UNDERSTANDING GENOMIC EVOLUTION AND SEGREGATION DISTORTION IN SOLANACEAE: A COSII LINKAGE MAP IN *NICOTIANA*

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

UNDERSTANDING GENOMIC EVOLUTION AND SEGREGATION DISTORTION IN SOLANACEAE: A COSII LINKAGE MAP IN *NICOTIANA*

presented by Paul J. Walker,

a candidate for the degree of master of art in biological sciences

and hereby certify that, in their opinion, it is worthy of acceptance.

Professor Tim Holtsford

Professor Chris Pires

Professor Michael McMullen

To Becky,
for her unconditional love and belief in me.

To Mom and Dad,
who always told me to shoot for the stars.

Finally, to Anthony Paul Guastello,
no matter where you are,
your Godfather will always be proud of you.

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"Two roads diverged in a wood and I, I took the one less traveled by, and that has made all the difference.[1]" Four years ago I began down a path that few have travelled. The path I chose was a difficult one littered with diverse challenges that tested every fiber of my being. Despite the obstacles I encountered over the years, I now find myself at the end of this arduous journey. It is at this time that I realize that I could not have traversed this difficult path were it not for the help and guidance of others.

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In spite of all the challenges I have faced, one thing that has been constant in my life is the support from my family. First, I would like to thank my wife, Becky Walker. Without her unending love, patience and support I would simply not be here. She has traveled every step of this journey with me and has been there through all the struggles. I

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[1] Excerpt from "*The Road Not Taken*" by Robert Frost. Frost, Robert. *Mountain Interval.* New York: Henry Holt and Company, 1920.

truly look forward to what lays before us on the next path. Second, I would like to thank my parents, Pete and Patty Walker. They've always encouraged me to do the best I can and have always given me their support and love in all my endeavors. Finally, I would like to thank my siblings, Anna, Joe and Peter, for their constant support through the years.

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

UNDERSTANDING GENOMIC EVOLUTION AND SEGREGATION DISTORTION IN SOLANACEAE: A COSII LINKAGE MAP IN *NICOTIANA*

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ABSTRACT

Genetic linkage maps are excellent tools to investigate genomic evolution, genomic structure and quantitative trait loci. In our study, we created comparative linkage maps in a reciprocal cross between *Nicotiana longiflora* and *N. plumbaginifolia*. We used genetic markers derived from the Conserved Ortholog Set II (COSII) to investigate synteny between *Nicotiana longiflora* and *N. plumbaginifolia* (n = 10) and other Solanaceae species (n = 12), such as tomato, pepper and eggplant. We produced two linkage maps; one in a maternal *Nicotiana longiflora* background (43 markers covering 808.1 cM) and a reciprocal map in the maternal *N. plumbaginifolia* background (54 markers covering 1110.4 cM). Segregation distortion was evident in both backgrounds, especially for the dominant ISSR markers, and was more prevalent in the maternal *N. longiflora* cross. In both backgrounds, there was a significant preference for the self-fertilizing *N. plumbaginifolia* allele for distorted markers. Finally, using the COSII markers, we infer many chromosomal rearrangements have occurred since the divergence of *Nicotiana* and tomato (*Solanum lycopersicum*) from a common ancestor.

I. Introduction

Genetic linkage maps are useful tools for studying the evolution of genomic organization and for mapping traits of interest (Tanksley et al. 1989; Fishman et al. 2001; Chen and Tanksley 2004). For instance, interspecific linkage mapping makes it possible to locate the genetic basis of reproductive isolating mechanisms in natural populations that may lead to speciation (Moyle and Nakazato 2008) and genetic regions that control mating system variation (Chen and Tanksley 2004; Chen et al. 2007).

In addition to locating quantitative trait loci (QTL), inter-specific linkage maps can serve an important purpose in genome evolution. Comparative genetic mapping between related organisms within a phylogenetic framework is a powerful method for understanding genome evolution (Schranz et al. 2007).

There have been numerous linkage maps created throughout Solanaceae (n = 12) that can be used for comparative purposes. These studies have shown much conservation of genomic sequences (synteny) throughout Solanaceae, although there have been many chromosomal rearrangements between species (Tanksley et al. 1992; Livingstone et al. 1999; Doganlar et al. 2002a; Doganlar et al. 2002b; Wu et al. 2009a; Wu et al. 2009b).

In this study, we constructed reciprocal linkage maps in an interspecific cross between *Nicotiana longiflora* and *N. plumbaginifolia* to study genomic evolution within Solanaceae. The karyotypes of *N. longiflora* and *N. plumbaginifolia* both possess ten pairs (n = 10) of acrocentric or telocentric chromosomes (Goodspeed 1954; Lin et al. 2001; Lim et al. 2006). *Nicotiana longiflora* and *N. plumbaginifolia* are part of *Nicotiana* section alatae which shows a variety of mating systems. The mating systems in alatae include two groups comprising of either obligate outcrossing through gametophytic self

incompatibility (GSI) or self-compatibility (SC) (Pandey 1973; Pandey 1979). Phylogenetic analysis shows the GSI and SC states are distinct clades (Lim et al. 2006). The SC clade is comprised of two sister species, *N. longiflora* and *N. plumbaginifolia* (n = 10), while the rest of alatae comprises the GSI clade (n = 9) (Goodspeed 1954).

Although both *N. longiflora* and *N. plumbaginifolia* are self-compatible, they have different mating systems. *Nicotiana plumbaginifolia* is generally autogamous, with self pollination occurring before anthesis. *Nicotiana longiflora* is also self-compatible, but is a facultative outcrosser (East 1916; Goodspeed 1954; Soule 2007). The species' geographic distributions overlap in northwest Argentina (Goodspeed 1954), where both allopatric and sympatric populations may be found (Figure 1, Figueroa-Castro 2008). *Nicotiana longiflora* and *N. plumbaginifolia* are inter-crossable and produce fertile hybrids (East 1916; Goodspeed 1954; Pandey 1973; Pandey 1979). Hawkmoths have been observed visiting both species in sympatric populations and gene flow between species has been suggested (Figueroa-Castro 2008).

To facilitate comparative mapping, we relied primarily on the Conserved Ortholog Set II (COSII) (Wu et al. 2006) and the SOL Genomics Network (Muller et al. 2005) to develop markers that were previously mapped in tomato. The COSII database serves as an excellent resource of genetic markers for comparative mapping. It includes most of the markers that comprised the first Conserved Otholog Set (COSI) (Fulton et al. 2002). The COSII database, which is derived originally from single copy genes in *Arabidopsis*, has been tested on Solanaceae species such as tomato, pepper and eggplant and provides an excellent resource for developing genetic markers in related Solanaceous species where very little genomic information is available (Wu et al. 2006). The fact that

all COSII genes have a single homologous match in *Arabidopsis* indicates the selection pressure to retain detectable homology or remain (or become) single copy after the divergence of Solanaceae from Arabidopsis, which is estimated to have occurred 94-125 MYA (Wu et al. 2006).

The creation of our interspecific linkage maps had multiple purposes. The main purposes of this study are: (1) to present the first diploid *Nicotiana* interspecific ISSR and SNP-based hybrid linkage map in *N. longiflora* and *N. plumbaginifolia*; (2) to examine the degree of synteny between other Solanaceae COSII maps with *Nicotiana*; and (3) use the reciprocal F2 mapping populations to test the hypothesis that cytoplasmic genomes affect segregation distortion.

II. Materials and Methods

A. Study System

The parental species, *N. longiflora* and *N. plumbaginifolia*, were grown from seeds that were collected in 2001 from a sympatric population found in Brazil. This population is referred to as "Mango" due to its proximity to a mango orchard. The sympatric Mango population presents an excellent source for this study because it is a natural sympatric population rich in genetic variation in mating system traits. The Mango seed collection consisted of five naturally-pollinated maternal families of *N. plumbaginifolia* and six families of *N. longiflora*.

There are other key characteristics that distinguish *N. longiflora* and *N. plumbaginifolia*. *Nicotiana plumbaginifolia* has similar floral morphology to *N. longiflora*, but has shorter corollas ~35 mm with 19.3 ± 1.1% solids in sucrose dominant nectar, versus long corollas, ~100 mm, with 21.3 ± 0.3% solids in sucrose dominant nectar (Kaczorowski 2005; Soule 2007).

The parental generation was grown in the Sears Greenhouse at the University of Missouri-Columbia. Since we had only a rough estimate of genetic diversity between species, we chose parents based on their corolla tube length and percent selfing in the glasshouse. These characters were chosen because they are correlated with self-fertilization (Soule 2007), a QTL we are interested to investigate in the future. A total of fifteen plants from each maternal family in the Mango population were grown in 3.5 inch pots, and uniformly arrayed over two benches. After the plants had reached maturity, we measured each plant and chose one parent from each species with the most extreme phenotypes (Figure 2). The *N. longiflora* parent produced zero selfed fruit, longer

corollas (~90 mm). The *N. plumbaginifolia* parent showed a 100% autogamous fruit set and short corollas (~30 mm, not shown).

B. Generation of F_2 Mapping Population

We made reciprocal F_1 crosses between *N. longiflora* and *N. plumbaginifolia* using one parent selected from each species (Figure 2). We then self-fertilized one plant in both of the F_1s to create two F_2 populations with cytoplasms from each species (Figure 2). In November 2007, we grew the parents, the reciprocal F_1s and F_2 populations (N = 200 for each cross) in the greenhouse. The reciprocal cross between *N. longiflora* and *N. plumbaginifolia* produced a total of 400 F_2 plants. A total of 96 F_2 plants were randomly chosen from each F_2 population for the mapping populations (Figure 2).

C. Tissue Collection and DNA Extraction

Several buds from each of the selected 96 F_2 individuals were collected into 1.5 ml Eppendorf tubes and placed on ice. DNA was extracted from the buds based on the following protocol: tissue was ground in 300 µl of extraction buffer containing β-mercaptoethanol, 300 µl lysis buffer and 100 µl of 5% sarkosyl; then incubated for 10 min at 65˚C; extracted twice with 24:1 chloroform: isoamyl alcohol; rinsed with 76% EtOH-10mM $NH_4C_2H_3O_2$ and stored in 1/10 TE/RNase.

D. Dominant Markers: ISSR Development and Analysis

Inter-simple sequence repeat markers (ISSRs) amplify regions between adjacent, inversely oriented microsatellites using single simple sequence repeat (SSR)-containing primers (Zietkiewics et al. 1994). ISSRs are dominant markers, but have the advantage in that they are more reproducible than AFLPs and RAPDs, produce large numbers of polymorphisms per primer and no genomic sequence data is required to use them (Ruas

et al. 2003). The ISSRs were amplified per 39 cycles at 2.75 min at 94˚C; 1 min at 60˚C, 1.5 min at 72˚C. Amplified products were ran on 1.2% agarose gels for 3 hours and visualized with UV light. The ISSR markers were photographed, stored as a tiff image and scored by eye.

Forty-two ISSR primers were screened in both of the selected *N. longiflora* and *N. plumbaginifolia* parents and their chosen reciprocal F_1 hybrid (Figure 2). Polymorphic markers were identified by the presence of a fragment in one parent and the F_1 hybrid, but absence in the other parent (Figure 3a). Only ISSR primers that showed at least two polymorphic markers were used for this study. A total of 112 ISSRs markers were scored; however, only 101 were usable for mapping (Appendix 1). ISSR products were scored for the presence (+) and absence (-) of homologous DNA bands. The presence/absence data was then converted into MAPMAKER 3.0 format (Lander et al. 1987) using Excel (*e.g.*., - $\rightarrow$ A, + $\rightarrow$ C, where A is homozygous for a recessive allele and C would be either a dominant homozygote or a heterozygote for band presence originally found in *N. plumbaginifolia*; - $\rightarrow$ B, + $\rightarrow$ D, where B is homozygous for a recessive allele and D would be either a dominant homozygote or a heterozygote for band presence originally found in *N. longiflora*).

E. Codominant Markers: SNPs/CAPS Development and Analysis

We relied primarily on the COSII database to develop codominant markers that were previously mapped in tomato (Fulton et al. 2002; Wu et al. 2006). In order to enhance our comparative mapping capabilities, we relied on the SolGenomics network (Muller et al. 2005) to verify if any of our COSII markers had also been mapped in other Solanaceae species, such as pepper or eggplant.

We amplified and sequenced select COSII primers based on their location in tomato so as to cover the whole tomato genome. We analyzed the sequence data using DNASTAR (DNASTAR Inc., Madison, WI) and searched for single nucleotide polymorphisms (SNPs) between bi-directional COSII sequences from *N. longiflora* and *N. plumbaginifolia*. We sequenced 112 different loci that were mapped throughout the tomato genome. We assayed at least five markers per tomato chromosome. Because *N. longiflora* and *N. plumbaginifolia* are so closely related and may even hybridize, we expected a low success rate for species-specific alleles. We then used DNASTAR to find restriction enzymes to assay the SNPs. All of the SNPs were scored as cleaved amplified polymorphic sequences (CAPS) (Konieczny and Ausubel 1993); except one locus with a large indel that was scored by fragment size.

We also used two codominant markers that were previously developed from *Nicotiana alata* (Table 1). These were also SNPs that were scored as CAPS. Two candidate CAPS markers were difficult to score, so they were excluded from the study, giving a total of 33 codominant markers (Table 1). The CAPS products were scored directly into MAPMAKER 3.0 format using Excel (*e.g.*, uncut/uncut $\rightarrow$ A, cut/cut $\rightarrow$ B, uncut/cut $\rightarrow$ H, where A and B are homozygotes for Plum and Long alleles and H is the heterozygote).

To assay the SNPs, we amplified DNA using 1:10 dilutions. COSII loci were amplified per 39 cycles at 2.75 min at 94˚C; 1 min at 55˚C, 1 min at 72˚C. Amplified products were digested over two hours to overnight, run on 1.5% agarose gels for 3 hours and visualized with UV light. The cleaved products were photographed, saved to a TIFF file and scored by eye (Figure 3b).

