Linkage map and analysis of a *Mimulus guttatus* and *Mimulus nasutus* interspecific cross

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ABSTRACT We constructed a linkage map for interspecific hybrids of the species *Mimulus guttatus* and *Mimulus nasutus*. We used information on 287 F_2 individuals, genotyped using four types of genetic markers: amplified fragment length polymorphisms (AFLPs), microsatellites, gene-based markers, and sequence-tagged sites (STS). Our map comprised 14 linkage groups, spanning 2375 cM. In accordance with the literature, our map showed markers with transmission ratio distortion clustering in specific regions.

KEYWORDS transmission ratio distortion; linkage map; *Mimulus guttatus*; *Mimulus nasutus*

Material and Methods

Study system

Species of the genus *Mimulus* (historically *Scrophulariaceae*, order *Lamiales*) are highly polymorphic and are geographically widespread across western North America (Pennell 1951; Vickery 1978). Here, we focus on the two species *M. nasutus* and *M. guttatus*, from the *M. guttatus* complex. The species *M. guttatus* (2n = 28) is the most common and presents a great morphological, life history strategy and habitat diversity. This species is predominantly outcrossing and pollinated by bees. *M. nasutus* (2n = 28) is the most common inbred species of the genus. Despite the existence of incompatibility mechanisms, wild hybrids of these species are frequently observed. However, populations from these interspecific crosses present some fertility issues.

Generation of F_2 mapping population

An inbred line of *M. guttatus* and an inbred line of *M. nasutus* were crossed. The parental line of the species *M. guttatus* (IM62) is highly fertile and was used as a pollen donor. The *M. nasutus* line (SF5.4) is highly inbred and homozygous at several loci which are highly diverse in *M. guttatus* populations. *M. guttatus* and *M. nasutus* plants were crossed to obtain F_1 generation plants, which were selfed to yield F_2 individuals. In March 1997, 100 IM62 plants and 100 SF5.4 plants were grown. Also, F_1 individuals and the F_2 mapping population were grown (N = 600 in total) in individual pots in a common garden experiment in the University of Oregon Department of Biology greenhouse. Plants were grown in 2.25-inch pots with a soilless substrate in a completely randomized design. The final sample for mapping consisted of 287 F_2 individuals.

Tissue collection and DNA extraction

Several corollas from F_2 individuals were collected in separate 1.5 ml Eppendorf tubes, immediately placed on dry ice and stored at -80°C. Genomic DNA was isolated from the corollas using a modified hexadecyl trimethyl-ammonium bromide chloroform extraction protocol (Lin and Ritland 1996; KELLY and WILLIS 1998). DNA concentration was quantified with a Hoechst fluorometer. From 600 F_2 plants, corolla tissue was collected from 526 individuals for genotyping.

Molecular markers

For genotyping, four different types of PCR-based molecular genetic markers were used, including amplified fragment length polymorphisms (AFLPs), microsatellites, gene-based markers, and sequence-tagged sites (STS).

Linkage map construction

Using molecular markers genotyped for 287 F_2 individuals, we constructed a linkage map using OneMap (Margarido *et al.* 2007). All distances between markers, two by two, were estimated using the Kosambi mapping function (Kosambi 1944) using the `rf_2pts` function. After calculating the two-point distances, markers were assigned to different linkage groups adopting a minimum LOD equal to 5.41 as a threshold, using the OneMap `group` function. Initially, the Rapid Chain Delineation heuristic

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was used to get a first idea of the marker positions. Then, we altered positions iteratively, removing from the analysis markers whose presence hindered a consistent linear order for the others.

Transmission rate distortion

It is possible that skewed genotypic ratios appear just by chance or as a result of linkage to an important locus for hybrid fitness. Thus, we tested each marker for significant deviations from the expected Mendelian genotypic frequencies using OneMap's `test_segregation` function (this was done using the chi-square test testing the segregation hypothesis 1:2:1 for dominant markers and 3:1 for codominant markers, $\alpha = 0.05$). Next, we performed Bonferroni's correction for multiple tests. Finally, to examine the distortion pattern in the map, we also analyzed the frequency deviation of the parental homozygotes from Mendel's Expectation of 0.25 for each locus in relation to its position on the map.

