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High resolution linkage maps of the model organism *Petunia* reveal substantial synteny decay with the related genome of tomato

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Abstract: Two linkage maps were constructed for the model plant *Petunia*. Mapping populations were obtained by crossing the wild species *Petunia axillaris* subsp. *axillaris* with *Petunia inflata*, and *Petunia axillaris* subsp. *parodii* with *Petunia exserta*. Both maps cover the seven chromosomes of *Petunia*, and span 970 centimorgans (cM) and 700 cM of the genomes, respectively. In total, 207 markers were mapped. Of these, 28 are multilocus amplified fragment length polymorphism (AFLP) markers and 179 are gene-derived markers. For the first time we report on the development and mapping of 83 *Petunia* microsatellites. The two maps retain the same marker order, but display significant differences of recombination frequencies at orthologous mapping intervals. A complex pattern of genomic rearrangements was detected with the related genome of tomato (*Solanum lycopersicum*), indicating that synteny between *Petunia* and other Solanaceae crops has been considerably disrupted. The newly developed markers will facilitate the genetic characterization of mutants and ecological studies on genetic diversity and speciation within the genus *Petunia*. The maps will provide a powerful tool to link genetic and genomic information and will be useful to support sequence assembly of the *Petunia* genome.

Key words: Petunia, linkage map, molecular markers, microsatellite markers, Solanaceae synteny.

Introduction

Petunia is an important horticulture crop cultivated for its flowers. It belongs to the Solanaceae family, which includes other important vegetable crops such as tomato, potato, pepper, and eggplant, as well as tobacco. *Petunia* is also a model plant for genetics and molecular biology. It has simple growth requirements, a short generation time, large genetic variability, and hundreds of seeds can be generated from a single cross. An efficient endogenous Ac/Ds-type transposon system has been successfully used to induce mutants and isolate the corresponding genes (Gerats et al. 1990; Stuurman and Kuhlemeier 2005). An easily screenable collection of transposon insertions has been created for reverse genetics (Vandenbussche et al. 2008). *Petunia* is easy to transform by stable *Agrobacterium*-mediated transformation (Conner et al. 2009), and an efficient virus-induced gene silencing system

has been successfully established for functional analysis (Reid et al. 2009). Bacterial artificial chromosome (BAC) libraries are available for *Petunia axillaris* (Bossolini and Kuhlemeier, unpublished) and *Petunia inflata* (McCubbin et al. 2000; Puerta et al. 2009). Genetic maps of *Petunia* have previously been constructed using phenotypic markers (Gerats et al. 1993; Strommer et al. 2009), restriction fragment length polymorphism (RFLP) markers (Strommer et al. 2000), and amplified fragment length polymorphism (AFLP) markers (Strommer et al. 2002; Stuurman et al. 2004). The relatively large genome size of *Petunia* (1200–1500 Mbp, Mishiba et al. 2000) and low marker density has made map-based gene isolation efforts difficult, and only one example of positional cloning has so far been described (Bentolila et al. 2002). A genome sequence initiative has recently been undertaken (F. Quattrocchio and T. Gerats, personal communication 2010). The rich molecular tool box, together with a high genetic diversity and interesting biology (Gerats and Strommer 2009), makes *Petunia* an attractive model system.

Until now genetic mapping in *Petunia* was restricted to the popular *Petunia hybrida*. The designation *P. hybrida* refers to a large collection of garden varieties thought to be derived from interspecific crosses between *Petunia integrifolia* and *P. axillaris*. *Petunia* interspecific hybrids in nature are rare. Only hybridization between *Petunia exserta* and *P. axillaris* has been observed to some extent (Lorenz-Lemke et al. 2006). Genetic and cytological maps of several *P. hybrida* accessions have previously shown that their genome is differentiated by a number of genomic rearrangements (reviewed by Strommer et al. 2009). It is not known whether these rearrangements arose before or after domestication.

The most detailed gene-based map of *Petunia* dates to 10 years ago (Strommer et al. 2000), it spans 368 centimorgans (cM), and consists of 36 RFLP markers. The importance of *Petunia* as a model system and as a horticultural commodity makes it imperative to increase the genetic coverage of its genome. The aim of this work was to extend genetic mapping of the *Petunia* genome to wild species.

Our interest in wild *Petunia* species stems from the presence of closely related, cross-fertile species that display distinct pollination syndromes. Pollination syndromes are suites of floral characters, such as petal color, fragrance, reward production, and morphological traits that are adaptations to specific pollinators. The genus *Petunia* counts 14 species, all endemic to southern South America (Stehmann et al. 2009). *Petunia axillaris* and *Petunia parodii* are pollinated by nocturnal hawkmoths, *P. inflata* is pollinated by solitary bees, and *P. exserta* displays a typical hummingbird pollination syndrome (Fig. 1).

Mapping populations were obtained by the crosses *P. axillaris* × *P. inflata* and *P. parodii* × *P. exserta.* We designed gene-derived codominant PCR markers that are highly reproducible, relatively inexpensive, and easily transferable across species. We describe for the first time the development and mapping of microsatellite markers in *Petunia* (Pm markers) derived from expressed sequence tags (EST) deposited in GenBank (www.ncbi.nlm.nih.gov/). Additional markers, including several genes encoding transcription factors and biosynthetic enzymes, were developed from sequences of genes with a putative role on floral development and color or scent biochemistry. Further marker saturation was achieved with multilocus AFLP (Vos et al. 1995) analysis or with markers designed to target specific *Petunia* chromosomal regions, relying on synteny with the related genome of tomato (Pt markers). We compared the mapping position of the markers of *Petunia* with that of tomato, and we provide a first insight into the extent of macrosynteny between these two representatives of the Solanaceae.

Materials and methods

Plant material and DNA extraction

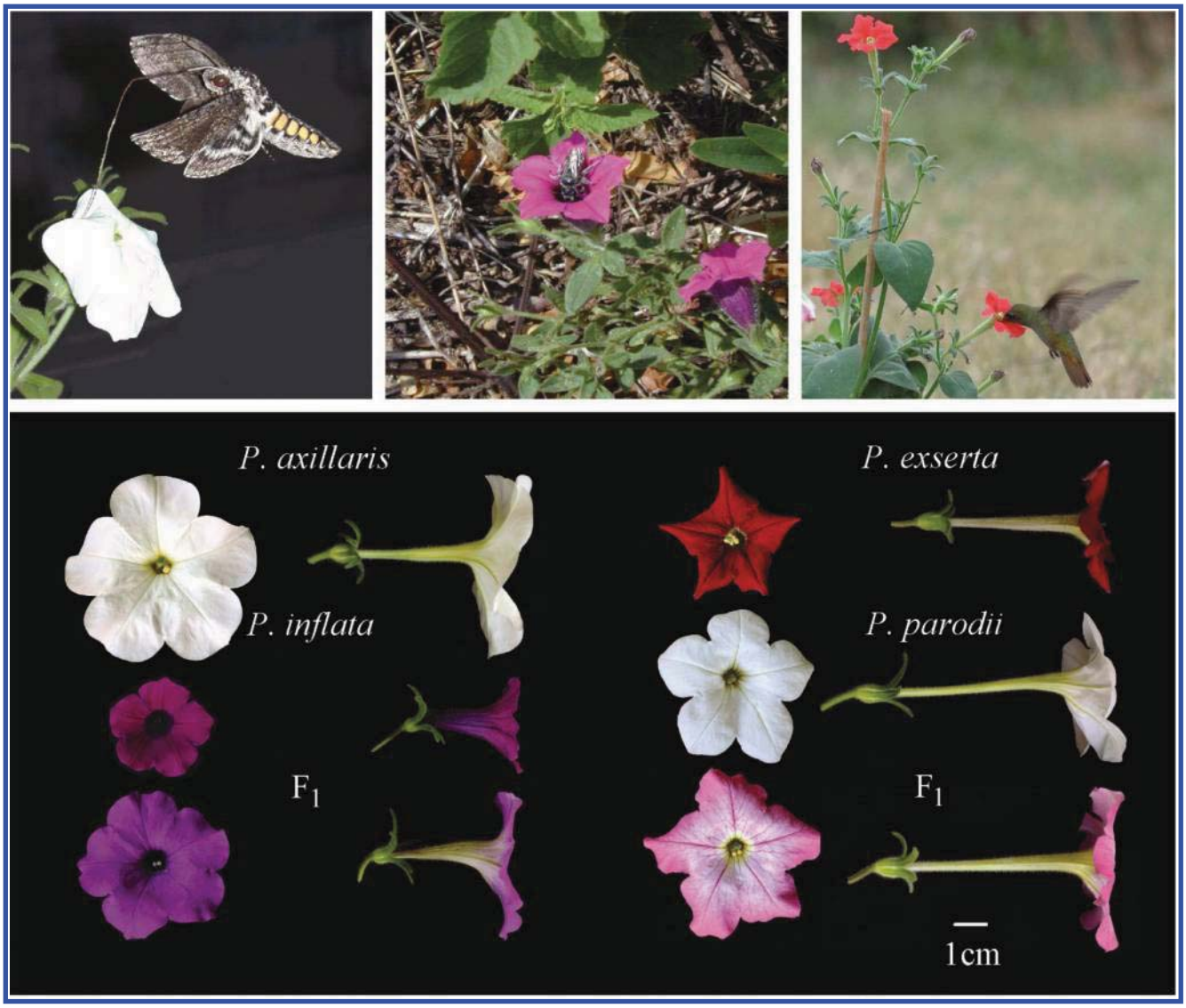
Petunia axillaris subsp. *axillaris* (accession N), *P. axillaris* subsp. *parodii* (accession S7), and *P. inflata* (accession S6) were kindly provided by R. Koes, Department of Genetics at the Free University Amsterdam, Amsterdam, the Netherlands. *Petunia exserta* was a gift from R. Griesbach, Beltsville Agricultural Research Center, USDA, Beltsville, Maryland. The plants were grown in the greenhouse and manually crossed. Two mapping populations derived from interspecific crosses were developed for genetic analysis. A first mapping population consisting of 173 F_2 plants was created by selfing a single F_1 plant that was obtained from the cross *P. exserta* × *P. parodii*. A second mapping population of 176 BC_1 plants was derived from backcrossing a F_1 plant (*P. axillaris* × *P. inflata*) with *P. axillaris* as a seed parent. In this second population, the backcross design was chosen to avoid segregation distortion at the self-incompatibility *S*-locus of *P. inflata*. All seedlings were sown in germination pots and transplanted into single pots at the stage of ~3 cm. DNA was extracted following a cetyltrimethylammonium bromide (CTAB) extraction (Murray and Thompson 1980) when the plants were 3 weeks old.

