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A molecular recombination map of *Antirrhinum majus*

Schwarz-Sommer *et al.*
A molecular recombination map of *Antirrhinum majus*

Zsuzsanna Schwarz-Sommer¹, Thomas Gübitz², Julia Weiss³, Perla Gómez-di-Marcò³, Luciana Delgado-Benarroch⁴, Andrew Hudson⁵, Marcos Egea-Cortines³*

**Abstract**

**Background:** Genetic recombination maps provide important frameworks for comparative genomics, identifying gene functions, assembling genome sequences and for breeding. The molecular recombination map currently available for the model eudicot *Antirrhinum majus* is the result of a cross with *Antirrhinum molle*, limiting its usefulness within *A. majus*.

**Results:** We created a molecular linkage map of *A. majus* based on segregation of markers in the F2 population of two inbred lab strains of *A. majus*. The resulting map consisted of over 300 markers in eight linkage groups, which could be aligned with a classical recombination map and the *A. majus* karyotype. The distribution of recombination frequencies and distorted transmission of parental alleles differed from those of a previous inter-species hybrid. The differences varied in magnitude and direction between chromosomes, suggesting that they had multiple causes. The map, which covered an estimated 95% of the genome with an average interval of 2 cM, was used to analyze the distribution of a newly discovered family of MITE transposons and tested for its utility in positioning seven mutations that affect aspects of plant size.

**Conclusions:** The current map has an estimated interval of 1.28 Mb between markers. It shows a lower level of transmission ratio distortion and a longer length than the previous inter-species map, making it potentially more useful. The molecular recombination map further indicates that the *IDLE* MITE transposons are distributed throughout the genome and are relatively stable. The map proved effective in mapping classical morphological mutations of *A. majus*.

**Background**

*Antirrhinum majus*, the garden snapdragon, has been used as a model system for genetics since the early 20th Century [1]. It is a member of a monophyletic group of about twenty five species that are native to the Mediterranean region share the same chromosome number (2n = 16) and are able to form fertile hybrids with each other [2]. The majority of species are allogamous, though cultivated *A. majus* lines and a few other wild species can self-fertilize.

A collection of *A. majus* mutants has been produced from some laboratory lines of *A. majus* selected for high transposon activity [3]. In several cases, these have been used to clone the corresponding genes by transposon tagging (e.g. [4-10]). In addition there is a collection of roughly four hundred classical mutants, mostly in an isogenic background (Sippe 50) [11,12]. The majority of these mutants does not show the genetic instability characteristic of transposon-induced mutations, and therefore have limited use for transposon tagging. The alternative approach of gene isolation by positional cloning is currently restricted by the availability of molecular recombination maps in *Antirrhinum*, though it has recently been successful in isolating the *fistulata* (*fis*) gene [13]. Though a classical *fis* mutation was genetically stable, it is caused by insertion of a miniature inverted-repeat transposable element (MITE), which is present in relatively low copy-number in all *Antirrhinum* species. Because the transposon family appeared relatively inactive it was called *IDLE*.

The existing molecular recombination map for *Antirrhinum* was built using the F2 of a cross between...
A. majus (line 165E) and a wild relative, A. molle [14]. The map identified eight linkage groups and use of common loci had allowed these to be related to a classical genetic map and to the A. majus chromosomes by in situ hybridization [15]. However, the majority of markers from the A. molle x A. majus hybrid showed significantly distorted transmission, which are likely to have affected the accuracy of the map, and the map also contained clusters of loci consistent with chromosome rearrangements between the species [12,15]. Such rearrangements were also suggested by observation of chromosomes [15]. These two factors would hinder attempts to map A. majus mutations in crosses to A. molle. A further disadvantage of using inter-species crosses to map A. majus mutations is that A. majus and A. molle differ in many morphological characters, including plant and organ size. Segregation of natural variation would therefore be likely to obscure the effects of mutations in hybrid mapping populations.