F. Segregation and Linkage Analysis

The mapping populations in each background consisted of 96 F_2 individuals genotyped for the 33 codominant and 112 dominant markers. We then tested for non-Mendelian genotype frequencies (segregation distortion) at each marker locus by X^2 tests in both codominant (1:2:1) and dominant (3:1) markers using separate X^2 tests (Table 1; Appendix 1). We used three significance thresholds ($p < 0.05$, 0.01 and 0.001) to provide conservative and liberal estimates of segregation distortion.

G. Transmission Bias

We examined the allelic transmission bias across the maps by using distorted markers, comparing the deviation between homozygote frequencies across each linkage group in both backgrounds and comparing allelic frequencies of the mapped codominant markers in each background. Segregation distortion was inferred when X^2 tests were significant.

H. Map Construction

Linkage analysis was processed through MAPMAKER/EXP 3.0 (Lander et al. 1987; Lincoln et al. 1992). We relied on the default settings of two-point linkage with a minimum LOD score of 3.0 and a maximum distance of 50 cM. We lowered the LOD score to 2.7 in one case; to estimate linkage between dominant and codominant markers for Group PL7 in the maternal *N. plumbaginifolia* (PL) cross.

We constructed a map in each background through several rounds of mapping and marker exclusion. To construct the two initial maps, we used the GROUP command to organize markers into linkage groups. For linkage groups with six or fewer markers, we then used the COMPARE command to find the likely order of the markers. We

examined the likelihood of error in each order and identified any unreliable markers, which were excluded. For linkage groups with seven or more markers, we used the ORDER function to identify a starting subset of five markers to place the other markers. These linked, but unplaced, markers were then individually evaluated using the TRY, COMPARE, MAP, and RIPPLE commands to produce alternative orders. This was repeated until we obtained a consistent order in each group using a subset with few potential genotype errors. To confirm the order of the codominant markers in both backgrounds, we combined the codominant marker data from both backgrounds and ran it in Mapmaker. We calculated the average marker spacing by dividing the summed length of all linkage groups by the number of intervals.

III. Results

A. Marker Segregation

The number of distorted markers varied in each background as the maternal *N. plumbaginifolia* (PL) background had more usable markers (Table 1; Appendix 1). All markers were used for mapping if they showed a X^2 significance of $p > 0.001$ (Table 1; Appendix 1). After removing badly distorted markers, we had a total of 125 markers (31 codominant, 94 dominant) in the PL background and 116 markers (31 codominant, 85 dominant) in the maternal *N. longiflora* background (LP).

There was very little segregation distortion in either background for codominant markers. Only 3 out of 33 (9%) codominant markers deviated significantly ($p < 0.01$) from the Mendelian expectation of 1:2:1 in both backgrounds (Table 1). There was more background-dependent distortion at lower levels of significance ($p < 0.05$). At this threshold, 6 of 33 (18.2%) codominant markers in the LP background and 4 of 33 (12.2%) in the PL background deviated significantly from their Mendelian expectations.

We also examined the directional allele frequency bias for the distorted codominant markers. There was a strong bias for the *N. plumbaginifolia* alleles in both backgrounds. Of the 6 LP and 4 PL markers that distorted at $p < 0.05$, 5 of 6 and 3 of 4 had an excess of *N. plumbaginifolia* alleles and a deficit of *N. longiflora* alleles (Table 1).

There was more segregation distortion evident in the dominant markers. There was background-dependent distortion ($p < 0.001$) in 27 of 112 (24.1%) dominant markers in the LP background and 18 of 112 (16.0%) in the PL background (Appendix 1). There was also directional allele frequency bias in the dominant markers. Of the 27 LP and 18

PL markers that distorted at $p < 0.001$, 20 (74.0%) and 13 (72.2%) showed an excess of *N. plumbaginifolia* genotypes and a deficit of *N. longiflora* genotypes (Appendix 1).

B. Linkage Analysis and Map Construction

A separate background-dependent linkage map was constructed for each cross. For the PL cross, 17 tentative linkage groups were inferred from a two-point analysis of allelic segregation of 94 ISSRs and 31 CAPS loci (45 ISSRs and 2 SNPs were unlinked). The map was constructed using the 10 linkage groups that contained 3 or more markers. The PL map consists of 54 markers and covers a total of 1110.4 cM with an average distance of 25.23 cM between markers (Figure 4). Linkage Groups 1 to 10 range from 168 cM to 66.4 cM and consist of 3 to 8 markers. Any markers that were unreliable, in that they exhibited non-linear two-point linkage patterns (increasing marker interval beyond 50 cM), were removed from the study. The naming of linkage groups (PL1 – PL10) was based on their estimated length (Figure 4).

The LP cross did not yield as many linkage groups and contained fewer usable markers. For the LP background, 20 tentative linkage groups were inferred from a two-point analysis of allelic segregation of 85 ISSRs and 31 CAPS loci (47 ISSRs and 5 SNPs were unlinked). The map was constructed using the 9 linkage groups that contained 3 or more markers. The LP map consists of 43 markers that covered a total of 808.1 cM with an average distance of 23.7 cM between markers (Figure 5). Linkage Groups 1 to 9 range from 199.4 cM to 24.7 cM and consist of 3-8 markers. The naming of linkage groups (LP1 – LP9) was based on their estimated length (Figure 5).

After construction of the background-dependent maps, we combined the codominant marker data in both backgrounds to verify our codominant marker orders in

both backgrounds. The combined codominant data produced 10 tentative linkage groups and produced a map consisting of 4 mapped linkage groups, which showed the same orders as both background-dependent maps (Figure 6). All 10 tentative linkage groups were also linked in the background-dependent maps.

Our marker orders could not be confirmed when we combined the codominant and dominant marker data from both backgrounds. The dominant markers, which did not transfer well between both backgrounds, would alter linkage orders when the data sets from both backgrounds were combined. Because we could not get reliable orders when we combined all of the marker data (codominant and dominant) from both backgrounds, we excluded the dominant markers from the combined data set.

C. Linkage Map Comparisons Between Cytoplasm Backgrounds (LP vs. PL)

Linkage maps from both backgrounds were compared and aligned based on synteny between markers in Figure 7 and Figure 8. A total of 10 linkage groups were confirmed through the comparison of both maps. This is the same number as the number of chromosomes for both *N. longiflora* and N. *plumbaginifolia* (n = 10). The order of some common markers also differed in both backgrounds (Figures 7 and 8).

Not all linkage groups were mapped in both backgrounds due to the variation in segregation distortion in common markers; however, the codominant two-point linkage in all linkage groups in Figure 7 and Figure 8 were confirmed in both backgrounds and the combined codominant data set (Figure 6). For example, Linkage Groups 3 and 9 do not have a comparative linkage group mapped in the LP background; however, all of the codominant markers in Linkage Groups 3 and 9 showed two-point linkage in the LP background and the combined codominant data. These linkage groups failed to map in

the LP background because a third marker, which is required for interval mapping, failed to show linkage with the codominant markers (Figure 7 and Figure 8). These codominant loci are marked with an * in Figure 7 and Figure 8.

Linkage group 1 showed linkage between two codominant markers (Tom 2.00 and Tom 12.03) in both backgrounds. There appears to be a background-dependent rearrangement between two dominant markers (D37 and D19) differentiating the maps (Figure 7).

Linkage group 2 showed linkage between two LP linkage groups (LP2 and LP7) and PL linkage group (PL3). It appears the only reason that a single linkage group did not appear in the LP background was the absence of codominant marker (Tom 11.04), which may serve as an allelic bridge between the two LP linkage groups (Figure 7).

Linkage group 3, while only mapped in the PL background, did show linkage in the LP background between the two sets of codominant markers (Tom 11.03 and Tom 11.00; Tom 9.09 and Tom 9.037). Therefore, the contents of this linkage group, but not necessarily the order, have been confirmed in both backgrounds.

Linkage group 4 showed consistent order between three common markers (Tom 8.07, Tom 8.05 and E19). No rearrangements were observed (Figure 7).

Linkage group 5 showed linkage between four common markers (Tom 10.00, D40, Tom 8.0035 and D28) with one rearrangement occurring between a codominant marker (Tom 10.00) and a dominant marker (D40).

Linkage group 6 showed linkage in the two common markers (Tom 5.037 and D22) (Figure 8).

Linkage group 7 showed linkage between three markers; however, it showed a minor background-dependent rearrangement between two codominant markers (Tom 2.07 and Tom 3.10) (Figure 8).

Linkage group 8 showed two rearrangements. One of these rearrangements involved only codominant markers (Tom 10.07 and Nic Alata 10), which are the most reliable markers regarding segregation distortion. These two codominant markers were linked in both backgrounds, but differed with regard to order (Figure 8). The other rearrangement occurred between a codominant marker (Tom 1.137) and a dominant marker (F20).

Linkage group 9, while only mapped in the PL background, did show linkage in the LP background between the two codominant markers (Tom 6.0392 and Tom 6.0395). Therefore, the codominant loci of this linkage group, not necessarily the order, have been confirmed in both backgrounds (Figure 8).

Linkage group 10 showed linkage between two common markers (F39 and Tom 3.133) in both backgrounds (Figure 8).

D. Comparative Mapping in Solanaceae: Tomato vs. *Nicotiana*

After constructing and comparing our linkage maps, we then observed the placement of COSII markers relative to their positions in tomato (Figure 7 and Figure 8). We observed numerous rearrangements between tomato and *Nicotiana*. In addition, 18 of the 31 mapped COSII markers have also been mapped in other Solanaceae species, such as eggplant or pepper, which allowed us to observe other possible rearrangements with regard to those markers (Muller et al. 2005; Wu et al. 2009a; Wu et al. 2009b).

Linkage Group 1 contains homeologous markers from tomato chromosomes 2 and 12 (2.00 and 12.03). Both markers mapped near the telomeres of both chromosomes in tomato, thus a possible fusion relative to tomato may have occurred. The marker Tom 2.00 did not link up with the other two markers on chromosome 2 (Tom 2.07 and Tom 2.135); however, this may simply be due to the lack of any markers close enough to link all three markers together. The COSII marker Tom 12.03 has been mapped to pepper chromosome 9 (9.106) (Wu et al. 2009a).

Linkage Group 2 shows partial conservation of marker content with homeologous tomato chromosome 4 in one section, but the other section shows markers that are homeologous to tomato chromosomes 1, 7, 10 and 11 (Figure 7). The COSII markers Tom 4.071 and Tom 7.003 have been mapped to pepper chromosomes 4 (4.098) and 7 (7.0214) (Table 1) (Wu et al. 2009a).

Linkage Group 3 shows partial conservation of marker content with homeologous tomato chromosomes 9 and 11 and may infer another fusion relative to tomato. This possible fusion is evident in only one background (PL); however, the dominant marker (G28) which served as an "allelic bridge" between chromosomes 9 and 11 was absent in the LP background (Figure 7). The COSII markers (Tom 9.0375; Tom 9.099; Tom 11; Tom 11.03; and Tom 11.08) have been mapped to pepper chromosomes 9 (9.00), 3 (3.012), 12 (12.054), 12 (12.01) and 11 (11.096), respectively (Table 1) (Wu et al. 2009a).

Linkage Group 4 showed partial conservation of marker content with respect to tomato chromosome 8 (Figure 7). Although unclear in our map, these loci have been part of an inversion that has been observed in pepper (Wu et al. 2009a). The COSII markers

Tom 8.054 has been mapped to pepper chromosome 1 (1.052) and Tom 8.077 has been mapped in pepper chromosome 1 (1.00) (Wu et al. 2009a).

Linkage Group 5 contains single markers from tomato chromosomes 8 and 10. The arrangement of this linkage group may indicate another chromosome fusion relative to tomato (Figure 8). The COSII markers Tom 8.0035 has been mapped to pepper chromosome 8 (8.00) and Tom 10.00 has been mapped to pepper chromosome 10 (10.00) and eggplant 4 (4.00) (Wu et al. 2009a; Wu et al. 2009b).

Linkage Group 6 showed partial conservation of marker content with respect to tomato chromosome 5. These loci have been part of an inversion that has been observed in pepper (Wu et al. 2009a). The COSII markers Tom 5.00 has been mapped in pepper chromosome 11 (11.057) and eggplant chromosome 3 (3.056) and Tom 5.037 has been mapped in pepper chromosome 11 (11.00) (Wu et al. 2009a; Wu et al. 2009b).

Linkage Group 7 indicates that a rearrangement involving loci homeologous to sections of tomato chromosomes 2 and 3 occurred (Figure 8). Two of the COSII markers in this linkage group, Tom 2.07 and Tom 2.135, have been mapped to pepper chromosome 2 (2.081) and eggplant chromosome 2 (2.054, respectively); and pepper chromosome 2 (2.107, Wu et al. 2009a; Wu et al. 2009b).

Linkage Group 8 indicates a rearrangement has occurred with loci that are homeologous to sections of tomato chromosomes 1 and 10 (Figure 8). The COSII marker, Tom 1.1372, mapped in pepper chromosome 1 (1.225) (Wu et al. 2009a).

Linkage Groups 9 shows conservation of marker order and content with two loci that is homeologous to segments of tomato chromosome 6 (Figure 8). The COSII marker, Tom 6.0395, mapped in pepper chromosome 6 (6.062) (Wu et al. 2009a).

Linkage Group 10 contained a single marker that is homeologous to a segment of tomato chromosome 3 (Figure 8). It is possible that this small group may combine with another linkage group (e.g. linkage group 7, where other tomato chromosome 3 markers are located) if further markers were to be added.

E. Localized Allelic Transmission Bias

As inferred in both maps, codominant markers in particular linkage groups showed localized allelic transmission bias based on their segregation rates (Figure 9; Figure 10; Figure 11; Figure 12). Localized allelic bias was inferred by the location of codominant markers throughout the linkage groups that distorted at $p < 0.05$. These markers are marked with a * and **, depending on their significance level, in Figure 11 and Figure 12. The codominant markers were more reliable because they show all three genotypes and were transferable between both backgrounds, unlike the dominant markers (ISSRs), and therefore were better for observing allelic bias.