Marker design and genotyping

Petunia EST sequences were downloaded from GenBank and mined for microsatellite motifs using the software SPUTNIK (Abajian 1994). To avoid amplification problems, repeats including cytosines and guanosines exclusively were not considered. We designed PCR primers preferentially targeting perfect repeats. Microsatellite markers were PCR amplified from petunia genomic DNA of the species *P. axillaris*, *P. inflata*, *P. exserta*, and *P. parodii*. Primers are listed in Table 1. A summary of the polymorphisms detected is provided in the Supplementary data[1] (Fig. S1). To reduce genotyping costs, forward primers were labeled as described by Schuelke (2000). The amplification products were electrophoresis separated and visualized on a LI–COR 4300 DNA sequencer (LI–COR Biosciences, Bad Homburg, Germany). All markers were amplified with the same PCR conditions in 10 μL volumes, containing approximately 20 ng of template DNA, 1 μL PCR buffer (60 mmol/L KCl, 12 mmol/L Tris–HCl, pH 9), 2 mmol/L $MgCl_2$, 0.35 pmol of the M13-tailed forward primer, 3.5 pmol reverse primer, 1.7 pmol labeled (IRD-700/800) M13 primer (5′-CACGACGTTGTAAAACGAC), 0.2 mmol/L dNTPs, and 0.5 U of *Taq* polymerase. Thermocycling started with a denaturation step for 3 min at 96 °C followed by 45 cycles of 15 s at 96 °C, 30 s at 50 °C,

[1]Supplementary data are available with the article

Fig. 1. *Petunia* species produce flowers adapted to different pollinators. *Petunia axillaris* (both subsp. *axillaris* and *parodii*) are adapted to nocturnal hawkmoth, while *Petunia inflata* attracts diurnal bees. *Petunia exserta* exhibits red flowers typical for hummingbird visitation. Pictures from A. Dell'Olivo and M. Gremillon.



and 1 min at 72 °C, and stopped after a final extension step of 72 °C for 7 min. After PCR, samples were denatured by adding 30 µL formamide stained with bromophenol blue. Chromosomal location of the polymorphic markers was determined by genotyping 173 F_2 segregant lines obtained from a cross between *P. exserta* and *P. parodii* and 176 BC_1 lines obtained from a cross between *P. axillaris* and *P. inflata*. The polymorphic index content (PIC) for the multiallelic microsatellite markers was inferred from parental tests on the four wild species *P. axillaris* N, *P. parodii* S7, *P. exserta*, and *P. inflata* S6. It was calculated as $\mathrm{PIC} = 1 - \sum_{i=1}^{n} p_i^2$, p_i being the frequency of the allele i at the marker locus p.

PCR primers used for genotyping the cleaved amplified polymorphic sequence (CAPS) markers were obtained from the literature or GenBank, and the sequences for the markers AN2, AN4, MYBB, and MYBX were kindly provided by F. Quattrocchio, Free University Amsterdam. Amplification was performed following a PCR protocol in 20 µL volume containing 50 ng of template DNA, 2 µL PCR buffer (60 mmol/L KCl, 12 mmol/L Tris–HCl, pH 9), 2 mmol/L $MgCl_2$, 20 pmol of forward and reverse primer, 0.2 mmol/L dNTPs, and 1 U of *Taq* polymerase. PCR products were digested with the appropriate endonucleases as indicated in Table 2, adding a mix of 4 µL water, 0.5 µL of the appropriate buffer, and 0.5 µL of the enzyme directly to 10 µL of PCR reaction. Digested fragments were electrophoresed on agarose gels and stained with ethidium bromide. AFLP markers were genotyped using the same settings as described by Strommer et al. (2002). We tested six AFLP primer combinations designed with *Eco*RI–*Mse*I and eight with *Pst*I–*Mse*I. Generally, primers designed on *Pst*I–*Mse*I resulted in fewer amplicons than *Eco*RI–*Mse*I. To compensate, the amplification reaction was performed with only two selective nucleotides at the *Mse*I primer. The AFLP markers were separated with a LI–COR sequencer. As the AFLP markers were dominant, they were added only to the *P. axillaris* × *P. inflata* BC_1 cross.

Chr.	SSR	Forward primer	Reverse primer	EST	Putative function	PIC	SSR motif
1	PM15	GTGGCTGGCAACATTGACTA	CACTTACCCCTCAGTCCTCG	CV297655	Unknown	0.75	$(CT)_{12}$
1	PM37	GGGGTGGGAATTCTAGTGGA	TGGATGAGCCATAATCTTTGC	EB174496	Transcription factor	0.625	$(ATG)_6$–$(CAA)_5$
1	PM41	GGCTCAAACACAATTTCCTC	CTCCAACAAAGTTACTTGCAG	CV297469	Jasmonate ZIM domain	0.625	$(TC)_{11}$
1	PM42	CGGCTCAAACACAATTTCCT	AATTCAACCGCCATGAAGTC	CV297469	Unknown	0.625	$(TC)_{11}$
1	PM81	ACTGAAATCGTTGGGCGTT	AAAAGGAGTTGCATATCCTGATTA	CV292797	Unknown	0.625	$(T)_{16}$
1	PM83	GCAAGTGTTCCATCTTGTC	CTCTGACCAAATAATGTG	CV299390	Unknown	0.625	$(T)_{19}$
1	PM85	TGCAAATGAATGTCCAGGAT	TGCTGCAACTTTCCCAATTA	CV294317	Unknown	0.625	$(TA)_7$
1	PM101	GAGAGAGAACCCTAACCC	GCAGAAGAAACAGAGATCC	FN001660	Unknown	0.667	$(CTT)_6$
1	PM120	GGTTTAGATACTGAAGTTG	CCAGCATTACACCAACCTG	FN005966	Unknown	0.625	$(T)_{16}$
1	PM149	CCTAATCAAACACGTAACTC	GGATGATGACACGTGGATCG	FN042637	Tyrosine phosphatase	0.625	$(CT)_{10}$
1	PM164	GGGGATGGCTACAGCAGC	CTTGCAGCTCATGGCAAAGC	FN014610	F-box family	0.375	$(CAG)_6$
1	PM169	GCAGAGAAACTACACTAATAGGG	CCTGAGGAAGAGCAGCAGC	FN016284	Nucleic acid binding	0.75	$(CA)_{12}$–$(CAG)_8$
1	PM188	CCCAACCATTGGCTACAGCC	GGACAACACAATACAATCTCTGC	FN037917	Singlet oxygen response	0.75	$(CTT)_8$
1	PM193	CGCAACATCACCACTATCAG	GCTGCCAAGTCCGACAATGG	FN030612	Unknown	0.625	$(CAT)_6$
1	PM195	GCCTTTCGCCGCTGTCACTG	GAGCAAATCGTGACCGTTGG	FN026706	Phosphatidylserine synthase	0.625	$(GAA)_6$
2	PM13	GAAGGCAAGAATAGTCACC	CCGATTACTGTTTGAGGAGG	CV296369	Unknown	0.375	$(CAC)_9$
2	PM21	CTACCGGTAGGCAGTAGTTGC	CCTCGACCTTCTTCCTGAC	CV297594	KDR transcription regulator	0.75	$(TAC)_8$
2	PM32	TTCTCTAAGAAGAAACAATAAAGCTCA	GGCTATGCCAGCTTTGGTAA	CV298848	Fascilin-like precursor	0.625	$(A)_{23}$
2	PM76	GATCGCAACCTGGATCCTAA	AGGGCTGCACTCTTGTTTGT	CV297778	Unknown	0.375	$(GAACCC)_6$
2	PM88	CTGTTTCCTAATTACCTG	GCCACTGGCATGGCTGCA	CV298718	Unknown	0.625	$(TA)_7$
2	PM93	GCACCTCAGGCTGGTGCACC	GCAGTTGAAACAGAGGGACC	DC243258	Unknown	0.667	$(TGC)_6$
2	PM94	CCGTGTTAGTATTGCCCAGG	CTCTAGATTGACCATAGC	CV300671	Photoassimilate response	0.625	$(GGT)_6$
2	PM111	CACCATGAGGAACATCAAGC	GGAACTGGCTGAGGGAAACC	FN000621	Unknown	0.375	$(CAC)_9$
2	PM113	GGCTCTGTCTGCAATGGACG	CTCTAGATTGACCATAGC	FN032542	RNA binding	0.375	$(TGG)_6$
2	PM119	CCGACACATACCAATTCAC	CACCTAACGTACATTAGC	FN004737	Inositol triphosphate kinase	0.75	$(TG)_9$
2	PM124	CCCACCACCACTTCCATTCAC	CGATGCTTGATTCCCCAC	FN005227	Unknown	0.375	$(TGA)_7$
2	PM142	GGTGGTGCTGAGCCAAAGC	CGCCAGCTGCTTCTGAG	FN008269	Transcription factor	0.375	$(ACT)_6$
2	PM165	CTCTACCTCTACATCTACC	GTGCAGCGACAACGAGTC	FN045187	UBX-domain	0.375	$(CAA)_6$–$(GAA)_6$
2	PM186	CCTTTACTAGTCTCAGAATTGC	GGATAATGATGATGACCC	FN036047	TCP-like transcription factor	0.375	$(GTT)_7$
2	PM190	CGAGTTGATGGTGCAATTGTG	CTAGAAAGTTCCTCCGG	FN038900	TraB family	0.625	$(GAA)_7$
2	PM200	CCTGACCCTCCCAGAACC	GGTAACATCTCCCTCACTTCC	FN022583	Unknown	0.667	$(GTT)_6$
2	PM202	CCCTGTTTCTTCTTCAC	CATCCACCACTTGTTGTTGAG	FN031565	Transcription factor	0.375	$(ATT)_7$
2	PM205	GGAGCCAAGTTGCTTGAGG	CCCTTCAACAACACCACC	FN035754	Transcription factor	0.625	$(TTG)_6$
3	PM12	GGAACCTTCACAGAATGG	GAAGTTGTTGGCTCAACC	CV293902	Unknown	0.5	$(CAA)_7$
3	PM79	TTGGCGAAACATGCTGTTAG	CAAGCTTCATAAACCAACAACC	CV299471	HTB4 DNA binding	0.625	$(GTT)_5$–$(A)_{11}$
3	PM99	CAAGTGTTGGAGCAGTGGAA	GCCCTGCAAAAATGTGAAAT	CV294997	Unknown	0.375	$(TTC)_5$
3	PM183	CCTATTTCAGTCCATGAGGC	GTTAGCTGTCTGCTGATCAC	FN041860	Phosphatase	0.75	$(GT)_{10}$
3	PM191	GGAGAAGATTGTTGGTAAC	GGGAAACGATCTCTTGCTG	FN034502	Unknown	0.75	$(TTG)_6$
3	PM197	CCATAAGTGAAGGATCCTGC	CTGACAACTTACACAGGAACAC	FN032593	Nucleic acid binding	0.375	$(CCA)_7$
3	PM219	GCTGTAACATGTAGCTGTG	GGCTGCCAATCCATGCAGTC	FN014488	Unknown	0.445	$(TGA)_8$
4	PM7	CGTTTTTCATTGCATTGTCG	CGTTTCCCTCCTTTGATCTG	CV298460	Squalene monooxygenase	0.75	$(AAC)_{10}$
4	PM8	TCTGCAAACTTCAAAGCCAA	ACATGCCATGCACTTTTGAG	CV298210	Transcription factor	0.75	$(AAGA)_{10}$