We therefore developed a linkage map of A. majus using the inbred lines Sippe50 and 165E. The map consists of 302 markers (protein coding sequences, AFLP and transposons), covering nearly 95% of the genome. As a proof of concept, we placed on the map six mutations affecting floral and overall size. We also mapped the distribution of IDLE transposons, revealing that they are allocated with coding genes in all Antirrhinum chromosomes.

Results & discussion
Construction of a molecular linkage map for A. majus
To construct a molecular linkage map for A. majus we crossed two inbred lines, 165E and Sippe 50. Line 165E originated from cultivated A. majus in the UK and is phenotypically distinct from Sippe 50, which was derived in Germany, possibly from a wild accession [11,16]. A single F1 progeny was self-pollinated to produce an F2 mapping population of 96 plants. This population therefore contains 192 recombinant copies of each chromosome, sufficient for mapping loci to a resolution of ~1 cM. The F2 population was genotyped at 377 loci. These included 90 protein-coding genes, in which polymorphisms were detected by sequencing the alleles from both parents. The identities of the protein-coding genes are given in Table 1. The remaining markers mainly comprised AFLP and insertions of the MITE transposon IDLE [13]. The genotype data were used to estimate a recombination map. Fifty-nine AFLP markers (28% of the total) and one dominant IDLE insertion were present in significantly more or fewer F2 plants than expected and could either not be mapped or mapped only by reducing support for linkage groups significantly. These markers were therefore rejected. The remaining markers formed a map comprising 90 protein-coding genes, 87 of which were mapped as co-dominant CAPS or size polymorphisms, 159 dominant AFLP and 53 IDLE insertions (10 with co-dominant alleles and 43 dominant markers). A complete list of primers for each marker and the corresponding map position can be found in Additional file 1. At nine loci AFLP bands from both parents showed complete linkage in repulsion and were subsequently treated as synthetic co-dominant markers.

The resulting map comprised eight linkage groups with a total length of 562 cM that was estimated to cover 95% of the genome (Figure 1). At this level of coverage, the average interval between markers was 2.0 cM, with 88% of the genome estimated to lie within 2.0 cM of a marker and 99% within 5 cM. Although the average interval between co-dominant markers was 6.0 cM, a similar proportion of the genome (83%) was within 2.0 cM of the nearest co-dominant marker. Assuming a haploid genome size of $3.6 \times 10^8$ bp for A. majus [17], a marker interval of 2.0 cM represents on average 1.28 Mbp of DNA.

Map comparison
A previous molecular map for Antirrhinum had been produced from the F2 progeny of a cross between A. majus (line 165E) and the wild species A. molle [14]. To allow identification and alignment of linkage groups in the two populations, the genotypes from the previous mapping population were used to reconstruct a map using the same parameters as for the A. majus x A. majus F2. Markers common to both maps allowed identification of corresponding linkage groups and their orientations (Additional file 2).

The total A. majus map was about 54% larger than for A. majus x A. molle. However the variations in length differed in magnitude and direction between chromosomes (Figure 2). Two linkage groups (5 and 3) were slightly smaller in map A, while the remainders were significantly longer, suggesting that the causes of length differences varied between chromosomes.