Only two linkage groups, PL7 and PL9, in the maternal *N. plumbaginifolia* background (PL) (Figure 11) show some localized allele bias. Linkage group PL7 seems to be skewed towards *N. plumbaginifolia* alleles; whereas Linkage group PL9, was skewed towards *N. longiflora* alleles. The rest of the linkage groups did not show bias in either direction.

Again, two linkage groups in the maternal *N. longiflora* background (LP) show some localized allelic bias (Figure 12). Linkage groups LP2 and LP7 are skewed towards *N. plumbaginifolia* genotypes. No linkage groups showed bias towards *N. longiflora* genotypes.

IV. Discussion

A. Linkage Analysis

We present two reciprocal maps in an interspecific cross between *N. longiflora* and *N. plumbaginifolia*. We produced two maps with some variation in order among dominant markers, but with mostly consistent ordering between codominant markers (Figure 7 and Figure 8). The codominant marker order in both maps was confirmed in our combined codominant data set (Figure 6).

Previously, a linkage map with RAPD markers was created in a cross between *N. plumbaginifolia* and *N. longiflora.* This map consisted of nine major linkage groups spanning a total of 1062 cM (Lin et al. 2001). The distance found by Lin et al. does not differ greatly from the results found in our PL background map. Our map spans 1110.4 cM and contains one more linkage group, which may represent the known chromosome number (n = 10) of *N. plumbaginifolia* and *N. longiflora* (Goodspeed 1954, Lim et al. 2006), although further mapping is necessary to confirm this.

Both background maps, although different in size due to number of distorted and unusable markers, produced similar linkage groups (Figure 7 and Figure 8) and common orders in the codominant markers. Possible reasons for the discrepancy between the two maps include the following: (1) the limited ability of the dominant markers to transfer between backgrounds affected the number of usable markers in both backgrounds; (2) different recombination frequencies may have occurred for the *N. plumbaginifolia* and *N. longiflora* gametes (Fishman et al. 2001); (3) imperfect pairing between the *N. longiflora* and *N. plumbaginifolia* chromosomes (Lim et al. 2006) and (4) mapping and experimental errors within the data (Schranz et al. 2007).

The rearrangements of order between both maps are indicated in Figure 7 and Figure 8. There were many linkage groups that showed strong synteny blocks, especially among codominant markers, in both backgrounds. In almost all linkage groups codominant markers aligned consistently in both backgrounds (except a small discrepancy in Linkage Group 8). Furthermore, the combined codominant marker data set (Figure 6) confirmed our codominant order in both maps.

The main difference between the background maps was the presence and distribution of the dominant markers. Due to the limited ability of the dominant markers to effectively transfer between backgrounds, both maps contained dominant markers that were absent in the other background (Figure 7 and Figure 8).

We observed rearrangements of order in Linkage Groups 1, 5 and 8. The rearrangements in Linkage Group 1, which involved only dominant markers, and in Linkage Groups 5 and 8, which involved a codominant and dominant marker, are not informative enough to infer a rearrangement. Due to the presence of a dominant marker in these rearrangements, it is very difficult to confirm if these are actual rearrangements, or merely a by-product of the limited dominant markers and mapping error.

The other two rearrangements evident in Linkage Groups 7 and 8 involved two codominant markers, which were proven to be more reliable than the dominant markers (Figure 8). Despite containing only codominant markers, both rearrangements are suspect. The rearrangement in Linkage 7 involved a small rearrangement between two very closely linked loci. If a larger sample size was used, it is feasible that this rearrangement could disappear due to the limited number of segregation events witnessed in this study. Finally, the rearrangement involving codominant markers in Linkage

Group 8 may be the product of mapping error (Figure 8). Furthermore, the combined codominant data set (Figure 6) confirmed the order of codominant markers found in the PL background (PL10). Therefore, both of these rearrangements involving codominant markers may not be reliable.

All chromosomes in *N. plumbaginifolia* a*nd N. longiflora* may not be represented in these maps. Because some linkage groups were very small (3 or 4 markers) and other tentative linkage groups failed to map, we cannot be sure that all 10 chromosomes are represented. In fact, we would expect some of the small linkage groups to coalesce with other groups and new linkage groups to appear in a larger mapping study. Finally, because neither map is fully saturated, there may be other rearrangements that are not included in this first map.

B. Background-Dependent Segregation Distortion

We analyzed the segregation distortion in both backgrounds and across all linkage groups. The amount of distortion varied moderately in both codominant and dominant markers and differed moderately between the maternal-cytoplasmic backgrounds, especially in the dominant markers (Appendix 1 and Table 1). This is the reason we implemented a reciprocal cross for the construction of our linkage maps, to detect nuclear-cytoplasmic influence on segregation distortion rates (Fishman et al. 2005).

A common theme in distorted markers ($p < 0.001$), especially in the LP background, was the overall preference of the *N. plumbaginifolia* alleles over the *N. longiflora* alleles (Table 1 and Appendix 1). The dominant markers in both backgrounds showed strong bias in the unusable distorted ($p < 0.001$) markers (Appendix 1). The majority of unusable distorted dominant markers in LP and PL (74.0% and 72.2%)

showed an excess of the *N. plumbaginifolia* genotypes over the *N. longiflora* genotypes (Appendix 1). This *N. plumbaginifolia* bias was also evident in the codominant markers.

In the LP background, most moderately distorted markers (20 of 27 dominant markers and the six codominant markers that showed a lower significance ($p < 0.05$)) were distorted because of an underrepresentation of the *N. longiflora* alleles (Table 1 and Appendix 1). This is surprising because *N. longiflora* was the maternal parent, suggesting that cytoplasmic incompatibility is not responsible for the distortion (Schranz et al. 2007). There seems to be a general preference in the LP background for the *N. plumbaginifolia* allele across most distorted markers.

This strong preference of the *N. plumbaginifolia* allele in this cross is not unprecedented. Lin et al. found most of their markers (84% RFLP loci and 63% RAPD loci) were skewed towards the *N. plumbaginifolia* allele in their cross of *N. plumbaginifolia* and *N. longiflora* (Lin et al. 2001). Furthermore, unequal segregation of genetic markers is a common feature of interspecific populations and may be attributed to structural differences or loci that affect gamete transmission in the region with distorted segregation (Doganlar et al. 2002b). There are several potential causes that may cause distortion from the expected Mendelian ratios. First, there may be active processes that influence the segregation of alleles immediately prior to, during, and immediately following meiosis (e.g. direct gamete competition or meiotic drive) (Fishman and Willis 2005; Moyle and Graham 2006). Alternatively, segregation distortion observed in distant crosses, such as those between a species, could be due to inadvertent selection against dysfunctional heterospecific allelic combinations. Hybrid incompatibility is known to be frequently caused by disrupted genetic interactions among loci that have diverged in

isolated parental lineages (Fishman and Willis 2001; Hall and Willis 2005; Moyle and Graham 2006). These specific allelic combinations can cause inviability and remove these specific allelic combinations from the mapping population. This nonrandom elimination of specific allelic combinations is the most common explanation for segregation distortion in mapping populations (Fishman and Willis 2001; Hall and Willis 2005; Moyle and Graham 2006).

Another explanation for the *N. plumbaginifolia* bias in the maternal *N. longiflora* background (LP) is due to the purging of deleterious mutations that is expected in selfing species (Byers and Waller 1999; Crnokrak and Barrett 2002). If mutational load can be purged through selection on deleterious alleles, then selfing may have an advantage over outcrossing. Nevertheless, complete purging is rare, at least over experimental time frames (Byers and Waller 1999; Crnokrak and Barrett 2002). If *N. longiflora* populations are still segregating for deleterious mutations, which have been selected out of *N. plumbaginifolia* that could explain the general *N. plumbaginifolia* preference. In contrast, Fishman et al. 2001 found in the one background they mapped, that the outcrossing species' allele was favored.

C. Localized Allelic Transmission Bias in Particular Linkage Groups

We analyzed which, if any, of the linkage groups showed localized transmission ratio distortion in each background (Figures 9, 10, 11 and 12) and whether these localized allelic biases were evident in both linkage groups that comprised the comparative map in Figures 7 and 8. The limitation to this analysis was the lack of complete genotype information across all linkage groups from the dominant markers. The use of dominant

markers allows only the observation of the homozygous recessive genotypes and fails to indicate the frequency of the other genotypes (Figure 9 and Figure 10).

All of the observed localized transmission bias was background-dependent. Linkage groups LP2 and LP7, which collectively comprise Linkage Group 2 with PL3, collectively showed a bias towards *N. plumbaginifolia* genotypes (Figure 7 and Figure 12). PL3 did not show bias. Another linkage group that exhibited bias, PL9, only showed bias in its background (PL) (Figure 11) and its background counterpart (LP8) (Figure 8) exhibited no general bias for either genotype (Figure 12). Finally, the bias in linkage group PL7 could not be compared to its maternal *N. longiflora* counterpart because it did not map in that background.

Much of this localized bias may be attributed to the significance threshold to distinguish distorted and usable markers ($p < 0.001$). This allowed for distortion-skewed markers to be mapped and infer bias throughout the linkage groups. This localized bias may also be caused by certain loci in linkage groups that are ultimately affecting gamete transmission (Doganlar et al. 2002b).

D. Comparative Mapping within Solanaceae

Use of genomic information gathered in model organisms greatly increases the number of genetic markers available in a related non-model system. The development of COSII database has identified putative orthologs across multiple species in Solanaceae that may be used for comparative mapping. Our use of COSII markers allows us the opportunity to further investigate genome evolution in Solanaceae (Fulton et al. 2002; Wu et al. 2006). *Nicotiana* is not in the same clade as *Solanum* (tomato, potato and eggplant) and *Capsicum* (pepper), which have been the subject of COSII maps, but it still

retains the primitive karyotype (n = 12) found throughout Solanaceae (Figure 13) (Knapp 2002; Olmstead et al. 2005; Wu et al. 2006).

Phylogenetic studies have attempted to estimate the time of divergence within Solanaceae (Figure 13). It has been predicted that within *Solanum*, it was ~12 MYA when tomato and eggplant diverged from a common ancestor (Wu et al. 2009b). Expanding further out on the phylogenetic tree, it has been estimated that it was ~20 MYA that *Capsicum* (pepper) and *Solanum* (tomato, pepper and eggplant) diverged from a common ancestor. Due to the limited fossil record that exists in Solanaceae, it has not been estimated how long it has been since the divergence between *Nicotiana* and *Solanum* or *Capsicum* (Knapp 2002; Olmstead et al. 2005).

There have been many comparative maps in Solanaceae, including those comparing potato, pepper and eggplant to tomato. Previous comparative studies have shown that tomato and potato differ by only five paracentric inversions (inversions that did not involve the centromere) (Tanksley et al. 1992); tomato and pepper differ by 25 rearrangements including several translocations and both pericentric (inversions involving the centromere) and paracentric inversions (Livingstone et al. 1999; Wu et al. 2009a). Eggplant and tomato differ by 24 paracentric inversions and five translocations (Doganlar et al. 2002a; Wu et al. 2009b). Our *Nicotiana* map, the first Solanaceaous n = 10 map, is expected to have at least 2 chromosomal fusions to account for the evolution of n = 12 to n = 10 karyotype. Many rearrangements are expected due to the greater distance between *Nicotiana* and *Solanum*.

In comparing map sizes among the Solanaceae maps, our map size is smaller than the greatly saturated linkage maps that exist in other Solanaceae species. For example, a

saturated COSII map of pepper (*Capsicum annuum* x *Capsicum frutscens*) contains 381 markers and is 1613 cM long (Wu et al. 2006; Wu et al. 2009a); eggplant (*S. linnaeanum* x *S. melongena*) contains 232 markers (110 are COSII) and is 1531 cM (Wu et al 2006; Wu et al. 2009b); finally, tomato (*Solanum lycopersicum* x *S. pennellii*) is the most saturated with 1459 markers (877 COSII) covering a distance of 1460 cM (Fulton et al. 2002). In comparison, our PL map (Figure 4) covered a distance of only 1110.4 cM and contained 54 markers.

E. Homeologous Tomato Segments in the Nicotiana Map

The linkage groups of tomato and *Nicotiana* show many possible chromosomal rearrangements since evolving from their common ancestor. Additionally, some of these rearrangements have been shown to occur in other Solanaceae species (Wu et al. 2009a; Wu et al. 2009b). The comparison of our background-dependent linkage maps (Figures 7 and 8) allowed us to observe possible synteny relative to tomato, potato and eggplant.

It appears that many chromosomal rearrangements have occurred between tomato and *Nicotiana*. For example, our three markers from tomato chromosome 10 were dispersed on three separate linkage groups (Figures 7 and 8). However, this is not unexpected, since karyotypes of these species differ significantly and much chromosomal reorganization has probably occurred during evolution (Ahn and Tanksley 1993). Furthermore, there has been considerable time since tomato and *Nicotiana* evolved from a common ancestor (Figure 13) (Knapp 2002; Olmstead et al. 2008).

Due to this long divergence between *Nicotiana* and tomato, it is feasible that many rearrangements have occurred. Although our maps appear to contain many chromosomal rearrangements relative to tomato, we are unable to completely infer what

type of rearrangements they are (translocations, fusions, or inversions) until the map becomes more saturated. Upon further saturation of the map, we would expect some types of chromosomal rearrangements to be more prevalent than others.

Previous studies have indicated that paracentric inversion of relatively conserved segments has been the primary mechanism for chromosome evolution in Solanaceae (Tanksley et al. 1992; Doglanar et al. 2002b; Wu et al. 2009a; Wu et al. 2009b). It has been proposed that inversions are more frequent than translocations in wild populations because chromosomal interchanges usually have negative effects on an organism's fertility (Doganlar et al. 2002b). In addition, telomere-telomere fusions have shown to be important factors in the evolution of Solanaceae genomes (Doganlar et al. 2002b). In tomato, telomeric sequences have been identified at the centromeres of eight tomato chromosomes (Presting et al. 1996) and influenced the rearrangements in the pepper genome (Livingstone et al. 1999).