Chr.	SSR	Forward primer	Reverse primer	EST	Putative function	PIC	SSR motif
4	PM40	AGCTTCCTTTTTGAGCCACA	TGGCTTAAGCAAGACAATGG	CV299350	Mannan synthase	0.75	$(T)_{14}$–$(A)_{10}$
4	PM63	TGGTACAATGGAGCAGAGG	ATGTGAGATTCCCAACGACC	NP1240021	Transcription factor	0.75	$(ACAGCA)_5$
4	PM68	GTCGAACGAGGATCATGTC	GTGTCCTCCTAATGCTTGCC	NP1240041	Transcription factor	0.375	$(CAA)_6$
4	PM77	ACCACGAGAAGAAGGAAGCA	CGAACAACGAGTTAAACCCC	CV298105	Glycine-rich protein	0.667	$(GT)_9$
4	PM98	ATGGAGGTAGCAAATGCAGG	CAACCAAATGCAGCTTCAGA	CV298392	Methionine sulfoxide reductase	0.5	$(ATT)_5$
4	PM107	GTCAAAGGTTGCAATCTCT	TGTTGCTGATGAGCAGTAG	FN001301	Transcription factor	0.625	$(CAA)_8$
4	PM155	GGCAACGACAATGGTGG	CCTTTGATCTGCATTCTCC	FN010739	Squalene epoxidase	0.667	$(AAC)_{10}$
4	PM166	GGCACTTGATTGTCCTTGTG	CCATGAATCGAATGCAG	FN014864	Unknown	0.625	$(A)_{32}$
4	PM173	CAGCGCTATCAACAGCAG	GTGAGAGGCAAGTGATTGG	FN039910	Unknown	0.667	$(GCA)_6$
4	PM181	CCAGCTCTCTCGGAGCTG	CATCAACTATGTAGGAC	FN000364	Harpin-induced protein	0.375	$(GAT)_6$
4	PM187	GAGGCATTGTCACAGGC	CTATGGATGCAGCTAGGCCAC	FN031819	Unknown	0.5	$(GCT)_6$
4	PM206	CAGTGGCAGATGGAAGAGC	CCAACGCAAGCAAACAAGGC	FN035807	Allantoate amidohydrolase	0.75	$(TC)_{12}$
4	PM218	GTAGGACAAAAGTAAAGGGTG	GTTTGAAGTTAGACAATCG	FN018261	NHL1 domain	0.375	$(CAT)_6$
5	PM19	ACCCTTGGAAAATGTCGTTG	TTCAAATTTCATCAGTGGCG	CV297851	Unknown	0.75	$(T)_{12}c$–$(T)_{16}$
5	PM44	AGAATCCCCATATGCTCCG	AGCAGCACCAACAACACAAG	CV298575	Pectinesterase	0.625	$(A)_{15}$–$(A)_{10}$
5	PM71	CACTAGGACTCCTATTTCAC	GCTTATAAGGGAAGAGACTG	CV298122	Unknown	0.75	$(CT)_8$
5	PM72	GTAAAGCCGTTTTGTTGGGA	CATTGAGGACTCTGCGATTG	CV295851	Unknown	0.375	$(CT)_9$
5	PM110	GGTACAGGGCTAGCAGG	CTAGTTGGGTGTTCACAG	FN006325	Disease resistance	0.667	$(AAC)_8$
5	PM114	GGGTAAGGTCTGTGTACG	CCCTTAGCTGGTATTCGCAG	FN003883	Unknown	0.625	$(TG)_9$
5	PM150	CGTCGAATGCCTTAACTGC	GGAACAACACAGAAACTGTC	FN009853	Sodium symporter	0.625	$(ATG)_{10}$
5	PM167	CTCACTAACCAACTTCACC	CTAAGAAGCTTAAGAGTG	FN015039	Glycosyl transferase	0.75	$(TTC)_{12}$
5	PM177	CCCTTACTCTCTTCTTCACC	GAACTATGAACCATAGCTCTC	FN016759	Unknown	0.667	$(CA)_{11}$
5	PM179	CGGAGGAGGAGGACAAGGC	CCAGTTGCTCAGCCGATTGC	FN019539	RCD1-like cell differentiation	0.445	$(AAT)_6$
5	PM192	GCTGCTTTAAGATTCAGAGGC	CTGAACTTTGCATTGGC	FN036401	Transcription factor	0.75	$(CAG)_8$
5	PM210	CCTTGTGGCATAAGCTGCC	CCAACAACTGCAACAGCAGC	FN042698	MYB transcription factor	0.625	$(TTG)_6$
6	PM9	GGAGGAGGAATATGAAGAAGC	CTCTTCTTCCTCCTCCGCAG	CV300118	Unknown	0.75	$(AGA)_{17}$
6	PM17	TCCATCTCGTTTAGCAACCA	GGCTTCCAGCAAGAGAAGTG	CV301045	Unknown	0.625	$(CTG)_8$
6	PM66	CTATGGGAACTTCTCCTAAC	GCATTTCTAGGTTCTAGAGG	CV301241	Unknown	0.667	$(AT)_7$
6	PM90	TGGCGCTGAAACATTCTATG	AGAAGACAACGACAACGCAA	CV297287	Acyltransferase	0.445	$(TC)_7$
6	PM91	AGCCAGGCAAAGACCATTTA	TTTCACGTCATAATCCACGG	CV298703	Transcription factor	0.667	$(TC)_7$
6	PM105	CAGTAGGAAGGGTGCAGTGG	GTGCACGGAAGTTCTCG	FN001497	Peptidyl-prolyl isomerase	0.667	$(A)_{18}$
6	PM106	GTTCCTCCAGGCACTTCTGG	CAGAGAGGACACAACTCCTC	FN004825	Gibberellin-regulated protein	0.75	$(T)_{17}$
6	PM117	CCATACCCCATCTTCCACTGG	GGTGGCAACCTTGAGCTCC	FN004482	Kinase	0.445	$(GTG)_7$
6	PM132	GCAGTAGGGCATTGCAG	CTGATTCCTCCTCCAGCTCGAG	FN010866	Cytochrome *b-f* synthesis	0.375	$(ATC)_6$
7	PM33	AAAATTCCTTTTTCTCTTTTCTTCC	GCAATACCGGTCCACTTGAT	CV298303	Serine-rich protein	0.625	$(A)_{24}$
7	PM54	CCGAAACCCAGGAAACGC	CCTGTTTGATGTCAAACCC	CV299353	Unknown	0.667	$(A)_{18}$
7	PM103	GTGGATGACAAACTTGAGG	GACAGCAGTGGTGTTTGG	FN006664	Cystathionine beta-lyase	0.75	$(A)_{20}$
7	PM144	GCAGCCCTTCTTCACTG	CCATTGAATCCACAAGG	FN008495	Unknown	0.625	$(A)_{20}$
7	PM157	GTAGTAGTAGTAACCCCACC	CATCAGAAGCTTCTGGAG	FN011441	Heat-shock protein	0.625	$(AC)_{10}$
7	PM184	GGACTTTTATCAACTACC	GCCTTGCCTTTATCGGAC	FN027453	Unknown	0.75	$(GAA)_6$
7	PM208	CGACGCGCGTTTTGAAGC	CCGTGTCGAAGCAGCGTAG	FN038297	VQ motif	0.375	$(TTG)_6$

