ORIGINAL PAPER

A high-density, integrated genetic linkage map of lettuce (*Lactuca* spp.)

**M. J. Truco · R. Antonise · D. Lavelle · O. Ochoa · A. Kozik · H. Witsenboer ·
S. B. Fort · M. J. W. Jeuken · R. V. Kesseli · P. Lindhout · R. W. Michelmore ·
J. Peleman**

Abstract An integrated map for lettuce comprising of 2,744 markers was developed from seven intra- and inter-specific mapping populations. A total of 560 markers that segregated in two or more populations were used to align the individual maps. 2,073 AFLP, 152 RFLP, 130 SSR, and 360 RAPD as well as 29 other markers were assigned to nine chromosomal linkage groups that spanned a total of 1,505 cM and ranged from 136 to 238 cM. The maximum interval between markers in the integrated map is 43 cM and the mean interval is 0.7 cM. The majority of markers segregated close to Mendelian expectations in the intra-specific crosses. In the two *L. saligna* × *L. sativa* inter-specific crosses, a total of 155 and 116 markers in 13 regions exhibited significant segregation distortion. Data visualization tools were developed to curate, display and query the data.

M. J. Truco (✉) · D. Lavelle · O. Ochoa · A. Kozik ·
H. Witsenboer · S. B. Fort · R. V. Kesseli · R. W. Michelmore
The Genome Center and The Department of Plant Sciences,
University of California, Davis, CA 95616, USA
e-mail: mjtruco@ucdavis.edu

R. Antonise · H. Witsenboer · J. Peleman
Keygene N.V, 6708 PW Wageningen, The Netherlands

M. J. W. Jeuken · P. Lindhout
Laboratory Plant Breeding, Graduate School for Experimental
Plant Sciences, Wageningen University, PO box 386,
6700 AJ Wageningen, The Netherlands

R. V. Kesseli
Department of Biology, University of Massachusetts,
Boston, MA 02125, USA

The integrated map provides a framework for mapping ESTs in one core mapping population relative to phenotypes that segregate in other populations. It also provides large numbers of markers for marker assisted selection, candidate gene identification, and studies of genome evolution in the Compositae.

Introduction

The development of several types of molecular markers in the last 20 years (Peters et al. 2003; Gupta and Rustgi 2004) has greatly facilitated the construction of genetic linkage maps for a variety of crop species. These linkage maps have been used for marker-assisted breeding (reviewed in Dekkers and Hospital 2002), map-based cloning strategies (Mayerhofer et al. 2005; van Os et al. 2006), comparative genomics (reviewed in Paterson et al. 2000) and dissection of quantitative traits (reviewed in Salvi and Tuberosa 2005).

Genetic maps have been developed using intra- or inter-specific crosses, mostly predicated on whether sufficient polymorphism exists within the species. Maps generated from intra-specific crosses can be more readily used for breeding purposes because they contain markers that are informative within the closely-related gene pool of the cultivated species. However, in self-pollinated species like lettuce, the development of comprehensive maps from intra-specific crosses has been obstructed by limited polymorphism within the cultivated species. Inter-specific crosses have a higher degree of polymorphism but segregation distortion for some crosses may be frequent and hybrid sterility precludes the development of permanent populations such as recombinant inbred lines. Furthermore, reduced recombination frequency or segregation distortion towards

the cultivated allele in certain regions may limit access to wild alleles in those regions of the genome.

Genetic maps developed using individual populations can be of limited use for other populations if markers are not frequently polymorphic among populations. Integrated maps that combine information from multiple populations increase the number of markers in a given genomic region and improve the chances of obtaining sufficient polymorphic markers in a specific population as well as allowing comparison of locations of genes of interest across maps. Integrated linkage maps have been developed for several crop species including pepper (Paran et al. 2004; Lefebvre et al. 2002), soybean (Song et al. 2004), melon (Périn et al. 2002), sunflower (Gedil et al. 2001), rapeseed (Lombard and Delourne 2001), tomato (Haanstra et al. 1999) and barley (Qi et al. 1996).

Lettuce (*Lactuca sativa* L.) is a diploid ($2n = 18$) species within the *Lactucae* tribe of the Asteraceae. There are four well-established species within the subsection Lactuca: the cultivated *L. sativa* and three wild species, *Lactuca serriola*, *Lactuca saligna*, and *Lactuca virosa* (in order of decreasing sexual compatibility with *L. sativa*). *L. serriola* is probably the progenitor of and con-specific with *L. sativa* (Kesseli et al. 1991; de Vries 1997). Lettuce is a major horticultural crop with a worldwide production of over 21 million tons in 2004 and accounts for $2.06 billion in farm value within the USA (Anonymous 2004). Lettuce has been the subject of classical and molecular studies. It is one of the species studied in the Compositae Genome Project (CGP; http://compgenomics.ucdavis.edu/). An objective of this project is the characterization of genes underlying agriculturally important and domestication traits. This includes genetic analyses to dissect qualitative and quantitative traits as well as the identification of candidate genes from extensive expressed sequence tag (EST) databases and the study of synteny among sunflower, lettuce and Arabidopsis (Timms et al. 2006; http://cgpdb.ucdavis.edu/).

Five genetic maps have been published previously for lettuce. Kesseli et al. (1994) and Waycott et al. (1999) reported the construction of two linkage maps assembled on populations derived from crosses within cultivated lettuce (*L. sativa*). These two maps are fragmented with multiple linkage groups exceeding the chromosomal number of *Lactuca* species. This is probably a consequence of the low intra-specific polymorphism and a high frequency of monomorphic regions between the parental lines. Two other maps are from crosses between cultivated lettuce and its closest wild relatives *L. serriola* and *L. saligna*. Johnson et al. (2000) described a framework map of AFLP® markers (a registered trademark of Keygene N.V.) for a QTL analysis of a population derived from *L. sativa* and *L. serriola*. Jeuken et al. (2001) used AFLP markers to generate a consensus map of nine chromosomes for two populations derived from crosses between *L. saligna* and *L. sativa*. Because these four maps have been constructed using different sets of markers, alignment of linkage groups across maps was not possible, excluding the possibility of integrating information between them. Recently, a map from a cross between *L. serriola* DH-M21 and *L. sativa* cv. Dynamite has been published (Syed et al. 2006). This map was developed using retrotransposon-based markers as well as some AFLP markers in common with the map of Jeuken et al. (2001).

Here we report on the construction of a consensus map in lettuce that integrates four previously published maps of lettuce with data from three new populations: two maps generated from crosses between *L. sativa* and *L. serriola* and an additional map derived from *L. saligna* × *L. sativa*. This map integrates data from all seven individual maps, thus providing for a better coverage of all genomic regions. It is also more representative of the *Lactuca* genus. *L. serriola* and *L. saligna* are the closest wild relatives of cultivated lettuce (*L. sativa*) and their inclusion allows for more universal utility of markers across different genetic backgrounds. Display of the data in the public Compositae Genome Database (http://cgpdb.ucdavis.edu/) provides a current resource for exploiting lettuce genetic and genomic data.