Previous studies have reported both smaller and larger maps for intra-specific crosses as compared to inter-specific crosses [18,19]. Several factors might contribute to variations in map lengths for Antirrhinum and might differ between chromosomes. Of particular relevance to the utility of the A. majus map is the possibility that the two marker sets cover different parts of the genome. However, this seems unlikely, because although the two maps contain a different number of loci (296 in map A and 227 in map B) they are estimated to cover a similar proportion of the genome (95% in A and 94% in B using Method 4 [20]). Moreover, randomly removing 69 markers from A, to make the numbers of markers the same in both
| Sequence number  | EST annotation                                           | Length in bp | min. eValue |
|-----------------|---------------------------------------------------------|--------------|-------------|
| EMAMAS8924      | thioredoxin peroxidase                                   | 723          | 1.0E-1.21206E-85 |
| EMAJ801757      | proline-rich apg-like protein                            | 670          | 1.0E-2.50815E-86 |
| EMAJ794773      | o-linked c-transferase                                   | 758          | 1.0E-2.06855E-101 |
| EMAJ804794      | histone h1                                               | 556          | 1.0E-1.27731E-27 |
| EMAJ794598      | at4g20410-like protein                                   | 736          | 1.0E-1.80515E-107 |
| EMAJ805499      | stearyl-acyl carrier protein desaturase                  | 481          | 1.0E-9.70457E-56 |
| EMXS7295        | TAP1 protein precursor                                   | 5280         | 1.0E-1.98821E-44 |
| EMAY072736      | HIRZINA KNOX protein                                     | 1462         | 1.0E-0.0     |
| EMAJ802708      | Eukaryotic translation initiation factor 6               | 630          | 1.0E-1.16373E-87 |
| EMAJ794444      | mcg108135 protein                                        | 675          | 1.0E-6.11236E-80 |
| EMAJ802293      | translational inhibitor protein                           | 589          | 1.0E-1.37912E-65 |
| EMAJ803243      | 60 s ribosomal protein I18                               | 731          | 1.0E-2.80747E-92 |
| EMAJ559267      | heavy-metal-associated domain-containing protein          | 504          | 1.0E-2.98463E-13 |
| EMAJ790658      | urease accessory protein g                                | 561          | 1.0E-3.52407E-97 |
| EMAJ792971      | gtp-binding protein                                       | 628          | 1.0E-1.89892E-82 |
| EMAJ560201      | iron-sulfur cluster assembly protein                     | 768          | 1.0E-3.45238E-59 |
| EMAJ620906      | STYLOSA co-repressor                                     | 2865         | 1.0E-0.0     |
| EMAJ806659      | atp-dependent clp protease proteolytic subunit           | 711          | 1.0E-1.54692E-116 |
| EMAJ560074      | leucine rich repeat protein                               | 754          | 1.0E-2.04947E-101 |
| EMAM422773      | YABBY4 transcription factor                              | 1395         | 1.0E-7.9976E-177 |
| EMX97639        | cyclin-dependent kinase, CDC2a                          | 1038         | 1.0E-2.78255E-170 |
| EMAY06654       | C2H2 zinc-finger protein                                  | 530          | 1.0E-4.79832E-31 |
| EMAM422772      | YABBY2 transcription factor                              | 1373         | 1.0E-3.22687E-114 |