Chr.	CAPS	Forward primer	Reverse primer	Putative function	Endonuclease
1	CCL	CACATAATAATGCCAATGG	GCATGATTAGAATTGCTGC	Coumarate-CoA ligase	*Hae*III–*Mse*I
1	CRC	CCAAACGGGCCATTGTCTGAG	AGCATAGACTAATCCTCCAAGAAGA	Transcription factor	Indel
1	HF1	TCCCTCATTAATTAACCATATCTC	CATGGATAGCTACCGAACG	Flavonoid hydroxylase	*Alu*I
1	SHO	TACATATTATTAGCATCACACGC	TCTTGACACTTGGTTCCACTAC	Isopentenyltransferase	*Eco*RI
2	13A14	GATGTACATTCCTTGAAGTTGC	GAGCCTCTCCTAACTCTC	Microtubule binding	*Dde*I
2	FLS	GCTTACACTGAAGGAAGAG	GTCAGAGTTAGGTCGGCC	Favonol synthase	*Hpy*188I
2	IGS	ATGACTACTGGGAAGGG	GGAAACGTGGTAACGCTC	Isoeugenol synthas	*Eco*RV
2	MYB14	CACCTACTAGTCCATGACC	TGAGGTAAAGTTGCTGCTG	Transcription factor	*Mwo*I
2	MYB58	GAGGTGGATTAATTACCTAAG	GACAAACTGGCTGATTGTCCTG	Transcription factor	*Dpn*II
2	MYBPH3PROT3	CTAGCAAAATGAAGGCATATTATTCCAGC	CTAGCAAAATGAAGGCATATTATTCCAGC	Transcription factor	*Hae*III
2	PAAS2	ACCCATTTGATCTCTAGC	GTATCCCGTTCGTAGCC	Phenylacetate synthase	*Msp*I
2	PAL1	TAACAACACATTGCCATATAACCAA	ATTTCCTGAGTTTGTTGAGCCAA	Phenylalanine ammonia-lyase	*Hpy*CH4V
2	PAL2a	ATGGTCATGCTAATGGTCATG	CATAACAAAATTAGATGAAAGG	Phenylalanine ammonia-lyase	*Eco*RV
3	ADH2	CGACAGGTACAGGCGAAACGACGATAGATTATG	AGCATTTTAATCGATCAAAAATCAGATTC	Alcohol dehydrogenase	*Tsp*509I–*Alu*I
3	ALDH1	CAGCTCTTAGTCCCCGAAC	AACTGGGAAAACAGTACTTGAAC	Aldehyde dehydrogenase	*Pvu*II
3	ALDH2	GCACATGTTGCTGAAGGTGATG	GATTTGCACACAAGTGAAAGC	Aldehyde dehydrogenase	*Hin*dIII
3	AN11	ATGGAAAATTCAAGTCAAGAATCAC	TTATACTTTAAGCAATTGCAACTT	Transcription factor	*Hae*III
3	C4H1	GCGCATTGTTGTCCATGCTC	GAGGTTGAAGCTGTTCAAGG	Cinnamate 4-hydroxylase	*Dde*I
3	EPF1	GAGTAGGGGTATTGATCCAA	ATCGATTGAGTCTTCGGAGAACTCC	Transcription factor	*Mse*I
3	MYB109	ATTGGAATGTCACATCCTAA	AGGTCTCTCGAGTTTACCGA	Transcription factor	*Alu*I
3	MYB111	CTGGCAAGAGTTGCAGATTACG	GATATCTTGATTGTCAAAG	Transcription factor	*Nco*I
3	MYB75	GAATAGTGTTCAATTTCCAAC	GTCACTAACATCTGCAGCTAATTCC	Transcription factor	Indel
4	ADH1	GATTGATCCACAGGCACC	CGTTAAGGCTCCATTAACAGC	Alcohol dehydrogenase	Hpy188I
4	DAHPS	ATGGCTCTTTCAACAAATAGCACCACC	CAGCACAATCACCACCTTGTAAC	DAHP synthase	*Tse*I–*Sau*3AI
4	EGS	ATGGCTGAGAAAAGCAAAATTC	ATGGCTGAGAAAAGCAAAATTC	Eugenol synthase	*Hae*III
4	F3H	GCGGTTTGACATGTCTGGTGGC	CCAATCTTGGACCACTTCACC	Flavonoid 3′-hydroxylase	*Xmn*I
4	MYB60	CAATGGGAAAGGACACTTCA	ATTGGAATGTCACATCCTAA	Transcription factor	*Hae*III
4	MYBPH3PROT1	CTCCAATGGGTCGATCTCCA	TGACCATCATGGGACAAGAG	Transcription factor	*Hpy*188I
4	SAMS	GACTTGCCCATGGCTCAGACCAG	CTGCTACTTAACAGTTAACAG	Salicylic acid carboxyl methyl-transferase	*Tsp*509I
5	BSMT	CAAATTTTCTCAAGTACCGTTCAG	CATAGTCTTATAATTAAGGGTG	Benzoic acid carboxyl methyl-transferase	*Msp*I
5	CHIA	ACACCAGTAAAAGTAGAGCAAAAA	ACAAGGGAATTCAGCACTAAAACA	Chalcone isomerase	*Hint*I
5	CHS	CTCGAGCCCTTGTCGTTTGT	TAATCGATCCACCCACGGTA	Chalcone synthase	*Hae*III
5	GT	TGGTGCAGCCTCATGTCATC	GAAGTGCAACTCGAAGACTC	Glucosyl transferase	*Bst*YI
5	MYBPH3PROT2	GTATGGCACACTCACTTG	AAATTCTGGTAAGTCTAATAAGTC	Transcription factor	*Mwo*I
5	RAT	GCTCCTAATTTTGCTGTACC	CACTCGCATAAGACTTCTCC	Rutenoside acetyl transferase	*Hae*III
6	3KAT	TGATACTAGTAGATGGAAA	GATCAAGATGAATTGTATTCTTC	3-ketoacyl thiolase	*Hpy*CH4IV
6	AN1	CCAGTCAAAAATCAAACCCCTTCA	AGCCTCCTCAGCACTAACTTCC	Transcription factor	*Tsp*RI
6	AN2	ATGGTCACTTATAGCTGG	CAAGAAACATGATTCATTGCCG	Transcription factor	*Sau*96I–Indel
6	C4H1B	GTTAGGTTTTAGAGCTTAG	GTGCCACATGCCTAACTCAC	Transcription factor	*Eco*RI
6	MYBX	TCTCATCCACTACTAGTCTTTCAAAC	AACTGATGACTAGGAATGAGCCTAA	Transcription factor	*Nsi*I
6	RT	CCAGCTAGTGCTGAGCTTCT	CTGAGTCCCGACTGTATACG	Rutinoside transferase	*Taq*I
7	AN4	CGTAGTATCTCATTATGTACTC	GTCTCCCAGCAATAAGTGACC	Transcription factor	Indel
7	MYBB	CTAGCAAAATGAAGGCATATTATTCCAGC	GGGACTGTATAAATTATATAGTCAAGGTGC	Transcription factor	*Kpn*I–*Hpy*CH4V
7	ODO1	GATCCACTACTAAGCTGCCTAC	CTCTAAGCAAATCTAACTTCCTAG	Transcription factor	*Hpy*CH4III–*Dpn*II
7	PAL2b	CTCCAGCAACACGGAATGCC	GGATGTAGGTGGTGAAATTATTGTCAATATCTG	Phenylalanine ammonia-lyase	*Hae*III

To further improve genetic mapping of some specific regions of the seven *Petunia* chromosomes, we relied on the genome of tomato (assembly 2.10, solgenomics.net, Müller et al. 2005). Sequences of the *Petunia* markers were located in the tomato genome with BLASTN. Site-specific *Petunia* markers (designated as Pt, Table 3) were designed from those regions that were poorly targeted in tomato. The Pt markers were genotyped with the same settings of the microsatellites when the source of polymorphism was a short insertion–deletion and with the same settings of the CAPS markers when a digestion was necessary. Gel pictures of the parental lines for the markers, DNA sequences, and additional genotyping details are available at www.botany.unibe.ch/deve/caps/index.html. The putative function of all the gene-derived markers relied on *Petunia* literature information when available or alternatively was inferred from a BLASTX search in the *Arabidopsis* protein database (TAIR9, www.arabidopsis.org/).

