

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

Eligio Bossolini, Ulrich Klahre, Anna Brandenburg, Didier Reinhardt, and Cris Kuhlemeier

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.

Résumé : Deux cartes génétiques ont été produites pour la plante modèle *Petunia*. Des populations en ségrégation ont été obtenues en croisant les espèces sauvages *Petunia axillaris* subsp. *axillaris* × *Petunia inflata* et *Petunia axillaris* subsp. *parodii* × *Petunia exserta*. Les deux cartes couvrent les sept chromosomes de *Petunia* et s'étendent sur 970 et 700 cM, respectivement, des génomes. Au total, 207 marqueurs ont été placés. De ce nombre, 28 sont des polymorphismes de longueur des produits amplifiés (AFLP) multilocus et 179 sont des marqueurs dérivés de gènes. Pour la première fois, les auteurs rapportent le développement et la cartographie de 83 marqueurs microsatellites chez *Petunia*. Les deux cartes conservent le même ordre des marqueurs, mais présentent des différences en ce qui a trait aux fréquences de recombinaison au sein d'intervalles orthologues. Des réarrangements génomiques complexes ont été détectés en comparant avec le génome de la tomate (*Solanum lycopersicum*), ce qui suggère que la synténie entre le genre *Petunia* et d'autres solanacées aurait été considérablement perturbée. Les marqueurs nouvellement développés faciliteront la caractérisation génétique de mutants et les études écologiques sur la diversité génétique et la spéciation au sein du genre *Petunia*. Les cartes constituent un outil puissant pour lier l'information génétique et génomique et seront utiles pour aider à l'assemblage de la séquence génomique du *Petunia*.

Mots-clés : *Petunia*, carte génétique, marqueurs moléculaires, marqueurs microsatellites, synténie chez les solanacées.

[Traduit par la Rédaction]

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

Received 19 August 2010. Accepted 30 November 2010. Published at www.nrcresearchpress.com/gen on 1 April 2011.

Paper handled by Associate Editor Francois Belzile.

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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

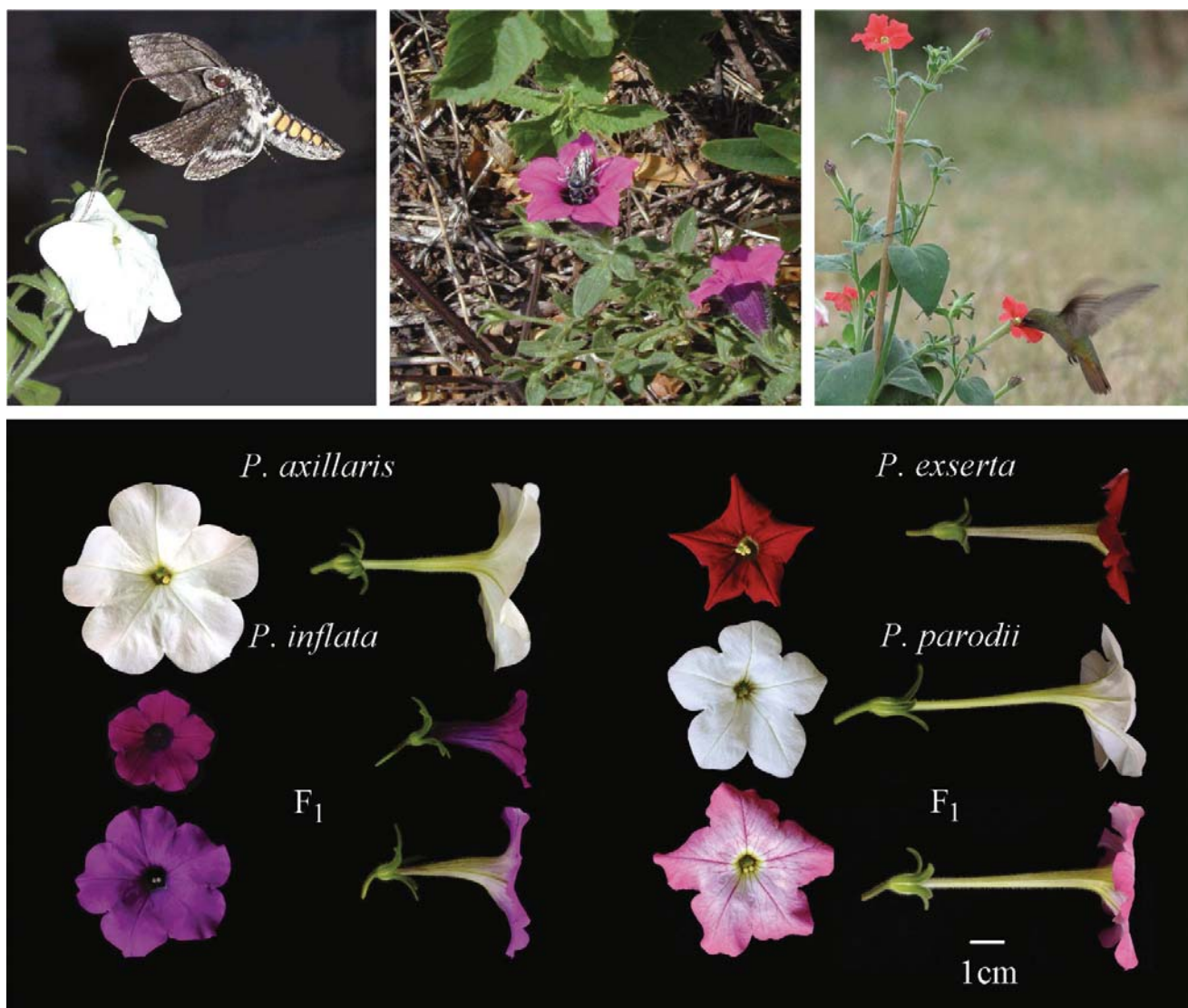
Petunia axillaris subsp. *axillaris* (accession N), *P. axillaris* subsp. *parodii* (accession S7), and *P. inflata* (accession S6) were kindly provided by R. Koes, Department of Genetics at the Free University Amsterdam, Amsterdam, the Netherlands. *Petunia exserta* was a gift from R. Griesbach, Beltsville 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 F₂ plants was created by selfing a single F₁ plant that was obtained from the cross *P. exserta* × *P. parodii*. A second mapping population of 176 BC₁ plants was derived from backcrossing a F₁ 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 data¹ (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 MgCl₂, 0.35 pmol of the M13-tailed forward primer, 3.5 pmol reverse primer, 1.7 pmol labeled (IRD-700/800) M13 primer (5'-CACGACGTTGTAACAAC-GAC), 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,

¹Supplementary data are available with the article at www.nrcresearchpress.com/gen

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 μ L 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 $PIC = 1 - \sum_{i=1}^n p_i^2$, 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 μ L volume containing

50 ng of template DNA, 2 μ L 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 μ L water, 0.5 μ L of the appropriate buffer, and 0.5 μ L of the enzyme directly to 10 μ L 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 *EcoRI*-*MseI* and eight with *PstI*-*MseI*. Generally, primers designed on *PstI*-*MseI* resulted in fewer amplicons than *EcoRI*-*MseI*. To compensate, the amplification reaction was performed with only two selective nucleotides at the *MseI* 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* \times *P. inflata* BC_1 cross.

Table 1. PCR primers, EST of origin, polymorphic index content (PIC), and SSR motif of the microsatellites used for mapping.

