An Expansion of the Genetic Map in the *Mimulus guttatus* Species Complex

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MATERIALS AND METHODS

Plant material and markers molecular The mapping population comprised 287 F_2 individuals derived from a cross between a single inbred line of *M. guttatus* and a single inbred *M. nasutus* genotype. The *M. guttatus* parental line was derived from an highly outcrossing population from Oregon Cascades (Iron Mountain; WILLIS 1993; SWEIGART *et al.*, 1999). The *M. nasutus* parental line was derived from a population in northwestern Oregon (Sherar's Falls) and maintained for several generations in the greenhouse through autonomous self-fertilization. The mapping population was planted in a common garden experiment at the University of Oregon Department of Biology greenhouse.

Total genomic DNA was extracted from the corollas from each F_2 individuals using a modified hexadecyl trimethyl-ammonium bromide (CTAB) chloroform extraction protocol (LIN, RITLAND 1996; KELLY, WILLIS 1998) and its concentration quantified with a Hoechst fluorometer. A total of 418 molecular markers were analyzed, which 213 markers had type 1:2:1 segregation (codominant) and 205 markers had type 3:1 segregation (dominant).

Transmission ratio distortion Occurrence of segregation distortion were determined by a chi-squared test at each marker locus in the full data set [χ^2 with 1 d.f. (dominant markers) or 2 d.f. (codominant markers); SOKAL, ROHLF 1995]. We used two significance thresholds ($\alpha = 0.05$ and 0.001) like in FISHMAN *et al.* (2001) and Bonferroni correction for multiple testing ($\alpha^* = 0.05$). Markers that did not fit the expected Mendelian ratio were treated as distorted. In the literature there is much disagreement about whether to use or not the markers with segregation distortion. Xu (2008) suggests that, when the segregation desviation is not pronounced, or when the markers are closely linked to the QTL, the identification of markers is not impaired. So, in this work all markers were used to construct the genetic map.

Linkage map construction The linkage map was constructed using the OneMap software (MARGARIDO *et al.* 2007; MARGARIDO *et al.* 2011) version 2.0-4 implemented in the statistical software R (R Development Core Time 2015). First, a two-point pairwise analysis was carried out between all pairs of markers using the rf.2pts command. Linkage groups were established with the group command and the criterias: minimum LOD score 6.0 and maximum recombination fraction of 0.4. Map distances were estimated using the Kosambi function (KOSAMBI 1944). At first, the algorithm RCD (DOERGE 1996) was used to order markers for each linkage group. To obtain the best order for each group the order.seq command was applied, thus a subset of five informative markers was used to create a framework (compare command) wherein the remaining markers were introduced using the try.seq function. This multi-point ordering methodology combining the compare and try functions of the MAPMAKER software (LANDER *et al.* 1987). A graphical representation of the recombination fraction and LOD score between markers within each group (heatmaps) were evaluated to identify markers incorrectly allocated and gaps between markers. The unreliable markers were individually evaluated using the drop and try.seq command to generate and compare alternative orders. Finally, linkage group marker orders were checked based on the Ripple algorithm (LANDER *et al.*, 1987). The linkage groups were numbered in correspondence to the map published for Fishman *et al.* (2001) based on common markers. Finally the map were visualized using MapChart software version 2.1 (VOORRIPS 2002).

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