Theor Appl Genet (2000) 100:899–905 © Springer-Verlag 2000

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A gene-based RFLP map of petunia

Received 14 July 1999 / Accepted: 30 July 1999

Abstract Due in large part to the data accumulated from years of classic genetic analysis, petunia (*Petunia hybrida* Vilm) has remained a useful model system, particularly for studies of gene regulation and genome structure. We have used three segregating populations of petunia, including those serving as the source of an earlier actin gene RFLP map, for RFLP mapping of several additional genes. Twenty-seven loci have been merged with 11 previously mapped morphological and biochemical markers. Our results contribute additional evidence to reports of a high degree of genome plasticity and segregation distortion in this species and suggest that petunia may be a useful plant system for detailed analysis of plant genome organization, activity and evolution.

Key words Petunia · RFLP · Genetic map · Genome · Segregation distortion

Introduction

While arabidopsis (*Arabidopsis thaliana* L.) is indisputably the model plant system for molecular genetics, and maize (*Zea mays* L.) – thanks to the vast reservoir of genetic information accumulated for nearly a century – remains close in pre-eminence, petunia (*Petunia hybrida* Vilm) continues to make contributions in the areas of molecular and classical genetics. Petunia is a relatively fast-breeding dicot that is easily maintained in the greenhouse and produces hundreds of seed from a single pollination. It is a commercially significant horticultural species, and years of commercial breeding have produced varieties with defined genes of interest to biotechnology. Molecular genetic analyses continue to advance our understanding of regulation of the anthocyanin pigment pathway in particular (Mol et al. 1985; Quattrocchio et al. 1993; 1998; Huits et al. 1994; Solani et al. 1995; Bradley et al. 1998; Davies et al. 1998). The seven chromosomes are manageable for the cytologist and geneticist and, like its close relatives tobacco and tomato, petunia is readily transformed. Consistent with its origins as an artificial hybrid, probably between *P. axillaris* and *P. integrifolia* (Wiering et al. 1979), and cultivation directed largely toward maximizing variation, it is rife with alternative alleles distinguishable by morphological, biochemical or molecular analysis. Petunia is of special interest in regard to a number of reported anomalies associated with meiotic segregation patterns and genome instability (Cornu and Maizonnier 1983; Baird and Meagher 1987; Koes et al. 1987; McLean et al. 1990; Fransz et al. 1996; Foster Atkinson 1996).

Seventy-four genes of petunia were described in 1979 (Wiering et al. 1979), and by 1993, 134 loci had been assigned to chromosomes (Gerats et al. 1993). In 1990 a restriction fragment length polymorphism (RFLP) map of actin genes was published (McLean et al. 1990). A few years ago, a number of researchers provided us with cDNA or genomic DNA probes for the petunia genes listed in Table 1. On the basis of RFLP analysis we have now added these genes to the actin gene map of McLean et al. (1990). The task was simplified by the availability of 73 of the same DNA samples used in the generation of the 1990 map; to these were added 79 DNA samples from another set of crosses. The availability of representatives from several gene families has permitted us to extend the observations and conclusions pertaining to genome plasticity and the evolution of gene families. As many of the original DNA samples remain, this map pro-

Communicated by R. Hagemann

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Table 1 Genes used as probes in RFLP mapping

Probe	Restriction enzyme	Gene product	Source	Reference
aco-3	*Hin*dIII	1-Aminocyclopropane-1-carboxylate oxidase	W. Woodson	Tang et al. 1993
aco-4	*Bcl*I	1-Aminocyclopropane-1-carboxylate oxidase	W. Woodson	Tang et al. 1993
acs-2	*Xba*I	1-Aminocyclopropane-synthase	W. Woodson	Unpublished
acs-3	*Xba*I	1-Aminocyclopropane-synthase	W. Woodson	Unpublished
acs-4	*Xba*I	1-Aminocyclopropane-synthase	W. Woodson	Unpublished
adh-1	*Hin*dIII	Alcohol dehydrogenase	J. Strommer	Gregerson et al. 1991
adh-2	*Hin*dIII	Alcohol dehydrogenase	J. Strommer	Gregerson et al. 1991
chiA	*Xba*I	Chalcone flavanone isomerase	R. Koes	van Tunen et al. 1988
chsC	*Hin*dIII	Chalcone synthase	R. Koes	Koes et al. 1987
chsF	*Eco*RI	Chalcone synthase	R. Koes	Koes et al. 1987
chsG	*Bcl*I	Chalcone synthase	R. Koes	Koes et al. 1987
dfrB	*Bam*HI	Dihydroflavonol-4-reductase	A. Gerats	Beld et al. 1989
f3 β h	*Xba*I	Flavanone-3-β-hydroxylase	L. Britsch	Britsch et al. 1992
fbp-1	*Bam*HI	MADS-box transcription factor	G. Angenent	Angenent et al. 1992
gad	*Bcl*I	Glutamate decarboxylase	H. Fromm	Baum et al. 1993
grp	*Eco*RI	Glycine-rich protein	M. McLean	Condit and Meagher 1987
hf-2	*Xba*I	Flavonoid-3′, 5′-hydroxylase	T. Holton	Holton et al. 1993
pac-1	*Hin*dIII	Actin	M. McLean	McLean et al. 1988
pac-2	*Xba*I	Actin	M. McLean	McLean et al. 1988
pac-3	*Xba*I	Actin	M. McLean	McLean et al. 1988
pac-4	*Xba*I	Actin	M. McLean	McLean et al. 1988
pac-7	*Xba*I	Actin	M. McLean	McLean et al. 1988
pac-9	*Hin*dIII	Actin	M. McLean	McLean et al. 1988
rdna-1	*Hin*dIII	Ribosomal RNA	M. McLean	Unpublished
rdna-2	*Hin*dIII	Ribosomal RNA	M. McLean	Unpublished
rnx-2	*Hin*dIII	Ribonuclease-X2	T. Kao	Lee et al. 1992
sx	*Bam*HI	S-protein	T. Kao	Ai et al. 1992