We observed three linkage groups, which upon further saturation of the map may contain inversions relative to tomato. The loci in Linkage Groups 3, 4 and 6 (Figures 7 and 8) were involved in paracentric inversions in other Solanaceae mapping studies. The inversions of tomato chromosome 11 (Linkage Group 3), the long arm of chromosome 8 (Linkage Group 4) and chromosome 5 (Linkage Group 6) relative to tomato have been observed in pepper (Wu et al. 2009a). In pepper, Tom 11.00 and Tom 11.031 were involved in a paracentric inversion to pepper chromosome 12 (12.054 and 12.012); Tom 8.077 and Tom 8.054 involved a paracentric inversion to pepper chromosome 1 (1.00 and 1.052); and Tom 5.00 and Tom 5.037 were involved in a paracentric inversion to pepper chromosome 11 (11.057 and 11.00) (Wu et al. 2009a).

We also observed rearrangements that may be chromosomal fusions relative to tomato. There were possible fusions relative to tomato in Linkage Groups 1 (tomato chromosomes 2 and 12), 3 (tomato chromosomes 9 and 11) and 5 (tomato chromosomes 8 and 10) (Figures 7 and 8). In Linkage Group 1, Tom 12.03 has been involved in a translocation to Pepper 9.106 (Wu et al. 2009a), so this substantiates our observation that this locus may have been involved in a rearrangement although it does not verify if this is a fusion. Finally, in Linkage Group 5, Tom 10.00 has been involved in a translocation to eggplant to chromosome 4 (Wu et al. 2009b), so it is feasible that it has been involved in a rearrangement although it does not verify if this is a fusion.

It is not possible at this point to infer how many translocations have occurred between tomato and *Nicotiana* based upon the current saturation of the map. Many of the possible translocations that we presently observe may ultimately be proven to be another type of rearrangement upon further saturation. However, it is not surprising finding particular tomato chromosomes scattered throughout our linkage map as most have been rearranged relative to other Solanaceae species in other studies, such as tomato chromosomes 3, 4, 5, 11 and 12 (Doganlar et al. 2002b; Wu et al. 2009a; Wu et al. 2009b). We would expect more chromosomal rearrangements to have occurred in *Nicotiana* because of its phylogenetic placement relative to other COSII-mapped Solanaceae (Figure 13). Furthermore, our species have n = 10, while all other maps were of species with n = 12 (Aoki and Ito 2000; Lim et al. 2006).

Finally, it is interesting to note that none of the three markers that are homeologous with tomato chromosome 10 showed linkage to each other despite their original close proximity in Tomato (10.00, 10.0032, and 10.07). Although it is too early

to tell, the ancestral form of tomato chromosome 10 may have been one of the chromosomes that was broken up and fused into several others during the evolution from n = 12 to n = 10. Further saturation of the *Nicotiana* map with COSII markers from tomato chromosome 10 would be needed to confirm this.

V. Conclusion

Although there appears to be some minor rearrangements between *N. plumbaginifolia* and *N. longiflora*, their genomes appear to be similar. Both linkage maps show strong blocks of synteny relative to their codominant markers. Although there was some segregation distortion and a slight allelic bias towards *N. plumbaginifolia* evident in our crosses, it was not strong enough to indicate any hybrid incompatibilities between the species.

Although the maps were not saturated enough to distinguish what chromosomal rearrangements have occurred since the divergence of *Nicotiana* and tomato, it has shown that many rearrangements are expected between the two. Future focus should be placed on the saturation of the map with more markers from the COSII set that are homeologous to tomato and other Solanaceae species. This will allow a stronger observation of any major chromosomal rearrangements that have occurred during the genomic evolution from n =12 to n =10 within Solanaceae. The more markers added to the map will enhance our ability to detect synteny throughout Solanaceae.

Finally, this study will help facilitate future comparative studies because it has shown the use and transferability of COSII markers within Solanaceae. By showing that genetic markers that have been developed in model crop systems within *Solanum* and *Capsicum*, such as COSII, may be readily used in non-model systems will greatly enhance marker development in non-model systems. Furthermore, the development and use of COSII markers in *Nicotiana* will help facilitate the detection of quantitative trait loci (QTL) of important mating system traits.

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Table 1: Cleaved Amplified Polymorphic Sequence (CAPS) markers (n = 31) used for linkage maps in the reciprocal *N. longiflora* X *N. plumbaginifolia* F_2 mapping populations. Markers are indicated by their original map location in tomato or unmapped origin from *Nicotiana alata*, plus their NCBI accession numbers. The loci used for mapping show a significance level of less than p = 0.001 in either or both backgrounds (PL = maternal *N. plumbaginifolia* background; LP = maternal *N. longiflora* background; B = indicates both backgrounds). Significance levels of X^2 tests are indicated by ns (no significance); * ($p < 0.05$); ** ($p < 0.01$). Background-specific allelic bias in all distorted markers ($p < 0.05$) is indicated by the allelic preference exhibited by the locus. RE: refers to restriction enzyme.

Locus	NCBI Name	Segregation Distortion	Allelic Bias	RE	Mapped
Nic Alata 10	AY159325	ns	None	MnI1	Yes
Nic Alata 6	AF304375	ns	None	AhdI	No
Tom 1.0492	At4g15520.1	LP**	Plum (LP)	TaqI	Yes
Tom 1.1372	At5g64350	ns	None	BsrI	Yes
Tom 2.00	At1g30580	ns	None	EcoRI	Yes
Tom 2.0701	At1g11430	ns	None	Csp 6I	Yes
Tom 2.1350	At4g37280	ns	None	TaqI	Yes (PL)
Tom 3.106	At1g61620.1	ns	None	HindIII	Yes
Tom 3.1335	At1g09760	ns	None	HinfI	Yes
Tom 4.0715	At1g71810	ns	None	EcoRV	Yes
Tom 4.1097	At1g74970.1	ns	None	Eco RI	Yes
Tom 4.1295	At2g45730	ns	None	EcoRI	Yes
Tom 5.00	At1g60440.1	ns	None	AluI	Yes (PL)
Tom 5.037	At2g01110.1	ns	None	FokI	Yes
Tom 6.0392	At1g44575.1	B*	Plum (B)	HaeIII	Yes (PL)
Tom 6.0395	At1g44760.1	PL*	None	TaqI	Yes (PL)
Tom 7.0003	At2g24270.1	LP**	Plum (LP)	HhaI	Yes
Tom 8.0035	At4g31130.1	ns	None	Csp 6I	Yes
Tom 8.054	At5g47010	ns	None	TaqI	Yes
Tom 8.0770	At1g63980.1	ns	None	SspI	Yes
Tom 9.03757	At5g06130.2	ns	None	Bst BI	Yes (PL)

Table 1	Continued				
Tom 9.045	At2g38025.1	ns	None	TaqI	Yes (PL)
Tom 9.099	At3g24010	LP*	Plum (LP)	BsI I	Yes (PL)
Tom 10.00	At3g13235.1	ns	None	MspI	Yes
Tom 10.0032	At5g06430	ns	None	AvaII	Yes (PL)
Tom 10.0715	At3g09740.1	ns	None	BsrI	Yes
Tom 11.00	At5g09880.1	LP*	Plum (LP)	BsrI	Yes (PL)
Tom 11.0314	At5g16710.1	ns	None	HaeIII	Yes (PL)
Tom 11.04	At1g44446	LP**	Plum (LP)	NdeI	Yes (PL)
Tom 11.0895	At5g25760	PL*	Plum (PL)	PCR	Yes (PL)
Tom 12.0327	At2g06530	ns	None	TaqI	Yes

FIGURE 1

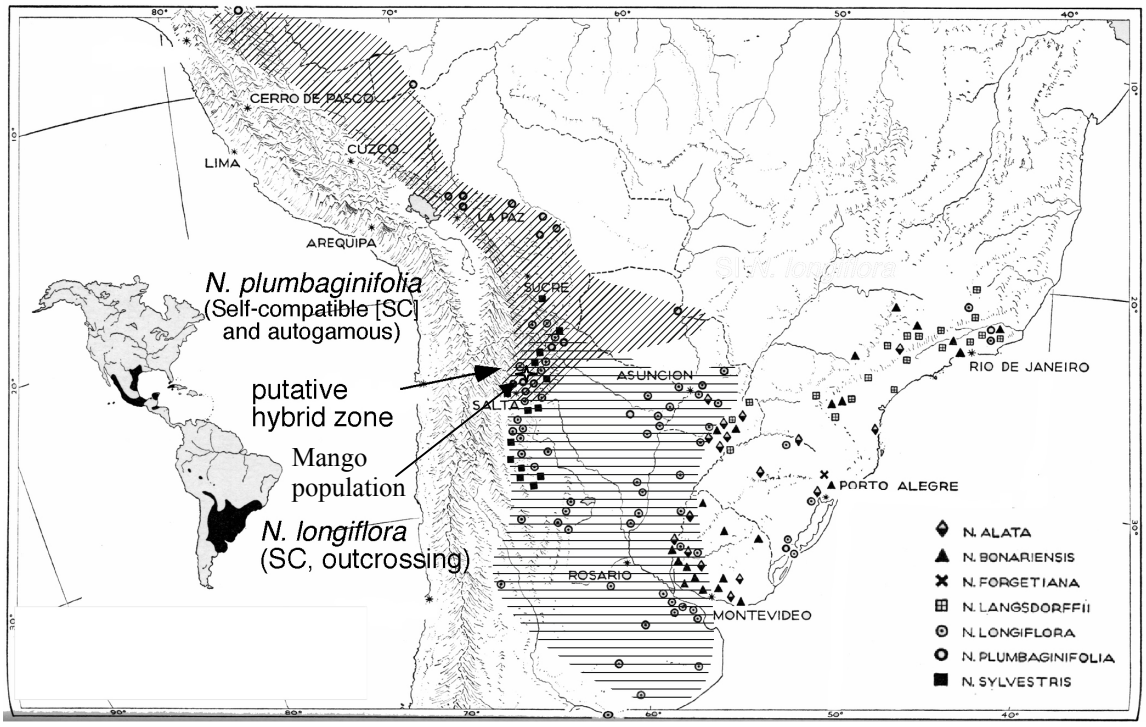

Figure 1: Geographical ranges of *N. longiflora* and *N. plumbaginifolia*. Populations overlap and form sympatric populations in Northern Argentina (Goodspeed 1954). Approximate location of the Mango population is indicated by arrow.

FIGURE 2

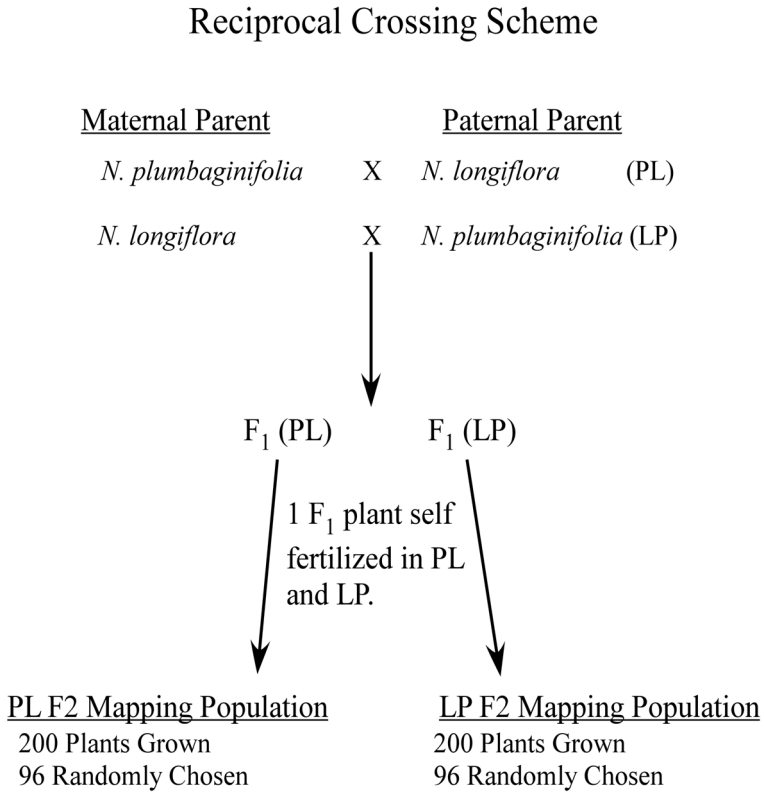

Figure 2: Reciprocal crossing scheme used for making the F_2 mapping populations. A single *N. longiflora* parent was reciprocally crossed with a single *N. plumbaginifolia* parent to produce the F_1 generations. A single F_1 plant was chosen from each reciprocal cross and self-fertilized to produce the F_2 mapping populations.