Linkage mapping

Linkage was determined with MAPMAKER 3.0b (Lander et al. 1987). Recombination fractions were converted into Kosambi centimorgan units (Kosambi 1944). The best marker order within each linkage group was resolved with the ripple command. Uncertain marker orders were manually refined after correcting genotyping errors and later confirmed with the ripple command of the R-library R/qtl (R Development Core Team 2010; Broman and Sen 2009). Linkage groups were drawn with MapChart (Voorrips 2002). Chromosome definition and orientation for the linkage groups was inferred from previously published cytological and genetic maps (Gerats et al. 1993; Strommer et al. 2000; Strommer et al. 2009).

Assessment of *Petunia*–tomato synteny

The degree of synteny between tomato and *Petunia* was estimated independently for each of the two *Petunia* maps with the assembled tomato genome (*Solanum lycopersicum* assembly 2.10, solgenomics.net). The maps of *Petunia* and the tomato genome sequence were linked together with a BLASTN search (Altschul et al. 1997). Synteny relationships were visualized with the software Circos (Krzywinski et al. 2009).

Results

Marker development and polymorphism analysis

EST sequences of *Petunia* were downloaded from GenBank and mined for SSRs. A total of 463 perfect and imperfect SSR motifs were detected. On average, 2.5% of the ESTs contained at least one microsatellite (one every 23 kb). Among the pool of possible SSRs, trinucleotides and mononucleotides were the most abundant (see Supplementary data, Fig. S1). These findings from *Petunia* are in line with the distribution and frequency of microsatellite motifs in the transcriptomes of maize, rice, and *Arabidopsis*, as described by Morgante et al. (2002). The over-representation of trinucleotides in the transcriptome is probably explained by the purifying selection acting on repeats whose instability would disrupt the open reading frame. PCR primers were tested on the four *Petunia* species *P. axillaris*, *P. exserta*, *P. parodii*, and *P. inflata* to check for polymorphisms. Table 1 contains the PCR primers of the microsatellite markers that were polymorphic and that were further genotyped for linkage analysis. CAPS markers were designed on *Petunia* EST sequences with a putative role in specifying floral traits (e.g., color, scent, nectar, or morphology; Stuurman et al. 2004). AFLP markers were genotyped only on the BC_1 cross *P. axillaris* × *P. inflata*. Eight primer combinations designed with the *Pst*I–*Mse*I restriction sites produced 75 polymorphic bands and six primer combinations for the pair *Eco*RI–*Mse*I amplified 78 polymorphisms. Because most of the AFLP markers clustered around a few loci (data not shown), only 28 nonclustering AFLP bands with high scoring quality were informative and were integrated into the *P. axillaris* × *P. inflata* map.

The Pt markers developed by relying on tomato positional information greatly improved mapping in *Petunia*, especially in chromosomes 4 and 7, which historically lacked molecular markers.

Construction of *Petunia* linkage maps

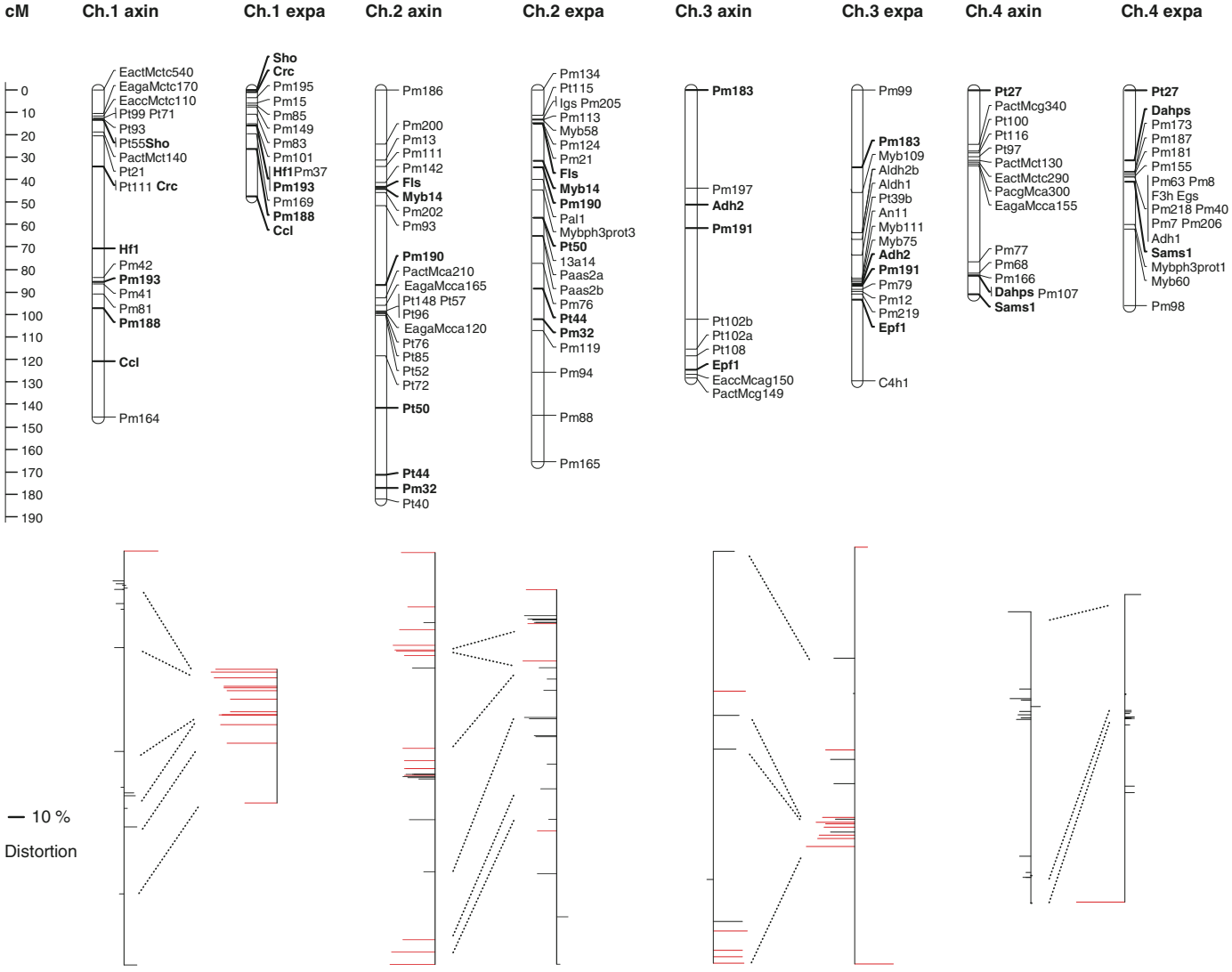
Two *Petunia* genetic maps were obtained by genotyping a set of 173 F_2 lines derived from *P. exserta* × *P. parodii* and 176 BC_1 lines from *P. axillaris* × *P. inflata* (Fig. 2, upper panel). Chromosome identity of each linkage group was established by mapping CAPS markers (Table 2) that were used in previous mapping studies (Strommer et al. 2000, 2009). Both maps span the seven chromosomes of the *Petunia* genome and cover 700 cM in *P. exserta* × *P. parodii* and 970 cM in *P. axillaris* × *P. inflata*. The *P. exserta* × *P. parodii* map consists of 119 markers, with an average marker density of one marker every 6 cM, whereas the map of *P. axillaris* × *P. inflata* was constructed with 125 markers (one every 8 cM). Using a set of 37 markers common to the two maps, it was possible to compare marker order and recombination rate at 30 orthologous genetic intervals. The two maps retained perfect collinearity of marker order (Fig. 2, lower panel), but substantial differences in recombination frequency at orthologous genetic intervals were observed. Generally, genetic distances in *P. axillaris* × *P. inflata* were significantly higher than in *P. exserta* × *P. parodii* (see Supplementary data, Fig. S2). In chromosome 5 the opposite was observed; markers Pm19, ChiA, Gt, and Pm44 cluster in the *P. axillaris* × *P. inflata* cross, but segregate in the *P. exserta* × *P. parodii* cross. On average we counted 1.15 crossovers per chromosome in BC_1 *P. axillaris* × *P. inflata* and 1.41 crossovers per chromosome in F_2 *P. exserta* × *P. parodii* (see Supplementary data, Fig. S3). Based on cytological observations, Rees and Durrant (1986) reported on average 1.7 chiasmata per chromosome in *Petunia*. Assuming transferability of this information, we are covering between 67% and 82% of the genome. Up to date, the maps presented here are the most complete, both in terms of the number of markers and genome coverage.