Chr.	SSR	Forward primer	Reverse primer	EST	Putative function	PIC	SSR motif
1	PM15	GTTGGCTGGCAACATTGACTA	CACTTACCCTCAGTCTCTCG	CV297655	Unknown	0.75	(CT) ₁₂
1	PM37	GGGTTGGGAATCTAGTGGGA	TGGATGAGCCATAATCTTTTGC	EB174496	Transcription factor	0.625	(ATG) ₆ -(CAA) ₅
1	PM41	GGCTCAAACACAATTTCTC	CTCCAACAAGATTACTTGCAG	CV297469	Jasmonate ZIM domain	0.625	(TC) ₁₁
1	PM42	GGCTCAAACACAATTTCTC	AATTCAACCGCCATGAAGTC	CV297469	Unknown	0.625	(TC) ₁₁
1	PM81	ACTGAAATCGTTGGCCGTT	AAAAGGAGTTGCATATCTTGATTA	CV292797	Unknown	0.625	(T) ₁₆
1	PM83	GCAAGTGTCCATCTTGT	CTCTGACCAATAAATGTG	CV299390	Unknown	0.625	(T) ₁₉
1	PM85	TGCAAAATGAATGTCCAGGAT	TGCTGCAACTTTCCCAATTA	CV294317	Unknown	0.625	(TA) ₇
1	PM101	GAGAGAAACCCTAACCC	GCAGAAGAACACAGAGATCC	FN001660	Unknown	0.667	(CTT) ₆
1	PM120	GGTTTAGATACTGAAAGTTG	CCAGCAATTACACCAACCTG	FN005966	Unknown	0.625	(T) ₁₆
1	PM149	CCTAATCAAACACGTAACCTC	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	CCCAACCAATGGCTACAGCC	GGACAACACAATACAATCTCTGC	FN037917	Singlet oxygen response	0.75	(CTT) ₈
1	PM193	CGAACATCACCACATCAG	GCTGCCAAGTCCGACAATGG	FN030612	Unknown	0.625	(CAT) ₆
1	PM195	GCCTTTCGCCGCTGTCACTG	GAGCAATCGTGACCCGTTGG	FN026706	Phosphatidylserine synthase	0.625	(GAA) ₆
2	PM13	GAAGGCAAGAATAGTCACC	CCGATTACTGTTTGAGGAGG	CV296369	Unknown	0.375	(CAC) ₉
2	PM21	CTACCGGTAGGCAGTAGTTGC	CCTCGACCTTCTTCTCTGAC	CV297594	KDR transcription regulator	0.75	(TAC) ₈
2	PM32	TTCCTAAGAAAGAAACAATAAAGCTCA	GGCTATGCCAGCTTTGGTAA	CV298848	Fascilin-like precursor	0.625	(A) ₂₃
2	PM76	GATCGCAACCTGGATCCTAA	AGGGCTGCACCTTGTGTTGT	CV297778	Unknown	0.375	(GAACCC) ₆
2	PM88	CTGTTTCTTAATFACCTG	GCCACTGGCATGGCTGCA	CV298718	Unknown	0.625	(TA) ₇
2	PM93	GCACCTCAGGTTGGTGCACC	GCAGTTGAAACAGAGGGACC	DC243258	Unknown	0.667	(TGC) ₆
2	PM94	CCGTGTTAGTATGGCCAGG	CTCTAGATTGACCATAGC	CV300671	Photoassimilate response	0.625	(GGT) ₆
2	PM111	CACCATGAGGAACATCAAGC	GGAACTGGCTGAGGGAAACC	FN000621	Unknown	0.375	(CAC) ₉
2	PM113	GGCTGTGTGCAATGGACG	CTCTAGATTGACCATAGC	FN032542	RNA binding	0.375	(TGG) ₆
2	PM119	CCGACACATACCAATTCAC	CACCTAACGTACATTAGC	FN004737	Inositol triphosphate kinase	0.75	(TG) ₉
2	PM124	CCCACACCACTTCCATTAC	CGATGCTTGATTTCCCCAC	FN005227	Unknown	0.375	(TGA) ₇
2	PM142	GTTGGTGTGAGCCAAAGC	CGCCAGCTGCTTCTGAG	FN008269	Transcription factor	0.375	(ACT) ₆
2	PM165	CTCTACCTTACATCTACC	GTGCAGCGACAACGAGTC	FN045187	UBX-domain	0.375	(CAA) ₆ -(GAA) ₆
2	PM186	CCTTACTAGTCTCAGAATJGC	GGATAATGATGATGACCC	FN036047	TCP-like transcription factor	0.375	(GTT) ₇
2	PM190	CGAGTTGATGGTGCAATJGTG	CTAGAAAAGTTCTCCCGG	FN038900	TraB family	0.625	(GAA) ₇
2	PM200	CCTGACCTCCAGAAAC	GGTAACATCTCCCTCACATTCC	FN022583	Unknown	0.667	(GTT) ₆
2	PM202	CCCTGTTCTTCTTCCAC	CATCCACCCTTGTGTTGAG	FN031565	Transcription factor	0.375	(ATT) ₇
2	PM205	GGAGCCAAGTTGCTTGAGG	CCCTTCAACAACACACC	FN035754	Transcription factor	0.625	(TTG) ₆
3	PM12	GGAACTTACAGAAATGG	GAAGTTGTTGGCTCAACC	CV293902	Unknown	0.5	(CAA) ₇
3	PM79	TTGGCGAAACATGCTGTATG	CAAGCTTCATAAACCAACAACC	CV299471	HTB4 DNA binding	0.625	(GTT) ₅ -(A) ₁₁
3	PM99	CAAGTGTGGAGCAGTGGAA	GCCCTGCAAAAATGTGAAAT	CV294997	Unknown	0.375	(TTC) ₅
3	PM183	CCTAATTCAGTCCATGAGGC	GTTAGCTGTCTGATCACC	FN041860	Phosphatase	0.75	(GT) ₁₀
3	PM191	GGAGAAGATGTTGGTAAC	GGGAAAACGATCTCTTGGCTG	FN034502	Unknown	0.75	(TTG) ₆
3	PM197	CCATAAGTGAAGGATCCTGC	CTGACAACCTTACACAGGAACAC	FN032593	Nucleic acid binding	0.375	(CCA) ₇
3	PM219	GCTGTAACATGTAGCTGTG	GGCTGCCAATCCATGCAGTC	FN014488	Unknown	0.445	(TGA) ₈
4	PM7	CGTTTTTCAITGCAATGTGC	CGTTTTCCCTTTTGTATCTG	CV298460	Squalene monooxygenase	0.75	(AAC) ₁₀
4	PM8	TCTGCAAACTTCAAAGCCAA	ACATGCCATGCATTTTGGAG	CV298210	Transcription factor	0.75	(AAG) ₁₀

Table 1 (continued).