FIGURE 3

Figure 3(a)

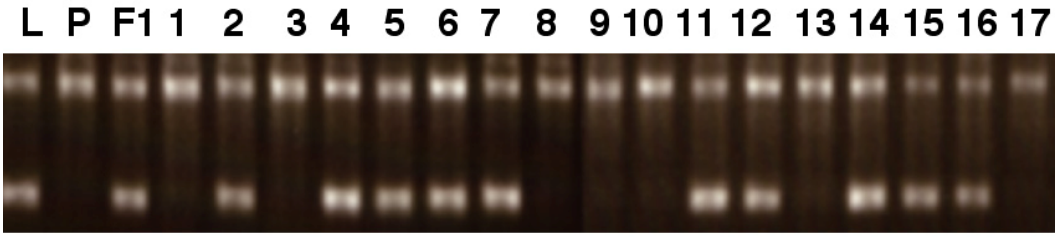

Figure 3(b)

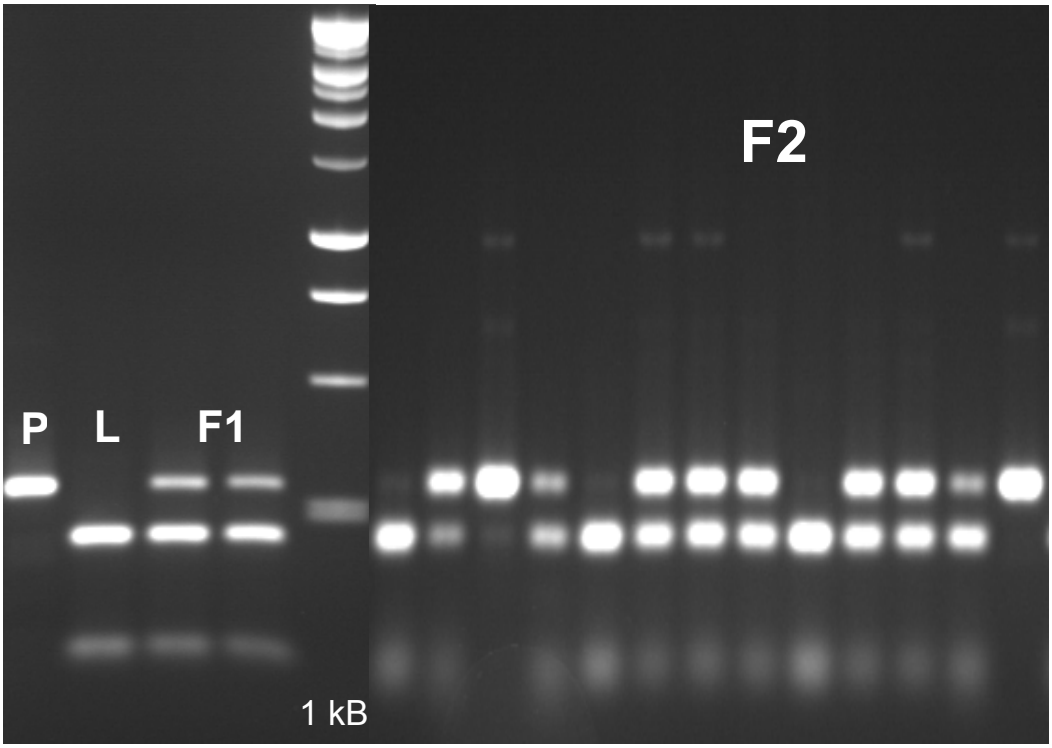

Figure 3: Agarose composite gels of the genetic markers used for hybrid linkage maps between *N. longiflora* and *N. plumbaginifolia*. Figure 2(a): An example of the dominant marker from Inter-Simple Sequence Repeats (ISSRs). Markers are scored based on band presence. In this example, the scorable band is present in *N. longiflora*, but absent in *N. plumbaginifolia*. Figure 2(b): A codominant marker from the COSII set (Tomato 11.04) digested with *NdeI* in *N. longiflora* and *N. plumbaginifolia* parents, F_1 and F_2 generations.

Genotypes are indicated by the presence of alleles 1 (300 bp/*N. plumbaginifolia*) and allele 2 (250 bp + 100 bp/*N. longiflora*). These alleles were scored against a 1 Kb ladder.

FIGURE 4

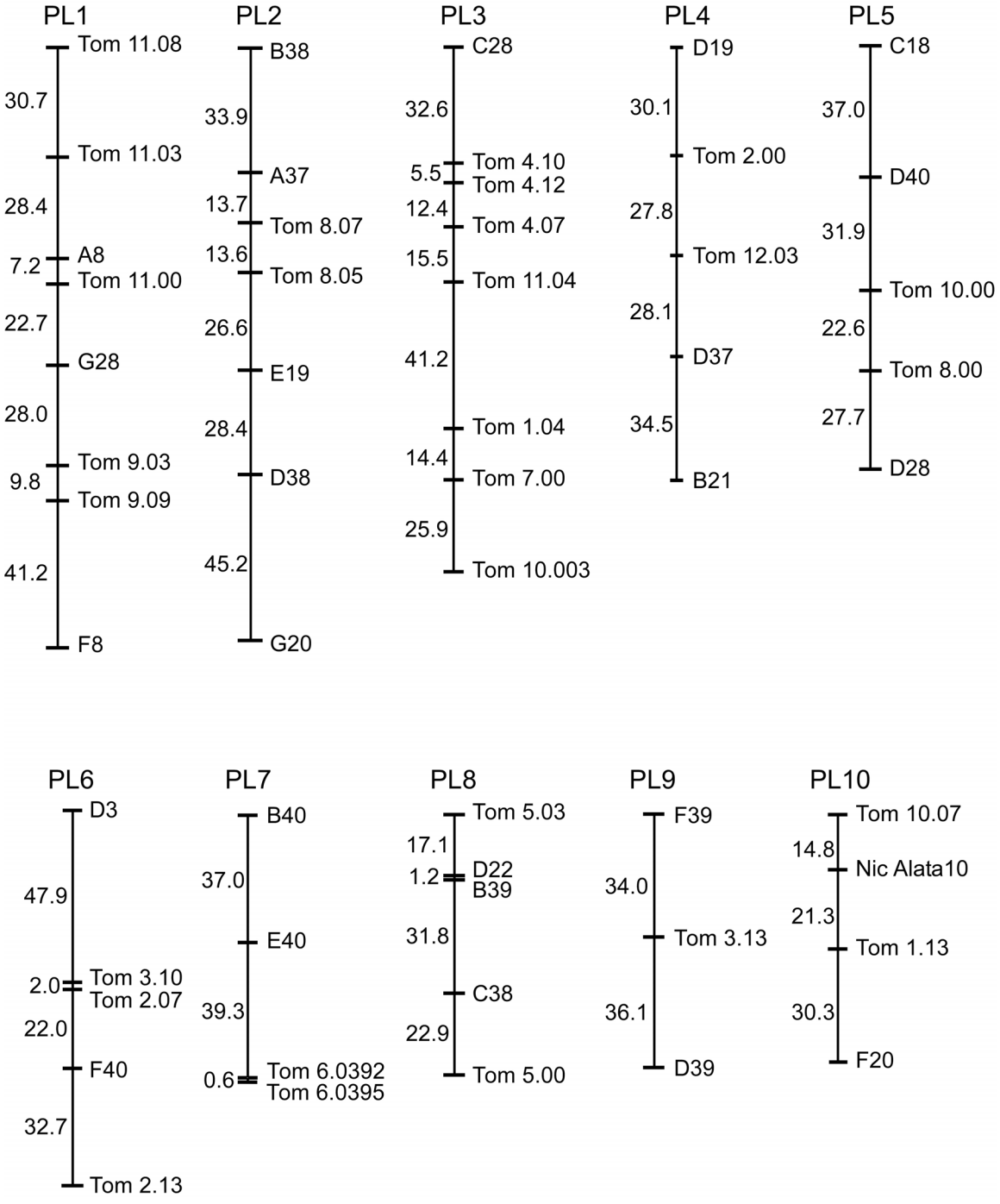

Figure 4: Linkage map of maternal *N. plumbaginifolia* F_2 population. The names of the codominant markers (CAPS) are indicated by either their location in tomato (e.g. Tomato 2.00) or their species of origin (Nic Alata 10). The names of the dominant markers

(ISSRS) are indicated by their band and primer (e.g. E1). Linkage groups are labeled PL(#) to indicate the maternal *N. plumbaginifolia* background. The map consists of 54 markers and covers a total of 1110.4 cM with an average distance of 25.23 cM between markers. Linkage Groups 1 to 10 range from 168 cM to 66.4 cM and consist of 3 to 8 markers.

FIGURE 5

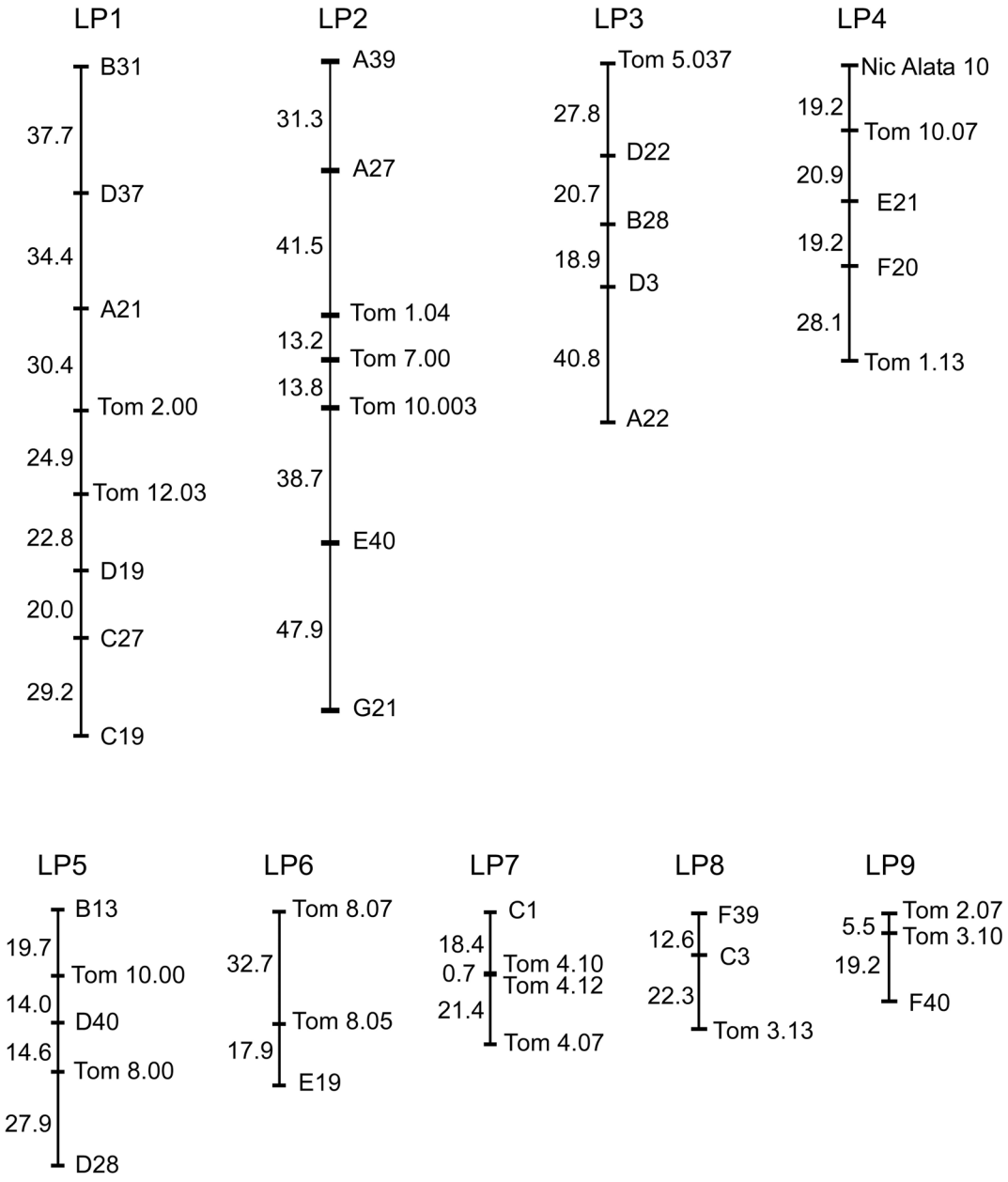

Figure 5: Linkage map of maternal N. longiflora F_2 population. The names of the codominant markers (CAPS) are indicated by either their location in tomato (e.g. Tomato 2.00) or their species of origin (Nic Alata). The names of the dominant markers (ISSRS) are indicated by their band and primer (e.g. E1). Linkage groups are labeled LP(#) to indicate the maternal *N. longiflora* background. The map consists of 43 markers and

covers a total of 808.1 cM with an average distance of 23.7 cM between markers. Linkage Groups 1 to 9 range from 199.4 cM to 24.7 cM and consist of 3 to 8 markers.

FIGURE 6

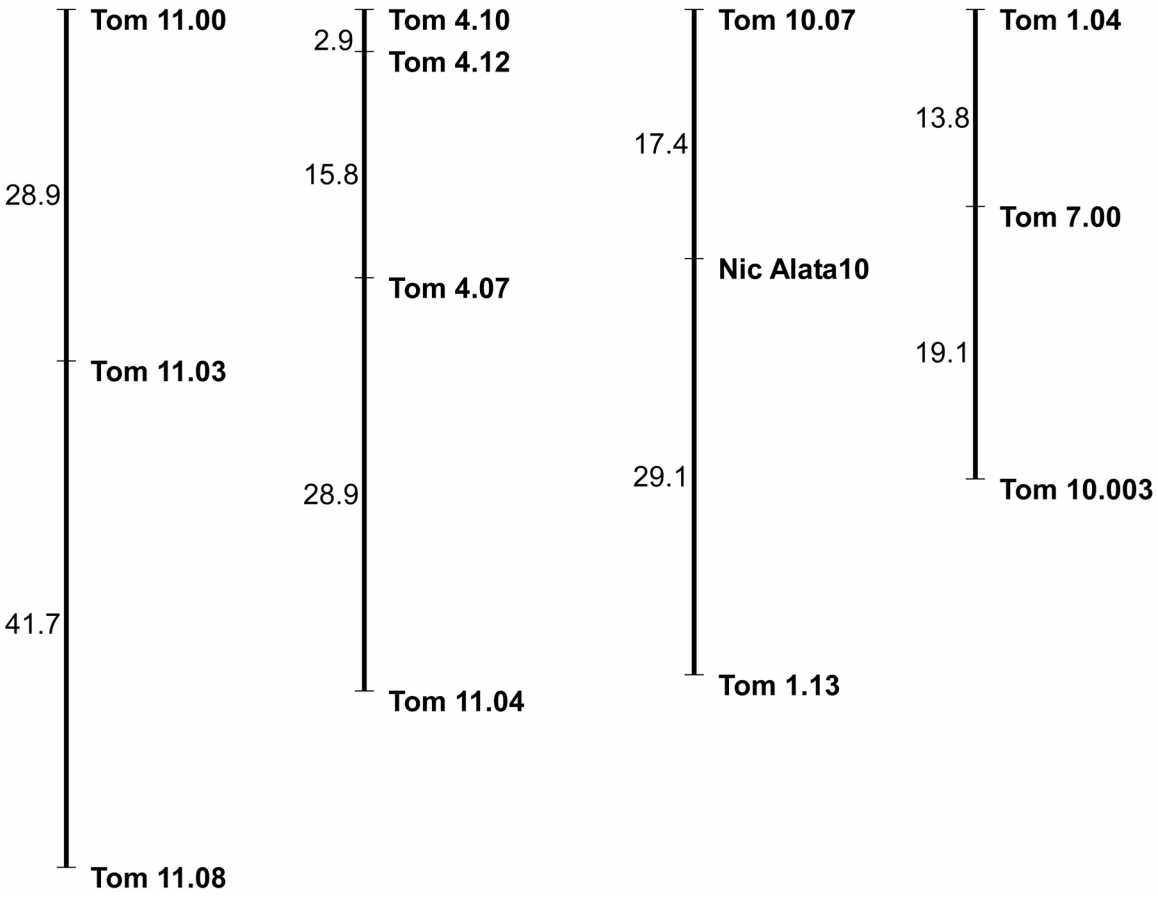

Figure 6: Combined codominant marker linkage groups. The four mapped linkage groups produced by the codominant marker data alone, combined from both backgrounds.

