

File S1

Nature of the Among-MA-Line Variance

The MA lines were established from an ancestral line that had been subjected to 13 generations of inbreeding, reducing the segregating genetic variation (McGuigan et al. 2011). The lack of among-line variance observed for most (71%) of the traits in this study (see Results) was consistent with successful elimination of standing variation in the common ancestor of the MA lines (see also McGuigan et al. 2011). Of particular note, 6,050 ESTs with significant among-line variance in an outbred population of *Drosophila serrata* had zero among-line variance in the MA experiment.

Nonetheless, segregating variation was detected in two cuticular hydrocarbon (CHC) traits assayed in the 3rd generation of the experiment, revealing the presence of some standing genetic variation in the ancestor. MA lines could be classified into two groups based on their CHC profiles in the 3rd generation. Preliminary analyses revealed difference between these two CHC groups in mean expression of some genes. To remove this effect, we fit “CHC group” as a fixed effect in all mixed models. Comparison of results from analyses with group fit versus not fit showed that the inclusion of the known standing variation inflated the estimate of mutational heritability for some traits. It is not known if there were other segregating variants at the start of the mutation accumulation experiment, and although we interpret the among-line variance components estimated in the mixed-model analyses as mutational in origin, this is unlikely to be strictly true.

References

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- McGuigan, K., D. Petfield, and M. W. Blows. 2011. Reducing mutation load through sexual selection on males. *Evolution* 65:2816-2829.