Materials and methods

Plant material

Seven different populations were used to develop an integrated map of lettuce (Table 1). Two were F_2 populations of intra-specific crosses between cultivars or breeding lines of *L. sativa*: Population DB0D was a F_2 of a cross between cv. Calmar (crisphead type) × cv. Kordaat (butterhead type); The RYDER population was a cross between F_4 breeding lines 87-25-1M × 87-1090M (for more detailed pedigrees see Waycott et al. 1999). Three other populations were inter-specific crosses between *L. sativa* and *L. serriola*. Population DB0H was a F_2 from a cross between *L. sativa* cv. Salinas (crisphead type) and *L. serriola* US96UC23; population DA0F was a RIL_7 of the same cross derived by single seed descent. Population DB9X was a F_2 from a cross between *L. sativa* cv. Salad Bowl (leaf type) and *L. serriola* CGN14263. The last two populations were inter-specific crosses between *L. saligna* and *L. sativa*. Population DB0F was a F_2 from *L. saligna* UC82US1 × *L. sativa* cv. Vanguard 75 (crisphead type). Population DB0T was a F_2 from *L. saligna* CGN5271 × *L. sativa* cv. Olof (butterhead type).

Construction of individual maps

Linkage maps for populations DB0D and RYDER utilizing restriction fragment length polymorphism (RFLP),

Table 1 Generation, number of individuals and markers of the seven populations integrated on the consensus map of lettuce

Code	Parentals[a]	Generation	No. of individuals	No. of mapped markers[b]					References[c]
				RFLP	AFLP	SSR	RAPD	others	
DB0D	*L. sativa* cv.Calmar × *L. sativa* cv. Kordaat	F_2	66	129	411	39	223	9	Kesseli et al. (1994); Witsenboer et al. (1997)
RYDER	*L. sativa* 87-25-1M × *L. sativa* 87-1090M	F_2	101	–	–	28	69	7	Waycott et al. (1999)
DB0H	*L. sativa* cv. Salinas × *L. serriola* US96UC23	F_2	92	–	643	70	–	2	Johnson et al. (2000)
DA0F	*L. sativa* cv. Salinas × *L. serriola* US96UC23	RIL_{F7}	119	–	729	18	–	–	
DB9X	*L. sativa* cv. Salad Bowl × *L. serriola* CGN14263	F_2	90	–	166	19	–	–	
DB0F	*L. saligna* UC82US1 × *L. sativa* cv. Vanguard 75	F_2	75	59	726	60	110	8	
DB0T	*L. saligna* CGN5271 × *L. sativa* cv. Olof	F_2	126	–	423	12	–	–	Jeuken et al. (2001)

[a] Parentals are ordered following standard procedures. First parental is the female parental

[b] *RFLP* restriction fragment length polymorphism, *AFLP* amplified fragment length polymorphism, *SSR* simple sequence repeats, *RAPD*, random amplification polymorphic DNA, others: isozymes, morphological traits, resistant genes and *SCAR* sequence characterized amplified region

[c] Additional information for individual maps for populations DB0D, DB0H, DA0F, DB9X and DB0F can be found at http://cgpdb.ucdavis.edu/database/genome_viewer/viewer/

random amplified polymorphic DNA (RAPD), resistant genes and morphological markers have been previously published (Kesseli et al. 1994; Waycott et al. 1999). Additional markers were run on these populations to assist in aligning and integrating the maps. AFLP markers were analyzed in all populations except RYDER using standard procedures (Vos et al. 1995). A total of 53 primer combinations were assayed in these populations. Of those, 13 were public primer combinations, the rest were proprietary (Keygene). Images of AFLP gels were electronically scanned and AFLP markers were scored co-dominantly by using the proprietary software developed for AFLP analysis at KeyGene. The KeyGene software analyses band intensity and accurately assigns genotypes using a mixture model of normal distributions as described by Jansen (2001). Therefore the majority of markers used for map integration were co-dominant. A set of proprietary simple sequence repeat (SSR) markers developed by Seminis Inc. (Woodland, CA, USA) were analyzed in all seven populations following standard procedures. Individuals were genotyped as "A" when they were homozygous for the parental female allele, "B" when they were homozygous for the parental male allele, "H" when they were heterozygous, "C" (not A) for dominant markers where the B allele was dominant and "D" (not B) for dominant markers where the A allele was dominant. Information for each marker and segregation data for each population is provided at http://cgpdb.ucdavis.edu/mjt_2006.

Individual linkage maps were constructed using Joinmap v 2.0 (Stam 1993; Stam 1995) and are displayed at the same website. Markers were assigned to linkage groups (LGs) by increasing the LOD score for grouping in steps of 0.5 LOD. This iterative process for each population selected a maximum LOD score for grouping on the basis of stability of groups over different LOD values and sufficiently large groups approaching the number of chromosomes for lettuce. In some cases, additional steps were required to split up some large groups at a higher LOD value. Mapping was carried out using the following thresholds for Joinmap; REC of 0.49, LOD of 0.10, RIPPLE of 1, JUMP of 4 and TRIPLET of 7. No order was forced during the linkage analysis. Recombination frequencies were converted to map distances in centimorgans (cM) using the Kosambi mapping function (Kosambi 1943). Using the proprietary Keygene software program "Genome Typer" graphical genotypes were generated for each population. These graphical genotypes provide an overview of the genotype configurations of all individuals. In this way, possible inconsistencies in the map, like the occurrence of frequent double crossovers in small cM intervals and "recombination hotspots", are readily detected. Markers which resulted in frequent apparent double crossovers (in at least 15% of the individuals) were removed from the map and the map was re-calculated and checked in a similar way. On average, the number of markers removed did not exceed 3%. The quality of the final maps were visualized using the publicly available CheckMatrix program (see data visualization below).

Marker distortion

Segregation ratios were calculated for all markers. Goodness of fit χ^2 tests were calculated with expected Mendelian ratios of 1:2:1 (A:H:B; co-dominant marker) or 3:1 (C:A or D:B; dominant marker) for F_2 populations and of 1:1 (A:B) with a residual heterozygosity of 1.5625% for the F_7 RIL population. Markers were considered distorted at a level of significance of $P < 0.05$. For co-dominant markers with individuals that were scored as "C" or "D" because they could not be unambiguously assigned a genotype, two χ^2 tests were calculated. One assumed a ratio of 1:2:1 (A:H:B) while not considering "C" or "D" scores. The other assumed a 3:1 ratio (C:A or D:B), where "H" genotypes were added to the dominant homozygous allele. Both χ^2 tests had to be significant for the marker to be considered skewed.

Recombination percentage

Recombination percentages were calculated for conserved marker intervals between individual maps. Markers defining conserved intervals were chosen from framework markers used for marker integration (see below). Conserved intervals among each pair of maps were identified and the number of recombinant individuals calculated as those individuals where there was a change on the genotype at the markers defining the interval; for example, if markers mk1 and mk2 defined a conserved interval in two populations, a recombination event was considered as present in those individuals where there was a change in the genotype at mk1 versus mk2 from A to H, B to H, A to C, B to D and vice versa and two recombination events were considered as present when there was a change in genotype from A to B and vice versa. Therefore, some recombination values may be slightly underestimated since in dominant markers changes between A to C (H or B) and B to D (H or A) can result from two recombination events; however, this situation is not frequent because most markers used were co-dominant rather than dominant.