vides a structure on which we shall be able to build additional forms of maps amplified; an AFLP map using a large subset of the same DNA samples is under construction.

Materials and Methods

Plants

V23 and R51, two varieties of *P. hybrida* carrying different alleles for 11 easily scored markers (Wallroth et al. 1986), together with *P. mitchell*, a doubled haploid generated from anther culture of a *P. hybrida-P. axillaris* hybrid (Mitchell et al. 1980), were used to generate the F_2 progeny used for mapping, as illustrated in Table 2. Progeny from three sets of crosses were used: the first, VRxR, resulted from the backcross of a V23/R51 F_1 hybrid to the R51 pollen parent; VRxV progeny resulted from backcrossing a V23/R51 hybrid to the V23 maternal parent. These F_2 progeny were previously scored for segregation of the morphological and biochemical markers listed in Table 3 as well as for actin gene family members (McLean et al. 1990). The DNA used for the 1990 study was stored at –20°C. for several years before the present work was undertaken. In 1993 a new V23/R51 hybrid was generated, and pollination by *P. mitchell* produced a third population of F_2 plants, VRxM. DNA from a total of 32 VRxR, 41 VRxV and 79 VRxM plants was used in this study. The newly acquired molecular data were merged with the data which formed the basis of the previous publication (McLean et al. 1990).

Table 2 F_2 populations used to construct map

Designation	Female parent	Pollen parent	Population size
VRxR	V23/R51 hybrid	R51	32
VRxV	V23/R51 hybrid	V23	41
VRxM	V23/R51 hybrid	*P. mitchell*	79

Table 3 Marker loci for V23/R51 hybrids

Marker	Gene	Chromosome
an4	Anthocyanin-4	VII
bl	Blind	IV
fl	Flavonol synthase	II
gpiB	Glucose phosphate isomerase-B	VII
hf-1	Flavonoid-3′, 5′-hydroxylase-1	I
hf-2	Flavonoid-3′, 5′-hydroxylase-2	V
ht-1	Flavonoid-3′-hydroxylase-1	III
ph-1	pH factor	I
po	Pollen color	V
prxA	Peroxidase-A	III
rt	Rhamnose at flavonoid position 3	VI

Plant DNA

Preparation of the VRxR and VRxV DNA samples is described in McLean et al. (1988). VRxM DNA was prepared from fresh leaf tissue essentially by the method of Murray and Thompson (1980), modified for use with fresh tissue by correcting for water content. DNA was stored in TE (10 m*M* TRIS-HCl, 1 m*M* Na-EDTA, pH 8.0) at –20°C for up to 6 years before digestion.

Four micrograms of plant DNA was digested with a fourfold excess of either *Bam*HI, *Bcl*I, *Eco*RI, *Hin*dIII, or *Xba*I, purchased from either Gibco-BRL (Gaithersburg, Md.), New England Biolabs (Beverly, Mass.), or Pharmacia (Uppsala, Sweden). Digested DNA was precipitated overnight at –20°C. in ethanol-0.3 *M* sodium acetate after the addition of 5 μg yeast tRNA carrier (Sigma,

