *S. cerevisiae* alone.

A Functional set (positives)



B Non-functional set (negatives)



Putative binding sites (*D. melanogaster* $p < 0.0001$) in alignments of enhancers from Berman et. al. 2004. A. The p-values of binding sites in functional enhancers decrease as expected, consistent with the hypothesis that they are under constraint. B. The p-values in the non-functional set behave somewhat more erratically, indicating that some fraction of them may be under functional constraint