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ORIGINAL PAPER

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# Genome-wide analysis of the frequency and distribution of crossovers at male and female meiosis in *Sinapis alba* L. (white mustard)

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**Abstract** We present the first genetic linkage maps of *Sinapis alba* (white mustard) and a rigorous analysis of sex effects on the frequency and distribution of crossovers at meiosis in this species. Sex-averaged maps representing recombination in two highly heterozygous parents were aligned to give a consensus map consisting of 382 loci defined by restriction fragment length polymorphisms and arranged in 12 linkage groups with no unlinked markers. The loci were distributed in a near-random manner across the genome, and there was little evidence of segregation distortion. From these dense maps, a subset of spaced informative markers was used to establish recombination frequencies assayed separately in male and female gametes and derived from two distinct genetic backgrounds. Analyses of 746 gametes indicated that recombination frequencies were greater in male gametes, with the greatest differences near the ends of linkage groups. Genetic background had a lesser effect on recombination frequencies, with no discernible pattern in the distribution of such differences. The possible causes of sex differences in recombination frequency and the implications for plant breeding are discussed.

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## Introduction

In many genetic systems there are differences in the frequency and distribution of the crossovers represented in male gametes compared to those represented in female gametes. In most vertebrates, such as human (Broman et al. 1998; Kong et al. 2002), mouse (Dietrich et al. 1996), zebrafish (Singer et al. 2002) and rainbow trout (Sakamoto et al. 2000), overall recombination frequencies are greater in female meioses than in male meioses, with female recombination frequencies most enhanced in the centromeric regions. However, this trend is not universal, even in vertebrates; for example, in chicken and opossum, male meiosis generates higher recombination frequencies than female meiosis (Groenen et al. 1998; Samollow et al. 2004). Differences between male and female recombination frequencies have also been reported in plant species. In rye (Benito et al. 1996), maize (Weber et al. 1999) and pine species (Plomion and O'Malley 1996; Sewell et al. 1999), recombination frequencies are greater in the male gametes, whereas in solanaceous species (de Vicente and Tanksley 1991; van Ooijen et al. 1994; Rivard et al. 1996), the reverse is true. Moreover, some plant species, such as oilseed rape (Kelly et al. 1997) and coffee (Lashermes et al. 2001), show no evidence of sex-specific differences.

The influence of the parental genotype on recombination rates has been demonstrated in a number of experimental systems, such as rye (Benito et al. 1996), *Solanum chacoense* (Rivard et al. 1996), human (Broman et al. 1998) and maize (Weber et al. 1999). Distinguishing the effects of genetic background from those of sex is difficult in dioecious organisms but is more straightforward in hermaphroditic plant species where reciprocal crosses can be employed. For inbred pedigrees, reciprocal backcrossing of an $F_1$ individual to one or both parents (van Ooijen et al. 1994; Kelly et al. 1997) or to a third inbred line (Busso et al. 1995) enables the analysis of recombination represented in male and female gametes from a single ($F_1$) individual under identical envi-

ronmental conditions. For out-breeding plant species, the reciprocal crossing of two heterozygous individuals allows characterisation of male and female gametes of both individuals (Rivard et al. 1996). However, in most published out-bred pedigrees, for example those of apple (Maliepaard et al. 1998) and loblolly pine (Sewell et al. 1999), detailed genetic studies employing reciprocal crosses have not been reported, leaving the influence of sex effects on recombination behaviour unresolved from the effects of genetic background.

Good experimental data are essential for accurate assessments of the frequency and distribution of crossovers. Ideally, a large number of recombinant individuals should be genetically characterised using numerous markers evenly distributed across the genome. The number of experiments fulfilling these criteria is quite small but is increasing slowly as efficient marker technologies become available in a wider range of species. Other factors, such as age and temperature, can influence recombination frequencies. Zetka and Rose (1990), using *Caenorhabditis elegans* males and hermaphrodites, observed a decrease in recombination frequency as parental age increased and an increase in recombination frequency as temperature increased. Controlling such factors is another important consideration when designing experiments to test differences in male and female recombination rates. The most rigorous investigations into sex differences in recombination that have been published to date have involved reciprocal backcrossing of an $F_1$ individual to a parent or to a third inbred line. Examples of this include *Brassica napus* (Kelly et al. 1997), *Pennisetum glaucum* (Busso et al. 1995), *Lycopersicon peruvianum* (van Ooijen et al. 1994) and *L. esculentum* and *L. pennellii* (de Vicente and Tanksley 1991). Since the genetic background was constant in each of these experiments, the observed differences in recombination could be attributed with confidence to differences in the meioses producing male gametes compared to those producing female gametes.