| EM AJ568130     | cyclic nucleotide-regulated ion channel                   | 620          | 1.0E-3.55048E-94 |
| EMAJ568031      | amino acid permease family expressed                     | 1459         | 1.0E-1.38642E-78 |
| EMAJ800340      | at3g04780 F7o18_27                                       | 721          | 1.0E-1.77924E-75 |
| EMAJ568062      | chloroplast translation initiation factor 2              | 415          | 1.0E-4.51282E-45 |
| EMSS3000        | PLENA MADS-box transcription factor                      | 1073         | 1.0E-1.61386E-120 |
| EMAY081384      | actin associated protein                                  | 538          | 1.0E-5.43416E-33 |
| EMAY00042       | multiple stress-responsive zinc-finger protein            | 781          | 1.0E-1.45498E-60 |
| EMAY05889       | centromere microtubule binding protein cbf5              | 692          | 1.0E-3.39584E-97 |
| EMAY08934       | transcription factor                                      | 884          | 1.0E-2.17902E-88 |
| EMAY568063      | polygalacturonase-inhibiting protein                      | 734          | 1.0E-1.19729E-66 |
| EMAY804237      | histidinol dehydrogenase                                 | 634          | 1.0E-2.00488E-95 |
| EMAY795662      | flavonoid 3-o-glucosyltransferase                        | 750          | 1.0E-9.60942E-83 |
| EMAY800415      | erwinia induced protein 2                                 | 615          | 1.0E-2.67616E-49 |
| EMAY796551      | Frigida                                                 | 666          | 1.0E-5.06947E-39 |
| EMAY796122      | p-p-bond-hydrolysis-driven protein transmembrane transporter | 747       | 1.0E-3.07514E-73 |
| EMAY800197      | atp-dependent protease clp atpase subunit                | 594          | 1.0E-3.10775E-69 |
| EMAY559052      | t-complex protein 1 epsilon tcp-1-                       | 690          | 1.0E-2.62699E-116 |
| EMAY03361       | monodehydroascorbate reductase                           | 730          | 1.0E-7.35389E-109 |
| EMAY02640       | isochorismatase hydrolase                               | 779          | 1.0E-1.51824E-94 |
| EMAY223518      | LIP1 apetala2-like transcription factor                  | 1845         | 1.0E-0.0     |
| EMAY805150      | photoassimilate-responsive protein par-like protein       | 530          | 1.0E-7.58999E-61 |
| EMAY620905      | STYLOSA1 co-repressor                                   | 3123         | 1.0E-0.0     |
| EMAY801986      | at-rich element binding factor 3                         | 590          | 1.0E-8.38628E-55 |
| EMX68831        | GLOBOSA MADS-box transcription factor                    | 6108         | 1.0E-5.69799E-27 |
| EMX76995        | polygalacturonase-inhibiting protein                      | 3545         | 1.0E-4.66003E-119 |
| EMAY558819      | psp psi-p ptc8 trp14 (thylakoid membrane phosphoprotein of 14 kda) dna binding | 762      | 1.0E-2.07028E-40 |
| EMAY793550      | trna-methyltransferase subunit                           | 535          | 1.0E-7.89349E-61 |
| EMAY620909      | SEUSS3A co-repressor                                    | 3683         | 1.0E-0.0     |
populations, reduced the length of map A by an average of only 2% (Additional file 3). Similarly maps made only with dominant protein-coding genes from each F2 population showed the same trends in map length differences (data not shown), suggesting that they are not dependent on the number or types of markers used.