Chr.	SSR	Forward primer	Reverse primer	EST	Putative function	PIC	SSR motif
4	PM40	AGCTTCCCTTTTGTAGCCACA	TGGCTTAAGCAAGACAATGG	CV299350	Mannan synthase	0.75	(T) ₁₄ -(A) ₁₀
4	PM63	TGTTACAATGGAGCAGAGG	ATGTGAGATTCCCAACGACC	NPI240021	Transcription factor	0.75	(ACAGCA) ₅
4	PM68	GTCGAACGAGGATCATGTC	GTGTCTCTTAATGCTTGCC	NPI240041	Transcription factor	0.375	(CAA) ₆
4	PM77	ACCACGAGAAGAAGGAAGCA	CGAACACGAGTTAAACCC	CV298105	Glycine-rich protein	0.667	(GT) ₉
4	PM98	ATGGAGGTAGCAAAATGCAGG	CAACCAATGCAGCTTCAGA	CV298392	Methionine sulfoxide reductase	0.5	(ATT) ₅
4	PM107	GTCAAAGGTTGCAATCTCT	TGTTGCTGATGAGCAGTAG	FN001301	Transcription factor	0.625	(CAA) ₈
4	PM155	GGCAACGACAAATGGTGG	CCTTTGATCTGCATTCTCC	FN010739	Squalene epoxidase	0.667	(AAC) ₁₀
4	PM166	GGCACTTGATGTCTTGTG	CCATGAATCGAATGCAG	FN014864	Unknown	0.625	(A) ₃₂
4	PM173	CAGCGCTATCAACAGCAG	GTGAGAGGCAAGTGAITGG	FN039910	Unknown	0.667	(GCA) ₆
4	PM181	CCAGCTCTCGGAGCTG	CATCAACTATGTAGGAC	FN000364	Harpin-induced protein	0.375	(GAT) ₆
4	PM187	GAGGCATTTGCACAGGC	CTATGGATGCAAGTAGGCCAC	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	TTCAAATTTTCATCAGTGGCG	CV297851	Unknown	0.75	(T) ₁₂ C-(T) ₁₆
5	PM44	AGAAATCCCATATGCTCCG	AGCAGCACAACAACAACAAG	CV298575	Pectinesterase	0.625	(A) ₁₅ -(A) ₁₀
5	PM71	CACTAGGACTCCATATTCAC	GCTTATAAGGGAAGAGACTG	CV298122	Unknown	0.75	(CT) ₈
5	PM72	GTAAAGCCGTTTTGTTGGGA	CATTGAGGACTTGCAGATTG	CV295851	Unknown	0.375	(CT) ₉
5	PM110	GGTACAGGGCTAGCAGG	CTAGTTGGGTGTTACACAG	FN006325	Disease resistance	0.667	(AAC) ₈
5	PM114	GGTAAAGGTCTGTGTAG	CCCTTAGCTGGTATTCGCAG	FN003883	Unknown	0.625	(TG) ₉
5	PM150	CGTCGAATGCCTTAACTGC	GGAAACAACACAGAAACTGTC	FN009853	Sodium symporter	0.625	(ATG) ₁₀
5	PM167	CTCACTAACCAACTTCACC	CTAAGAAGCTTAAAGAGTGG	FN015039	Glycosyl transferase	0.75	(TTC) ₁₂
5	PM177	CCCTTACTCTTCTTCTCACC	GAACTATGAACCAATAGCTCTC	FN016759	Unknown	0.667	(CA) ₁₁
5	PM179	CGGAGAGGAGGACAAAGGC	CCAGTTGCTCAGCCGATTCG	FN019539	RCD1-like cell differentiation	0.445	(AAT) ₆
5	PM192	CCTGTCTTAAAGATTCAGAGGC	CTGAACCTTGGCAATGGC	FN036401	Transcription factor	0.75	(CAG) ₈
5	PM210	CCTTGTGGCATAAGCTGCC	CCAACAACCTGCAACAGCAGC	FN042698	MYB transcription factor	0.625	(TTG) ₆
6	PM9	GGAGGAGAAATATGAAGAAGC	CTCTTCTCTCTCTCCGCAG	CV300118	Unknown	0.75	(AGA) ₁₇
6	PM17	TCCATCTCGTTTACCAACCA	GGCTTCCAGCAAGAGAAGTG	CV301045	Unknown	0.625	(CTG) ₈
6	PM66	CTATGGAACTTCTCCTAAC	GCAATTTCTAGGTTCTAGAGG	CV301241	Unknown	0.667	(AT) ₇
6	PM90	TGGCGCTGAAACATCTATG	AGAAGACAACGACAACGCAA	CV297287	Acyltransferase	0.445	(TC) ₇
6	PM91	AGCCAGGCAAAAGACCAITTA	TTTACGTCATAATCCACGG	CV298703	Transcription factor	0.667	(TC) ₇
6	PM105	CAGTAGGAAGGTTGCAGTGG	GTGCACGGAAAGTTCTCG	FN001497	Peptidyl-prolyl isomerase	0.667	(A) ₁₈
6	PM106	GTTCTCCAGGCACCTTCTGG	CAGAGAGGACACAACCTCTC	FN004825	Gibberellin-regulated protein	0.75	(T) ₁₇
6	PM117	CCATACCCCAATCTCCACTGG	GGTGGCAACCTTGAGCTCC	FN004482	Kinase	0.445	(GTG) ₇
6	PM132	GCAGTAGGCAATTCGAG	CTGATTCCTCCAGCTCGAG	FN010866	Cytochrome <i>b-f</i> synthesis	0.375	(ATC) ₆
7	PM33	AAAATCTTTTCTCTTTTCTTCC	GCAATACCCGTTCCACTTAT	CV298303	Serine-rich protein	0.625	(A) ₂₄
7	PM54	CCGAAACCCAGGAAACGC	CCTGTTGATGTCAAACCC	CV299353	Unknown	0.667	(A) ₁₈
7	PM103	GTGGATGACAAACTTGAGG	GACAGCAGTGGTGTGTTGG	FN006664	Cystathionine beta-lyase	0.75	(A) ₂₀
7	PM144	GCAGCCCTCTTCACTG	CCATTGAATCCCAAGG	FN008495	Unknown	0.625	(A) ₂₀
7	PM157	GTAGTAGTAGTAAACCCACC	CATCAGAAGCTTCTGGAG	FN011441	Heat-shock protein	0.625	(AC) ₁₀
7	PM184	GGACTTTTATCAACTACC	GCCTTGCCTTATCGGAC	FN027453	Unknown	0.75	(GAA) ₆
7	PM208	CGACGCGGCTTTTGAAGC	CCGTGTGCAAGCAGCGTAG	FN038297	VQ motif	0.375	(TTG) ₆

Table 2. PCR primers and restriction enzyme used to map the CAPS markers.