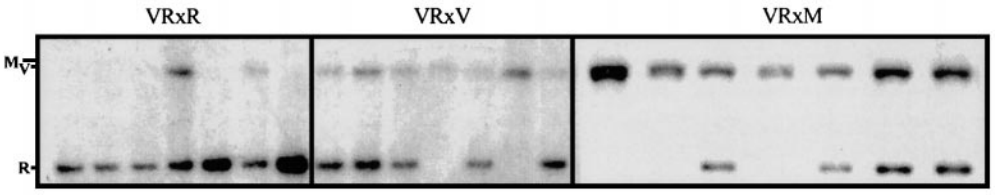

Fig. 1 Autoradiogram of DNA obtained from 21 individuals, 7 from each family (VRxR, VRxV and VRxM), digested with *Xba*1 and probed with *acs4*. All VRxR progeny bore at least one R allele, and were scored for presence or absence of a V allele; similarly VRxV progeny were scored for presence of an R allele. In VRxM progeny, both V and R alleles from the VR hybrid parent could be scored directly. Reading from left, results were scored as HHHAHAH... for MAPMAKER analysis and 2221212... for easy visual scoring

St. Louis, Mo.). After pelleting and resuspending in 25 µl of TE containing 5% glycerol, 0.05% bromophenol blue and 0.05% xylene cyanol, one-tenth of the sample was used in a test gel to verify DNA concentration and extent of digestion. The remaining DNA, approximately 3.5 µg, was subjected to slow electrophoresis (16 h) through 0.7% agarose in TBE buffer (Sambrook et al. 1989). After ethidium bromide staining and photography, the DNA was transferred to a nylon membrane (MSI, Westboro, Mass.) with a Pharmacia LKB VacuGene XL blotting system, as recommended, by the supplier. DNA was bound to the filter with a Hoefer Model UVC-500 UV crosslinker as recommended, then baked in vacuo at 60°–80°C. for 2 h. Filters were sealed in plastic and stored at 4°C.

Probes and Hybridizations

The probes used in this study are listed in Table 1, along with diagnostic restriction endonucleases, DNA sources and references. Two of them – *hf-2* and *chiA*, the latter identical to *po* (van Tunen et al. 1991) – represent genes for biochemical markers previously scored phenotypically using the VRxV and VRxR plants. Plasmid DNA isolation and electrophoresis techniques were generally as described in Sambrook et al. (1989). Plasmid DNAs as supplied were introduced into *E. coli* DH5α and recovered from alkaline lysates of 20-ml cultures. Cloned regions were excised from gels and recovered using Gene Clean (Bio 101, Vista Calif.) as recommended by the supplier.

Twenty-five nanograms of probe was radioactively labeled by random priming using a kit obtained from Boehringer Mannheim (Laval, Quebec) and 50 1.8×10^6 Bq of [^{32}P]-dCTP, SA 3,000 Ci/mmol (Amersham Canada, Oakville, ON). The labeled probe was purified by chromatography through Sephadex G25 (Pharmacia), and recovered in approximately 0.4 ml TE-0.1 *M* NaCl at a specific activity of $1–3\times10^9$ dpm/µg input DNA.

Gel hybridization and high-stringency wash conditions were as described in Gregerson et al. (1991). Blots were exposed to XAR 5 film (Kodak) at –70°C with two intensifying screens for 2–3 days before development of the autoradiograms. Bound probe was removed by immersing filters in 50% formamide-10 m*M* sodium phosphate pH 6.5 at 65°C for 1 h, followed with 10 min in 2×SSC-0.1% SDS at room temperature.

Data analysis

Preliminary hybridization of each probe to parental V23, R51 and Mitchell DNA samples defined useful RFLPs. Parental DNA samples included on each subsequent blot then permitted ready assignment of an F_2 plant's genotype.

Independent documents were created for data from each segregating population, and a fourth document formed by merging data from the three populations. The MAPMAKER Macintosh V2.0 program, provided by E.I. Dupont (Wilmington, Del.), was used initially to define linkage groups by grouping the data. Orphan loci, not meeting the default linkage criteria, were assigned a linkage group based on loci with which they exhibited minimal recombination. Three-point crosses were used to determine the highest likelihood maps for each of the seven derived linkage groups, again using MAPMAKER.

To minimize the probability of errors in data entry, we also entered all RFLP data into a master Excel program and screened by eye for apparent double and triple crossovers. These were then verified by reexamination of the autoradiograms.

Maps generated from each of the three F_2 populations were compared to the multi-population map to reveal any inter-population variability of potential significance. Chromosomal assignments for the linkage groups were made possible from the earlier mapping of morphological and biochemical traits in the VRxR and VRxV populations (McLean et al. 1990).

Results

An early surprise was the excellent condition of DNA samples from VRxR and VRxV crosses, which had been stored at –20°C for several years. DNA isolation had included ultracentrifugation in cesium chloride, but no special care had been taken, and DNA samples were frozen and thawed many times. Figure 1 illustrates banding patterns seen in a subset of the VRxV and VRXR families, the patterns as clear as those seen for freshly isolated DNA of the VRxM family.