In both crosses, many loci displayed significant segregation distortion for one of the parents. The degree of distortion was stronger in the *P. exserta* × *P. parodii* population (Fig. 2, lower panel), where the alleles of *P. exserta* were overrepresented for half of the markers. In this cross, distorted markers were mostly localized to chromosomes 1 and 3 and to the top of chromosome 6. In the *P. axillaris* × *P. inflata* cross, the distortion was lower and affected a smaller number of

Chr.	Marker	Forward primer	Reverse primer	Putative function	Polymorphism
1	PT21	CAAAGGGGTGGAGCAGCAG	CAACTACCATAAGTTCCTTG	Unknown	Indel
1	PT55	GGACTTCCACAGAGAATTGG	GCTGAAACTACATTCAGATAC	Unknown	Indel
1	PT71	GTGCTCTCATCCAACATGAG	CCCACGAGTTTGCACCACTAG	Anion channel	Indel
1	PT93	CCTCTCTATTCTAAATTGCTTG	GCTGAAACTACATTCAGATAC	Unknown	Indel
1	PT99	CTTGATGACTCAGACATATGGC	TCACTTCTCAACCACATA	BolA-like family	Indel
1	PT111	GGACAGTGAGAATCATGCTAC	CTATGGAGGTCAGTCGACCCAC	Armadillo motif	Indel
2	PT40	CTCTCTGGTAAGATGGGCTG	TGATGCTCTAATTACTGGC	Unknown	Indel
2	PT44	CTTCTCTTGTACTTGGAGG	CACATTTACGCCCAATCTCAG	RAB GDP-dissociation inhibitor	Indel
2	PT50	GATGAGCTTGGGGACACCAG	CCAATGCTACACAACACAGTC	Coumarate-CoA ligase	Indel
2	PT52	GCAGTGGAAACTAGTGTCAAC	CACCCCACTAATCAACATTAAC	Ethylene forming enzyme	Indel
2	PT57	GGAGGCTTTGGTCATGTGAG	TCCTATTCAGATGTCATGA	Peptidase	Indel
2	PT72	GTGGTACAAGTACATTGAG	CTCTAGTCTCCTTGCACTAC	Proton-dependent oligopeptide transporter	Indel
2	PT76	GTTGCCCATGTTTGGGTG	GCCCTTCACCTTTAAGGG	Unknown	Indel
2	PT85	CAATACCTATGGAAGCTCTTAG	GTCCATTGATCTGCCTGAAAC	Exoribonuclease	Indel
2	PT96	CTAACCGGCACAACTAATTGC	ACGTGAAACATCAGCATTG	Unknown	Indel
2	PT115	GGGTTGGAGACTCACTCAAC	CGTCAAAAGAACCAATTCT	Aldo/keto reductase	Indel
2	PT148	CTTGCCCCCAACAGCTGGTG	GTTAAGAGTTTGAGAGATC	Unknown	Indel
3	PT39b	CTCCCTTCCGGATCATTGGG	GCAGAATTCATCACACTTCC	Heme binding	Indel
3	PT102a	GAAGAGCCCTCTAGTATCAG	GCACCGCAGAATGATTTGCTG	Chlorophyll binding	Indel
3	PT102b	GAAGAGCCCTCTAGTATCAG	GCACCGCAGAATGATTTGCTG	Chlorophyll binding	Indel
3	PT108	GCCTAGATCGCATCAGAC	GCTGAAAATTCGAATCATCAGAC	Leucin-rich repeat kinase	Indel
4	PT27	CAGGATTGGGATGACGATTGG	CCGTGGTGTATGTACCTCGTG	Peptidoglycan-binding kinase	Indel
4	PT97	CTGATGTATGCTAAGCGTGCT	CCAGCAGAATTCATGTCAGC	Cytoskeleton structural constituent	Indel
4	PT100	GAACTTGGAGAAGCCGTAAGG	AGATATAACGGCTGTCACC	UDP-glucoronosyl transferase	Indel
4	PT116	GGTCACATTCTCAATCTTGG	CATGGAAATAACAAGCTGCTG	Uroporphyrinogen decarboxylase	Indel
5	PT22	CAAGCTTCCTGTGCAGTC	TGGAATGACCATTCAACTG	Unknown	Indel
5	PT26	CAGATGAGGGATTATCTCC	TTCAGAACTACTTTACA	Carbohydrate kinase	Indel
5	PT36	CCTTCAAGCCCCTATGACAAG	CTCATCTCAAACGAAAACC	Calmoduline	Indel
5	PT37	CGAGTGGCTATAGCGAGGG	CTCATTCAGCAAAATTCACAAGATC	Unknown	Indel
5	PT104	GATGTTGGAAACAGCCTCAAG	GTACATCCGTTTGTCTCTCT	Cinnamyl-alcohol dehydrogenase	Indel
5	PT113	GGCACTTCTTCAAGAAATGG	CACATTGAAGTCTTTACAC	Diacylglycerol kinase	Indel
5	PT114	GGCGCCTTTCGAAGCACTTTC	CACACCACCGGGTGGCTCGCC	Unknown	Indel
5	PT140	GGAGCTGAGAAATCAGTGT	CTCCACTAAAGTGGGAAGGAG	ATP synthase	Indel
6	PT25	CAGAGCCTTGGGAGCTAGAGC	AGAAGCTTGTGGCAGCC	Unknown	Indel
6	PT84	CAAGGAGAGCTTATGTGAGC	TGTGAAGATACAGCTACCAG	Unknown	Indel
6	PT105	GCATCTTGTTTAGGACAACC	CAAGTAATGAATCGCTAAGTTCC	Unknown	Indel
6	PT110	GGAATTGCAGAGTGGCAGAGC	CAAAAGCCAAAACTCATACG	Nucleoside diphosphate kinase	Indel
6	PT149	GAGATGTACTAGTACTATG	GTACATTGTTGTCGAAC	Unknown	Indel
7	PT3	CTTCCCCTCCTTCAACGCATGTACG	CGGAAACGGCCTCTCACCC	Unknown	Indel
7	PT5	CAGGATCCTAAGTATTGGAC	GCATGACTCCTTTATCGAC	Exonuclease	*Dpn*II
7	PT6	CTCGGTCTGGACTTGATTCAG	CCTTTGTAAGATAATCCCCTG	Unknown	Indel
7	PT7	GTGGAGTCTGCATCTATGG	CTTCAGATCATCCTCAGTGAG	Unknown	*Nsi*I
7	PT8	CCTTAGGACCTGCATCACCC	CAGCGGCTATCTTTGGAGC	Heat-shock protein	Indel
7	PT11	GAATGTGGATGTGGACCTCG	GCTGCTCCCCTCGTCAGATCC	Superoxide dismutase	*Rsa*I
7	PT13	CATGGCCTTGATGTCTCAGG	CCGCGAAGAAGTATGCAC	Glutamate–cysteine ligase	Indel
7	PT15	CTAAAGATTCCCATGAATCAGC	GGGGAAGATGTAGTTTTATAACC	Unknown	Indel
7	PT30	CCAAGTGATTCCACCATCTC	GAGATATTCCACCACCC	RNA polymerase	Indel
7	PT39a	CTCCCTTCCGGATCATTGGG	GCAGAATTCATCACACTTCC	Heme binding	Indel
7	PT87	CATGTGATTCTATAATCCGAG	TGAGGCAATCCCGGGTCTTTTG	Gamma carbonic anydrase	Indel
7	PT134	CCAAGTTACTAGGAGTACC	GTAATGCCCAATGGTTC	*O*-Methyltransferase	Indel

Note: Markers presenting an insertion–deletion polymorphism were mapped like the microsatellites; if a restriction digestion was required, they were genotyped as the CAPS markers.

Fig. 2. Genetic maps of *Petunia* interspecific crosses. Linkage maps of *Petunia* obtained from two interspecific crosses. For each chromosome, the *Petunia axillaris* × *Petunia inflata* map is represented on the left (denoted by axin) and the *Petunia exserta* × *Petunia parodii* on the right (denoted by expa). The markers in bold are shared between the two maps. The diagram below each chromosome displays the segregation distortion at each marker locus. Distortion towards *P. axillaris* or *P. exserta* is to the left and towards *P. inflata* or *P. parodii* is to the right. Black horizontal lines correspond to markers that are not significantly distorted. Red color refers to a distortion in favour of *P. exserta* or *P. inflata*. The length of the line is proportional to the degree of the distortion, as indicated in the legend. Dotted lines in the middle connect the markers shared between the two crosses.