Chr.	CAPS	Forward primer	Reverse primer	Putative function	Endonuclease
1	CCL	CACATAATAATGCCAATGG	GCATGATAGAAATTGCTGC	Coumarate-CoA ligase	<i>HaeIII</i> - <i>MseI</i>
1	CRC	CCAAACGGGCCAATTGTCTGAG	AGCATAGACATAATCTCTCCAAGAAGA	Transcription factor	Indel
1	HFI	TCCCTCAITTAITTAACCATATCTC	CATGGATAGTACCAGAACG	Flavonoid hydroxylase	<i>AluI</i>
1	SHO	TACATAITTAITAGCATCACAGC	TCTTGACACTTGGTTCCACTAC	Isopentenyltransferase	<i>EcoRI</i>
2	13A14	GATGTACATTCCTTGAAGTTGC	GAGCCTCTCCTAACTCTC	Microtubule binding	<i>DdeI</i>
2	FLS	GCTTACACTGAAGGAAGAG	GTCAGAGTTAGTCTGGCC	Favonol synthase	<i>Hpy188I</i>
2	IGS	ATGACTACTGGGAAGGG	GGAAACGTGGTAACGCTC	Isoeugenol synthase	<i>EcoRV</i>
2	MYB14	CACCTACTAGTCCATGACC	TGAGTAAAGTTGCTGCTG	Transcription factor	<i>MwoI</i>
2	MYB58	GAGTGGATTAATTAAGCTAAG	GACAAACTGGCTGATTTGCTCTG	Transcription factor	<i>DpnII</i>
2	MYBPH3PROT3	CTAGCAAAATGAAGGCATATTATCCAGC	CTAGCAAAATGAAGGCATATTATCCAGC	Transcription factor	<i>HaeIII</i>
2	PAAS2	ACCCATTTGATCTCTAGC	GTATCCCGTTCTGCTAGCC	Phenylacetate synthase	<i>MspI</i>
2	PAL1	TAACAACACATGCGCATATAACCAA	AITTCCTGAGTTGTTGAGCCAA	Phenylalanine ammonia-lyase	<i>HpyCH4V</i>
2	PAL2a	ATGGTCAATGCTAATGGTCAATG	CATAACAAAATTAAGATGAAAGG	Phenylalanine ammonia-lyase	<i>EcoRV</i>
3	ADH2	CGACAGGTACAGGCGAAACGAGCATAGATTATG	AGCATTTTAATCGATCAAAAATCAGATTTC	Alcohol dehydrogenase	<i>Tsp509I</i> - <i>AluI</i>
3	ALDH1	CAGCTCTTAGTCCCGAAC	AACCTGGAAAACAGTACTTGAAC	Aldehyde dehydrogenase	<i>PvuII</i>
3	ALDH2	GCCATGTTGCTGAAGGTGATG	GATTTGCACACAAGTGAAGC	Aldehyde dehydrogenase	<i>HindIII</i>
3	AN11	ATGGAAAATTCAAAGTCAAGAAATCAC	TTATACITTTAAGCAATTTGCAACTT	Transcription factor	<i>HaeIII</i>
3	C4H1	GCGCATTTGTTGCTCATGCTC	GAGGTGAAGCTGTTCAAGG	Cinnamate 4-hydroxylase	<i>DdeI</i>
3	EPF1	GAGTAGGGTATTGATCCAA	ATCGATTGAGTCTCGGAGAACTCC	Transcription factor	<i>MseI</i>
3	MYB109	ATTGGAATGTCACATCCTAA	AGGCTCTCGAGTTTACCGA	Transcription factor	<i>AluI</i>
3	MYB111	CTGGCAAGAGTTGCAGATTACG	GATAICTTGAITGTCAAAG	Transcription factor	<i>NcoI</i>
3	MYB75	GAAATAGTITCAAITTTCCAAC	GTCACATAACATTCGACGCTAATTC	Transcription factor	Indel
4	ADH1	GATTGATCCACAGGCACC	CGTTAAGGCTCCATTAACAGC	Alcohol dehydrogenase	<i>Hpy188I</i>
4	DAHPS	ATGGCTCTTCAACAAATAGCACACC	CAGCACAAATCACCACTTGTAAAC	DAHPS synthase	<i>XseI</i> - <i>SauI3A1</i>
4	EGS	ATGGCTGAGAAAAGCAAATTC	ATGGCTGAGAAAAGCAAATTC	Eugenol synthase	<i>HaeIII</i>
4	F3H	GCGGTTTGACATGCTGGTGGC	CCAACTTGGACCACTTCCACC	Flavonoid 3'-hydroxylase	<i>XmnI</i>
4	MYB60	CAATGGGAAAGGACACTTCA	ATTGGAATGTCAACATCTAA	Transcription factor	<i>HaeIII</i>
4	MYBPH3PROT1	CTCCAATGGGTCGATCTCCA	TGACCATCATGGGACAAGAG	Transcription factor	<i>Hpy188I</i>
4	SAMS	GACTTGCCCATGGCTCAGACCAG	CTGCTACTTAAACAGTTAAACAG	Salicylic acid carboxyl methyl-transferase	<i>Tsp509I</i>
5	BSMT	CAAAATTTCTCAAGTACCCTGTCAG	CATAGCTTATAATTAAGGGTG	Benzoic acid carboxyl methyl-transferase	<i>MspI</i>
5	CHIA	ACACCAGTAAAAGTAGAGCAAAAA	ACAAGGGAATTCAGCACTAAAACA	Chalcone isomerase	<i>HinfI</i>
5	CHS	CTCGAGCCCTTGCTGTTGT	TAATCGATCCACCCACGGTA	Chalcone synthase	<i>HaeIII</i>
5	GT	TGGTGCAGCCTCATGTCATC	GAAGTCAACTCGAAGACTC	Glucosyl transferase	<i>BsrYI</i>
5	MYBPH3PROT2	GTATGGCACACTCACTTG	AAATTCCTGGTAAAGTCTAATAAGTC	Transcription factor	<i>MwoI</i>
5	RAT	GCTCCTAATTTTGGTGTACC	CACCTGCATAAAGACTTCTCC	Rutinoside acetyl transferase	<i>HaeIII</i>
6	3KAT	TGATACTAGTAGTGGAAA	GATCAAGATGAATGTATTTCTTC	3-ketoacyl thiolase	<i>HpyCH4IV</i>
6	AN1	CCAGTCAAAAATCAAAACCCCTTCA	AGCCTCTCAGCACTAATCTCC	Transcription factor	<i>TspRI</i>
6	AN2	ATGGTCACTTATAGCTGG	CAAGAAACATGATTCATTGGCG	Transcription factor	<i>Sau96I</i> -Indel
6	C4H1B	GTTAGGTTTTAGAGCTTAG	GTGCCACATGCCTAACTCAC	Transcription factor	<i>EcoRI</i>
6	MYBX	TCTCATCCACTACTAGTCTTTTCAAAC	AACTGATGACTAGGAATGAGCCTAA	Transcription factor	<i>NsiI</i>
6	RT	CCAGCTAGTGTGAGCTTCT	CTGAGTCCCAGCTGTATAGC	Rutinoside transferase	<i>TaqI</i>
7	AN4	CGTAGTATCTCATATGTACTC	GTCTCCCAGCAATAAGTGACC	Transcription factor	Indel
7	MYBB	CTAGCAAAATGAAGGCATATTATCCAGC	GGGACTGTATAAATATATAGTCAAAGGTGC	Transcription factor	<i>KpnI</i> - <i>HpyCH4V</i>
7	ODO1	GATCCACTACTAAGCTGCCTAC	CTCTAAAGCAAATCTAATCTCTAG	Transcription factor	<i>HpyCH4III</i> - <i>DpnII</i>
7	PAL2b	CTCCAGCAACACGGAATGCC	GGATGTAGGTGGTGAATAATTTGTCAATATCTG	Phenylalanine ammonia-lyase	<i>HaeIII</i>

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 BC₁ 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 F₂ lines derived from *P. exserta* × *P. parodii* and 176 BC₁ 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 BC₁ *P. axillaris* × *P. inflata* and 1.41 crossovers per chromosome in F₂ *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.

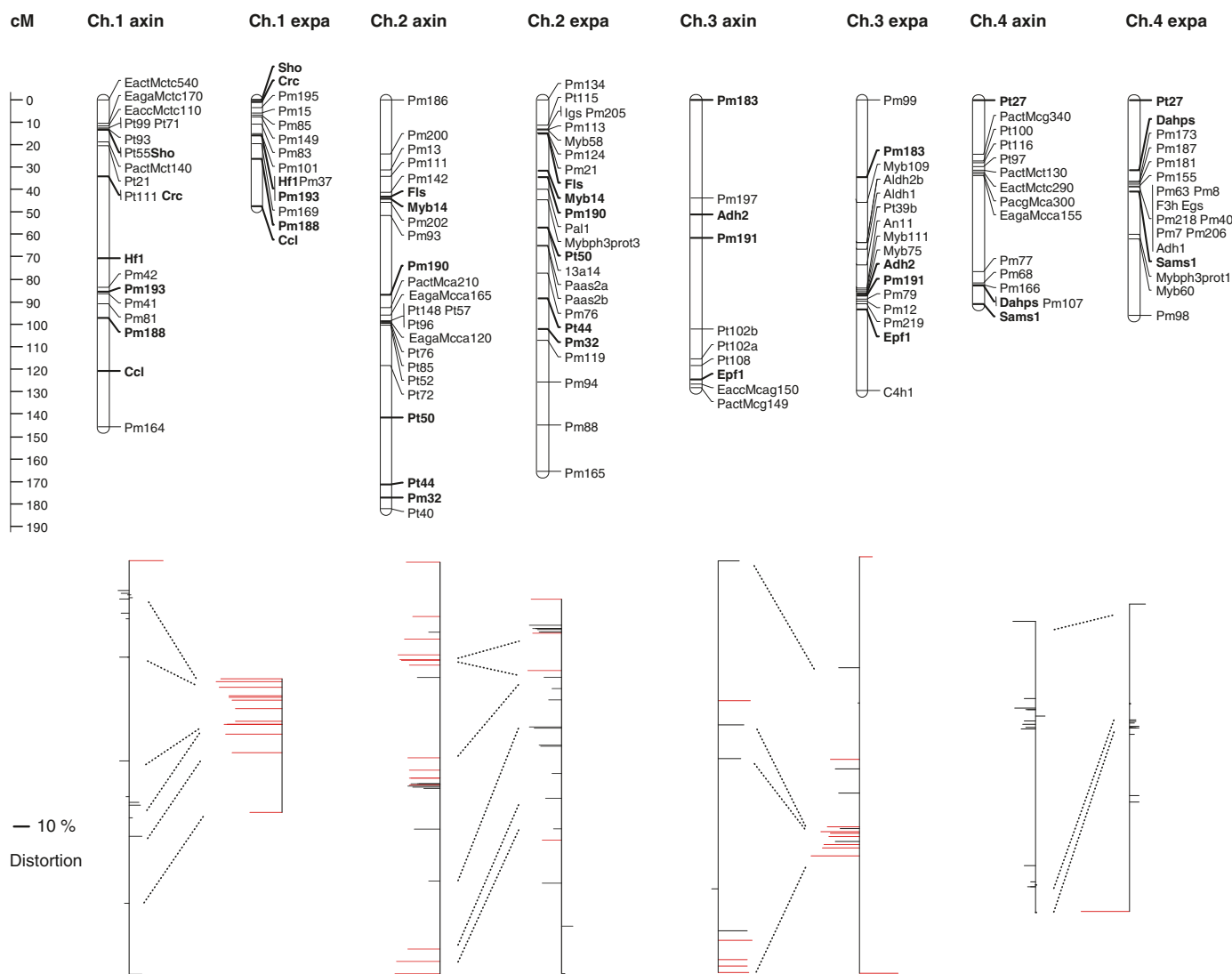
In both crosses, many loci displayed significant segregation distortion for one of the parents. The degree of distortion was stronger in the *P. exserta* × *P. parodii* population (Fig. 2, lower panel), where the alleles of *P. exserta* were overrepresented for half of the markers. In this cross, distorted 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

