

## File S1 - Methodological details of msms simulations

### *msms simulations under the neutral model*

Using the demographic history of the GA and GA-R lines and the design of our sequencing experiment as our guide, we simulated  $F_{ST}$  values ( $n = 10,000$  simulations) under the neutral model along a 40 kb genomic window for five diploid individuals (10 haplotypes) from each of the two *H. zea* lines. Our goal was to estimate the probability that a neutral process like genetic drift caused  $F_{ST}$  values  $> 0.44$ , like those observed for the genomic windows containing *cad-86C*. We assumed the individuals sampled from the field were drawn from an effective population size of  $N_e = 100,000$  (Anderson et al. 2018) at 72 generations in the past. We modeled an initial population size of 180, followed by two generations with 600 individuals per generation. After these two generations, the population was split into two lines of 600 individuals each, representing the GA and GA-R lines. In the first 35 generations post-colonization, the population size of GA-R deviated from 600 nine times, while the size of GA remained constant at 600. These population size deviations corresponded to the actual population size changes that occurred in the first nine GA-R selection events ( $n = 97, 106, 156, 203, 320, 214, 406, 1050, \text{ and } 1440$ , respectively). We emphasize that our demographic model did not include selection, however. At generation 36, one unidirectional migration event, reflecting the crossing of GA with GA-R, allowed for exchange of genetic material from one line into the other at a rate of 50%. In the following generation, each line was split into two replicates with 600 individuals each. To simulate the interbreeding between replicates of each line that occurred every other generation (causing 50% migration per two generations), we used a 25% migration rate between replicates of each line per generation. The present population size ( $N_0$ ) was 600 individuals per replicate, and we calculated theta using a mutation rate of  $2 \times 10^{-9}$  (Anderson et al. 2018) along the 40 kb region. Throughout the simulations, we used a recombination rate of 4 cM/Mb (Martin et al. 2019). At present day, or 72 generations from the initial sampling (matching the duration of the experiment), we sampled five haplotypes from each replicate, generating a sample size of 10 haplotypes per line, which were used to calculate Weir and Cockerham's  $F_{ST}$  along a 40kb genomic window using the R (v. 3.4.4) package hierfstat (v. 0.04-22).

After this initial simulation, we simulated additional genotypic datasets for which: 1) the post-colonization generation sizes were 50% and 25% of the sizes of GA and GA-R in our experiments, and 2) the initial collection sizes were 50% and 25% of the size compared to our initial collection event. In both cases, these datasets could represent variability in the percentage of the population that mate and/or contribute viable offspring to subsequent generations. Distributions of  $F_{ST}$  values were compared with one another, and the proportion of times a simulated dataset produced an  $F_{ST}$  value greater than 0.44 was calculated for each simulated dataset.

### References:

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Martin SH, Davey JW, Salazar C, Jiggins CD (2019) Recombination rate variation shapes barriers to introgression across butterfly genomes. *PLoS Biol* 17(2): e2006288.  
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