The map produced from three-point MAPMAKER analysis is presented in Fig. 2. Beside each chromosome's RFLP map is a cytological diagram, illustrating where phenotypic markers and centromeres have been placed (Cornu and Maizonnier 1983). The total length of the map derived from RFLP data is 368.2 cM, with no general correlation between the number of loci per chromosome or total map lengths and physical chromosome length. Gene orders derived from individual backcross populations were consistent with those based on data merged from all three populations.

The map represents the analysis of 3,270 alleles, 1,689 of the R form, 1,581 of V. A χ^2-test assessed the probability of this deviation from the expected 1:1 occurring by chance as greater than 0.05. Similarly, if one compares the alleles recovered from individual populations, there is no significant deviation from randomness. There is, however, a statistically significant preponderance of the R allele for Ch II (537/960, $P<0.01$) and Ch VI (167/287, $P<0.05$). The basis for this distortion can be narrowed by examining the population-specific inheritance of alleles for specific chromosomes: among VRxM progeny, Ch II of the R type is preferred (242/402, $P<0.01$); for progeny of both VRxM and

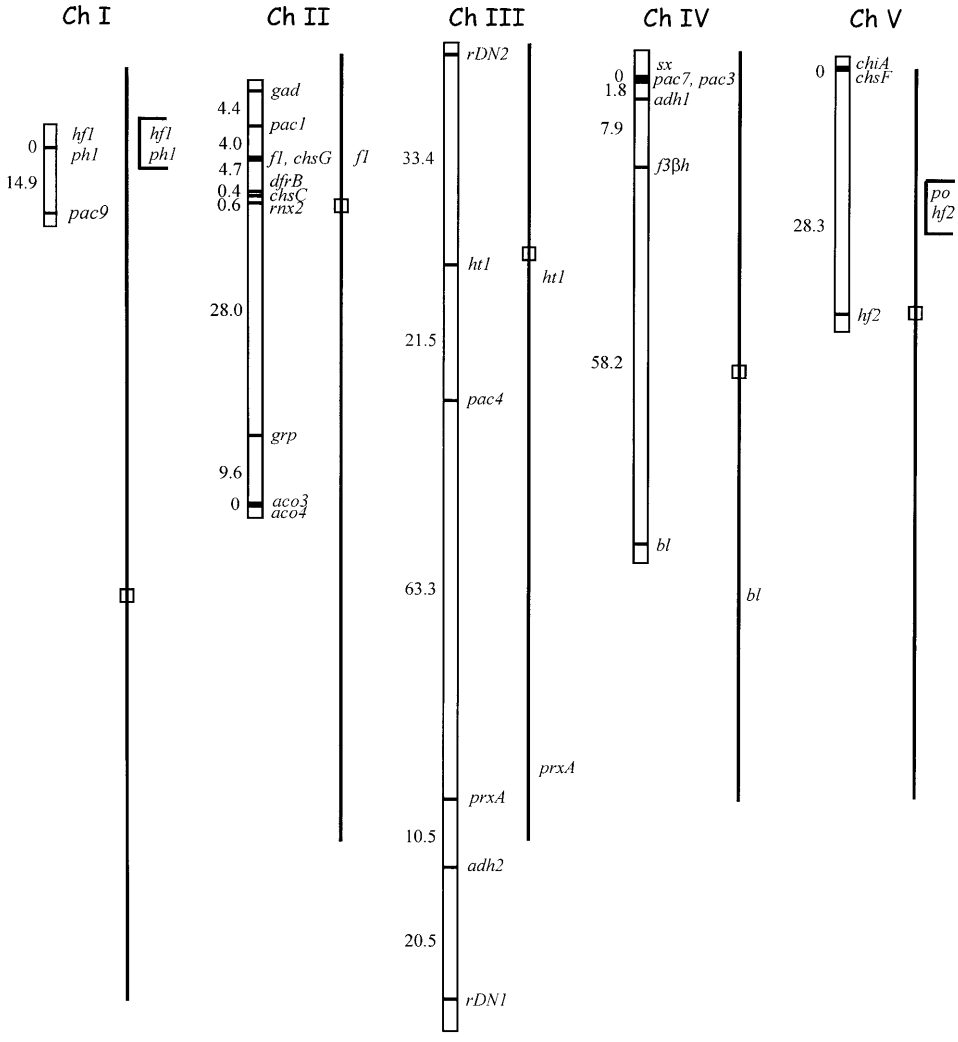

Fig. 2 RFLP map (left) together with morphological and biochemical chromosome markers. Cytological maps (*right*) with known arm placements for marker loci are depicted adjacent to each genetic map (Cornu and Maizonnier 1983). Only in the case of Ch III do we know orientation with regard to the centromere, which lies above *ht1*. *Numbers* to the *left* of each chromosome represent map distances in centi-Morgans, corrected for multiple crossovers by the MAPMAKER program (the indicated 63.3 cM on Ch III, for example, is derived from a raw crossover frequency of 35.9%). For Ch V, *chiA* and *po*, diagrammed on RFLP and cytological maps, respectively, represent the same gene. □ Centromere