Integrated map construction

Individual maps were integrated using INT_MAP (Peleman et al. 2000). This proprietary software integrates individual genetic linkage maps into a single consensus map by using common markers between populations as anchor markers. Map integration consisted of three separate steps. First, markers were identified that were common to at least two populations. These common markers were identified *a priori* as framework markers and then used to associate the corresponding linkage groups in the individual maps. Second, the consensus order of framework markers within a linkage group was calculated from the relative positions of the framework markers in each individual map. Starting sequentially with the most common marker, additional markers were added one at the time following the rule of most proximate, most common marker. Therefore within a set of equally common markers, markers are added in order depending on their distance to the already placed markers. Subsequently, the next step is made with less common markers. The consensus position of each framework marker was calculated as a weighted average of the individual map distances between new markers and the previously positioned markers. Two parameters controlled map integration: (1) The minimum fraction of overlap between groups (the minimum fraction of markers that are common to at least two groups, for example 3 out of 10 common markers) and (2) the maximum disagreement allowed in the distance between two adjacent framework markers on the individual maps. We used 0.30 and 10.0 cM for these two parameters respectively for the construction of the integrated lettuce map. Markers deviating from these criteria were not used as framework markers for map integration and were not included on the integrated map but remained on the individual maps. Lastly, markers unique to individual populations were positioned on the integrated map using interpolation and extrapolation to the framework markers. Markers that were unique to a map were placed in a "bin" between framework markers; the accuracy of their position depended on the size of the interval between the framework markers in the individual map.

Framework RFLP markers were identified by the use of the same probe name. Common RAPD markers were identified when the same size of polymorphic fragment was amplified with the same primer. Common AFLP markers were identified when, using the same enzyme/primer combination, bands co-migrated on reference gels including all the parents (Rouppe van der Voort et al. 1997). Framework SSRs were recognized by the use of the same set of primers.

Linkage groups in the integrated map were assigned numbers corresponding as close as possible to the linkage group designations in the previously published maps; priority was given to the first map published (Kesseli et al. 1994).

Nomenclature

Each of the populations and the maps derived from them were identified by an alpha-numeric code in upper case (see section "Plant Material" above). The same code in lower case followed by a number was used to identify the linkage groups within each of the maps. For example, the F_2 population derived from *L. sativa* cv. Calmar × *L. sativa* cv. Kordaat was identified by the code DB0D and linkage group 3 in the map derived from this population was designated Db0d-3. The code for the integrated map was INT (Table 2).

Table 2 Number of markers and size in cM for linkage groups (LG) in all populations integrated on the lettuce map. For previously reported maps the correspondence among published linkage groups and the linkage groups in the integrated map is given in the PM (previous map) column. In populations DB0D and RYDER, linkage groups designated between quotations indicated those LG where numbers were not assigned on the published map. For the purpose of this table numbers have been assigned corresponding to the order of the linkage groups in the previously published map

INT LG	#mk	cM	DB0D LG	#mk	cM	PM[a]	RYDER LG	#mk	cM	PM	DB0H LG	#mk	cM	PM	DA0F LG	#mk	cM	DB9X LG	#mk	cM	DB0F LG	#mk	cM	DB0T LG	#mk	cM	PM
			1a	38	42.4	1																					
			1b	129	57.5	1																					
1	360	141.3	1	167	99.9		1	16	59.2	'1a'	1	57	121.3	10	1	81	131.5	1	24	114.3	1	94	108	1	37	99.9	1
			2a	72	54.9	2																					
			2b	24	50.8	2																					
2	294	136.4	2	96	106		2	14	51.4	'2a'	2	75	143.1	6	2	64	113	2	20	124	2	101	130	2	50	93.1	2
			3a	38	58.5	3					3a	29	85.5	8				3a	6	64.5							
			3b	48	36.8	3					3b	17	33.8	9				3b	26	96.6							
3	266	193	3	86	95.3						3	46	119.3		3	60	175.8	3	32	161.1	3	101	91.5	3	23	77	8
			4a	78	58.6	4	4a	6	28.4	'5a'								4a	19	94							
			4b	52	58.8	4	4b	11	117	'2b'+'5c'								4b	8	54.2							
			4c	15	16.6	'17'		17	145										27	148.2							
4	507	238	4	145	134						4	136	207.9	4	4	142	197.6				4	179	168	4	84	163	4
5	369	194.1	5	106	124	5+'15'+'16'	5	7	40.9	'4b'	5	120	194.2	2	5	112	187.6	5	12	53.2	5	99	153	5	51	117	5
			6a	14	16.5	2																					
			6b	21	44	'13'																					
6	217	128.2	6	35	60.5						6	70	137.9	3	6	71	151.3	6	15	99.1	6	79	107	6	49	98.1	3
			7a	22	24.4	'11'																					
			7b	11	27.3	7																					
			7c	22	20.2	7																					
7	204	125.5	7	55	72		7	11	83.7	'8a'	7	55	134.8	5	7	65	137.2	7	12	118.6	7	71	122	7	30	67.3	7
			8a	35	30.5	8																					
			8b	53	47.7	'12'																					
8	342	150.4	8	88	78.2		8	10	49.1	'6a'	8	97	182.9	7	8	101	153.6	8	23	156.5	8	97	81.5	8	67	99	6
			9a	24	44.1	'10'																					
			9b	19	56.6	'9'																					
9	185	197.6	9	43	101		9	7	43.9	'7b'+'16'	9	60	126.9	1	9	51	98.4	9	20	139.1	9	89	112	9	44	102	9
not in INT			add1	5	12.6	'14'	add1	7	43.7	'3a'																	
							add2	9	55.6	'7a'																	
							add3	6	44.6	'3b'																	
9	2744	1504.5	20	823	883		13	104	618		10	716	1368		9	747	1346	11	185	1114	9	910	1073	9	435	917	

Data curation and visualization

In order to curate and query all the genetic data, we developed several modules and utilities for the Compositae database (http://cgpdb.ucdavis.edu/). This website and database was created originally to display and query EST data for lettuce and sunflower generated by the Compositae Genome Project. Segregation data is available for all loci at http://cgpdb.ucdavis.edu/mjt_2006. Enabling information and images are also available for most public markers in a mySQL relational database.

Lettuce Genetic Map Viewer is a PHP program that allows the visualization of markers on the integrated lettuce map as well as the individual maps (http://cgpdb.ucda-vis.edu/mjt_2006). It uses GD graphic libraries and the PHP scripts interact with the mySQL database dynamically to generate graphical images "on the fly" upon a user's query via the web interface. Each map can be displayed separately allowing the search for specific markers within a specific map or linkage groups can be compared between maps to identify common markers and to align them to the integrated map. The types of markers displayed and the appearance of the map can be customized depending on the needs of the researcher.

A CheckMatrix plot is displayed for each linkage group. CheckMatrix (http://cgpdb.ucdavis.edu/XLinkage/) is a Python script designed to assess the quality of genetic maps by generating 2D plots (heat maps) of the degree of linkage between all markers. Markers are ordered according to their genetic position. The colored pattern of the linkage relationships indicates the quality of the map and helps identify problematic markers and regions. Checkmatrix also displays the segregation ratios for each marker, which facilitates the analysis of segregation distortion.