Results

Linkage map construction

Linkage map was constructed based on information from 288 markers with greater reliability regarding their position in the linkage groups, thus building a map of 2375 cM, with 14 linkage groups (Figure 1). The heatmaps in Figure 2 show that our final map had a consistent linear order for the markers. It is possible to observe that the recombination fraction is close to zero around the diagonal, demonstrating the proximity between the loci on the chromosome, and at the extremes the recombination fraction is close to 0.5, indicating a greater distance. This indicates that the markers are placed in a consistent linear order.

Transmission rate distortion

In Figure 3, it is observed that the transmission ratio is favoring the loci from *M. guttatus* over *M. nasutus*, with some type of selection, not occurring according to Mendelian segregation. In total dataset, 213 markers are codominant (51.0%) and 205 (49.0%) are dominant. Of 205 dominants, 113 have *M. guttatus* allele as dominant and in 92 *M. nasutus* allele is dominant. Of these 418 markers, 62 have transmission rate distortion (14.8%). Furthermore, 39 markers (9.3% of the total) present $p < 0.001$, among which 29 are codominant (13.6% of total number of codominants) and 10 are dominant (4.9% of total number of dominants). Most of those with distortion are codominant (45 markers, 21.1%) while the rest is dominant (17 markers, 8.3%). Of 288 markers that were mapped, 172 are codominant (59.7%) and 116 are dominant (40.3%). Of this total, 14.2% differ from the expected Mendelian segregation ($p < 0.05$), a number close to those that present distortion among the totals (14.8%). Furthermore, 28 markers (9.7% of the total) have $p < 0.001$, 22 of which are codominant (12.8% of mapped codominants) and 6 are dominant (5.2% of mapped dominants). Most of those with distortion are codominant (33 markers, or 19.2%), while the rest are dominant (6.9%). Of the 33 SNPs that were mapped, they are codominant and present significant distortion ($p < 0.05$), 24 favor the *M. guttatus* allele (72.7%). Furthermore, all 6 markers with the dominant *M. guttatus* allele favor this allele (75% of the 8 dominant markers). Note, in Figure 3, that markers are grouped in large quantities (more than 2 in a row) in the linking groups LG1, LG11 and LG14. In groups LG1, LG5, LG10 and LG11, significant markers all favor the *M. guttatus* allele.

Discussion

As shown, segregation did not occur according to the Mendelian expectation for several loci, *M. gusttatus* alleles being favored over *M. nasutus*, with many distorted markers grouping together in the genetic map. It is unlikely that this transmission ratio distortion was caused by inbreeding depression, since both inbred lines can be obtained and are normally fit. Other studies aimed to investigate this phenomenon, pointing to a possible chromosomic region associated to zyygotic lethality of hybrids when F_1 individuals served as maternal parents in backcrosses with *M. nasutus* or *M. guttatus* (Fishman and Willis 2005; Hall and Willis 2005). By using this new dataset, it was possible to build a genetic map more suited to reality. However, even with the improvement of the genetic map through the insertion of new loci, it is necessary to continue studies in this area. The presence of errors is an intrinsic factor when analyzing data, such as genotyping and building genetic maps, with the development of technologies it is possible to obtain more accurate results and better detection and characterization of genomic regions associated with phenotypic diversity.