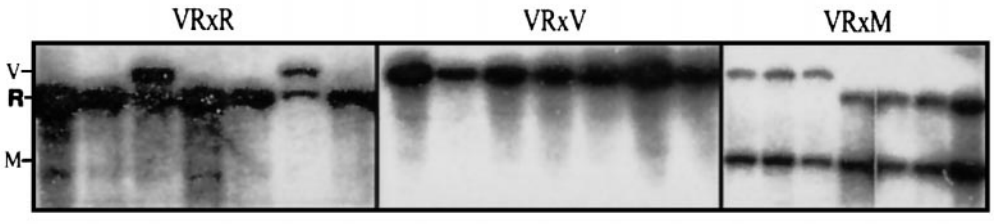

Fig. 3 Example of an unexpected R-allele polymorphism found in a Ch IV gene cluster. The *adh1*-RFLP seen between V and R alleles in the VRxR and VRxM populations is lost in the VRxV population, where only the V-type band was seen in DNA samples digested with *Hin*d III. Unexpected variations in R alleles were also seen for *sx*, *pac-3* and *pac-7*, which lie within 2 cM of *adh1* (see Results for details)

VRxV crosses, Ch VI of the R form is preferred (52/78, $P<.01$ and 39/61, $P<0.05$, respectively). For Ch VII, there was a significant preponderance of the V allele among VRxM progeny (117/193, $P<0.01$). Differences in allelic frequencies for other chromosomes and families were not statistically significant by the χ^2 test.

Members of six gene families were included in this mapping effort: actin, chalcone synthase (CHS), alcohol dehydrogenase (ADH), aminocyclopropane-1-carboxylate oxidase (ACO), aminocyclopropane-1-carboxylic acid synthase (ACS) and rDNA.

Ten chalcone synthase genes have previously been assigned to loci on two chromosomes in another variety, V30, with some gene family members tightly clustered and others dispersed. The number of *chs* genes was found to vary among varieties of *P. hybrida* (Koes et al. 1987). Six actin gene loci were previously mapped to five chromosomes using a subset of the DNA samples used to generate the current map, again with examples of both tightly clustered and dispersed genes (McLean et al. 1990). This map adds two *adh* loci on two chromosomes; two closely linked ACO genes; and three ACS genes, two tightly linked and the third on a different

chromosome. These data provide additional evidence for the general pattern of gene families in petunia: dispersed loci, with multiple gene copies clustered at some of them.

In the case of 4 loci, all on chromosome IV, unexpected allelic variation was detected in the VRxV mapping population. For the *adh1* locus, expected RFLP patterns were seen for the VRxR and VRxM DNA samples, but with VRxV there was no evidence of a segregating R allele (Fig. 3). For the *sx* locus, only the VRxV family exhibited a polymorphism for the R allele. In the same family, *pac-3* and *pac-7*, which map between *adh1* and *sx*, failed to exhibit the expected R polymorphism in *Xba* I-digested samples but they did retain it in *Hin*d III-digested samples.

Discussion

The contrast between chromosome size and the clustering of markers in our map is striking. Rees and Durrant (1986) reported, on the basis of cytological examination, an average of 1.7 chiasmata per chromosome in a single meiosis of *P. hybrida*. Deriving data from the individuals contributing maximal information for a given chromosome, we calculate a frequency of 0.4 crossovers per chromosome (151/379), or about one-quarter the expected number. This suggests that we have managed to cover only about a quarter of the genome. A potential complication of this simple view is the evidence in petunia for ”linkage blocks” of loci exhibiting very low levels of recombination despite significant physical separation (Cornu and Maizonnier 1983), especially in wide crosses (Robbins et al. 1995).

A good correlation between chromosome length and chiasma frequency within a chromosomal complement has been reported, along with a general negative correlation between chiasma frequency and overall genome size (Rees and Durrant 1986). In arabidopsis, however, where *tetrad* mutants have allowed direct measurement of crossover frequency, the tendency is for fewer chiasmata per unit length on longer chromosomes (Copenhaver et al. 1998). We can make no assumptions, therefore, about the relationship between measured crossover frequencies and chromosome length, or about the locations of loci on chromosomes, beyond the earlier studies assigning markers to arms (Cornu and Maizonnier 1983).