The investigation described herein provides the most compelling evidence to date for sex differences in the frequency and the distribution of crossovers in a plant species. The experimental organism employed in this investigation was *Sinapis alba* L. (white mustard, $2n = 24$) an out-crossing plant species closely related to *Brassica* crops (Warwick and Black 1991). *S. alba* cultivars are usually highly heterozygous and heterogeneous, and sensitive to inbreeding depression. However, by reciprocally crossing two genetically distinct and highly heterozygous plants, we were able to produce large segregating $F_1$ populations in which the recombination represented in male and female gametes, and produced by two distinct genotypes, could be evaluated with high precision.

*S. alba* has considerable promise as an alternative cruciferous oilseed crop because it performs better than oilseed rape (*B. napus*) in areas with short, dry growing seasons (such as those found in the semi-arid regions of western Canada and Australia) and requires only minimal inputs (Rakow et al. 2000). The development of effective marker technologies and dense linkage maps for *S. alba* will accelerate the improvement of this crop through marker-assisted selection.

## Materials and methods

### Plant material and growth conditions

Four diverse lines of *Sinapis alba* differing in seed quality characteristics were used as starting material: T096-1046, zero erucic acid, low glucosinolate (G. Rakow, Saskatoon Research Centre, Canada); Sel-01, a high oil line developed in Canada (G. Rakow, Saskatoon Research Centre, Canada); Svalöf, a high oil line developed in Sweden (Olsson 1974); *Sabre*, a high erucic acid, high glucosinolate variety developed in Canada (D. Woods, Beaverlodge Research Centre, Canada). Plants were grown, and all sexual crosses were performed in controlled environment rooms maintained at 18°/15°C (light/dark) and a 16/8-h (light/dark) photoperiod. The parents and segregating populations were developed using the three-generation pedigree outlined in Fig. 1. Single individuals from each of four diverse *S. alba* lines were crossed in the pairwise combinations indicated (using Se1-01 and *Sabre* as females) yielding parents P1 and P2 (single plants). Self-seed was also produced from each of the four grandparents for allele screening purposes. A pair of the resulting highly heterozygous parents was crossed reciprocally to produce a total of 382 $F_1$ individuals, 188 with

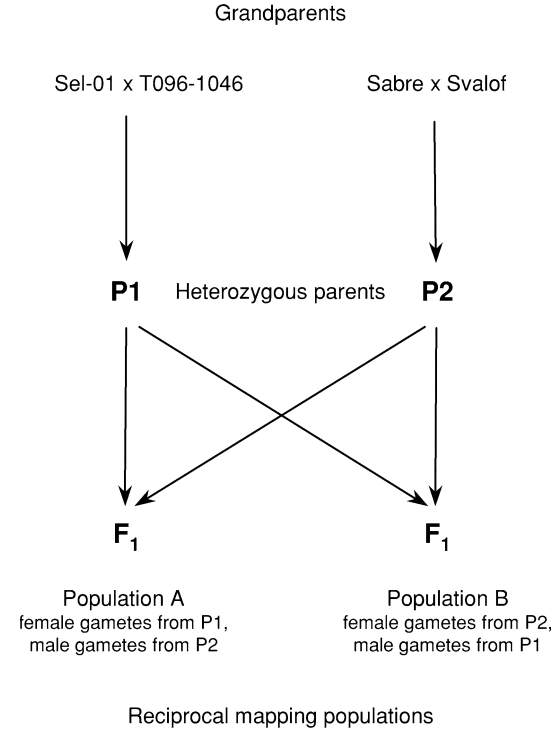



**Fig. 1** Crossing strategy used to produce the two reciprocal *Sinapis alba* $F_1$ mapping populations. Population A consisted of 188 individuals, while population B consisted of 194 individuals

P1 as the female parent (population A) and 194 with P2 as the female parent (population B).

### RFLP screening

The DNA extraction, restriction enzyme digestion, gel electrophoresis, alkaline transfer and Southern hybridisation were carried out according to the methods of Sharpe et al. (1995), using the DNA size-standard ladder described by Lydiate et al. (1986).

A total of 214 *Brassica* genomic clones and six *Brassica* cDNA clones (Thormann et al. 1994; Sharpe et al. 1995) were used in Southern hybridisation to blots of DNA from two separate self-progeny from each of the four founder plants (grandparents) of the mapping populations. The DNA was digested separately with each of three restriction enzymes (*Eco*RI, *Eco*RV and *Hin*dIII). The vast majority of *Brassica* probes produced strong signals on Southern hybridisation filters of *S. alba* DNA. All 220 probes detected the expected restriction fragment length polymorphism (RFLP) alleles in *Brassica oleracea* and *B. rapa* controls, and only one probe failed to hybridise to the *S. alba* DNA. Of the 219 remaining probes, eight (3.5%) produced only monomorphic patterns and a further nine (4%) produced patterns characteristic of dispersed or tandemly repeated elements. Of the 202 informative probes, 160 that gave the most easily scored banding patterns were chosen for analysis of the mapping populations. This polymorphism screening also demonstrated that the four founder plants were themselves highly heterozygous.