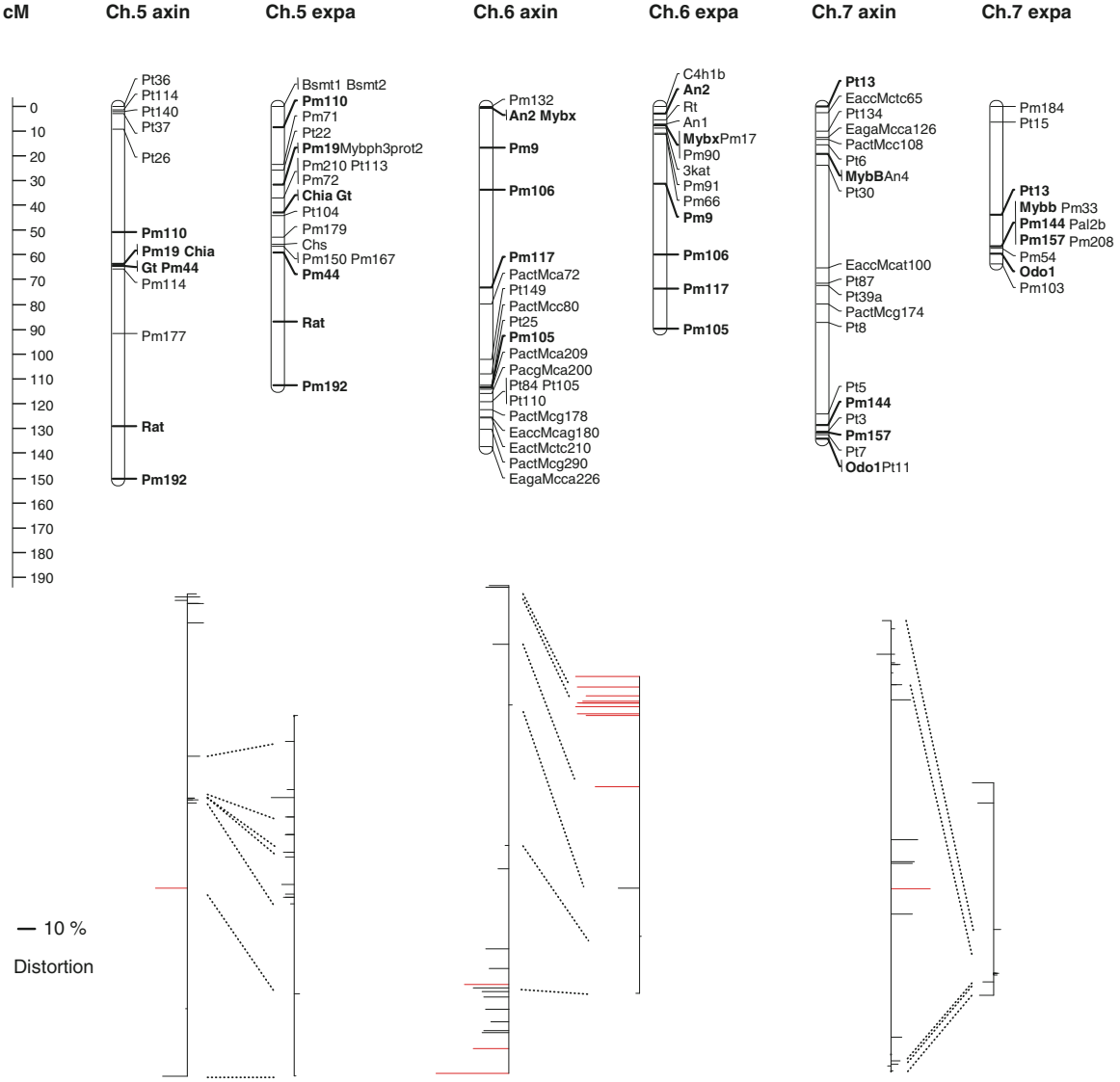


markers (30%), mostly in chromosome 2 towards the *P. axillaris* alleles and in chromosome 3 towards the *P. inflata* alleles. Interestingly, the chromosomal regions subject to segregation distortion were different in the two crosses.

Comparative mapping of *Petunia* and tomato reveals a complex mosaic of rearrangements

We tested synteny between the two genetic maps of *Petunia* and the physical map of tomato (assembly 2.10, solgenomics.net, Müller et al. 2005) with a BLASTN search of the *Petunia* marker sequences in the tomato genome database. Conservation of macrosynteny within the Solanaceae has been described between the genomes of tomato, potato, pepper, eggplant, and diploid tobacco (reviewed by Wu and Tanksley 2010). The genomes of these solanaceous crops all have the same chromosome number ($2n = 24$). In contrast, *Petunia* has a chromosome number of $2n = 14$, necessitating a number of rearrangements. A tabular summary of the BLASTN output is provided in the Supplementary data (Table S1). The pattern of synteny varies greatly for different chromosomes (Fig. 3). Chromosome 7 of *Petunia* retains most of the markers from the tomato chromosome 8. Chromosome 5 retains most of the markers from the tomato chromosome 12. Chromosome 1 and 6 of *Petunia* contain segments of the tomato chromosomes 5 and 6, and 1 and 9, respectively. Chromosomes 3 and 4 of *Petunia* retain synteny with chromosomes 3 and 4 of tomato, but only for a segment of the terminal portions of the long arms. Within orthologous syntenic blocks, we observed little correlation between genetic distances of *Petunia* and physical distances in tomato. In *Petunia* chromosome 2 synteny was more disrupted. This chromosome contains segments of tomato chromosomes 2, 7,

Fig. 2. (*concluded*).



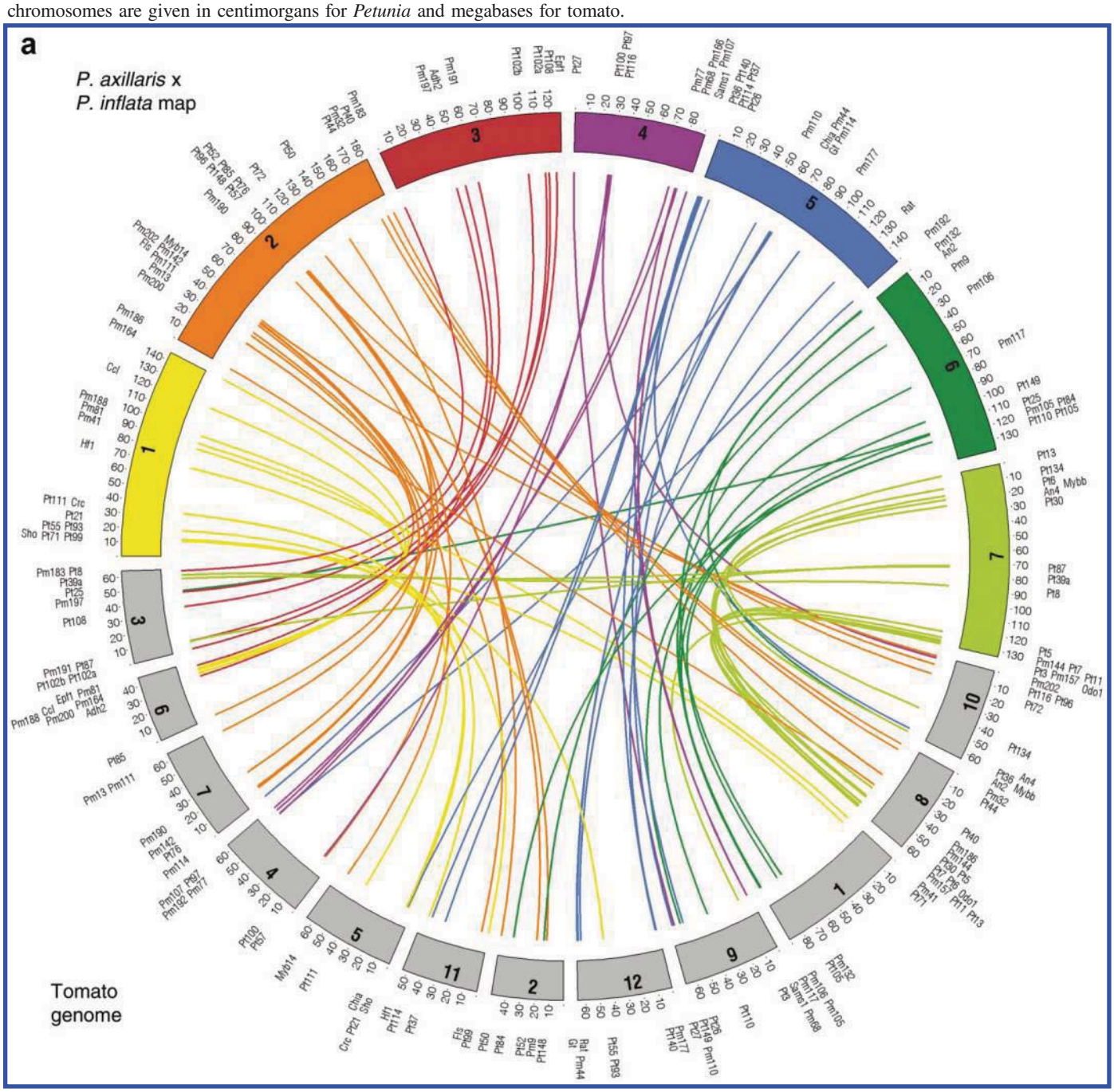
8, and 10. Most of these observations are consistent in the two crosses; small-scale incongruence (e.g., in chromosome 4) is explained by the presence of different marker sequences in the two maps.

Discussion

Distortion of the segregation frequencies as a result of recent species radiation

For half of the marker loci mapped in the *P. exserta* × *P. parodii* cross we observed significantly more alleles of *P. exserta*. For all loci but one (*Pm99*), distortion was towards *P. exserta* alleles. In chromosomes 1 and 6, 40% more *P. exserta* alleles were observed than expected. Conversely, segregation distortion in the *P. axillaris* × *P. inflata* cross was more localized, more moderate, and in the direction of both parents. Distortion is localized to specific chromosomal segments that are different in the two crosses. This indicates that distortion originates from species-specific locus interactions. These interactions may be prezygotic, resulting from differences in the growth rates of recombinant pollen in the styles of the F_1 cross. Because the F_1 pollen has a recombinant genotypic constitution, the loci influencing pollen growth would be segregating. Thus, distortion affects only specific chromosomal segments and not all of the genome. Distortion may also result from postzygotic selection against specific allelic combinations that determine lower fitness in interspecific hybrids. While growing the recombinant plants, several seeds failed to germinate and the seedlings differed greatly in growth rate, fitness, and survival. Segregation distortion in interspecific hybrids has previously been observed in *Petunia* (Robbins et al. 1995; Strommer et al. 2000) and more generally within the Solanaceae and other plant families (Zamir and Tadmor 1986). In *Arabidopsis*, temperature-dependent lethal allelic combinations of the Bateson–Dobzhansky–Müller type were ascribed to autoimmune-like responses (Bomblies et al. 2007). *Petunia exserta* is a rare species endemic to a restricted area (500 km^2) of Brazil. In this area it grows sympatrically with the highly abundant *P. axillaris* and interspecific hybridization has been observed (Lorenz-Lemke et al. 2006). From an evolutionary perspective, hybridization represents a threat to spe-

Fig. 3. Synteny relationship with the tomato genome. The seven chromosomes of *Petunia* are depicted with different colors. Lines of the same color of the chromosomes connect *Petunia* marker sequences with their physical position in the genome of tomato. The chromosomes of tomato are represented in grey and have been reordered to minimize line overlapping. The synteny relationships with tomato have been plotted independently for the maps of *Petunia axillaris* × *Petunia inflata* (*a*) and *Petunia exserta* × *Petunia parodii* (*b*). The units on the chromosomes are given in centimorgans for *Petunia* and megabases for tomato.