Results

Individual genetic maps

Individual linkage maps for populations DB0D, RYDER, DB0H and DB0T have been previously published (Table 1). Additional AFLP and SSR markers were analyzed on populations DB0D, RYDER and DB0H to provide markers common to multiple maps. AFLP markers were analyzed for six of the seven populations (Table 1). RFLP markers were analyzed only for populations DB0D and DB0F and RAPD markers were analyzed for populations DB0D, RYDER and DB0F. SSR markers were the only marker type assayed in all seven populations. Limited numbers of isozymes, morphological markers, resistant genes and SCAR markers were also assayed in populations DB0D, RYDER, DB0H and DB0F.

New individual maps were constructed for all populations, except DB0T, integrating the old and new marker data. Segregation data and individual maps for the DB0D, RYDER, DB0H, DA0F, DB9X and DB0F populations are available at http://cgpdb.ucdavis.edu/mjt_2006. The two maps constructed on crosses between *L. saligna* × *L. sativa* (DB0F and DB0T) and the RIL map (DA0F) comprised nine chromosomal linkage groups. Maps constructed on crosses within *L. sativa* had higher numbers of linkage

groups (20 linkage groups in DB0D and 11 on RYDER). Population DB0T was used for construction of the consensus map without any modifications (Jeuken et al. 2001). The seven maps ranged from 618 to 1368 cM and comprised of 104 to 910 markers.

Different types of markers were distributed uniformly through the linkage groups in the individual maps. Clustering of AFLP markers was evident in most of the maps. RFLPs and RAPDs were also clustered in the map from population DB0D.

Marker order of the individual maps was generally consistent between the maps as compared using Lettuce Genetic Map Viewer. Markers showing inconsistent positions were not subsequently used for map integration. Comparisons of linkage groups among individual maps and with the integrated map revealed that some individual maps were missing large segments of the integrated map. For example, in map DB9X, linkage group Db9x-5 is only 53.2 cM and corresponds to a terminal third of Int-5. The other two thirds of Int-5 are missing from map DB9X (supplementary material S1). In map DB0D, linkage groups Db0d-6a and Db0d-6b correspond to three quarters of Int-6 (supplementary material S2); similarly, linkage groups Db0d-8a and Db0d-8b correspond to two thirds of Int-8 (supplementary material S3). However, linkage group Db0d-add1 could not be integrated in the consensus INT map for lack of markers in common with other maps and this linkage group may correspond to the missing portions of either Int-6 or Int-8.

Segregation distortion

All populations had some markers with segregation ratios that deviated from Mendelian expectations. Populations DB0F and DB0T had markers with distorted segregation in almost all the linkage groups (Table 3). On the individual maps most of these markers were associated in groups, indicating that the distortion was due to biased transmission rather than mis-scoring of individual markers. Segregation distortion was highest in the *L. saligna* × *L. sativa* crosses. Some regions showed segregation distortion in multiple populations with the same direction of the distortion (Table 3). Linkage group Int-7 was distorted in DB0D (linkage groups Db0d-7b and Db0d-7c), DB0F (linkage group Db0f-7) and in DB0T (linkage group Db0t-7) with a maximum number of distorted markers in Db0t-7 where the segregation of all but one of the markers was distorted (Table 3). In all these populations, the distortion of linkage group 7 markers was towards the *L. sativa* haplotype. Linkage groups Int-5, Int-6 and Int-9 were distorted only in populations DB0F and DB0T; in these groups, even though the region of distortion was similar, the distortion was towards different parental haplotypes (Table 3) except for one of the regions in linkage group 9 distorted towards the cultivated haplotype in both maps. Linkage group Int-3 had groups of distorted markers in populations DB0H, DA0F, DB9X, DB0F and DB0T. All the distorted regions were coincident except the one in DB0F map (linkage group Db0f-3) that did not overlap with the others (Table 3) Distortion was towards the *L. sativa* haplotype in DB0H, DA0F and DB9X and towards the heterozygous haplotype in DB0F and DB0T.

Recombination percentage

We calculated recombination percentages in all the F$_2$ populations except RYDER (that could not be included due to the small number of conserved intervals between this map and any of the others). Recombination percentages for conserved intervals between pairs of maps are presented in Table 6. In most of the comparisons when more than one

Table 3 Distribution of groups of distorted markers across linkage groups in the different populations considered in this study. A group of skewed markers is defined as a group of at least three linked distorted markers with gaps of non-distorted markers of three or less

INT LG	DB0D *L. sativa* x *L. sativa* (6.32% distorted markers)					RYDER *L. sativa* x *L. sativa* (7.89% distorted markers)					DB0H *L. sativa* x *L. serriola* (3.21% distorted markers)					DA0F *L. sativa* x *L. serriola* (11.28% distorted markers)					DB9X *L. sativa* x *L. serriola* (7.98% distorted markers)					DB0F *L. saligna* x *L. sativa* (24.42% distorted markers)					DB0T *L. saligna* x *L. sativa* (37.78% distorted markers)				
	LG	#m	s/g	i	d	LG	#m	s/g	i	d	LG	#m	s/g	i	d	LG	#m	s/g	i	d	LG	#m	s/g	i	d	LG	#m	s/g	i	d	LG	#m	s/g	i	d
1																										1	94	10/16	20.9	B					
																												5/7	12.2	B					
						1	16	5/9	37.3	D																									
2																										2	101	7/12	12.9	A					
																												8/13	8.5	A	2	50	4/9	8.8	A
3											3a	29	4/6	19.6	A	3	60	29/30	77.7	A	3b	26	12/16	36	A	3	101	5/13	27.6	D					
											3b	17	8/11	22.1	A																3	23	5/7	30.7	H
4																4	142	11/11	10.3	B						4	179	5/11	3.1	B	4	84	9/11	22.6	B
																																	15/22	56	A
5																										5	99	42/44	70.8	B	5	51	19/22	45.7	A
6																										6	79	25/33	20.8	A	6	49	3/3	5.4	B
7	7c	22	9/11	7.7	B																					7	71	14/26	45.1	B	7	30	29/30	67.3	B
	7b	11	3/5	9.9	B																														
8																8	101	10/13	16.1	A						8	97	3/5	4.2	A	8	67	13/18	32.6	B
9																										9	89	19/26	44.4	A	9	44	10/10	27.8	B
																												12/16	15.3	B			12/17	49.1	B

LG linkage group, *# m* total number of markers on linkage group, *s/g s* number of distorted markers, *g* number of total markers on the distorted group, *i* interval in cM of the distorted group, *d* direction of the distortion. A towards the mother's allele, B towards the father's allele, H towards the heterozygous, D not B and C not A. Crosses are shown female × male

interval was considered, populations where *L. saligna* was one of the parents had always a lower number of recombination events. For the four conserved intervals between DB9X and DB0F in LG2 the same rate of recombination was observed (Table 6).