### Linkage analysis

Complex patterns of segregating alleles were resolved into separate P1 and P2 bi-allelic scoring strings using alleles that uniquely identified each parent, and the linkage phase of each scoring string was determined using grandparent allele information (see the Results section for further details). Two distinct sets of scoring strings corresponding to separate P1 and P2 meioses were used for the initial development of independent P1 and P2 maps using MAPMAKER 3.0 (Lander et al. 1987). Two-point analysis (critical LOD score = 4.0) identified linkage groups, which were then ordered by multi-point analysis (log-likelihood threshold = 3.0), followed by proof reading of the scoring data and subsequent re-mapping. The Kosambi mapping function (Kosambi 1944) was used to calculate interval sizes.

### Statistical tests

#### *Distribution of crossovers and marker loci*

The observed frequency distribution of the size of marker intervals (in centiMorgans) was compared with the expected distribution of interval sizes based on a random distribution of both markers and crossovers for the combined population of male and female gametes for P1 and independently for P2. The expected distributions of interval sizes were calculated as described by Dietrich et al. (1992) using the average interval size ($d$) for each population and the Kosambi mapping function. In order to extend the method described by Dietrich et al. (1992) to allow for an arbitrary pattern of missing data, the value $n$ (the number of informative gametes) was averaged over the observed distribution of informative assays, with an informative assay being an interval where both flanking loci were scored. The significance of the deviation between the observed and expected cumulative probability distributions of recombination frequency was tested in each map using the Kolmogorov-Smirnov statistic (Pratt and Gibbons 1981).

#### *Differences in recombination frequencies*

Recombination frequencies in the four classes of gametes were compared in four pairwise comparisons (male versus female in P1; male versus female in P2; P1 males versus P2 males; P1 females versus P2 females) at the level of individual genetic intervals, individual chromatids and whole gametes.

For the comparisons at the level of the chromatids, the statistical procedure was as follows. For each of the four classes of gametes, a count was made of (1) the total number of individuals (chromatids) that were successfully scored for each marker in the chosen marker set for the particular linkage group and (2) the total number of crossovers detected on those individuals. The number of crossovers per chromatid ($r$) was then calculated for each of these four sets of chromatids. To carry out the comparison tests, we calculated the corrected number of crossovers ($rN$) accepting the supposition that the total population of chromatids involved in the comparison ($2N$) was equally divided between the two subpopulations. These corrected numbers were compared for equality using the chi-square test with two classes (1 $df$). To carry out the comparisons for the whole genome, we obtained the total numbers of chromatids and crossovers by totalling the above numbers over all of the linkage groups and applying the same statistical test.

For the comparison of individual intervals, we used Fisher's exact test (Fisher 1935) to detect significant differences in recombination frequencies in corresponding intervals in each of the four pairwise comparisons. The data used for the test were the numbers of recombinant and non-recombinant individuals at each interval in each population.

## Results

### Dense genetic maps of meioses in P1 and P2

The two large populations (A and B) of $F_1$ progeny derived from reciprocal crosses between a pair of