Table 3. PCR primers and polymorphism for the Pt markers.

Chr.	Marker	Forward primer	Reverse primer	Putative function	Polymorphism
1	PT21	CAAAAGGGTGGAGCAGCAG	CAACTACATAAGTTCCTTG	Unknown	Indel
1	PT55	GGACTTCCACAGAGAAATGG	GCTGAAACTACAITTCAGATAC	Unknown	Indel
1	PT71	GTGCTCTCATCCAAATGAG	CCCACGAGTTGCACCACTAG	Anion channel	Indel
1	PT93	CCTCTCTATTCTAAATGCTTG	GCTGAAACTACAITTCAGATAC	Unknown	Indel
1	PT99	CTTGATGACTCAGACATATGGC	TCACTTCTCAACCACATA	BoiA-like family	Indel
1	PT111	GGACAGTGAGAATCATGCTAC	CTATGGAGTTCAGTCGACCCAC	Armadillo motif	Indel
2	PT140	CTCTCTGGTAAGATGGGCTG	TGATGCTCTAAITTAATGGC	Unknown	Indel
2	PT144	CTTCTCTTGTACTTGGAGG	CACATTTACGCCAATCTCAG	RAB GDP-dissociation inhibitor	Indel
2	PT150	GATGAGCTTGGGACACACAG	CCAATGCTACACACACACAGTC	Coumarate-CoA ligase	Indel
2	PT152	GCAGTGGAAACTAGTGTCAAC	CACCCCACTAATCAACATTAAC	Ethylene forming enzyme	Indel
2	PT157	GGAGCTTGTGCTCATGTGAG	TCCTAITTCAGATGTCATGA	Peptidase	Indel
2	PT172	GTGGTACAAATGATATTGAG	CTCTAGTCTCTTGCACATAC	Proton-dependent oligopeptide transporter	Indel
2	PT176	GTTCGCCATGTTGGGGTG	GGCCTTCACCTTTAAAGGG	Unknown	Indel
2	PT185	CAATACCTATGGAAGCTCTTATG	GTCCATTTGATCTGCCCTGAAAC	Exoribonuclease	Indel
2	PT196	CTAACCCGGCACAACTAATTCG	ACGTGAAACATCAGCATTG	Unknown	Indel
2	PT115	GGGTTGGAGACTCACTCAAC	CGTCAAAAGAAACCAATCT	Aldo/keto reductase	Indel
2	PT148	CTTGCCCCAACAGCTGGTG	GTTAAGAGTTTGAGAGATC	Unknown	Indel
3	PT139b	CTCCCTCCGGATCAITGGG	GCAGAATTCATCACACTCC	Heme binding	Indel
3	PT102a	GAAGAGCCCTCTAGTATCAG	GCACCGCAGAATGATTTGGCTG	Chlorophyll binding	Indel
3	PT102b	GAAGAGCCCTCTAGTATCAG	GCACCGCAGAATGATTTGGCTG	Chlorophyll binding	Indel
3	PT108	GCCTAGATCGCATCAGAC	GCTGAAAATTCGAAATCATCAGAC	Leucin-rich repeat kinase	Indel
4	PT27	CAGGATGGATGACGATTTGG	CCGTGGTGTATGTACCTCGTG	Peptidoglycan-binding kinase	Indel
4	PT197	CTGATGTATGTAAGCGTGT	CCAGCAGAATTCATGTCCAGC	Cytoskeleton structural constituent	Indel
4	PT100	GAACCTGGAGAAGCCGTAAAG	AGATATAAGCGCTGCACC	UDP-glucuronosyl transferase	Indel
4	PT116	GGTCACATCTCAATCTTGG	CATGGAAATPAACAAGCTGCTG	Uroporphyrinogen decarboxylase	Indel
5	PT22	CAAGCTTCTGTGGAGTC	TGGAATGACCATTCAACTG	Unknown	Indel
5	PT26	CAGATGAGGATTAATCTCC	TTCAGAACTACTTTTACA	Carbohydrate kinase	Indel
5	PT36	CTTCAAGCCCTATGACAAG	CTCATCTCAAACGAAAACC	Calmoduline	Indel
5	PT37	CGAGTGGCTATAGCGAGGG	CTCATTCAGCAAAATTCACAAGATC	Unknown	Indel
5	PT104	GATGTTGAAAACAGCCTCAAG	GTACATCCGTTTGTCTCTCT	Cinnamyl-alcohol dehydrogenase	Indel
5	PT113	GGCACCTTCAAGAAATGG	CACATTTGAAGTCTTTACAC	Diacylglycerol kinase	Indel
5	PT114	GGCCCTTTCGAAGCACTTTC	CACACCACCGGGTGGCTCGCC	Unknown	Indel
5	PT140	GGAGCTGAGAAATCAGTGT	CTCCACTAAAGTGGGAAGGAG	ATP synthase	Indel
6	PT25	CAGAGCCTTGGGAGCTAGAGC	AGAAAGCTTGTGGCAGCC	Unknown	Indel
6	PT84	CAAGGAGGCTTATGTGAGC	TGTGAAGATACAGTACCAG	Unknown	Indel
6	PT105	GCATCTTGTAGGACAACC	CAAGTAATGAATCGCTAAGTTCC	Unknown	Indel
6	PT110	GGAAATGACAGAGTGGCAGAGC	CAAAGGCCAAAACACTCATPACG	Nucleoside diphosphate kinase	Indel
6	PT149	GAGATGTACTAGTACTATG	GTACATTTGTTGTGGAAC	Unknown	Indel
7	PT3	CTTCCCTCTTCAACGCCAATGATAG	CGGAAACGGCTCTCACCC	Unknown	Indel
7	PT15	CAGGATCTTAAGTATGGAC	GCATGACTCTTTATCGAC	Exonuclease	Indel
7	PT16	CTCGGTCTGGACTTGAATTCAG	CCTTTGTAAGATAAATCCCTCG	Unknown	DpnII
7	PT17	GTGGAGCTGCATCTATGG	CTTCAGATCATCTTCAGTGAG	Unknown	Indel
7	PT8	CCTTAGGACTTGCATCACCC	CAGCGGCTATCTTTGGAGC	Heat-shock protein	NsiI
7	PT11	GAATGTGGATGTGGACCTCG	GCTGTCCCTCTGTCAGATCC	Superoxide dismutase	RsaI
7	PT13	CATGGCTTGTATGTCCTCAGG	CCGCGAAGAAGATATGCAC	Glutamate-cysteine ligase	Indel
7	PT15	CTAAAGATTCCCAATGAAATCAGC	GGGGAAGATGTAGTTTTATAACC	Unknown	Indel
7	PT30	CCAAGTATTCCACCATCTC	GAGATATCCACCAACC	RNA polymerase	Indel
7	PT39a	CTCCCTTCCGGATCAITGGG	GCAGAATTCATCACACTTCC	Heme binding	Indel
7	PT87	CATGTGATCTATAATCCGAG	TCAGGCAATCCCGGCTCTTTTG	Gamma carbonic anhydrase	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.



