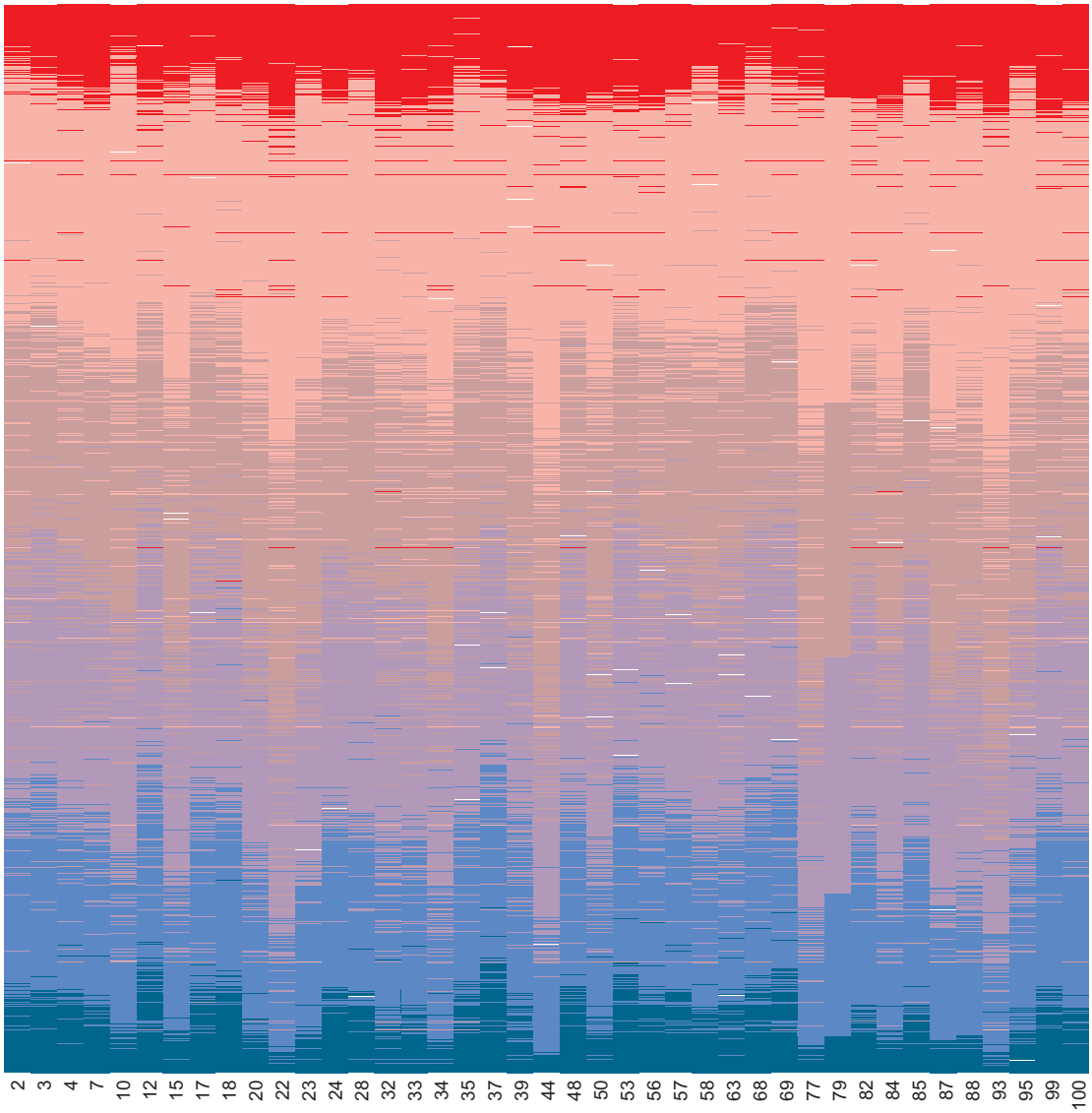

Figure S1. Mean $\log_2$ transcript abundance in each MA line. Expression ranged from not expressed (red; $\log_2$ ratio to background < 1) up to highly expressed features (deep blue; $\log_2$ ratio to background >6; maximum expression 364 times background) ($\log_2$ ratio of: orange = 1-3; grey = 3-4; lilac = 4-5; blue = 5-6). Note, this heat map illustrates only which genes are expressed versus not expressed (interpreted as greater or less than 1 fold $\log_2$ signal intensity above the mean signal intensity of the 20,000 random probes on each array), not whether genes were up versus down regulated among MA lines. Genes were ordered by their expression in MA line 79, which had the least genes expressed (10,602 genes had signal intensity >1 fold above the mean of the random probes). This was done to aid visualization of the relative numbers of unexpressed genes. Between 231 and 1,002 genes in a MA line (on average, 5.6% per line) had expression levels that were not distinguishable from the background signal. We nevertheless included these genes in the analyses because low (no) expression in most lines, but increased expression in one line could reflect a mutation in that line.

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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