The distortion in recovery of V and R alleles of Ch II, VI and VII suggests that something about the R (Ch II and VI) and V (Ch VII) chromosomes may have offered an advantage, at least in specific crosses: preferred combinations were M pollen parent with the R form of Ch II; M and V pollen parents with the R form of Ch VI; and M pollen parent with the V form of Ch VII. In every case we were mapping the results of exchanges occurring in Meiosis I of the VR hybrid, so that effects of the maternal genotype should have been irrelevant. There are two simple explanations: R alleles on Ch II and Ch VI (and V alleles on Ch VII) enhance the probability of successful fertilization by specific pollen donors, or specific allelic combinations are preferred, *e.g.* RM combinations of some alleles on Ch II confer an advantage over VM combinations. Significant distortions of segregation ratios have been previously noted in other Solanaceous species including pepper and tomato (Zamir and Todmor 1986) as well as petunia (Wallroth et al. 1986, Cornu et al. 1989, McLean et al. 1990; Robbins et al. 1995).

RFLP differences for single alleles which were thought to be shared by the three populations are all for R alleles of genes clustered at one end of Ch IV (*adh1, sx, pac-3* and *pac-7*) and were found only among progeny of the VRxV cross. We conclude that the R chromosome donated by the VR maternal parent in the VRxV cross had undergone some rearrangement prior to formation of the flower from which the VRxV capsule was recovered. The origins and the nature of a rearrangement cannot be adduced, but we can rule out a simple deletion, in which case no polymorphism would have been seen around the *sx* and *pac* loci of the same population.

Based on evidence from studies of actin (Baird and Meagher 1987; McLean et al. 1990) chalcone synthase (Koes et al. 1987; Fransz et al. 1996) and alcohol dehydrogenase (Foster Atkinson 1996), gene copy number and location are surprisingly fluid in the petunia genome. General instability of the petunia genome was the subject of a number of early investigations described in Cornu and Maizonnier (1983) and has been ascribed to the verified activity of mobile genetic elements in petunia, the interspecific origin of *Petunia hybrida* and/or other factors beyond our knowledge.

The previous chromosomal assignments for actin and chalcone synthase genes were verified, with some changes in position relative to markers. These differences are probably the consequence of using a larger mapping population in the current study. The shift in position of the *adh2* locus is more curious. Previously *adh 2* was assigned to Ch IV, based on the lack of co-segregation between *blind*, a Ch IV marker, and an ADH2 isozyme of *Petunia mitchell*, the only ADH variant detected to date (Gregerson et al. 1993). Linkage between *blind* and *adh2* in that study was clear (eight recombinant progeny among 85 individuals). The current map, relying on RFLP mapping in V23 and R51 lines of *P. hybrida*, places *adh2* on Ch III near *prxA*, a marker locus for Ch III (six recombinant progeny among 66 individuals). The position of the *adh2* locus thus varies among the mapping populations. Simply with regard to *adh* genes, *P. mitchell* differs from its parent species, *P hybrida* and *P. axillaris* (itself a progenitor of *P. hybrida*), in a number of ways: the absence of the *adh1* gene product in vegetative tissues and a distinct electrophoretic mobility of ADH2, as well as the map position of *adh2* (Foster Atkinson 1996). These anomalies are likely to reflect the presence of a very high level of genetic alteration in *P. mitchell*.

Variations in locus positions have also been reported for the *chs* gene family in V26/V30 hybrids using fluo-

rescent in situ hybridization (FISH, Fransz et al. 1996) as well as the for *S* locus in V23/V26 hybrids using indirect methods (Ten Hoopen et al. 1998). One of the rDNA loci mapping to Ch III in this study was previously mapped to Ch II (Fransz et al. 1996). Both sets of results are clear; in our mapping, neither rDNA locus exhibited evidence of Ch II linkage (recombination frequencies between rDNA loci and all Ch II markers ≥30.5) but clearly cosegregated with Ch III markers (*e.g.* raw recombination frequency between *rdna1* and a*dh2*, 0.15; *rdna2* and *ht1*, 0.25). These results emphasize the importance of specifying the varieties or lines used for the mapping of petunia loci and the need for caution in applying an established map to a new variety.

The additional mapping corroborates early suggestions that petunia gene families commonly exist in a combination of gene clusters and dispersed loci (Koes et al. 1987, McLean et al. 1988). This pattern has been interpreted as evidence for ancient duplications, reflected in dispersed loci for members of a gene family, followed with recent tandem duplications of individual genes (McLean et al. 1988). The nearly ubiquitous duplication of *adh* genes in plants suggests an ancient duplication event, and in accordance with the proposed model, there are two distinct loci for these genes. Of the three *acs* genes mapped in this work, the two for which sequence data are available are tightly linked (0% recombination) and exhibit 99% identity in the coding region (Genbank Accessions Z18953 and AF049711). For the *aco* gene family, we know that the two mapped loci (*aco3* and *aco4*) are very close physically (6 kb apart) but that the DNA sequence of *aco3* is closer to that of *aco1* than *aco4*. This suggests that the *aco3*-*aco4* duplication preceded the *aco3*-*aco1* duplication, contrary to an easily presumed correlation between tight linkage and tight evolutionary relationship. We do not know, however, whether the third gene (*aco1*) also lies nearby, or whether the organization of genes might have differed between the cloning and mapping lines.