The insensitivity of map length to the type of marker also suggests that the 51 mapped IDLE transposons were relatively stable, because excision of an IDLE in members of the mapping population would result in the wrong parental origin being assigned to its locus and an over-estimation of recombination frequencies. The relative stability of IDLE markers was further supported by the finding that they were no more likely than other marker types to have an apparent recombination break-point immediately next to them, as would be expected if excision had resulted in an incorrect genotype.

Although many transposon families are predominant components of heterochromatin, MITE transposons have commonly been found associated with gene-rich regions [21,22]. This is consistent with the observed distribution of IDLE insertions in Antirrhinum, which are interspersed with protein-coding genes and do not appear to be clustered in centromeric or telomeric regions.

Table 1 List of EST-based markers and functional annotation (Continued)

| Accession | Description | Log10 of P-Value | E-value |
|-----------|-------------|-----------------|--------|
| EM:AJ800998 | Transcription factor lim | 710 | 1.0E-3.7918E-99 |
| EM:AJ620910 | seu3b protein | 2004 | 1.0E-2.36396E-82 |
| EM:AY451399 | CRABSCLOW-like YABBY transcription factor | 732 | 1.0E-1.78657E-70 |
| EM:AJ794665 | nuclear cap binding protein subunit 2 | 673 | 1.0E-1.25817E-85 |
| EM:AJ802365 | ——NA—— | 583 | 1.0E-1.68821E-23 |
| EM:AJ801224 | ——NA—— | 613 | 1.0E-2.33555E-13 |
| EM:AJ794216 | nuclear RNA binding | 758 | 1.0E-1.84607E-33 |
| EM:AJ794472 | serine threonine protein kinase | 746 | 1.0E-1.35578E-75 |
| EM:AJ680999 | pgr5-like a | 1478 | 1.0E-9.46041E-75 |
| EM:AJ791655 | bzo2h3 (arabidopsis thaliana basic leucine zipper 63) dna binding transcription factor | 758 | 1.0E-9.80266E-27 |
| EM:AJ790549 | immunophilin | 566 | 1.0E-1.28799E-54 |
| EM:M55525 | FLORICAULA transcription factor | 1545 | 1.0E-0.0 |
| EM:AJ802861 | ——NA—— | 728 | 1.0E-1.22273E-47 |
| EM:AJ799233 | delta-12 oleate desaturase | 736 | 1.0E-8.2567E-116 |
| EM:AJ804300 | protein | 683 | 1.0E-6.22658E-88 |
| EM:AJ803800 | af31947S_1 alpha-expansin 9 precursor | 590 | 1.0E-5.56035E-91 |
| EM:AJ803115 | protein | 654 | 1.0E-9.83593E-72 |
| EM:AJ790136 | tfl1b-related protein | 677 | 1.0E-1.59963E-96 |
| EM:AJ805759 | eukaryotic translation initiation factor 6 | 689 | 1.0E-1.55434E-110 |
| EM:AJ559280 | tic20-like protein | 710 | 1.0E-6.95833E-77 |
| EM:AJ620907 | SEUS51 protein | 3495 | 1.0E-0.0 |
| EM:AJ789733 | ——NA—— | 675 | 1.0E-6.54566E-74 |
| EM:AJ800463 | tubulin alpha-6 chain | 605 | 1.0E-4.42202E-40 |
| EM:AJ791186 | Glutaredoxin-like protein | 684 | 1.0E-1.57483E-30 |
| EM:AJ790836 | b12 d protein | 564 | 1.0E-1.21222E-36 |
| EM:AJ794452 | duf1230-containing protein | 763 | 1.0E-1.97117E-91 |
| EM:AJ793362 | phosducin-like protein 3 | 578 | 1.0E-6.36625E-60 |
| EM:Y16313 | CYCLOIDEA TCP transcription factor | 861 | 1.0E-1.34443E-129 |
| EM:AY223519 | LIP2 apetala2-like transcription factor | 1914 | 1.0E-0.0 |
| EM:AJ800866 | polycomb protein ez1 | 507 | 1.0E-1.11628E-31 |
| EM:AJ805209 | rer1a protein | 582 | 1.0E-1.72796E-60 |
| EM:AJ68117 | phenylalanine ammonia-lyase | 619 | 1.0E-7.46548E-60 |
| EM:AJ791765 | microtubule motor | 745 | 1.0E-7.82606E-45 |
| EM:AJ808258 | flu (fluorescent in blue light) binding | 663 | 1.0E-2.25085E-39 |
| EM:AJ93379 | rpm1-interacting protein 4 | 580 | 1.0E-1.07793E-22 |

The ESTs were automatically annotated using BLAST2GO. The corresponding EST annotation was performed using a minimal threshold of e-6. Those genes with high homology to genes with unknown function were annotated as NA. Sequences with known mutant phenotypes in Antirrhinum are given their Antirrhinum gene names.
Transmission ratio distortion differences between inter and intra-specific maps

At least some of the length variation between maps might be attributed to transmission ratio distortion (TRD). This was more marked in the interspecies cross, in which loci representing most of the genome deviated significantly from their expected Mendelian ratios (Figure 3). It was most severe for LG6, in which \textit{A. molle} carries a functional gametophytic self-incompatibility (S) locus. This prevented recovery of F2 plants homozygous for \textit{A. molle} alleles at LG6 unless recombination had occurred between the marker and the S locus. In contrast, \textit{A. majus} lacks a functional S locus and shows only mildly distorted transmission of markers from LG6 (Figure 3). TRD can lead to under-estimation of map distances [23] and loss of marker information, for example no dominant markers closely linked to the \textit{A. molle} S allele were identified in map B. It can also lead to markers being wrongly assigned to linkage groups. The lower level of TRD in map A therefore justifies the use of mapping populations of \textit{A. majus} rather than interspecies hybrids.

It was previously suggested that the clustering of markers in map B may be caused by chromosome inversions that distinguished \textit{A. majus} from \textit{A. molle} [14], preventing mapping of loci that lie within inversions. However, there is significant clustering (p < 0.0001) of markers in both maps and significantly more clustering in map A than map B. Since fewer rearrangements are expected between two \textit{A. majus} lines than between \textit{A. majus} and \textit{A. molle} clusters of markers appear unlikely to represent inversions.

