Produced by: Msa Cristiane Taniguti, Ms Fernando Correr, Ms Fernando Espolador and Ms Jhonathan Pedroso.

Material and Methods

In this investigation we used genotypic data from a F_2 population derived from selfing F_1 hybrids obtained from an interspecific biparental cross between two pure inbred lines, in which the pollen donor parent was IM62 (*Mimulus guttatus*), and the maternal parent SF5.4 (*Mimulus nasutus*) (Fishman et al., 2001). The F_2 population was designed with 526 individuals that were genotyped in a previous work with 255 polymorphic molecular markers, and here we added more 163 markers to the previous dataset, totalizing 418 polymorphic molecular markers that include codominant and dominant ones.

We build the genetic map with the software onemap (Margarido et al., 2007) available in https://github.com/augusto-garcia/onemap. Initially, we did a descriptive graphical analysis with the onemap plot function plot.onemap() of the types of molecular markers (codominant and dominant), their segregation pattern, and the presence of missing genotypic data. After this step, we performed a segregation test with the function test_segregation() to identify molecular markers with distortion from the expected mendelian segregation pattern. Molecular markers with mendelian segregation were selected with the function select_segregation(). The selected molecular markers were subjected to two point recombination rate estimation analysis using the one map function rf_2pts(). We obtained the LOD criterion to establish significance of linkage between pair of markers with the function suggest_lod(). To build our reference map, we used the mapping function “Haldane” (Haldane, 1919) implemented in the function set_map_fun(). The linkage groups were formed with the function group(), using as input the results from the two point recombination analysis. As a first try to order molecular markers within each linkage group, we used the function rcd(), which applies the rapid chain delineation algorithm (Doerge, 1996). After this step, we refined our genetic map using the multipoint ordering approach based on a Hidden Markov Model (HMM) (Lander and Green, 1987) implemented in the function order_seq(). We plotted heatmaps with the upper off-diagonal elements being the ordered LOD scores measuring significance of linkage signal between pair or markers, and with lower off-diagonal elements representing the recombination fraction between pair of markers. These heatmaps were plotted with the function rf_graph_table(), using as input the results obtained from the HMM analysis. The graphical inspection of the heatmaps allowed us to identify some ordering pitfalls, like the visualization of unexpected recombination hotspots, and absence of appropriate recombination gradient as markers distantiate. Markers showing these ordering pitfalls were removed and reinserted individually with try_seq() function depending on the pattern observed in the heatmap and the possible increase of group size and the global likelihood. After establishing well ordered linkage groups, we included the markers with segregation distortion, and we builded consistent linkage groups. We included these markers always evaluating the ordering of the previous Mimulus genetic map (Fishman et al., 2001). The linkage groups were represented visually using MapChart (Voorrips 2002).

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