The current mapping effort also calls attention to a number of complications contributed by the petunia genome, with evidence for a high degree of segregation distortion and genome plasticity. The ability of such studies to reveal genetic quagmires may be the most valuable aspect of work such as that described here. Molecular genetic analysis of the petunia genome may prove an excellent source of information to enhance our general understanding of plant genome organization, activity and evolution.

The DNA samples used in construction of this map are also being used as the basis of an AFLP map, which will allow for superimposition of the maps. With the mapping of 250 AFLP loci, coverage of the petunia genome should be greatly extended, thereby maximizing utility of the genome for evolutionary studies and facilitating future mapping and gene cloning efforts.

Acknowledgements We would like to thank the many scientists, listed in Table 1, who generously shared probes with us; Vance Baird, who dug deep into his freezer for the VRxV and VRxR samples; Mike McLean for sharing his data and expertise; Brian Miki for supplying many of the chemicals required for this work; Owen van Cauwenberge for patient assistance with figures; and, finally, Hélène Labbé and Adèle MacKay for their inexhaustible good spirits and helpfulness in the laboratory. Tim Robbins' thoughtful review of the manuscript has been greatly appreciated. The major portion of this work was carried out by JS on research leave in the laboratory of SM, who deserves special thanks for being such a gracious and generous host.

References

Ai Y, Tsai D-S, Kao T-h (1992) Cloning and sequencing of cDNAs encoding two *S* proteins of a self-compatible cultivar of *Petunia hybrida*. Plant Mol Biol 19:523–528

Angenent GC, Busscher M, Franken J, Mol JMN, van Tunen AJ (1992) Differential expression of two MADS box genes I wild-type and mutant petunia flowers. Plant Cell 4:983–993

Baird WV, Meagher RB (1987) A complex gene superfamily encodes actin in *Petunia*. EMBO J 6:3223–3231

Baum G, Chen Y, Arazi T, Takatsuji H, Fromm H (1993) A plant glutamate decarboxylase containing a calmodulin binding domain: cloning, sequence and functional analysis. J Biol Chem 268:1961–19617

Beld M, Martin C, Huits H, Stuitje AR, Gerats AGM (1989) Flavonoid synthesis in *Petunia hybrida*: partial characterization of dihydroflavonol-4-reductase genes. Plant Mol Biol 13:491–502

Bradley JM, Davies KM, Deroles SC, Bloor SJ, Lewis DH (1998) The maize *Lc* regulatory gene up-regulates the flavonoid biosynthetic pathway of *Petunia*. Plant J 13:381–393

Britsch L, Ruhnau-Brich B, Forkmann G (1992) Molecular cloning, sequence analysis, and in vitro expression of flavanone 3β-hydroxylase from *Petunia hybrida.* J Biol Chem 267:5380–5387

Condit CM, Meagher RB (1987) Expression of a gene encoding a glycine-rich protein in petunia. Mol Cell Biol 7:4273–4279

Copenhaver GP, Browne WE, Preuss D (1998) Assaying genome-wide recombination and centromere functions with *Arabidopsis* tetrads. Proc Natl Acad Sci USA 95:247–252

Cornu A, Maizonnier D (1983) The genetics of Petunia. Plant Breed Rev 1:11–58

Cornu A, Farcy E, Maizonnier D (1989) A genetic basis for variations in meiotic recombination in *Petunia hybrida*. Genome 32:46–53

Davies KM, Bloor SJ, Spiller GB, Deroles SC (1998) Production of yellow colour in flowers: redirection of flavonoid biosynthesis in petunia. Plant J 13:259–266

Foster Atkinson E (1996) Alcohol dehydrogenase gene expression in petunia. PhD thesis, University of Guelph, Guelph, Ontario, Canada

Fransz PF, Stam M, Montign B, ten Hoopen R, Wiegant J, Kooter K, Oud O, Nanninga N (1996) Detection of single copy genes and chromosome rearrangements in *Petunia hybrida* by fluorescent *in situ* hybridization. Plant J 9:767–774

Gerats AGM, Souver E, Kroon J, McLean M, Farcy E, Maizonnier D (1993) *Petunia hybrida.* In O'Brien S (ed) Genetic maps: locus maps of complex genomes, 6th ed. Cold Spring Harbor Laboratory Press, New York, pp 6.13–6.23