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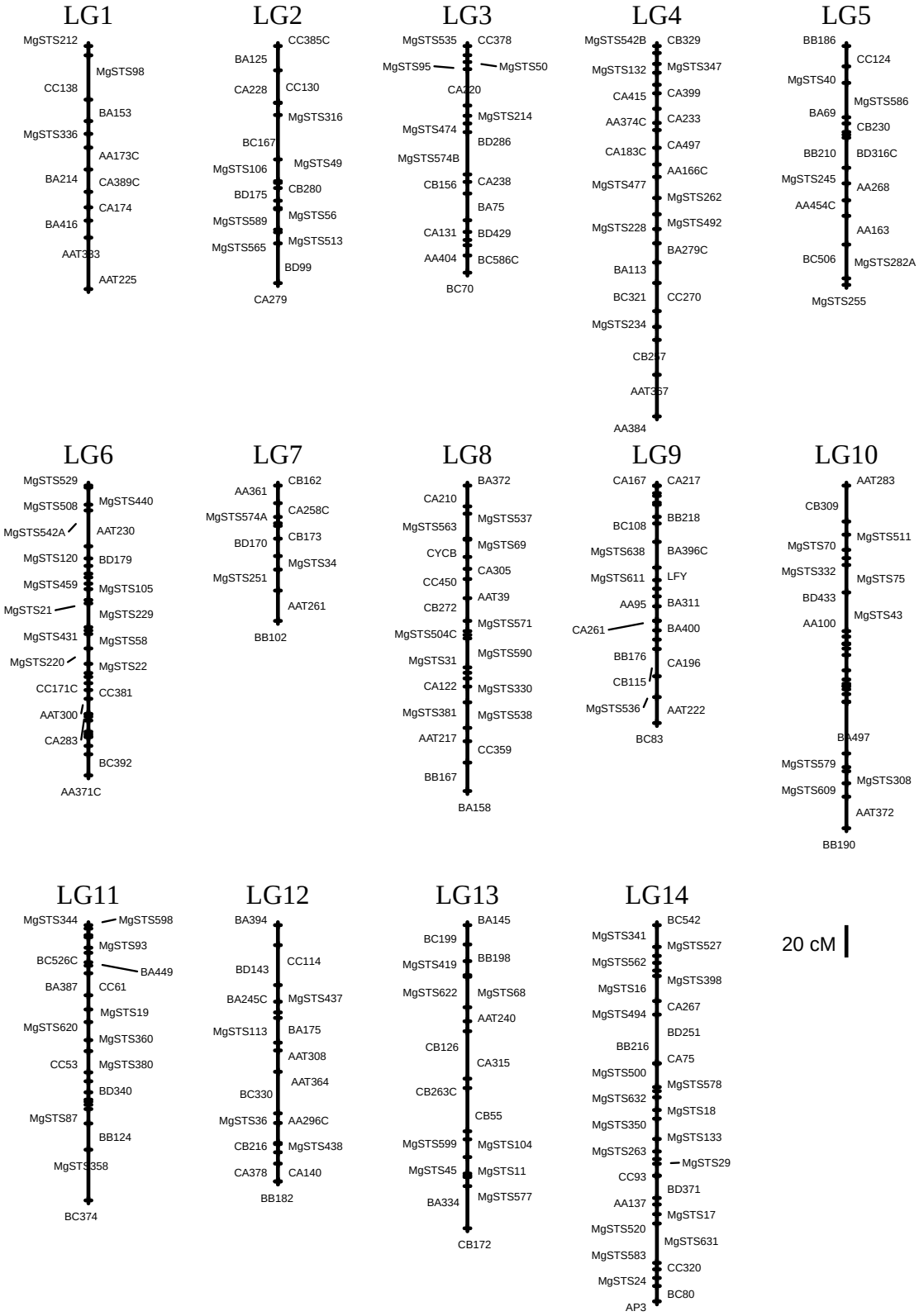

Figure 1 Linkage map of *M. guttatus*×*M. nasutus* F_2 hybrid population. Hatch marks indicate marker placement.