When comparing the maps from the two inter-specific crosses with *L. saligna* (DB0F and DB0T, Table 6), DB0T had a more severe suppression of recombination. This tendency of suppressed recombination was also apparent in the smaller sizes of the linkage groups in these two maps when compared to the other individual maps or to the integrated map (Table 2; http://cgpdb.ucdavis.edu/mjt_2006).

Map integration

A total of 695 markers common to two or more maps were available for map integration. Of those, 62 (8.9%) were discarded as having inconsistent grouping (mapping in different linkage groups in the individual maps). A total of 47 markers were discarded for having contradictory genetic positions (exceeding the 10 cM maximum distance disagreement allowed between two adjacent framework markers). A total of 560 (80.6% of the original framework markers) were used for map integration. Of these 560 markers, 474 were AFLP, 24 RFLP and 25 SSR markers (Table 4; http://cgpdb.ucdavis.edu/database/genome_viewer/viewer). Only 16 RAPD markers were used as framework markers, mostly to allow the integration of the RYDER linkage groups. As expected, maps DB0H and DA0F had the most common framework markers between them (274) since these maps are from different generations derived from the same cross. Maps DB0F and DB0T derived from crosses between *L. saligna* and *L. sativa* had the second most common number of markers (136). The RYDER map had the fewest common markers (18) with any of the other maps (Table 5).

Most of the linkage groups from the individual maps were successfully integrated into the consensus map. The correspondence among linkage groups in the individual maps and the linkage groups in the integrated map is shown

Table 4 Number of common framework markers among populations used for linkage group integration

	RFLP	AFLP	SSR	RAPD	Morphological	Total
All framework markers	24	474	45	16	1	560
Between 2 populations	24	349	24	16	1	414
Among 3 populations	–	88	13	–	–	101
Among 4 populations	–	22	5	–	–	27
Among 5 populations	–	15	2	–	–	17
Among 7 populations	–	–	1	–	–	1

Table 5 Distribution of common framework markers between populations

	DB0D	RYDER	DB0H	DA0F	DB9X	DB0F	DB0T
RYDER	18						
DB0H	68	12					
DA0F	51	5	274				
DB9X	15	4	47	55			
DB0F	58	7	63	64	42		
DB0T	22	1	44	56	28	136	
Total	141	26	333	328	87	237	180

in Table 2. When possible, linkage groups were designated to be consistent across maps with priority given to the earliest report. Only linkage groups add1, add2 and add3 in the RYDER map and linkage group add1 in the DB0D map were not integrated on the consensus map for lack of common framework markers (Table 2).

## Integrated map characteristics

The integrated lettuce map was composed of 2,744 markers assembled into nine linkage groups, corresponding to the nine chromosomes of lettuce, with a total map length of 1,505 cM (Table 2; Fig. 1). The integrated map was ~150 cM larger than the largest individual maps from populations DB0H and DA0F (1,368 cM and 1,346 cM). This was the result of the expansion of linkage groups 3 and 9 by intercalating new markers from the other maps (Table 2). Average interval size between markers was greatly reduced on the integrated map (Fig. 2). Less than 1% of the intervals were larger than 5 cM.

Marker distribution in the integrated map was a reflection of the individual maps distributions (Fig. 1). AFLP markers were obviously clustered in multiple regions and few regions exhibited clustering of RFLP and RAPD markers. Morphological markers that only segregated in populations DB0D and RYDER (DM resistance genes etc.) were integrated into the linkage groups using anonymous markers.

Discussion

Our integrated map of lettuce integrates four previously published maps of lettuce with data from three previously unreported datasets. The seven maps were constructed from crosses within and between *L. sativa* (cultivated lettuce) and its closest wild relatives *L. serriola* and *L. saligna*. Consequently it provides markers for accessing and exploiting variability within the sexually-compatible gene pool of cultivated lettuce.

Fig. 1 Integrated map of lettuce. AFLP and SSR markers are displayed below the linkage group line. RFLP, RAPD and morphological markers are displayed above the linkage group line. For details, see http://cgpdb.ucdavis.edu/mjt_2006
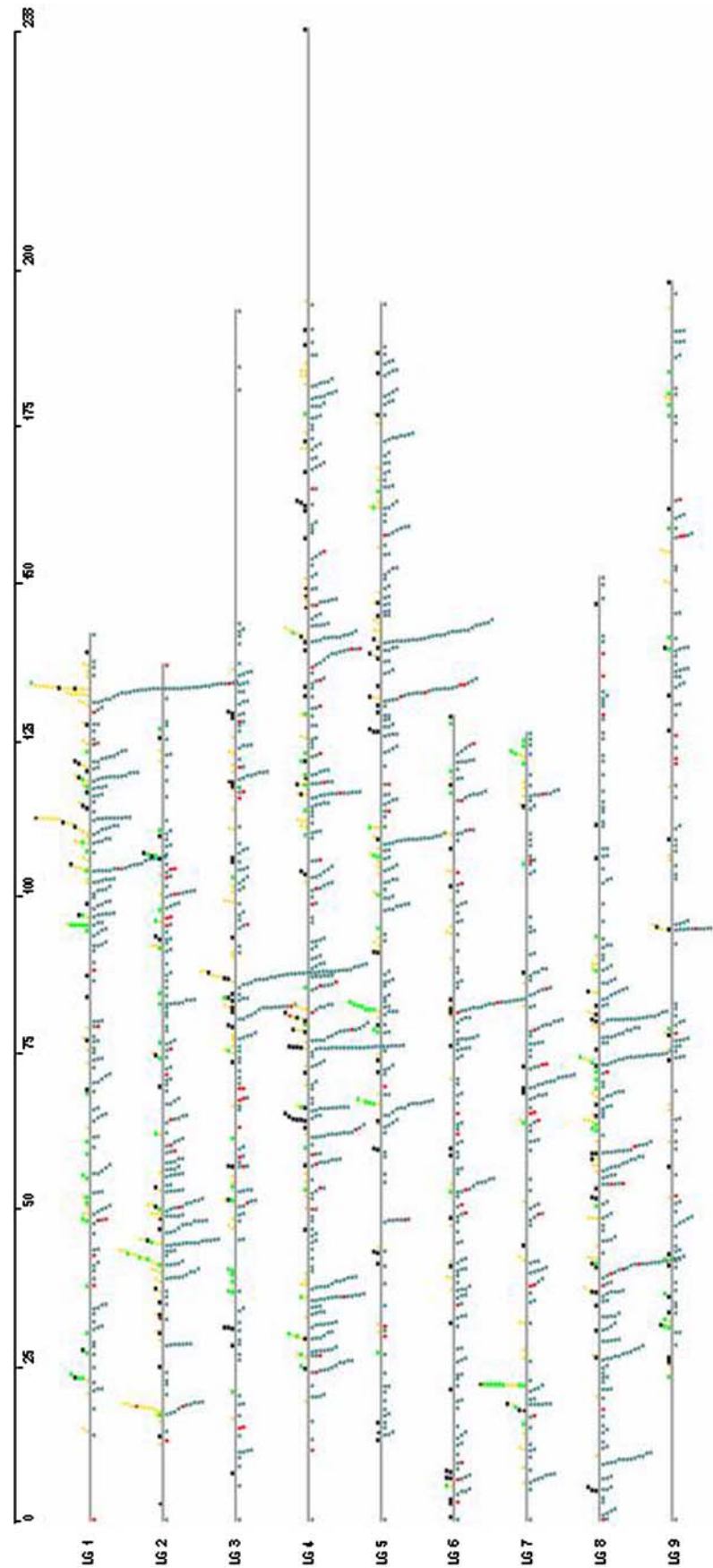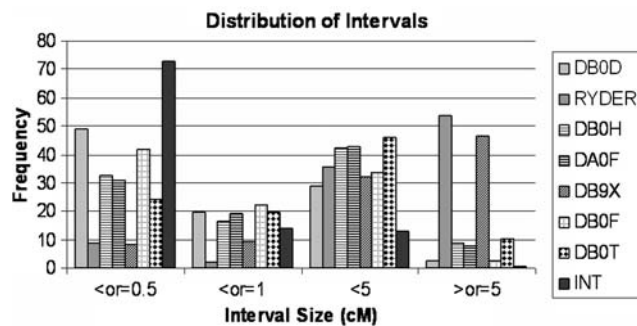
Fig. 2 Distribution of size intervals among populations. Comparison with INT map