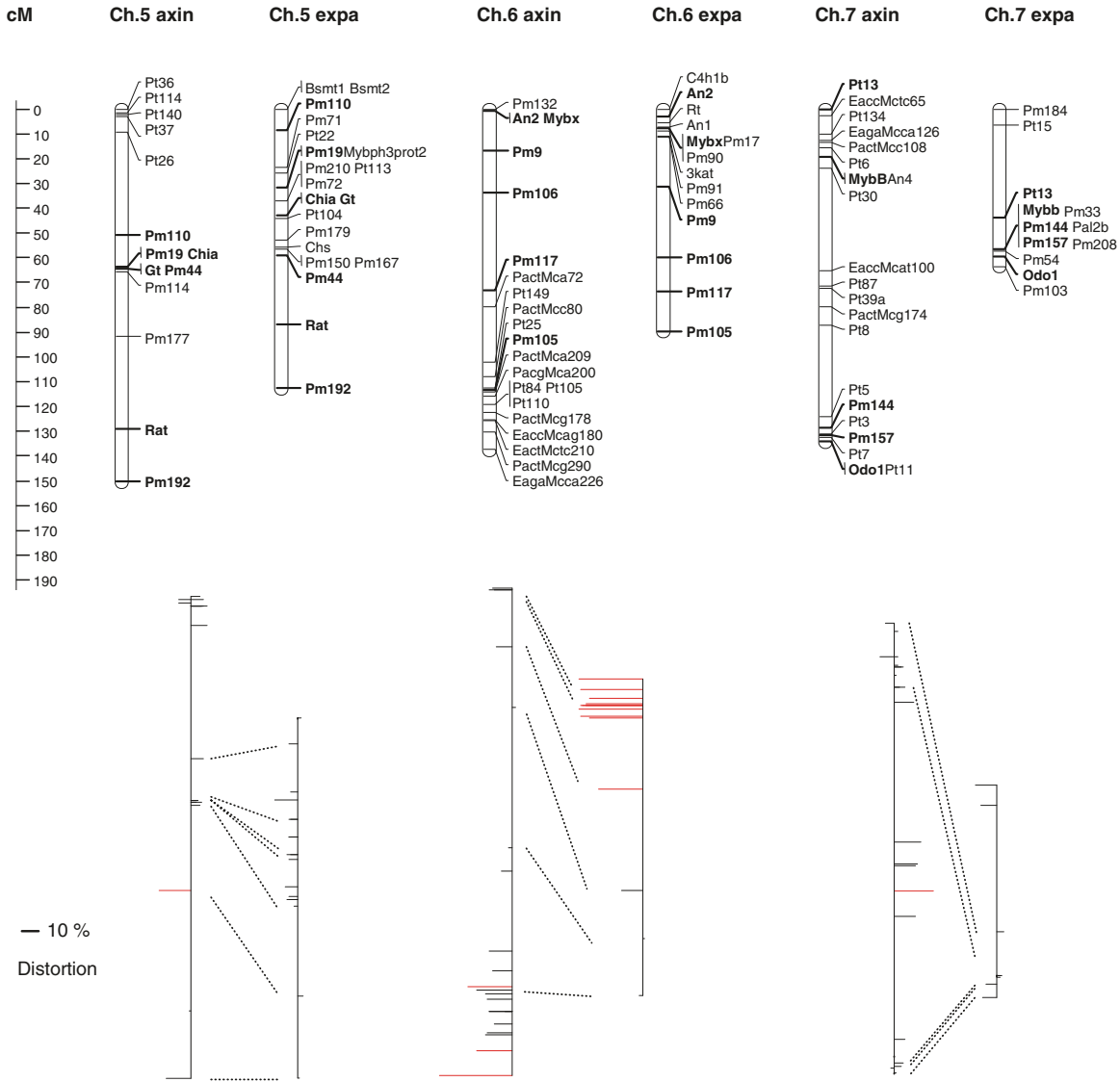
markers (30%), mostly in chromosome 2 towards the *P. axillaris* alleles and in chromosome 3 towards the *P. inflata* alleles. Interestingly, the chromosomal regions subject to segregation distortion were different in the two crosses.

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.

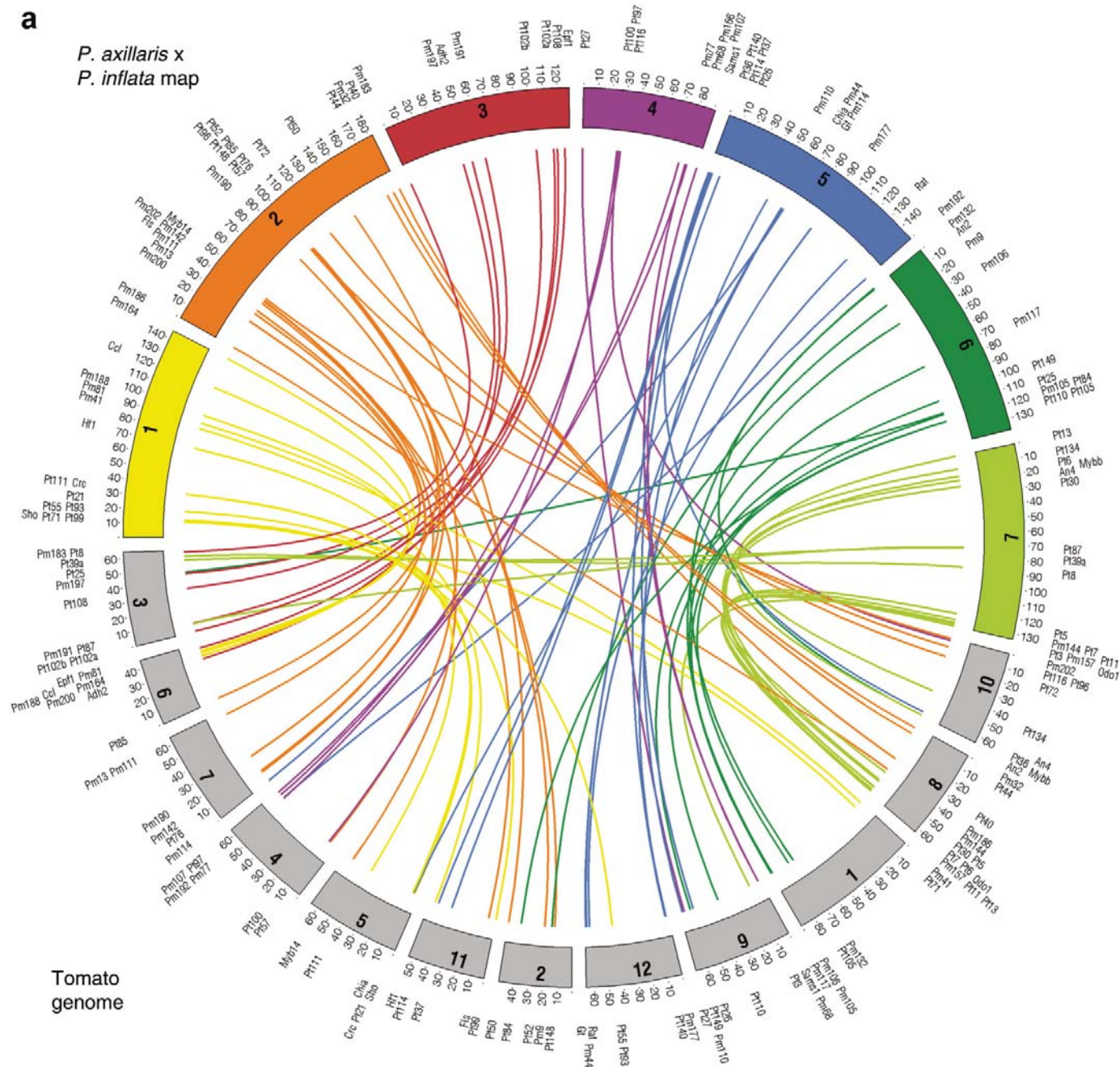
Discussion

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 F₁ cross. Because the F₁ 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 (Bombliés et al. 2007). *Petunia exserta* is a rare species endemic to a restricted area (500 km²) 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