Map validation by mapping mutations affecting size

One possible use of a molecular map is to determine the chromosomal positions of loci that have been identified by mutation. This can, for example, classify mutations that are potentially allelic, which is particularly useful for dominant mutations, and allows isolation of the affected genes on the basis of their positions. We therefore tested the utility of the \textit{A. majus} map in determining the position of six classical mutations affecting aspects of plant size (Figure 4). All six mutations were in the Sippe 50 mutant background and therefore crossed to wild-type 165E to generate F2 mapping populations.

The \textit{nana} (\textit{na}) mutation described at the end of the XIXth century in the Vilmorin catalog [24], reduces plant size in a recessive fashion and flowers early irrespective of photoperiod. The \textit{na} mutant phenotype
segregated as expected for a recessive mutation in the F2 generation of the cross to 165E. However, a second allele *nana* *largiflora*, which caused a somewhat weaker phenotype in the Sippe 50 genetic background [12], could not be distinguished from wild-type in F2 populations produced by crossing to 165E line. This highlights a potential problem arising from suppression of a weak mutant phenotype in a cross between two lines that differ, albeit slightly, in morphology. Another difficulty was identified in the case of the recessive *muscoides* (*mus*) mutation, which causes dwarfism. No *mus* mutants were initially identified in the F2 of the cross with 165E. However, *mus* mutants were recovered at a low frequency (2 out of 60 plants) when F2 seeds were germinated in Petri dishes, suggesting that the *mus* mutation can be lethal in the 165E genetic background.

The mutant *hero* affected stem thickness, a trait that seems to be partly controlled by genes affecting floral size in Arabidopsis like *Bigbrother* and *Kluh* [25,26]. However *hero* did not show a statistical difference from wild-type in lateral organ size, either in the original Sippe 50 background or in F2 populations, and segregated as expected of a recessive mutation in the F2 (data not shown).

Four mutations affecting floral size also segregated as expected in the F2 populations produced by crossing to 165E with *compacta* (*co*), *compacta-ähnlich* (*coan*) and *formosa* (*fo*) mutations appearing fully recessive, and *Nitida* (*Ni*) as semi-dominant [11]. In the case of *Ni* and *co* mutants, their phenotypes in the F2 were similar to those of the original background while *coan* and *fo* mutants showed slightly larger differences from wild-type.

The mutants affect floral size in different ways, *coan* decreases flower size without affecting vegetative body size [27], while the *co* mutation reduces both flower and lateral organ size. The *fo* mutation increases floral size, [28] while the *na* mutation reduces plant height and leaf width without significantly affecting flower size while the *Ni* mutation reduces the sizes of flowers, leaves and internodes in a dosage-dependent fashion.

As an initial approach, the mutations were mapped by bulk-segregant analysis [29]. DNA was extracted from several pools of four plants that shared the same phenotype and screened with a CAPS marker located in a middle region of each chromosome arm (a total of 16
markers). Markers that $\chi^2$ tests suggested were not linked to the size mutation were rejected. Where evidence for linkage was found, additional CAPS markers from the same chromosome regions were used to analyze individual F2 plants to refine map positions. Statistically significant linkage was found between the additional markers and the mutations in all cases (Table 2). The distance between a mutation and the closest marker ranged from the $coan$ locus and the marker AJ790136 in LG3, which showed no recombination in 43 homozygous mutants, to $na$ and AJ568062 in LG2 which were separated by about 26 cM.

We have therefore shown that it is feasible to map mutations in crosses between these two Antirrhinum species is that they differ widely from each other in size. Several genes underlying this size variation have been mapped as quantitative trait loci (QTL), e.g. [30]. Like the size mutants analyzed here, the QTL can affect a single type of organ or have more pleiotropic effects. It should now be possible to identify whether any classical size mutations might correspond to size QTL on the basis of map positions and so select candidate mutations for more allelism tests. A corresponding classical mutation can facilitate QTL isolation and the understanding of QTL gene function.

Conclusions

We have constructed a molecular linkage map using two inbred lines of Antirrhinum majus, 165E and Sippe 50. The newly developed map has eight linkage groups and a total length of 562 cM with an estimated coverage of 95% of the genome. There is an average interval of 2 cM between codominant markers in 88% of cases and 5 cM in 99%, and assuming a genome size of $3.6 \times 10^8$ bp, an interval of 2 cM represents on average 1.28 Mbp of DNA.