FIGURE 7

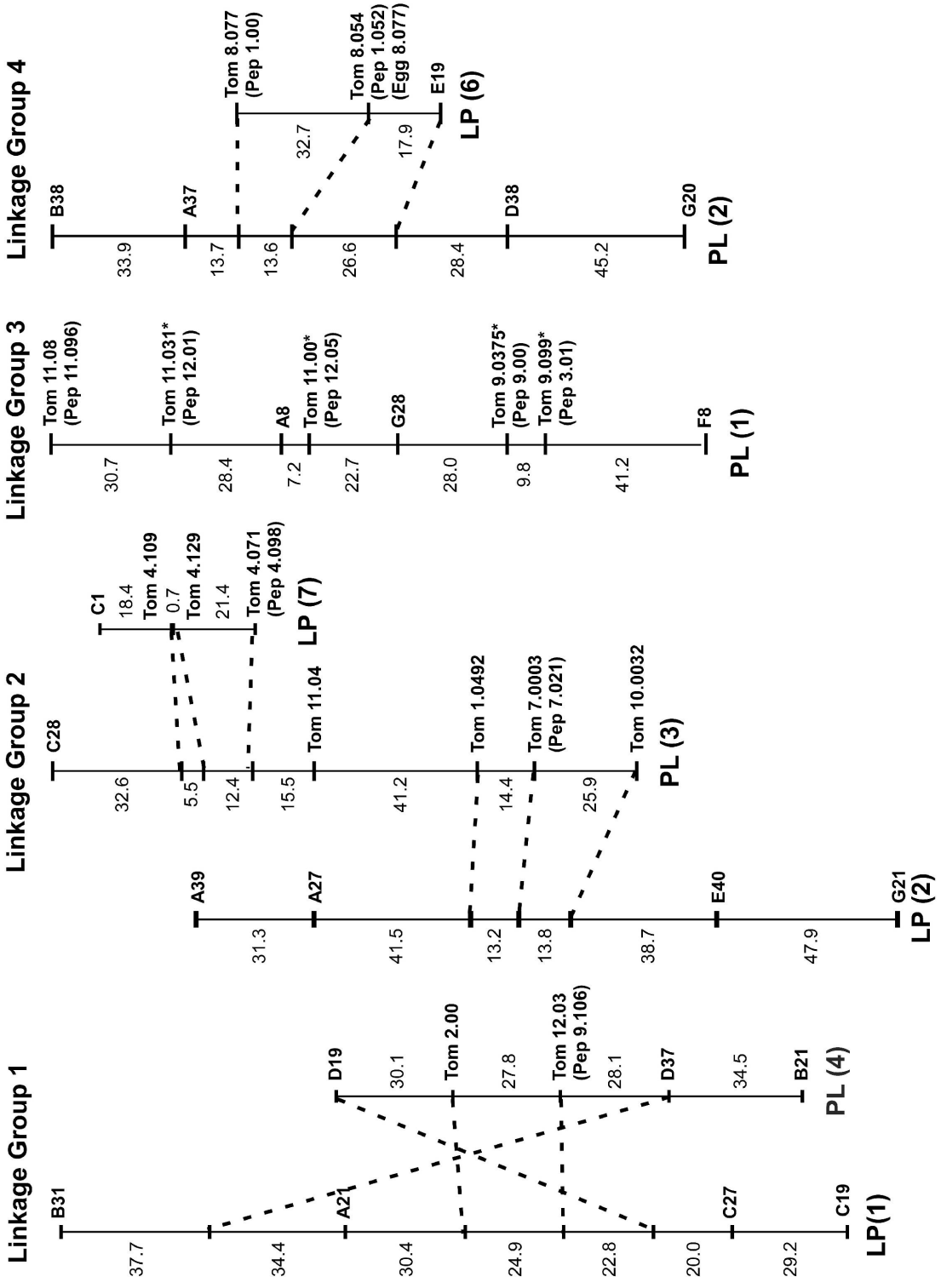
Linkage Group 4
B38
33.9
A37
13.7
Tom 8.077
(Pep 1.00)
13.6
32.7
Tom 8.054
(Pep 1.052)
(Egg 8.077)
26.6
17.9
E19
LP (6)
28.4
D38
45.2
G20
PL (2)
Linkage Group 3
Tom 11.08
(Pep 11.096)
30.7
Tom 11.031*
(Pep 12.01)
28.4
A8
7.2
Tom 11.00*
(Pep 12.05)
22.7
G28
28.0
Tom 9.0375*
(Pep 9.00)
9.8
Tom 9.099*
(Pep 3.01)
41.2
F8
PL (1)
Linkage Group 2
C1
18.4
Tom 4.109
0.7
Tom 4.129
21.4
Tom 4.071
(Pep 4.098)
LP (7)
C28
32.6
5.5
12.4
15.5
Tom 11.04
41.2
Tom 1.0492
14.4
Tom 7.0003
(Pep 7.021)
25.9
Tom 10.0032
PL (3)
A39
31.3
A27
41.5
13.2
13.8
38.7
E40
47.9
G21
LP (2)
Linkage Group 1
B31
37.7
34.4
A21
30.4
D19
30.1
Tom 2.00
27.8
Tom 12.03
(Pep 9.106)
24.9
28.1
D37
22.8
34.5
B21
PL (4)
20.0
C27
29.2
C19
LP(1)

Figure 7: Comparison of linkage groups (1-4) made from a reciprocal cross of *N. longiflora* X *N. plumbaginifolia* F_2 population. Linkage groups are ordered by relative size and grouped based on comparative linkage groups in both backgrounds. Horizontal lines connecting the pairs of linkage groups indicate markers mapped in both backgrounds. Estimated Haldane map distances (cM) between adjacent markers are shown to the left of the vertical line representing each linkage group. Codominant markers marked with an * denotes those markers that were linked in the other background, but failed to map in the other background. Comparative linkage groups are denoted by their order in their respective background map (e.g. PL(3) is the third linkage group in the PL map). Markers that are also mapped in Pepper (Pep) and Eggplant (Egg) are marked in parenthesis with the mapped location (e.g. (Pep 2.107) refers to Pepper chromosome 2).

FIGURE 8

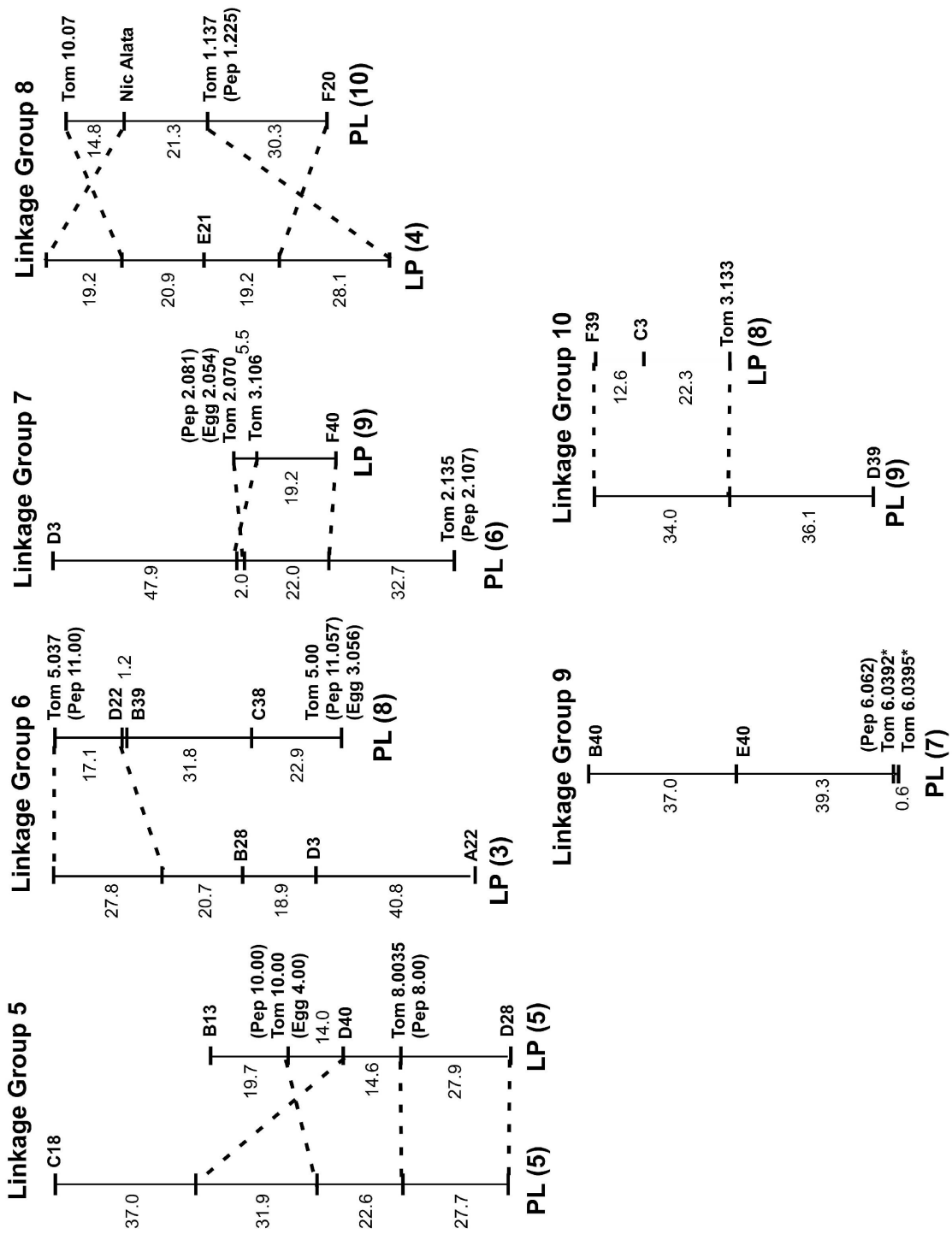
Linkage Group 5
C18
37.0
B13
(Pep 10.00)
Tom 10.00
(Egg 4.00)
19.7
31.9
14.0
D40
14.6
22.6
Tom 8.0035
(Pep 8.00)
27.9
27.7
D28
PL (5)
LP (5)
Linkage Group 6
27.8
Tom 5.037
(Pep 11.00)
17.1
D22
1.2
B39
20.7
31.8
B28
18.9
C38
Tom 5.00
(Pep 11.057)
(Egg 3.056)
22.9
D3
40.8
PL (8)
A22
LP (3)
Linkage Group 7
D3
47.9
(Pep 2.081)
(Egg 2.054)
Tom 2.070
2.0
Tom 3.106
5.5
22.0
19.2
F40
LP (9)
32.7
Tom 2.135
(Pep 2.107)
PL (6)
Linkage Group 8
19.2
Tom 10.07
14.8
Nic Alata
20.9
21.3
E21
19.2
Tom 1.137
(Pep 1.225)
30.3
28.1
F20
PL (10)
LP (4)
Linkage Group 9
B40
37.0
E40
39.3
(Pep 6.062)
Tom 6.0392*
0.6
Tom 6.0395*
PL (7)
Linkage Group 10
F39
12.6
C3
34.0
22.3
Tom 3.133
LP (8)
36.1
D39
PL (9)

Figure 8: Comparison of linkage groups (5-10) made from a reciprocal cross of *N. longiflora* X *N. plumbaginifolia* F_2 population. Linkage groups are ordered by relative size and grouped based on comparative linkage groups in both backgrounds. Horizontal lines connecting the pairs of linkage groups indicate markers mapped in both backgrounds. Estimated Haldane map distances (cM) between adjacent markers are shown to the left of the vertical line representing each linkage group. Codominant markers marked with an * denotes those markers that were linked in the other background, but failed to map in the other background. Comparative linkage groups are denoted by their order in their respective background map (e.g. PL(3) is the third linkage group in the PL map). Markers that are also mapped in Pepper (Pep) and Eggplant (Egg) are marked in parenthesis with the mapped location (e.g. (Pep 2.107) refers to Pepper chromosome 2).