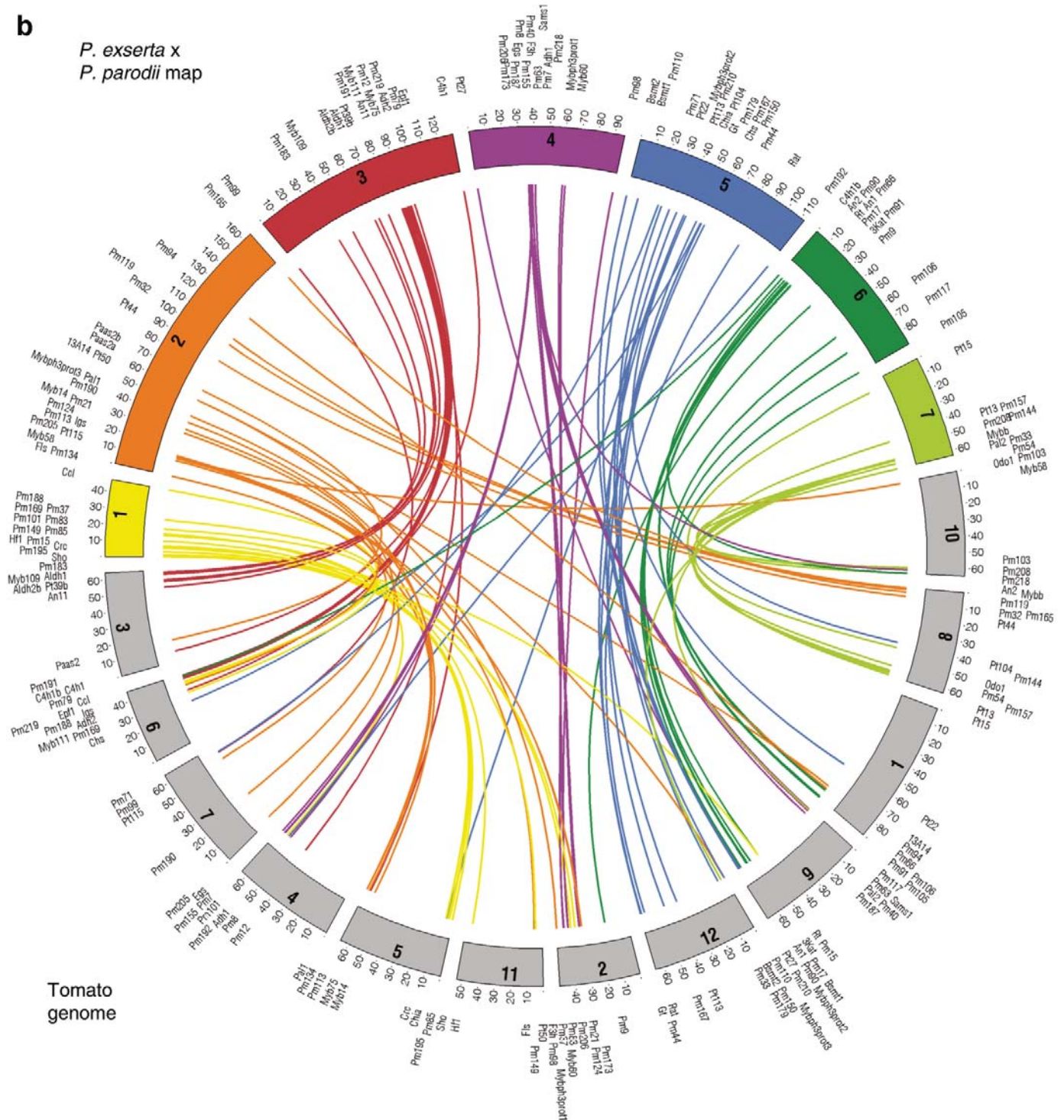
In the two interspecific crosses we observed significant differences in recombination frequency. Previous genetic maps of *Petunia* were done with accessions of the garden line *P. hybrida* (Strommer et al. 2000, 2002; Stuurman et al.

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).

b

P. exserta x
P. parodii map



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. axillarlis* × *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 suppres-

sion 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. axillarlis* × *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 proto-repeats. 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.

Acknowledgments

We thank E. Jakab and M. Grémillon for their technical assistance, and C. Ball and N. Signer for greenhouse help. We gratefully acknowledge the contribution of the Solanaceae Genomics Network for maintaining an online database with the tomato genome sequences. Finally, we thank R. Koes and F. Quattrocchio for generously supplying materials and T. Gerats for a critical reading of the manuscript. This work was supported by the University of Bern and by the National Centre for Competence in Research “Plant Survival”.

References

- Abajian, C. 1994. SPUTNIK [online]. Available from cbib.u-bordeaux2.fr/pise/sputnik.html [accessed 19 October 2008].
- Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J.H., Zhang, Z., Miller, W., and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**(17): 3389–3402. doi:10.1093/nar/25.17.3389. PMID:9254694.
- Bentolila, S., Alfonso, A.A., and Hanson, M.R. 2002. A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. *Proc. Natl. Acad. Sci. U.S.A.* **99**(16): 10887–10892. doi:10.1073/pnas.102301599. PMID:12136123.
- Bomblyes, K., Lempe, J., Epple, P., Warthmann, N., Lanz, C., Dangl, J.L., and Weigel, D. 2007. Autoimmune response as a mechanism for a Dobzhansky–Müller-type incompatibility syndrome in plants. *PLoS Biol.* **5**(9): e236. doi:10.1371/journal.pbio.0050236.
- Broman, K.W., and Sen, S. 2009. A guide to QTL mapping with R/qtl. Springer, New York.
- Conner, A.J., Albert, N.W., and Derolles, S.C. 2009. Transformation and regeneration of *Petunia*. In *Petunia: evolutionary, developmental and physiological genetics*. 2nd ed. Edited by T. Gerats and J. Strommer. Springer, New York. pp. 395–416.
- Cornu, A., Farcy, E., and Mousset, C. 1989. A genetic basis for variations in meiotic recombination in *Petunia hybrida*. *Genome*, **32**: 46–53. doi:10.1139/g89-409.
- Fondon, J.W., III, and Garner, H.R. 2004. Molecular origins of rapid and continuous morphological evolution. *Proc. Natl. Acad. Sci. U.S.A.* **101**(52): 18058–18063. doi:10.1073/pnas.0408118101. PMID:15596718.
- Gerats, T., and Strommer, J. 2009. *Petunia: evolutionary, developmental and physiological genetics*. 2nd ed. Springer, New York.
- Gerats, A.G.M., Huits, H., Vrijlandt, E., Marañón, C., Souer, E., and Beld, M. 1990. Molecular characterization of a nonautonomous transposable element (*dTph1*) of *Petunia*. *Plant Cell*, **2**(11): 1121–1128. doi:10.1105/tpc.2.11.1121. PMID:1967052.
- Gerats, A.G.M., Souer, E., Kroon, J., McLean, M., Farcy, E., and Maizonnier, D. 1993. *Petunia hybrida*. In *Genetic maps: locus maps of complex genomes*. 6th ed. Edited by S. O’Brien. Cold Spring Harbor Laboratory Press, New York. pp. 6.13–6.23.