Segregation distortion

We found two different types of distortion: specific regions distorted in multiple populations and distortion of multiple linkage groups in a single population suggesting multiple reasons for segregation distortion. Markers with segregation distortion were found restricted to certain genomic positions in maps within *L. sativa* and between *L. sativa* and *L. serriola*. Factors that affect the segregation of alleles during meiosis (e.g. direct gamete competition, male killing) can produce distorted ratios at affected loci (Moyle and Graham 2006). A region in Int-3 was consistently distorted in populations DB9X, DB0H and DA0F towards the *L. sativa* maternal allele. In tomato and rice, distortion towards one of the parental alleles was the result of selective abortion of gametes carrying a specific allele at a gamete eliminator locus (Rick 1966; Sano 1990).

In crosses between *L. saligna* and *L. sativa*, distorted markers were widely distributed through the genome present in almost all the linkage groups. In a comparison among 58 intra and inter-specific crosses in different agricultural crop species, Jenczewski et al. (1997) reported an increase of distorted markers in inter-specific crosses versus intra-specific crosses in agreement with our results. The degree of distortion in a genetic mapping population is thought to be correlated with the level of genomic divergence between parental taxa (Taylor and Ingvarsson 2003). Hybrid inviability or sterility is known to occur by the accumulation of inter-locus incompatibilities between divergent populations (Sweigart et al. 2006). F_1 hybrids between *L. saligna* and *L. sativa* are partially sterile (de Vries 1990; Koopman et al. 1998). Possibly disruptive genetic interactions among divergent loci between *L. saligna* and *L. sativa* are responsible for hybrid sterility and observed distorted ratios in the F_2. Distorted segregation during the development of a set of 29 lettuce backcross inbred lines that introgressed single segments from *L. saligna* into *L. sativa* over more than five generations resulted in six regions in which the *L. saligna* segment could not be obtained as homozygous (Jeuken and

Lindhout 2004). This extreme preference for *L. sativa* alleles was observed for segments that mapped to Int-6, Int-8, Int-7 (two regions) and Int-9 (two regions). One segment corresponding to a region on Int-7 showed complete distortion as only the *L. sativa* haplotype was obtained. These segments are similar to those that showed segregation distortion in the *L. saligna* × *L. sativa* F_2 populations DBOT and DBOF (Table 3).

Wild ancestors of crop plants have been useful sources of genetic variation that has been lost during domestication of crop plants (Zamir 2001). Wild species in the *Lactuca* genus have been successfully used in the introgression of horticulturally important traits in breeding programs, particularly disease resistance (Crute and Dickinson 1976). Understanding the mechanisms responsible for segregation distortion in a given cross is important since such distortion can obstruct gene flow between species and hamper the introgression of horticulturally interesting alleles in certain regions of the genome. In contrast to the development of BILs (Jeuken and Lindhout 2004), the distortion observed in our interspecific populations was never complete (i.e. only one of the parental haplotypes was recovered); all the possible haplotypes were recovered in the progeny for all regions, albeit at different frequencies. The consequence for selection of genes in these skewed regions is the increased progeny sizes required to recover wild alleles in regions that become distorted towards the cultivated allele. Horticulturally, interesting genes can be located in regions exhibiting segregation distortion; quantitative trait locus (QTL) analysis of numerous horticulturally important traits segregating in population DA0F identified QTLs for leaf shape and heading in a distorted region of Da0f-3 (MJ Truco, unpublished data).

Recombination rates

Observed recombination frequencies are dependent on the accuracy of the dataset as well as the actual rate of recombination. Mis-scored markers are especially problematic on dense maps, where a few errors can greatly increase apparent marker distances. This type of error was reduced by searching for and removing individual markers flanked by recombination events. The maps from *L. sativa* × *L. sativa* and between *L. sativa* and *L. serriola* have similar rates of recombination supporting the idea that these taxa are conspecific (Kesseli et al. 1991) (Table 6).

Recombination was reduced in crosses between *L. saligna* and *L. sativa* resulting in more compressed linkage maps (Table 2; Supplementary material S5). This was probably the consequence of the greater sequence divergence between these parents. The higher levels of divergence were reflected by the mean numbers of AFLP markers per primer combination; populations DB0T and

Table 6 Comparison of recombination frequencies among DB0D, DB0H, DB9X, DB0F and DB0T populations for conserved intervals within linkage groups

Populations (p)	LG1			LG2			LG3			LG4			LG5			LG6			LG7			LG8			LG9		
	# in	1st p	2nd p	# in	1st p	2nd p	# in	1st p	2nd p	# in	1st p	2nd p	# in	1st p	2nd p	# in	1st p	2nd p	# in	1st p	2nd p	# in	1st p	2nd p	# in	1st p	2nd p
DB0D-DB0H	7	95	62	7	101	136	5	60	128	22	263	247	6	151	155	1	43	51	2	12	10	1	2	19	0	–	–
DB0D-DB9X	3	87	64	0	–	–	0	–	–	1	38	20	0	–	–	0	–	–	0	–	–	1	48	58	0	–	–
DB0D-DB0F	11	189	99	11	197	121	2	35	17	13	225	184	2	81	46	1	43	13	0	–	–	1	7	18	2	40	27
DB0D-DB0T	2	53	47	1	4	11	0	–	–	4	124	82	3	97	78	0	–	–	0	–	–	1	48	12	0	–	–
DB0H-DB9X	4	119	140	4	129	118	0	–	–	8	175	185	2	57	47	6	175	149	5	100	115	7	212	223	0	–	–
DB0H-DB0F	7	127	109	7	212	103	0	–	–	11	234	230	8	188	166	10	203	139	8	191	176	5	160	45	0	–	–
DB0H-DB0T	5	95	46	2	40	25	0	–	–	10	240	143	5	130	71	6	162	66	2	81	21	6	170	55	0	–	–
DB9X-DB0F	0	–	–	4	121	120	2	20	14	6	161	138	3	48	38	7	130	74	2	93	77	4	105	86	2	55	27
DB9X-DB0T	2	80	5	1	4	0	0	–	–	6	161	62	2	47	27	5	131	66	0	–	–	4	137	59	0	–	–
DB0F-DB0T	10	132	116	11	130	69	8	109	69	29	298	189	16	173	122	17	161	137	9	132	98	20	163	85	9	111	93