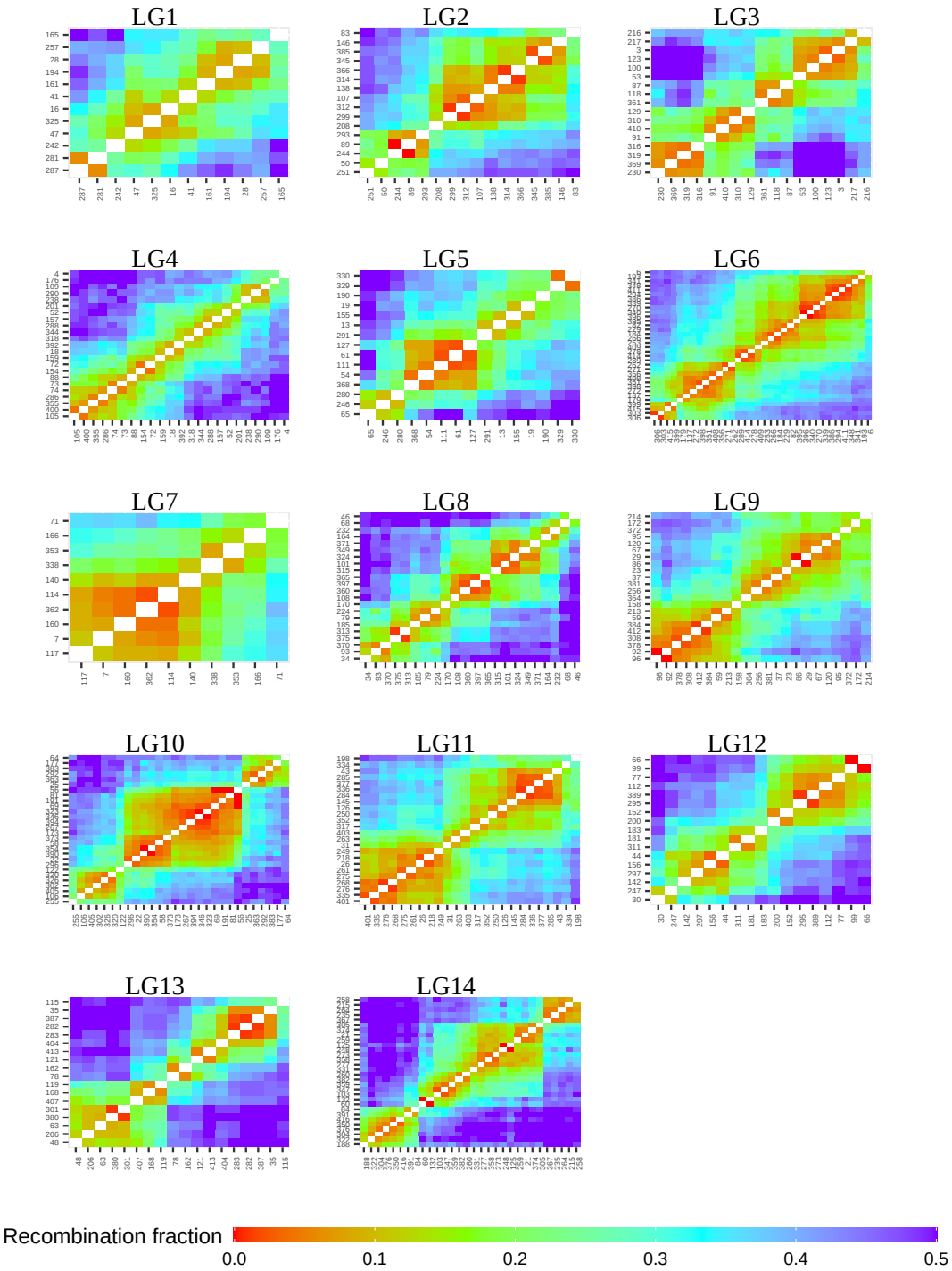

Figure 2 Heatmaps of the recombination fraction between markers for the fourteen linkage groups.