FIGURE 9

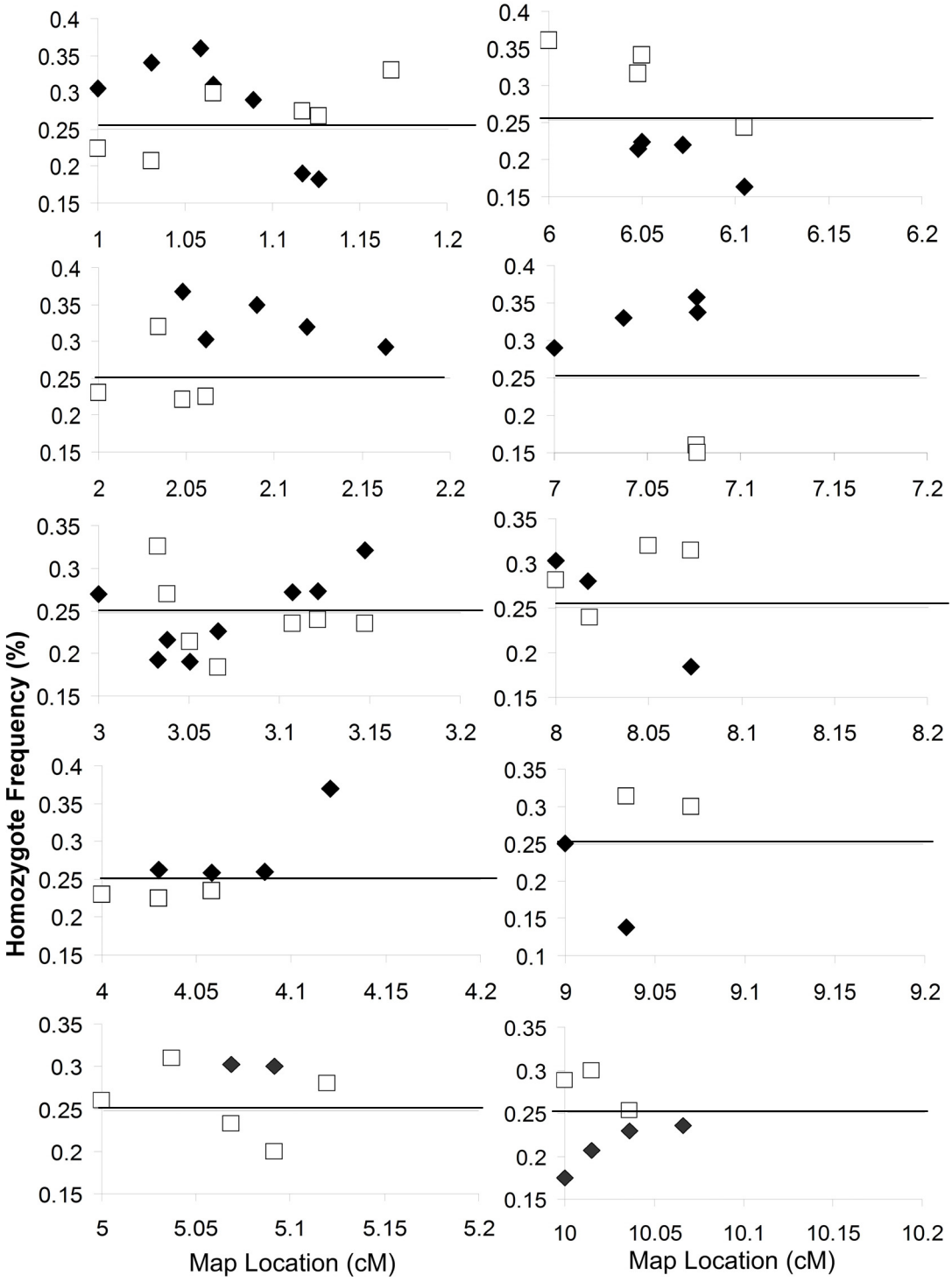

Figure 9: Transmission ratio distortion (TRD) across the maternal *N. plumbaginifolia* F_2 linkage map. The □ and ◊ symbols represent the two homozygous parental genotypes (*N. longiflora* and *N. plumbaginifolia*, respectively) at marker loci on each of the 10 linkage groups. The vertical position of each symbol shows the magnitude and direction of the deviation from the Mendelian expectation (0.25, denoted by the horizontal lines in each panel). Graphs are ordered by their respective linkage group's length, beginning with PL1 and ending with PL10. Codominant markers have two symbols at each map position. Dominant markers do not have a corresponding symbol since only the recessive homozygotes can be scored.

FIGURE 10

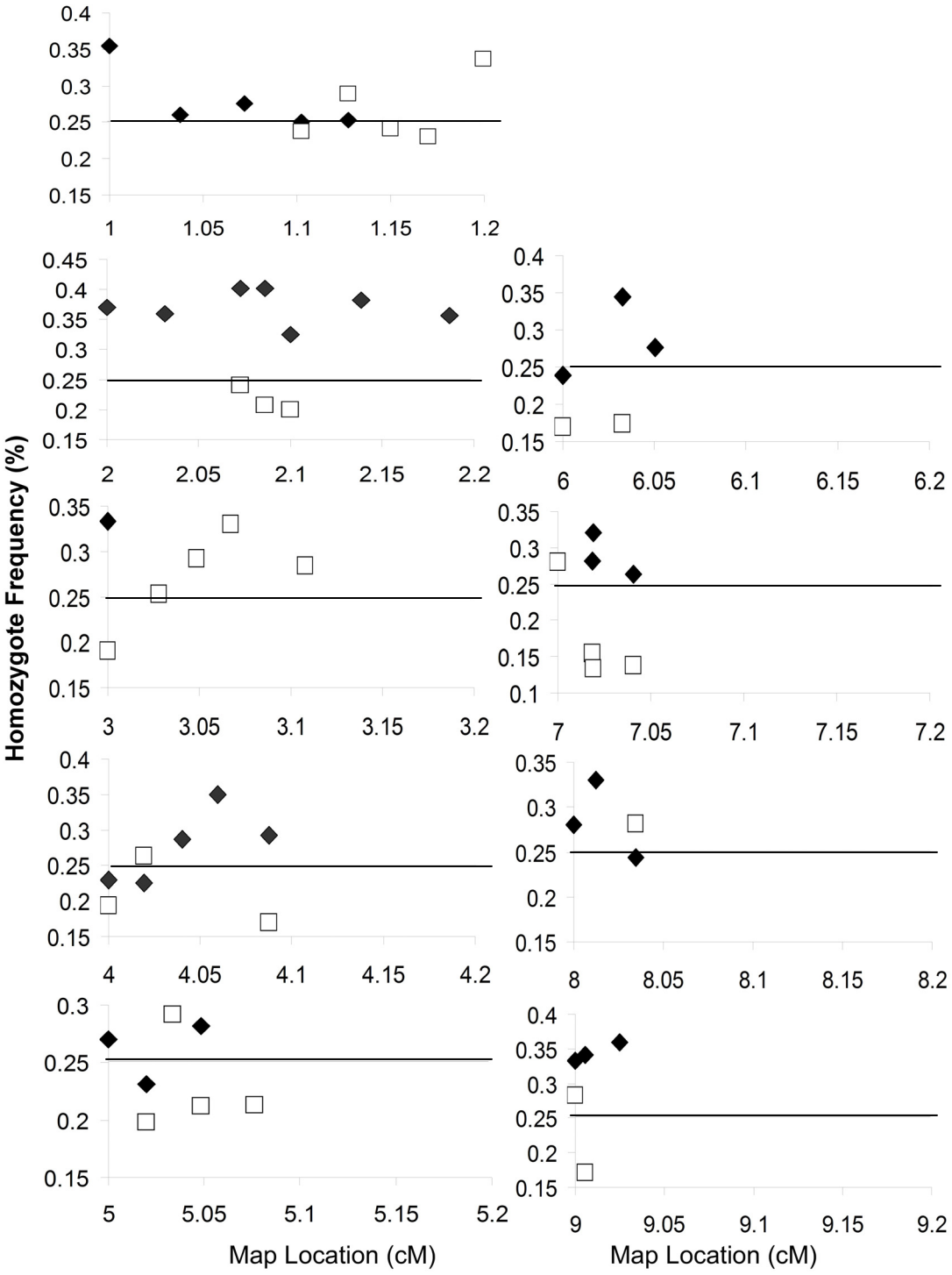

Figure 10: Transmission ratio distortion (TRD) across the maternal *N. longiflora* F_2 linkage map. The □ and ◊ symbols represent the two homozygous parental genotypes (*N. longiflora* and *N. plumbaginifolia*, respectively) at marker loci on each of the 10 linkage groups. The vertical position of each symbol shows the magnitude and direction of the deviation from the Mendelian expectation (0.25), which is demonstrated by a line. Graphs are ordered by their respective linkage group's length, beginning with LP1 and ending with LP9. Codominant markers have two symbols at each map position. Dominant markers do not have a corresponding symbol since only the recessive homozygotes can be scored.

FIGURE 11

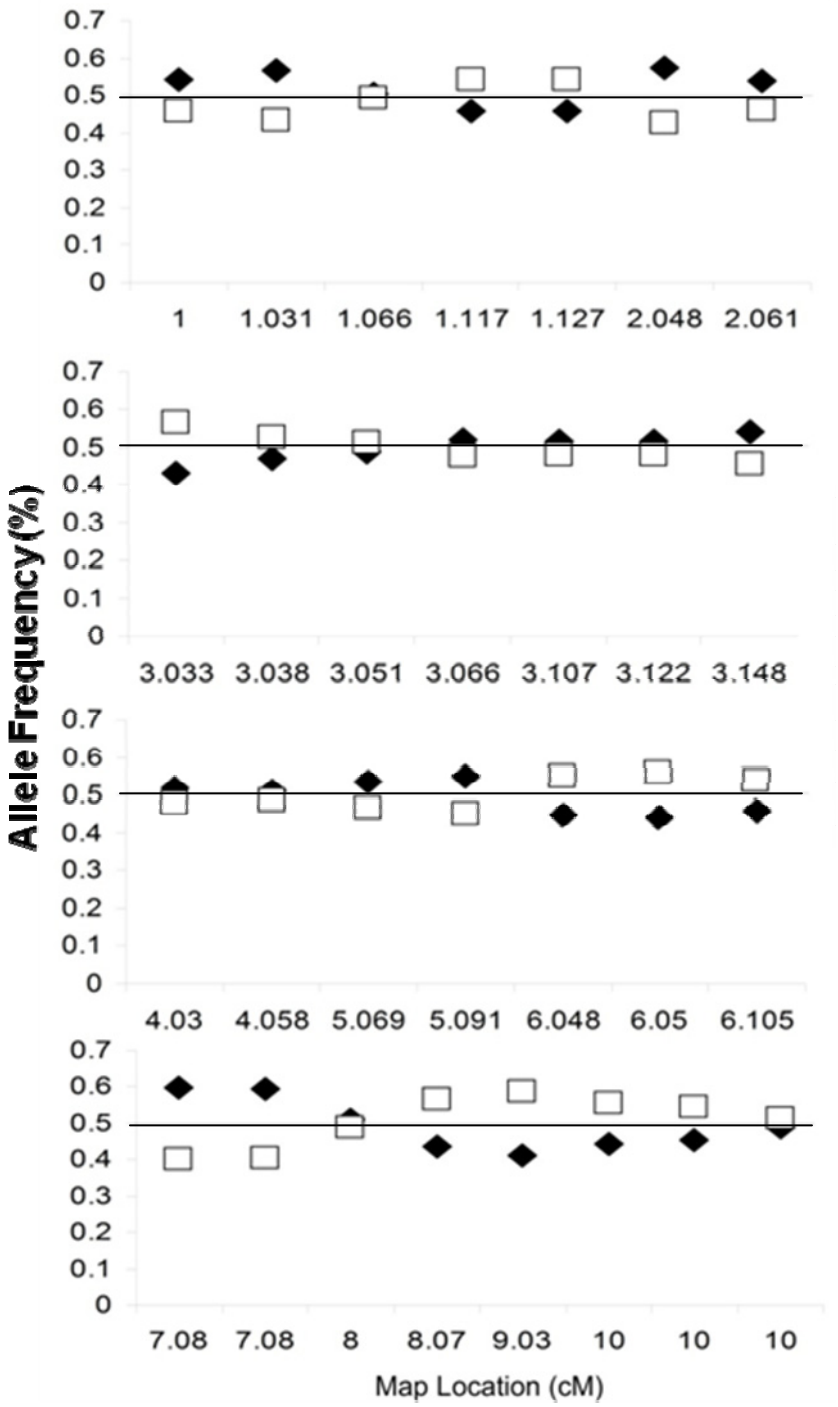
Allele Frequency (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 1.031 1.066 1.117 1.127 2.048 2.061
3.033 3.038 3.051 3.066 3.107 3.122 3.148
4.03 4.058 5.069 5.091 6.048 6.05 6.105
7.08 7.08 8 8.07 9.03 10 10 10
Map Location (cM)

Figure 11: Allelic preference of codominant markers only, across the maternal *N. plumbaginifolia* F_2 linkage map. The □ and ◊ symbols represent the two parental alleles (*N. longiflora* and *N. plumbaginifolia*, respectively). The vertical position of each symbol shows the magnitude and direction of the deviation from the Mendelian expectation (0.50), which is demonstrated by a line. The X-axis indicates the linkage group and map position (e.g. 1.117) of the codominant markers from the PL map (Figure 4). Asterisks indicate significance levels of X^2 tests and are indicated as * ($p < 0.05$) and ** ($p < 0.01$).