The new map is 54% longer than the previously published map of A. majus x A. molle, and this difference is caused by increased length of the different linkage groups, except 3 and 5 that were slightly shorter indicating that map length differences were the result of differences between chromosomes in the two crosses.

We have mapped 51 IDLE transposons that are interspersed with EST-based markers indicating that MITE transposons, like in other plants, are found in gene-rich regions. Determination of EST-based markers will allow future use of the A. majus map for comparative genomic studies with other plants.

The new map has fewer regions of TRD reinforcing its usefulness to determine genome positions with higher accuracy. This has been achieved by validating the map with six classic mutants affecting floral size (Ni, $co$, $coah$, and $fo$), body size ($Hero$ and $na$) and flowering time ($na$). We have been able to obtain map positions for each mutant using F2 mapping populations.

Methods

Plant material
Seeds of Antirrhinum majus L. were germinated and grown as described by [31].

The A. majus line Sippe 50 [11] was obtained originally from the IPK Gatersleben and maintained by self-pollination while the second wild-type line 165E was produced by several generations of self-pollination from line H.98 [16,32]. An F2 population ($n = 96$) for mapping molecular markers was selected at random from the progeny of a single F1 hybrid of Sippe 50 × 165E. The mutants compacta ($co$) [33], compacta ähnlich ($coan$) [12], formosa ($fo$), Grandiflora (Graf), heroina ($Hero$), Nitida (Ni) [11] and Nana ($na$) [24] were obtained from the IPK Gatersleben collection. All the mutants are in the Sippe 50 genetic background. Mutations were mapped in F2 populations produced by crossing mutants to the 165E wild-type.

DNA was extracted using a NucleoSpin® kit (Macherey-Nagel) from 100 mg of frozen leaf samples that had been ground to a powder in liquid $N_2$.
Mapping transcribed genes
Sequence tagged sites (STS) were generated using primers able to amplify regions from a collection of *A. majus* EST sequences that showed differences between Sippe50 and 165E [34]. The identities of PCR products were confirmed by sequencing. For six genes, both parental alleles could be distinguished by differences in the sizes of their amplified products in agarose gels without digestion. A further three loci amplified from only one parent and were therefore treated as dominant markers. For the remaining genes, restriction site polymorphisms were identified by comparing sequences of amplified products and the loci scored as co-dominant CAPS resolved in agarose gels. The ESTs used to develop markers were annotated automatically using the BLAST2GO program [35,36].

AFLP analysis
AFLPs were amplified from DNA that had been digested with *Pst*I and *Mse*I using eight combinations of selective primers. Primers for the *Pst*I ends of fragments had 3' selective di-nucleotides AA (P11), AC (P12) or AT (P14) and were labeled with one of four different fluorescent dyes (6-FAM, VIC, NED or PET) while those at the *Mse*I ends had 3' extensions of ACA, AGC, AGT, CAC or CAT. Products were separated with the LIZ-500 internal size standard (ABI) using an ABI 3730 DNA Analyzer. Output files were converted to fsa format using the program obtained from http://dna.biotech.wisc.edu/ABRF/3730toGSconverter.exe, processed using Genescan software (ABI) and the presence or absence of bands scored from virtual gels created using a version of the program Genographer http://hordeum.oscs.montana.edu/genographer/ that had been modified by its authors to accommodate the five different color channels. AFLPs were scored as dominant markers. They were named according to the primers used to generate them, their size and their parent of origin - e.g. locus 11AGA141J amplified with selective primers P11 and Mse-AGA as a band of 141 nt and originated from parent 165E.