- Hammock, E.A.D., and Young, L.J. 2005. Microsatellite instability generates diversity in brain and sociobehavioral traits. *Science*, **308**(5728): 1630–1634. doi:10.1126/science.1111427. PMID:15947188.
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Ann. Eugen.* **12**: 172–175.
- Krzywinski, M., Schein, J.E., Birol, I., Connors, J., Gascoyne, R., Horsman, D., 2009. Circo: an information aesthetic for comparative genomics. *Genome Res.* **19**(9): 1639–1645. doi:10.1101/gr.092759.109. PMID:19541911.
- Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., and Newberg, L.A. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics*, **1**(2): 174–181. doi:10.1016/0888-7543(87)90010-3. PMID:3692487.
- Lorenz-Lemke, A.P., Mäder, G., Muschner, V.C., Stehmann, J.R., Bonatto, S.L., Salzano, F.M., and Freitas, L.B. 2006. Diversity and natural hybridization in a highly endemic species of *Petunia* (Solanaceae): a molecular and ecological analysis. *Mol. Ecol.* **15** (14): 4487–4497. doi:10.1111/j.1365-294X.2006.03100.x. PMID:17107478.
- McCubbin, A.G., Zuniga, C., and Kao, T. 2000. Construction of a binary bacterial artificial chromosome library of *Petunia inflata* and the isolation of large genomic fragments linked to the self-incompatibility (*S*-) locus. *Genome*, **43**(5): 820–826. doi:10.1139/gen-43-5-820. PMID:11081972.
- Mishiba, K.I., Ando, T., Mii, M., Watanabe, H., Kokubun, H., Hashimoto, G., and Marchesi, E. 2000. Nuclear DNA content as an index character discriminating taxa in the genus *Petunia* sensu Jussieu (Solanaceae). *Ann. Bot.* **85**(5): 665–673. doi:10.1006/anbo.2000.1122.
- Morgante, M., Hanafey, M., and Powell, W. 2002. Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nat. Genet.* **30**(2): 194–200. doi:10.1038/ng822. PMID:11799393.
- Müller, L.A., Solow, T.H., Taylor, N., Skwarecki, B., Buels, R., Binns, J., 2005. The SOL Genomics Network: a comparative resource for Solanaceae biology and beyond. *Plant Physiol.* **138** (3): 1310–1317. doi:10.1104/pp.105.060707. PMID:16010005.
- Murray, M.G., and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* **8**(19): 4321–4326. doi:10.1093/nar/8.19.4321. PMID:7433111.
- Puerta, A.R., Ushijima, K., Koba, T., and Sassa, H. 2009. Identification and functional analysis of pistil self-incompatibility factor HT-B of *Petunia*. *J. Exp. Bot.* **60**(4): 1309–1318. doi:10.1093/jxb/erp005. PMID:19282427.
- R Development Core Team. 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rees, H., and Durrant, A. 1986. Recombination and genome size. *Theor. Appl. Genet.* **73**(1): 72–76. doi:10.1007/BF00273721.
- Reid, M., Chen, J.C., and Jiang, C.-Z. 2009. Virus-induced gene silencing for functional characterization of genes in *Petunia*. In *Petunia: evolutionary, developmental and physiological genetics*. 2nd ed. Edited by T. Gerats and J. Strommer. Springer, New York. pp. 395–416.
- Robbins, T.P., Gerats, A.G.M., Fiske, H., and Jorgensen, R.A. 1995. Suppression of recombination in wide hybrids of *Petunia hybrida* as revealed by genetic mapping of marker transgenes. *Theor. Appl. Genet.* **90**(7–8): 957–968. doi:10.1007/BF00222909.
- Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nat. Biotechnol.* **18**(2): 233–234. doi:10.1038/72708. PMID:10657137.
- Stehmann, J.R., Lorenz-Lemke, A.P., Freitas, L.B., and Semir, J. 2009. The genus *Petunia*. In *Petunia: evolutionary, developmental and physiological genetics*. 2nd ed. Edited by T. Gerats and J. Strommer. Springer, New York. pp. 1–28.
- Strommer, J., Gerats, A.G.M., Sanago, M., and Molnar, S.J. 2000. A gene-based RFLP map of *Petunia*. *Theor. Appl. Genet.* **100**(6): 899–905. doi:10.1007/s001220051368.
- Strommer, J., Peters, J., Zethof, J., De Keukeleire, P., and Gerats, T. 2002. AFLP maps of *Petunia hybrida*: building maps when markers cluster. *Theor. Appl. Genet.* **105**(6–7): 1000–1009. doi:10.1007/s00122-002-1009-y. PMID:12582927.
- Strommer, J., Peters, J.L., and Gerats, T. 2009. Genetic recombination and mapping in *Petunia*. In *Petunia: evolutionary, developmental and physiological genetics*. 2nd ed. Edited by T. Gerats and J. Strommer. Springer, New York. pp. 395–416.
- Stuurman, J., and Kuhlemeier, C. 2005. Stable two-element control of *dTph1* transposition in mutator strains of *Petunia* by an inactive *ACT1* introgression from a wild species. *Plant J.* **41**(6): 945–955. doi:10.1111/j.1365-313X.2005.02340.x. PMID:15743456.
- Stuurman, J., Hoballah, M.E., Broger, L., Moore, J., Basten, C., and Kuhlemeier, C. 2004. Dissection of floral pollination syndromes in *Petunia*. *Genetics*, **168**(3): 1585–1599. doi:10.1534/genetics.104.031138. PMID:15579709.
- Sutherland, G.R., and Richards, R.I. 1995. Simple tandem DNA repeats and human genetic disease. *Proc. Natl. Acad. Sci. U.S.A.* **92**(9): 3636–3641. doi:10.1073/pnas.92.9.3636. PMID:7731957.
- ten Hoopen, R., Robbins, T.P., Fransz, P.F., Montijn, B.M., Oud, O., Gerats, A.G.M., and Nanninga, N. 1996. Localization of T-DNA insertions in *Petunia* by fluorescence in situ hybridization: physical evidence for suppression of recombination. *Plant Cell*, **8** (5): 823–830. doi:10.1105/tpc.8.5.823. PMID:12239403.
- Vandenbussche, M., Janssen, A., Zethof, J., van Orsouw, N., Peters, J., van Eijk, M.J.T., 2008. Generation of a 3D indexed *Petunia* insertion database for reverse genetics. *Plant J.* **54**(6): 1105–1114. doi:10.1111/j.1365-313X.2008.03482.x. PMID:18346192.
- Voorrips, R.E. 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. *J. Hered.* **93**(1): 77–78. doi:10.1093/jhered/93.1.77. PMID:12011185.
- Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Friters, A., Pot, J., Paleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* **23**(21): 4407–4414. doi:10.1093/nar/23.21.4407. PMID:7501463.
- Wang, Y., Diehl, A., Wu, F.N., Vrebalov, J., Giovannoni, J., Siepel, A., and Tanksley, S.D. 2008. Sequencing and comparative analysis of a conserved syntenic segment in the Solanaceae. *Genetics*, **180** (1): 391–408. doi:10.1534/genetics.108.087981. PMID:18723883.
- Wu, F.N., and Tanksley, S.D. 2010. Chromosomal evolution in the plant family Solanaceae. *BMC Genomics*, **11**(1): 182. doi:10.1186/1471-2164-11-182. PMID:20236516.
- Zamir, D., and Tadmor, Y. 1986. Unequal segregation of nuclear genes in plants. *Bot. Gaz.* **147**(3): 355–358. doi:10.1086/337602.