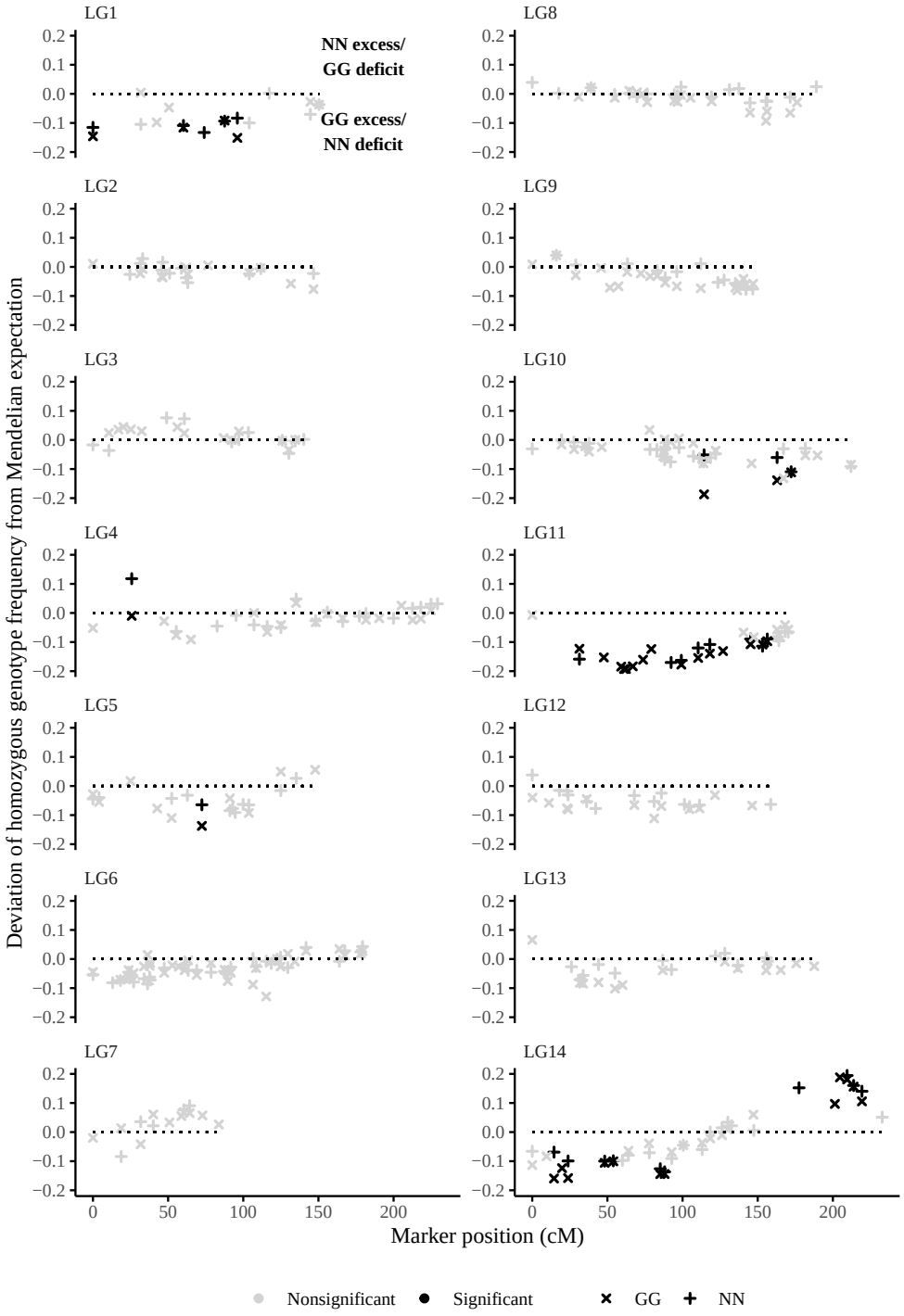

Figure 3 Transmission ratio distortion accross the *M. guttatus*×*M. nasutus* linkage map.