Mapping MITE transposons
Different copies of the *IDLE* transposon were identified by homology to the insertion in the *fistulata-2* mutation in *A. majus* [13] either by hybridization to genomic clones or comparison to *A. majus* BAC clones. The host sequences to both sides of 10 *IDLE* insertions were identified. In these cases, primers from the two flanking regions were used to distinguish the presence or absence of the transposon on the basis of size polymorphism allowing these loci to be scored as co-dominant markers. For 43 insertions only one flanking sequence was obtained and a flanking primer was used with an *IDLE* primer to detect the presence of an insertion, which was treated as a dominant marker.

Map construction
To construct the molecular recombination map for the F2 population, co-dominant markers were scored as one of three allelic states (homozygous 165E, homozygous Sippe 50 or heterozygous) while dominant markers were assigned to one or other parent and scored for the presence or absence in F2 individuals. A map was estimated from the genotype data at 377 loci using Joinmap 3.0 [37], using a minimum LOD score of 6.0 to identify potential linkage groups. Maps of each linkage group were then established using the default thresholds for elimination of markers and establishing marker order and the Kosambi mapping function [38] to calculate genetic distances. Transmission ratio distortion was represented for loci with co-dominant alleles by plotting the frequencies of each homozygote and half the frequency of heterozygotes and for dominant loci by the frequency of homozygotes lacking the dominant allele. Each class was expected with a frequency of 0.25 and significant deviations from this expectation were assessed with \( \chi^2 \) tests.

Total genome size was estimated using Method 4 from [20] or by adding twice the average marker spacing to each chromosome, with both methods providing very similar estimates. The percentage of the genome within a particular map distance of the nearest molecular marker was estimated with the method used by [39]. To analyze whether markers showed non-random clustering, the number of 1 cM intervals expected to contain a particular number of markers was calculated from the total number of markers and map length, assuming that the markers were distributed randomly (i.e. that the number of markers per 1 cM interval followed a Poisson distribution). This null hypothesis was tested against the observed frequency distribution of markers, using a \( \chi^2 \) test. The frequency distributions of marker densities for the two maps were also compared directly, using a \( \chi^2 \) test.

Mutant mapping
To map mutations, F2 plants were selected for genotyping on the basis of their phenotype. Four pools, each containing a similar amount of DNA from four homozygous plants, were first used to scan for linkage to one of 16 markers-representing both arms of all eight chromosomes. Linkage to a marker locus was suggested by an enrichment of one of its parental alleles in more than one of the pools (i.e. enrichment of the Sippe 50 allele in pools of recessive mutations or the 165E allele in wild-type pools in the case of dominant mutants). Suspected linkage was investigated further by genotyping
between 20 and 60 F2 individuals for the original locus and at additional loci linked to it. Linkage was assessed using $\chi^2$ tests to identify significant deviations from random segregation in the mutant population and the Kosambi function used to estimate map distances between mutations and markers from recombination frequencies.

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**Additional material**

**List of abbreviations**

AFLP: Amplified fragment length polymorphism; cM: centiMorgan; MITE: Miniature Inverted Repeat Transposable element; TRD: transmission ratio distortion

**Author details**

1Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, 50829 Köln, Germany.
2Deutsche Forschungsgemeinschaft (DFG)-Wissenschaftliche Geräte und Informationstechnik, D-53170 Bonn, Germany.
3Institute of Plant Biotechnology (IBB), Technical University of Cartagena, Campus Muralla del Mar, 30202 Cartagena, Spain.
4Instituto de Botánica del Nordeste (IBONE)-CONICET-Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste (UNNE) CC 209, Corrientes 3400 Argentina.
5Institute of Molecular Plant Sciences, University of Edinburgh, King’s Buildings, Mayfield Rd., Edinburgh EH9 3JH, UK.

**Authors’ contributions**

ZsSS, TG, AH and MEC designed experiments. ZsSS developed the EST and IDLE markers, TG and AH did the AFLP markers, TG, ZsSS and AH made the mutant pictures. PGC, LDG and JW grew the F2 populations and scored the phenotypes. MEC did the mutant pictures. LDG, JW and MEC grew the F2 populations and at additional loci linked to it. Linkage was assessed using $\chi^2$ tests to identify significant deviations from random segregation in the mutant population and the Kosambi function used to estimate map distances between mutations and markers from recombination frequencies.

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