FIGURE 12

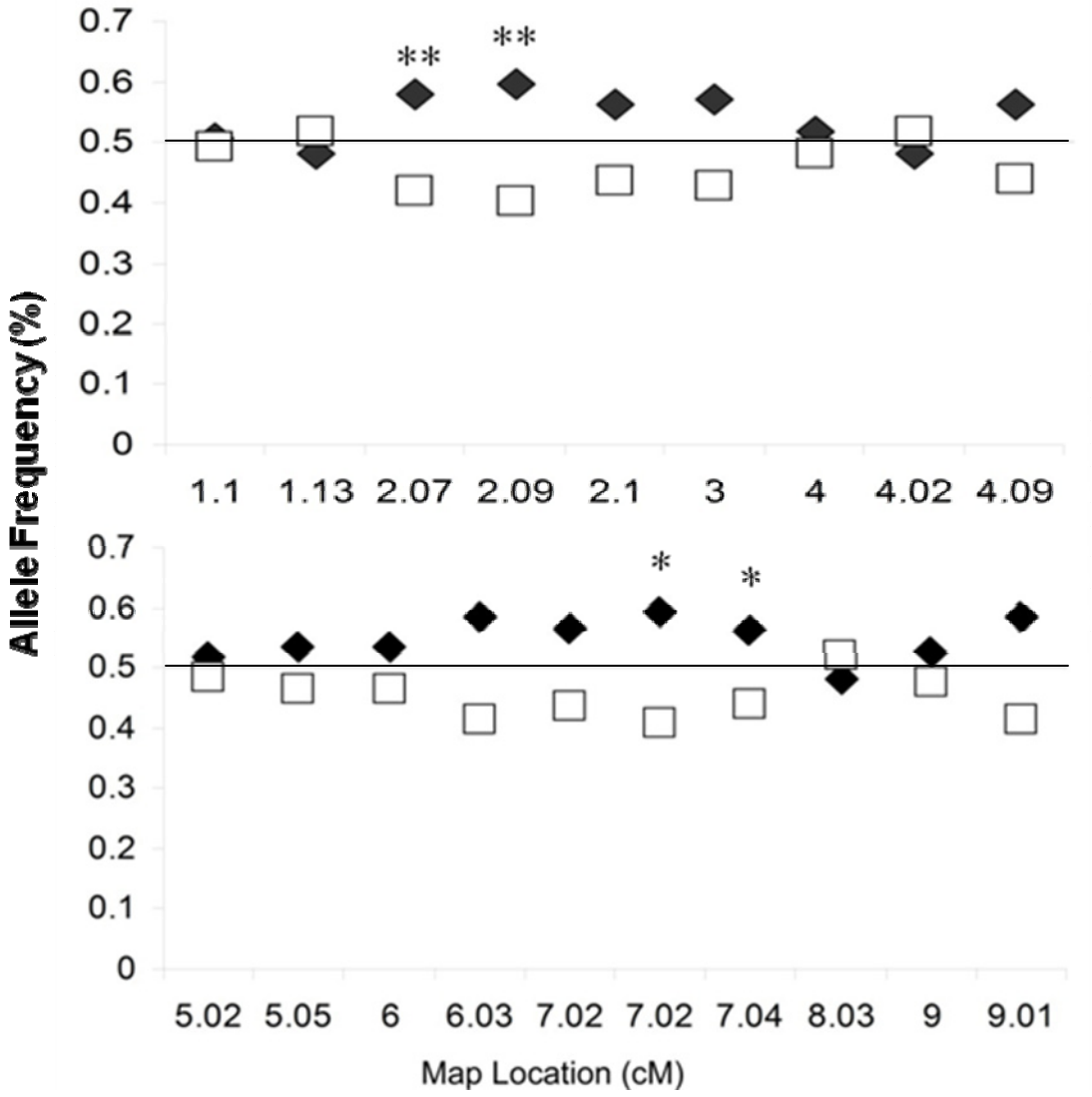

Figure 12: Allelic preference of codominant markers only, across the maternal *N. longiflora* F_2 hybrid linkage map. The □ and ◊ symbols represent the two parental alleles (*N. longiflora* and *N. plumbaginifolia*, respectively). The vertical position of each symbol shows the magnitude and direction of the deviation from the Mendelian expectation (0.50), which is demonstrated by a line. The X-axis indicates the linkage group and map position (e.g. 1.117) of the codominant markers from the LP map (Figure

5). Asterisks indicate significance levels of X^2 tests and are indicated as * ($p < 0.05$) and ** ($p < 0.01$).

FIGURE 13

Solanaceae Phylogeny: Divergence between *Solanum*, *Capsicum* and *Nicotiana*.

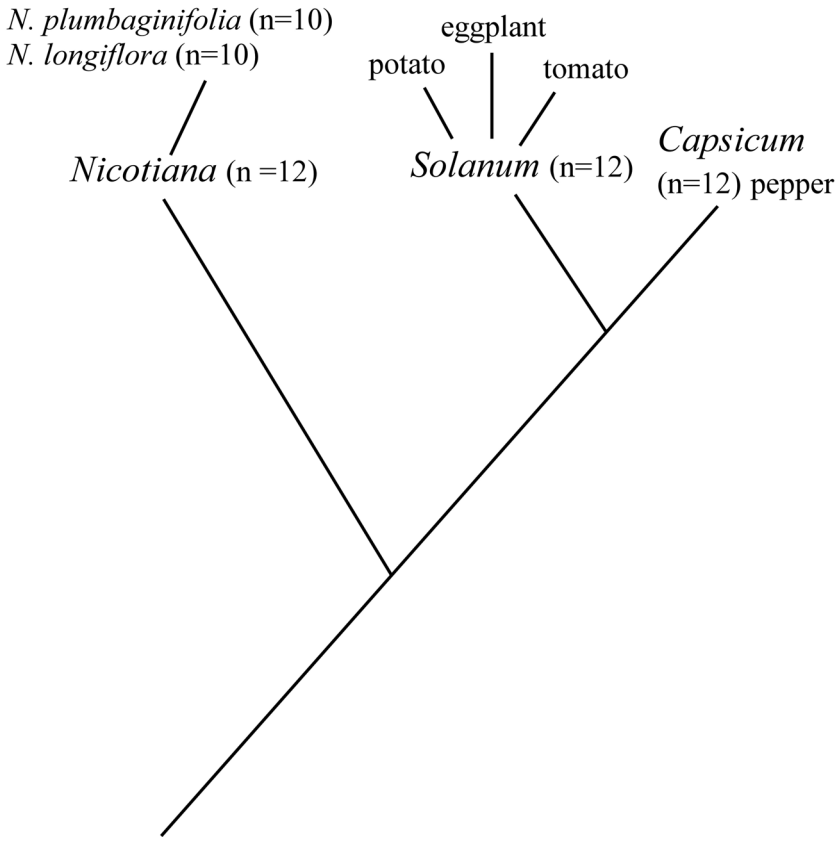

Figure 13: Phylogeny tree showing the divergence between *Nicotiana*, *Solanum* and *Capsicum*. The phylogeny gives the base chromosome number and relevant species in each genus. Phylogeny was simplified from Knapp (2002) and Olmstead et al. (2008).

Appendix 1: Inter-Simple Sequence Repeats (ISSR) markers (n = 101) used for linkage maps in the reciprocal *N. longiflora* X *N. plumbaginifolia* F_2 mapping populations. ISSRs are indicated by their primer name and band size of the polymorphic marker. Markers were screened in both backgrounds (PL = maternal *N. plumbaginifolia* background; LP = maternal *N. longiflora* background; B = indicates both backgrounds). Significance levels are indicated by ns (no significance); * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$). *** markers were considered badly distorted and not mapped. Background-specific allelic bias in distorted mapping markers ($0.05 > p \geq 0.001$) is indicated by the genotype preference exhibited by the marker.

Band	Primer	Band Size	Seg. Distortion	Bias
C1	N(GT)6-YR	730	B(ns)	None
D1	N(GT)6-YR	545	LP(ns); PL*	Plum (PL)
E1	N(GT)6-YR	465	PL*; LP***	Plum (B)
A2	N(GT)6-AY	1500	PL***; LP(ns)	Long (PL)
B2	N(GT)6-AY	500	PL***; LP(ns)	Long (PL)
B3	N(CA)6-RY	960	PL***; LP(ns)	Long (PL)
C3	N(CA)6-RY	603	B(ns)	None
D3	N(CA)6-RY	456	PL*; LP(ns)	Long (PL)
B5	(AC)8-G	1746	LP**	Plum (LP)
E5	(AC)8-G	1366	PL(ns); LP*	Long (LP)
G5	(AC)8-G	1073	PL(ns); LP***	Long (LP)
H5	(AC)8-G	906	PL(ns); LP***	Long (LP)
J5	(AC)8-G	545	PL(ns); LP***	Plum (LP)
A8	(GA)8-C	302	PL*; LP***	Plum (B)
C8	(GA)8-C	402	PL(ns); LP**	Plum (LP)
D8	(GA)8-C	627	PL(ns); LP***	Plum (LP)
E8	(GA)8-C	866	PL*; LP***	Plum (B)
F8	(GA)8-C	1139	PL(ns); LP**	Long (LP)
G8	(GA)8-C	1239	PL**; LP***	Plum (B)
H8	(GA)8-C	1455	PL(ns); LP*	Long (LP)
A13	(GACA)4	1514	B(ns)	None
B13	(GACA)4	1114	PL*; LP(ns)	Plum (PL)
C13	(GACA)4	715	B(ns)	None

Appendix	1 continued			
D13	(GACA)4	556	PL*; LP(ns)	Long (PL)
E13	(GACA)5	331	PL***; LP*	Plum (LP)
A18	(CA)7-GT	1512	PL(ns); LP**	Long (LP)
B18	(CA)7-GT	1307	PL(ns); LP*	Plum (LP)
C18	(CA)7-GT	774	PL(ns); LP**	Long (LP)
D18	(CA)7-GT	600	PL*; LP***	Long (B)
E18	(CA)7-GT	508	PL*; LP***	Plum (B)
A19	(CA)7-AG	1674	PL***; LP*	Plum (B)
B19	(CA)7-AG	887	B(ns)	None
C19	(CA)7-AG	768	B(ns)	None
D19	(CA)7-AG	694	B(ns)	None
E19	(CA)7-AG	610	B(ns)	None
A20	(CA)7-GG	2225	LP***; PL*	Plum (B)
B20	(CA)7-GG	1740	PL*; LP(ns)	Plum (PL)
C20	(CA)7-GG	1488	B(ns)	None
D20	(CA)7-GG	1336	B(ns)	None
E20	(CA)7-GG	1180	B(ns)	None
F20	(CA)7-GG	1017	PL(ns); LP*	Plum (LP)
G20	(CA)7-GG	880	B(ns)	None
A21	(GA)7-GG	1500	PL**; LP(ns)	Plum (PL)
B21	(GA)7-GG	1333	PL**; LP***	Plum (B)
C21	(GA)7-GG	1256	B(ns)	Long (B)
D21	(GA)7-GG	1116	B(ns)	None
E21	(GA)7-GG	756	B(ns)	None
F21	(GA)7-GG	345	B(ns)	None
G21	(GA)7-GG	295	B(ns)	Plum (B)
A22	(GT)7-GG	1865	PL**; LP(ns)	Long (PL)
B22	(GT)7-GG	1450	B(ns)	None
C22	(GT)7-GG	1267	PL***; LP(ns)	Long (PL)
D22	(GT)7-GG	1034	B(ns)	None
A27	(GT)7-CC	1599	PL(ns); LP*	Plum (LP)
B27	(GT)7-CC	1392	PL(ns); LP*	Long (LP)
C27	(GT)7-CC	686	B(ns)	None
D27	(GT)7-CC	372	B(ns)	None
A28	(CAC)5-GC	2476	PL**; LP***	Long (B)
B28	(CAC)5-GC	2120	PL**; LP(ns)	Long (PL)
C28	(CAC)5-GC	1705	PL(ns); LP*	Plum (LP)
D28	(CAC)5-GC	1292	B(ns)	None
E28	(CAC)5-GC	1017	B(ns)	None
F28	(CAC)5-GC	787	B(ns)	None

Appendix	1 continued			
G28	(CAC)5-GC	495	B(ns)	None
A37	(GAC)5	1371	B(ns)	None
B37	(GAC)5	893	B(ns)	None
C37	(GAC)6	703	PL*; LP***	Plum (B)
D37	(GAC)5	624	B(ns)	None
A38	N(TGT)5-RG	1878	PL**; LP(ns)	Plum (PL)
B38	N(TGT)5-RG	1583	B(ns)	None
C38	N(TGT)5-RG	1346	B(ns)	None
D38	N(TGT)5-RG	1104	B(ns)	None
A39	NNAAC-(GT)5	1406	PL(ns); LP*	Plum (LP)
B39	NNAAC-(GT)5	991	B(ns)	None
C39	NNAAC-(GT)5	866	B(ns)	None
D39	NNAAC-(GT)5	743	B(ns)	None
E39	NNAAC-(GT)5	696	PL(ns); LP**	Long (LP)
F39	NNAAC-(GT)5	500	B(ns)	None
A31	N(AG)8	1451	PL(ns); LP*	Long (LP)
B31	N(AG)8	1220	PL(ns); LP*	Plum (LP)
C31	N(AG)8	756	PL*; LP**	Plum (B)
D31	N(AG)8	475	B(ns)	None
A35	N(AC)6-RG	1148	PL*; LP***	Long (B)
B35	N(AC)6-RG	483	B(ns)	None
A40	NNTTC-(AC)5	1331	B(ns)	None
B40	NNTTC-(AC)5	1202	B(ns)	None
C40	NNTTC-(AC)5	1038	PL*; LP***	Long (B)
D40	NNTTC-(AC)5	689	B(ns)	None
E40	NNTTC-(AC)6	621	PL(ns); LP**	Plum (B)
F40	NNTTC-(AC)5	445	PL(ns); LP*	Plum (LP)
A4	(AG)7-YC	1540	B(ns)	None
B4	(AG)7-YC	1381	PL*; LP(ns)	Long (PL)
C4	(AG)7-YC	768	B(ns)	None
D4	(AG)7-YC	664	B(ns)	None
E4	(AG)7-YC	578	B(ns)	None
A12	NNCAA-(GA)5	1541	PL(ns); LP**	Long (LP)
B12	NNCAA-(GA)5	1336	B(ns)	None
C12	NNCAA-(GA)5	887	PL(ns); LP*	Long (LP)
D12	NNCAA-(GA)5	391	B(ns)	None
A9	N(CAC)4-RC	716	B(ns)	None
B9	N(CAC)4-RC	520	PL(ns); LP*	Plum (LP)