Values are calculated by summing the recombination frequency of each conserved interval within a linkage group. *p* pair of populations being compared, *#in* number of intervals, *1st p* and *2nd p* percent recombination observed in first and second populations, respectively, of the pair being compared

DB0F averaged 35 and 45 AFLP markers respectively, while DB0H and DB9X averaged 23 and DBOD only 12 AFLP markers per primer combination. This is similar to inter-specific hybrids in tomato with a high degree of marker polymorphism that showed severe suppression of recombination between homeologous chromosomal segments due to reduced chiasma frequency in F_1 hybrids (Chetelat et al. 2000). Sequence divergence is known to repress homologous recombination. The recombination rate in Arabidopsis was reduced threefold by single nucleotide mismatches (Opperman et al. 2004). Extensive recombination studies in maize have documented reduced recombination associated with sequence heterogeneity (Schnable et al. 1998). Within the *bronze* gene, a hot spot for recombination in maize, recombination occurs randomly within the gene in the absence of sequence divergence but is reduced by point mutations or single base pair mismatches (Dooner and Martínez-Férrez 1997).

Integrated map

Of the 695 potential framework markers available for map integration, over 90% were readily assembled into nine chromosomal linkage groups. Only 62 (8.9%) had to be discarded because of inconsistent grouping. This proportion is consistent with map integration studies with other species (Keygene unpublished). The reason for inconsistent grouping of markers has been studied in maize by re-sequencing bands that gave inconsistent grouping in different maps; one third of these bands were the result of different sequences with very close mobility being called the same. The remaining two thirds of the inconsistent markers had the same or similar sequence and were presumed to be duplicated sequences. The inconsistent positions may reflect the segregation of one or more polymorphisms in different paralogs.

The integrated map of lettuce reported here is a more informative genetic resource than any of the individual maps previously available. It combines 2,744 markers into nine linkage groups corresponding to the nine chromosomes of *Lactuca* spp. with better marker coverage (1,505 cM) than any of the other individual maps with nine linkage groups (DA0F, 1,346 cM; DB0F, 1,073 cM; DB0T, 9,17 cM). Genomic regions that were either split into separated linkage groups or missing in the individual maps for lack of polymorphism are now combined into single linkage groups. For example, linkage group Int-3 is split into two linkage groups in DB0D (36.8 and 58.5 cM), DB0H (85.5 and 33.8 cM) and DB9X (96.6 and 64.5 cM) and has missing regions in DB0F (91.5 cM) and DB0T (77 cM). Int-3 has a total length of 193 cM and is the result of combining regions from all the populations (Int-3, supplemen-

tary material S4). Some of the maps, for example DB9X, contribute to this linkage group with unique regions not present in any of the other maps.

Even marker distributions through the linkage groups were observed for most of the marker types except for clustering of AFLP markers. The same situation has been reported previously in an ultradense map of potato (van Os et al. 2006). Clustering of AFLP markers may be due to a more even distribution of AFLPs over the physical map. Consequently, clusters of AFLPs may be indicative of heterochromatic regions near centromeres (Haanstra et al. 1999); confirmation of this for lettuce awaits cytological analysis.

The integrated map of lettuce combines diverse types of markers into a single map. Combining information from multiple crosses increases the chances of identifying putative polymorphic markers in a given region. *Dm13*, a disease resistance gene against lettuce downy mildew, was previously mapped in DB0D to a region of Db0d-3b with only dominant RAPD markers linked to it (Kesseli et al. 1994). In the integrated map this gene is linked to a suite of AFLP, SSR and RAPD markers. To map new traits that segregate in previously unmapped populations, bulked segregant analysis (BSA, Michelmore et al. 1991) could be used to identify a few markers linked to the gene of interest. These markers would then be aligned to the integrated map to identify other candidate markers in the region avoiding the time-consuming construction of a detailed map in the new population.

Comparisons among individual maps and the integrated map of lettuce were greatly facilitated by Lettuce Map Viewer. This program provided graphical displays of the comparisons and allowed easy access of the raw data behind the construction of the individual and integrated maps including genotypes and details of markers. These tools are publicly available for application to other species. These tools and the integrated map data will greatly facilitate analyses of synteny with Arabidopsis (Timms et al. 2006), and marker assisted selection as well as genetic dissection (QTL analysis) and map-based cloning of agriculturally important traits.

References

Anonymous (2004) http://usda.mannlib.cornell.edu/usda/ers/Lettuce/tab61wrldprodn.xls; http://usda.mannlib.cornell.edu/reports/nassr/fruit/pvg-bban/vgan0105.pdf

Chetelat RT, Meglic V, Cisneros P (2000) A genetic map of tomato based on a BC_1 *Lycopersicon esculentum* x *Solanum lycopersicoides* reveals overall synteny but suppressed recombination between these homeologous genomes. Genetics 154:857–867

Crute IR, Dickinson CH (1976) Behaviour of *Bremia lactucae* on cultivars of *Lactuca sativa* and other composites. Ann Appl Biol 82:433

De Vries IM (1990) Crossing experiments of lettuce cultivars and species (*Lactuca* sect. *Lactuca*, *Compositae*). Plant Syst Evol 171:233–248

De Vries IM (1997) Origin and domestication of *Lactuca sativa* L. Genet Resources Crop Evol 44:165–174

Dekkers JCM, Hospital F (2002) The use of molecular genetics in the improvement of agricultural populations. Nat Rev Genet 3:22–32

Dooner HK, Martínez-Férez IM (1997) Recombination occurs uniformly within the *bronze* gene, a meiotic recombination hotspot in the maize genome. Plant Cell 9:1633–1646

Gedil MA, Wye C, Berry S, Segers B, Peleman J, Jones R, Leon A, Slabaugh MB, Knapp S (2001) An integrated restriction fragment length polymorphism—amplified fragment length polymorphism linkage map for cultivated sunflower. Genome 44:213–221

Gupta PK, Rustgi S (2004) Molecular markers from the transcribed/expressed region of the genome in higher plants. Funct Integr Genomics 4:139–162

Haanstra JPW, Wye C, Verbakel H, Meijer-Dekens F, van denBerg P, Odinot P, van Heusden AW, Tanksley S, Lindhout P, Peleman J (1999) An integrated high-density RFLP-AFLP map of tomato based on two *Lycopersicon esculentum* x *L. pennellii* F_2 populations. Theor Appl Genet 99:254–271

Jansen R, Geerlings H, van Oeveren AJ, van Schaik RC (2001) A comment on codominant scoring of AFLP markers. Genetics 158:925–926

Jenczewski E, Gherardi M, Bonnin I, Prosperi JM, Olivieri I, Huguet T (1997) Insight on segregation distortions in two intraspecific crosses between annual species of *Medicago* (Leguminosae). Theor Appl Genet 94:682–691

Jeuken MJW, Lindhout P (2004) The development of lettuce backcross inbred lines (BILs) for exploitation of the *Lactuca saligna* (wild lettuce) germplasm. Theor Appl Genet 109:394–401