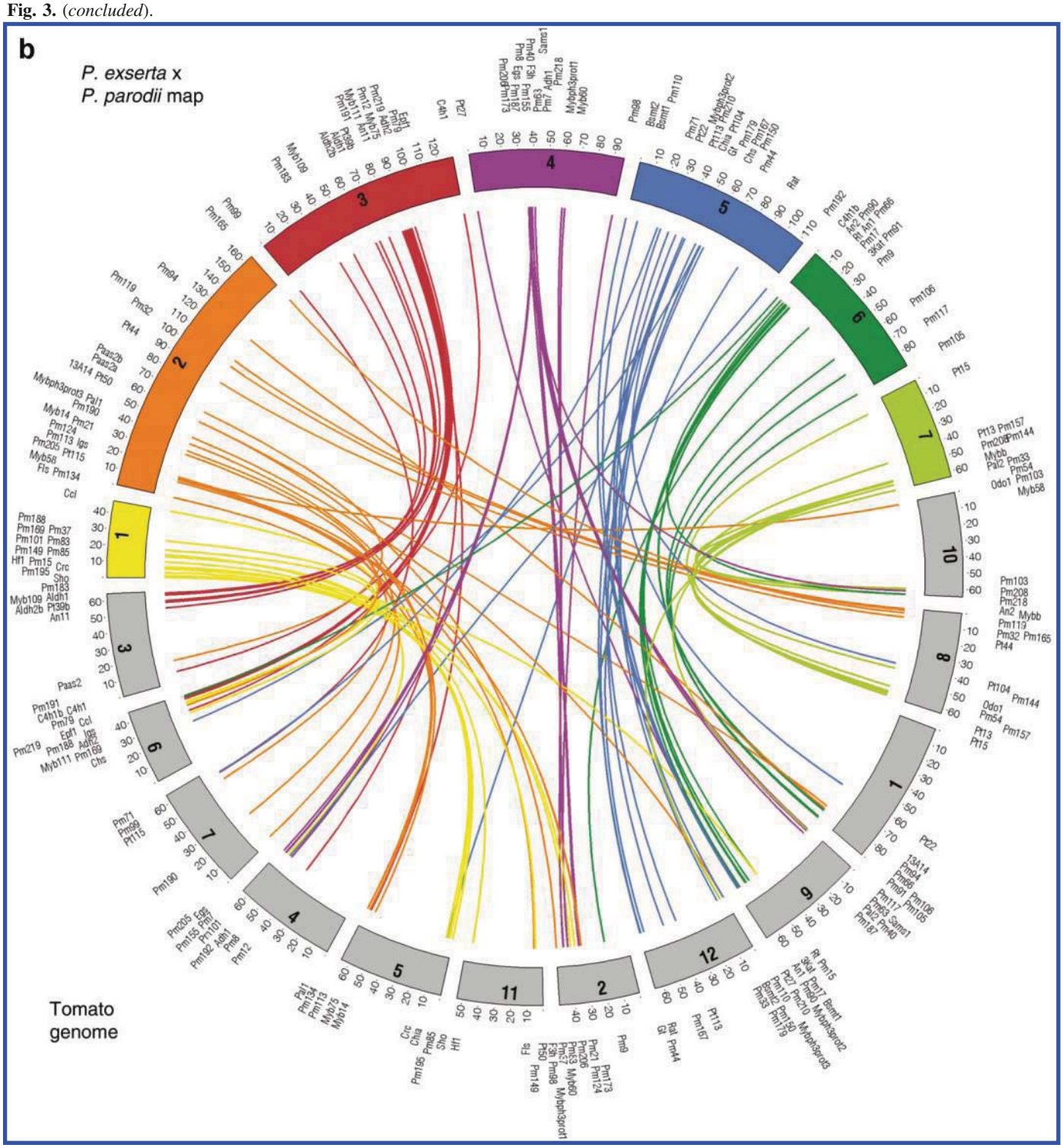


cies identity and segregation distortion in favour of *P. exserta* may be an advantage contributing to the maintenance of species identity.

Genetic recombination in *Petunia* is influenced by the genetic background

In the two interspecific crosses we observed significant differences in recombination frequency. Previous genetic maps of *Petunia* were done with accessions of the garden line *P. hybrida* (Strommer et al. 2000, 2002; Stuurman et al. 2004). In these previous studies great differences in recombination frequency at orthologous genetic intervals were also observed. While counting recombination frequency in different crosses, Cornu et al. (1989) postulated the presence of a major modulator of recombination (*Rm1*) present on chromosome 2 of the *P. hybrida* line St43. This locus is described as dominant with complete penetrance. It is not clear whether this gene of *P. hybrida* can be traced back to the *P. axillaris* or *P. inflata* germplasm. However, as *P. parodii* has a genetic background very similar to that of *P. axillaris*, but exhibits

Fig. 3. (*concluded*).



large differences in recombination rate, the *Rm1* locus is more likely to have been derived from *P. inflata*; this would explain the higher recombination rate observed in the *P. axillaris* × *P. inflata* cross. Robbins et al. (1995), using T-DNA insertions, observed that extensive suppression of recombination occurred in hybrid genetic backgrounds, but recombination levels were three times higher in an inbred background. Recombination suppression was therefore attributed to physical rearrangements in hybrids. Physical evidence for suppression of recombination was later confirmed by ten Hoopen et al. (1996) with fluorescence in situ hybridization. To some extent we also observed that in orthologous genetic intervals recombination can vary greatly. In some cases suppression of recombination led to complete clustering of genetic markers. In chromosomes 2, 5, and 7 of the *P. axillaris* × *P. inflata* population, clusters of genetic markers occurred in more than one chromosomal segment, indicating that clustering cannot be explained only by the presence of the centromere.

An intriguing hypothesis is the presence of residual paleocentromeres or heterochromatic regions remaining after chromosome fusion during the radiation of the Petunioideae clade. We speculate that $x = 12$ may represent the ancestral chromosome number of the Solanaceae family. Some of these chromosome fusions in *Petunia* may have occurred only recently, as the *Petunia* sister taxon *Calibrachoa* has the karyotype $n = x = 9$ (reviewed by Stehmann et al. 2009). Generally, chromosome fragmentation and fusion are known to happen and represent an important speciation factor in plants. However, more cytological work is needed to shed light on the role of chromosomal rearrangements for species radiation within the Solanaceae.

Despite the differences in recombination rate observed between the two crosses, the order of the 37 shared markers was identical, indicating that the genome structure within the genus is well conserved. These results are consistent with the findings from Strommer et al. (2000, 2002), where despite big differences in recombination frequency, marker order between different crosses was largely retained.

Distribution of *Petunia* genic microsatellites and their conservation in tomato

In the *Petunia* transcriptome, we detected on average one microsatellite every 23 kb. Generally, microsatellites in plants are preferentially associated with nonrepetitive DNA and the number of microsatellite motifs detected in expressed sequences is considerably higher than that observed in intergenic DNA (Morgante et al. 2002). Within the *Arabidopsis* transcriptome, the untranslated regions (UTRs) are the sequences with the highest dinucleotides abundance, whereas trinucleotides are most abundant in coding sequences (Morgante et al. 2002). Using unigenes and predicted proteins from tomato, we could annotate the position of 29 SSRs in the *Petunia* transcripts (see Supplementary data, Fig. S4). Although the data set is small for a conclusive statement, the distribution observed in *Petunia* is similar to what Morgante et al. (2002) found in *Arabidopsis*. Despite 35 million years of divergent evolution with tomato (Wang et al. 2008), 60% of the *Petunia* microsatellites were also detected in the orthologous tomato EST, either as fully developed motifs or as short protorepeats. Microsatellite positions were better conserved in the coding portion of the ESTs. EST-microsatellites may play a role in the generation of allelic variation, e.g., by modifying the protein primary structure or by altering mRNA stability. In animals, numerous studies correlate the generation of rapid phenotypic variation with the instability of EST-microsatellites (Sutherland and Richards 1995; Fondon and Garner 2004; Hammock and Young 2005). Although at present a thorough investigation does not exist, microsatellites are likely to provide a substrate for rapid phenotypic variation also in plants.

Conclusions

We have constructed two linkage maps of *Petunia* with gene-derived markers using two interspecific crosses, and we report for the first time the development and mapping of multiallelic microsatellite markers of *Petunia*. Genetic analysis has shown that the structure of the *Petunia* genome is well conserved within the genus. Local segregation distortion within the interspecific crosses hints at partial genetic barriers that arose after recent speciation. Comparative mapping with tomato suggests that numerous genomic rearrangements occurred during the radiation of these two Solanaceae species. Given the decay of synteny between tomato and *Petunia*, the usefulness of the tomato genome as a template for comparative genomics is limited to a few chromosomal segments, and a *Petunia* genome sequence would be needed to support genomic research on this model plant. A *Petunia* sequencing initiative has been undertaken. The maps reported here will facilitate the assembly of its large genome.

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