Gregerson R, McLean M, Beld M, Gerats AGM, Strommer J (1991) Structure, expression, chromosomal location and product of the gene encoding ADH1 in *Petunia*. Plant Mol Biol 17:37–48

Gregerson RG, Cameron L, McLean M, Dennis P, Strommer J (1993) Structure, expression, chromosomal location and product of the gene encoding ADH2 in *Petunia*. Genetics 133: 999–1007

Holton TA, Brugliera F, Lester DR, Tanaka Y, Hyland CD, Menting JGT, Lu C-Y, Farcy E, Stevenson TW, Cornish EC (1993) Cloning and expression of cytochrome P450 genes controlling flower colour. Nature 366:276–278

Huits HSM, Gerats AGM, Kreike MM, Mol HNM, Koes RE (1994) Genetic control of dihydrdoflavonol 4-reductase gene expression in *Petunia hybrida*. Plant J 6:295–310

Koes RE, Spelt CE, Mol JNM, Gerats AGM (1987) The chalcone synthase multigene family of *Petunia hybrida* (V30): sequence homology, chromosomal location and evolutionary aspects. Plant Mol Biol 10:159–169

Lee H-S, Singh A, Kao T-h (1992) Rnase X2, a pistil-specific ribonuclease from *Petunia inflata*, shares sequence similarity with solanaceous S proteins. Plant Mol Biol 20:1131–1141

McLean M, Gerats AGM, Baird WV, Meagher RB (1990) Six actin gene subfamilies map to five chromosomes of *Petunia hybrida*. J Hered 81:341–346

McLean MD, Baird WV, Gerats AGM, Meagher RB (1988) Determination of copy number and linkage relationships among five actin gene subfamilies in *Petunia hybrida*. Plant Mol Biol 11:663–672

Mitchell AZ, Hanson MR, Skvirsky RC, Ausubel FM (1980) Anther culture of *Petunia*: genotypes with high frequency of callus, root or plantlet formation. Z Pflanzenphysiol 100:131–146

Mol JMN, Koes R, van den Berg EA, Reif HJ, Kreuzaler F, Veltkamp E (1985) The genetics of secondary metabolite production in higher plants: the flavonoid genes of *Petunia* as a model system. Adv Agric Biotechnol 13:122–123

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4326

Quattrocchio F, Wing JF, Leppen HTC, Mol JNM, Koes RE (1993) Regulatory genes controlling anthocyanin pigmentation are functionally conserved among plant specieis and have distinct sets of target genes. Plant Cell 5:1497–1512

Quattrocchio F, Wing JF, van der Woude K, Mol JNM, Koes R (1998) Analysis of bHLH and MYB domain proteins: species-specific regulatory differences are caused by divergent evolution of target anthocyanin genes. Plant J 13:475–488

Rees H, Durrant A (1986) Recombination and genome size. Theor Appl Genet 73:72–76

Robbins TP, Gerats AGM, Fiske H, Jorgensen RA (1995) Suppression of recombination in wide hybrids of *Petunia hybrida* as revealed by genetic mapping of marker transgenes. Theor Appl Genet 90:957–968

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York

Solano R, Nieto C, Avila J, Canas L, Diaz I, Paz Ares J (1995) Dual DNA binding specificity of a petal epidermis-specific MYB transcription factor from *Petunia hybrida*. EMBO J 14: 1773–1784

Tang X, Wang H, Brandt AS, Woodson WR (1993) Organization and structure of the 1-aminocyclopropane-1-carboxylate oxidase gene family from *Petunia hybrida*. Plant Mol Biol 23: 1151–1164

Ten Hoopen R, Harbord RM, Maes T, Nanninge N, Robins T (1998) The self-incompatibility (*S*) locus in *Petunia hybrida* is located on Ch III in a region syntenic for the Solanaceae. Plant J 16:729–734

van Tunen AJ, Koes RE, Spelt CE, van der Krol AR, Stuitje AR, Mol JNM (1988) Cloning of the two chalcone flavanone isomerase genes from *Petunia hybrida:* coordinate, light-regulated and differential expression of flavonoid genes. EMBO J 7:1257–1263

van Tunen, Mur LA, Recourt K, Gerats AGM, Mol JNM (1991) Regulation and manipulation of flavonoid gene expression in anthers of petunia: the molecular basis of the *Po* mutation. Plant Cell 3:39–48

Wallroth M, Gerats AGM, Rogers SG, Fraley RT, Horsch RB (1986) Chromosomal location of foreign genes in *Petunia hybrida*. Mol Gen Genet 202:6–15

Wiering H, deValming P, Cornu A, Maizonnier D (1979) Petunia genetics. I. List of genes. Ann Amél Plant 29:611–622

Zamir D, Tadmor Y (1986) Unequal segregation of nuclear genes in plants. Bot Gaz 147:355–358