Jeuken M, van Wijk R, Peleman J, Lindhout P (2001) An integrated interspecific AFLP map of lettuce (*Lactuca*) based on two *L. sativa* x *L. saligna* F_2 populations. Theor Appl Genet 103:638–647

Johnson WC, Jackson LE, Ochoa O, Peleman J, van Wijk R, St.Clair DA, Michelmore RW (2000) A shallow-rooted crop and its wild progenitor differ at loci determining root architecture and deep soil water exploitation. Theor Appl Genet 101:1066–1073

Kesseli R, Ochoa O, Michelmore R (1991) Variation at RFLP loci in *Lactuca* sps. and origin of cultivated lettuce (*L. sativa*). Genome 34:430–436

Kesseli RV, Paran I, Michelmore RW (1994) Analysis of a detailed genetic linkage map of *Lactuca sativa* (lettuce) constructed from RFLP and RAPD markers. Genetics 136:1435–1446

Koopman WJM, Guetta E, van de Wiel CCM, Vosman B, van den Berg RG (1998) Phylogenetic relationships among *Lactuca* (Asteraceae) species and related genera based on ITS-1 DNA sequences. Am J Bot 85:1517–1530

Kosambi DD (1943) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lefebvre V, Pflieger S, Thabuis A, Caranta C, Blattes A, Chauvet JC, Daubeze AM, Palloix A (2002) Towards the saturation of the pepper linkage map by alignment of three intraspecific maps including known-function genes. Genome 45:839–854

Lombard V, Delourme R (2001) A consensus linkage map for rapeseed (*Brassica napus* L.): construction and integration of three individual maps from DH populations. Theor Appl Genet 103:491–507

Mayerhofer R, Wilde K, Mayerhofer M, Lydiate D, Bansal VK, Good AG, Parkin IAP (2005) Complexities of chromosome landing in a highly duplicated genome: toward map-based cloning of a gene controlling blackleg resistance in *Brassica napus*. Genetics 171:1977–1988

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by

using segregating popultions. Proc Natl Acad Sci USA 88:9828–9832

Moyle LC, Graham EB (2006) Genome-wide associations between hybrid sterility QTL and marker transmission ratio distortion. Mol Biol Evol 23:973–980

Opperman R, Emmanuel E, Levy AA (2004) The effect of sequence divergence on recombination between direct repeats in Arabidopsis. Genetics 168:2207–2215

Paterson AH, Bowers JE, Burow MD, Draye X, Elsik CG, Jiang CX, Katsar CS, Lan TH, Lin YR, Ming RG, Wright RJ (2000) Comparative genomics of plant chromosomes. Plant Cell 12:1523–1539

Paran I, van der Voort JR, Lefebvre V, Jahn M, Landry L, van Schhriek M, Tanyolac B, Caranta C, Ben Chaim A, Livingstone K, Palloix A, Peleman J (2004) An integrated genetic linkage map of pepper (*Capsicum* spp.). Mol Breed 13:251–261

Peleman J, van Wijk R, van Oeveren J, van Schaik R (2000) Linkage map integration: an integrated genetic map of *Zea mays* L. Poster P472. Plant and animal genome conference VIII, San Diego, California USA

Périn C, Hagen LS, De Conto V, Katzir N, Danin-Poleg Y, Portnoy V, Baudracco-Arnas S, Chadoeuf J, Dogimont C, Pitrat M (2002) A reference map of *Cucumis melo* based on two recombinant inbred line populations. Theor Appl Genet 104:1017–1034

Peters JL, Cnudde F, Gerats T (2003) Forward genetics and map-based cloning approaches. Trends Pl Sci 8:484–491

Qi X, Stam P, Lindhout P (1996) Comparison and integration of four barley RFLP-maps. Genome 39:379–394

Rick CM (1966) Abortion of male and female gametes in the tomato determined by allelic interaction. Genetics 53:85–96

Rouppe van der Voort JNAM, van Zandvoort HJ, van Eck HJ, Folkertsma RT, Hutten RCB, Draaistra J, Gommers FJ, Jacobsen E, Helder J, Bakker J (1997) Use of allele specificity of comigrating AFLP markers to align genetic maps from different potato genotypes. Mol Gen Genet 255:438–447

Salvi S, Tuberosa R (2005) To clone or not to clone plant QTLs: present and future challenges. Trends Plant Sci 10:297–304

Sano Y (1990) The genetic nature of gamete eliminator in rice. Genetics 125:183–191

Schnable PS, Hsia AP, Nikolau BJ (1998) Genetic recombination in plants. Curr Opin Plant Biol 1:123–129

Song QJ, Marek LF, Shoemaker RC, Lark KG, Concibido VC, Delannay X, Specht JE, Cregan PB (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122–128

Stam P (1993) Construction of integrated genetic-linkage maps by means of a new computer package–Joinmap. Plant J 3:739–744

Stam P (1995) JoinMap 2.0 deals with all types of plant mapping populations. Plant Genome III Abstracts, World Wide Web site: http://www.int-pag.org

Sweigart AL, Fishman L, Willis JH (2006) A simple genetic incompatibility causes hybrid male sterility in *Mimulus*. Genetics 172:2465–2479

Syed NH, Sorensen AP, Antonise R, van de Wiel C, van der Linden CG, van't Westende W, Hooftman DA, den Nijs HC, Flavell AJ (2006) A detailed linkage map of lettuce based on SSAP, AFLP and NBS markers. Theor Appl Genet 112:517–527

Taylor DR, Ingvarsson PK (2003) Common features of segregation distortion in plants and animals. Genetica 117:27–35

Timms L, Jimenez R, Chase M, Lavelle D, McHale L, Kozik A, Lai Z, Heesacker A, Knapp S, Rieseberg L, Michelmore R, Kesseli R (2006) Analyses of synteny between *Arabidopsis thaliana* and species in the Asteraceae reveal a complex network of small syntenic segments and major chromosomal rearrangements. Genetics 173:2227–2235

van Os H, Andrzejewski S, Bakker E, Barrena I, Bryan GJ, Caromel B, Ghareeb B, Isidore E, de Jong W, van Koert P, Lefebvre V, Milbourne D, Ritter E, van der Voort JNAMR, Rousselle-Bourgeois F, van Vliet J, Waugh R, Visser RGF, Bakker J, van Eck HJ (2006) Construction of a 10,000-marker ultradense genetic recombination map of potato: providing a framework for accelerated gene isolation and a genomewide physical map. Genetics 173:1075–1087

Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP—a new technique for DNA-fingerprinting. Nucleic Acids Res 23:4407–4414

Waycott W, Fort SB, Ryder EJ, Michelmore RW (1999) Mapping morphological genes relative to molecular markers in lettuce (*Lactuca sativa* L.). Heredity 82:245–251

Witsenboer H, Vogel J, Michelmore RW (1997) Identification, genetic localization, and allelic diversity of selectively amplified microsatellite polymorphic loci in lettuce and wild relatives (*Lactuca* spp.). Genome 40:923–936

Zamir D (2001) Improving plant breeding with exotic libraries. Nat Rev Genet 2:983–989