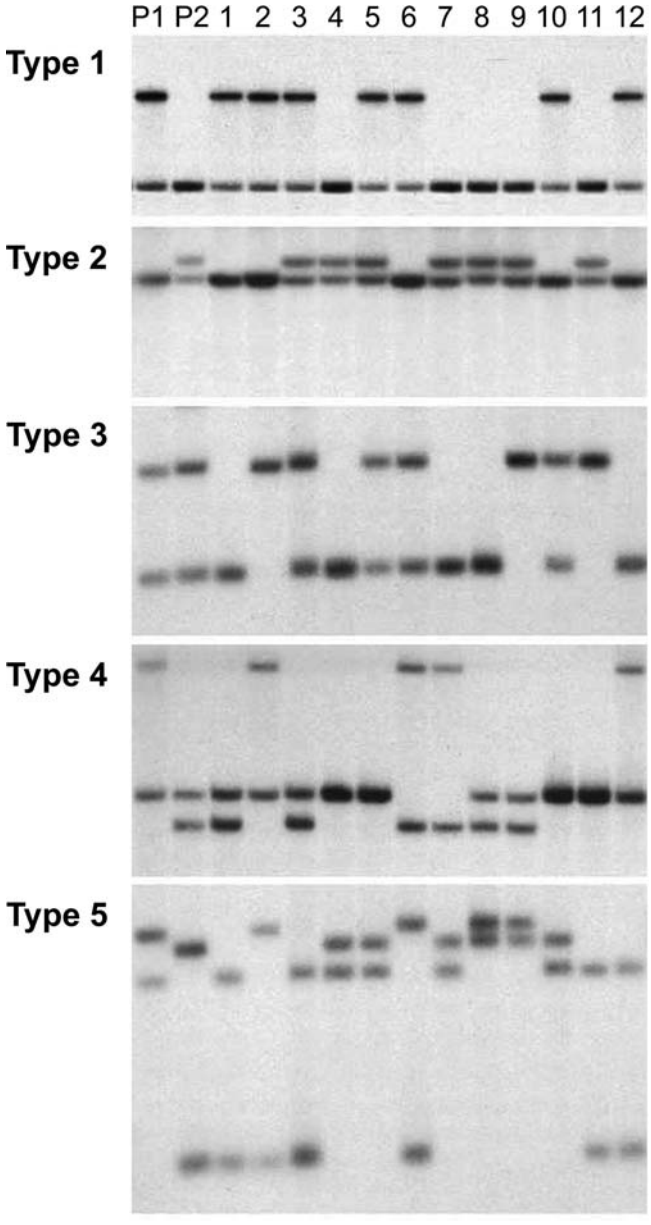



**Fig. 2** Five types of segregation patterns were observed in the $F_1$ populations: types 1–5. These segregation patterns represented loci where: P1 was heterozygous while P2 was homozygous and the parents shared a common allele (type 1); P2 was heterozygous while P1 was homozygous and the parents shared a common allele (type 2); P1 and P2 were both heterozygous for the same two alleles (type 3); P1 and P2 were both heterozygous with a single allele in common (type 4); P1 and P2 were both heterozygous with no alleles in common (type 5). The autoradiographs representing types 1–5 segregation patterns were derived from Southern hybridisation filters carrying DNA from parent 1 (*P1*), parent 2 (*P2*) and 12 $F_1$, individuals (*lanes 1–12*) digested with *Hin*dIII (types 1, 3 and 5), *Eco*RV (type 2) or *Eco*R1 (type 4) and probed with pR116, pN64, pW152, pN194 or pN173, respectively

unrelated and highly heterozygous parents (Fig. 1) were ideal for genetic mapping when combined with genetic marker data. The population design also allowed the comparative analysis of four classes of gametes; female gametes derived from P1, male gametes derived from P1, female gametes derived from P2 and male gametes from P2.

Random subsets of $F_1$ individuals from populations A (60 plants) and B (68 plants) were used to develop the initial high-density genetic map of *S. alba*. Five hundred and fifty-one segregation patterns for marker loci detected by 160 *Brassica* RFLP probes were analysed in the 128 $F_1$ individuals. The products of meiosis in P1 and of meiosis in P2 were each expected to produce separate, sex-averaged genetic linkage maps of the *S. alba* genome. The products of both meioses were assayed in each of the $F_1$ individuals. A total of 263 segregation patterns were derived from meiosis in the P1 parent, and 288 segregation patterns were derived from meiosis in the P2 parent. A high proportion of RFLP probes hybridised to multiple loci, resulting in probes detecting an average of 1.64 polymorphisms in the P1 parent and 1.80 polymorphisms in the P2 parent.

Five general types of segregation pattern were identified, as shown in Fig. 2. Marker patterns type-1 and type-2 represented single segregation patterns derived from meiosis in P1 and P2, respectively, while segregation patterns type-3 and type-4 represented overlaid pairs of segregation patterns, one derived from P1 and the other derived from P2. The most informative marker patterns were derived from pairs of equivalent, polymorphic loci that shared one allele common to both parents (type-4). The unique alleles in each parent identified the grandparental origin and, therefore, the linkage phase of each marker, while shared alleles identified equivalent loci in the two maps. $F_2$-like marker patterns (type-3), produced when both P1 and P2 were heterozygous with the same alleles, also identified equivalent loci in the two maps. However, type-3 markers were only 50% as informative as type-4 markers because it was impossible to identify the parental origin of alleles in heterozygous $F_1$ individuals without recourse to the genetic linkage map and segregation data for flanking loci. Type-5 marker patterns, produced when P1 and P2 together possessed four unique alleles, usually also represented segregation at the same loci in P1 and P2, but this could only be established using the aligned P1 and P2 maps.

Of the 382 loci finally positioned on the integrated *S. alba* genetic map (Fig. 3), an estimated 33%, 35%, 53% and 54% were heterozygous in the founder grandparents T096-1046, Svalöf, Sel-01 and *Sabre*, respectively (Nelson 2000). Despite this heterozygosity, it was still possible to assign at least one of the parental alleles to a particular grandparent unambiguously, and so assign the scoring phase systematically in 488 of the 551 segregation patterns derived from the P1 and P2 meioses. These 488 segregation patterns were used to form the backbone of the P1 and P2 maps. Approximately one-half of the 63 remaining segregation patterns represented loci that could be positioned in the initial linkage analysis, thereby demonstrating that these patterns had been scored in the correct linkage phase. The remaining seg-

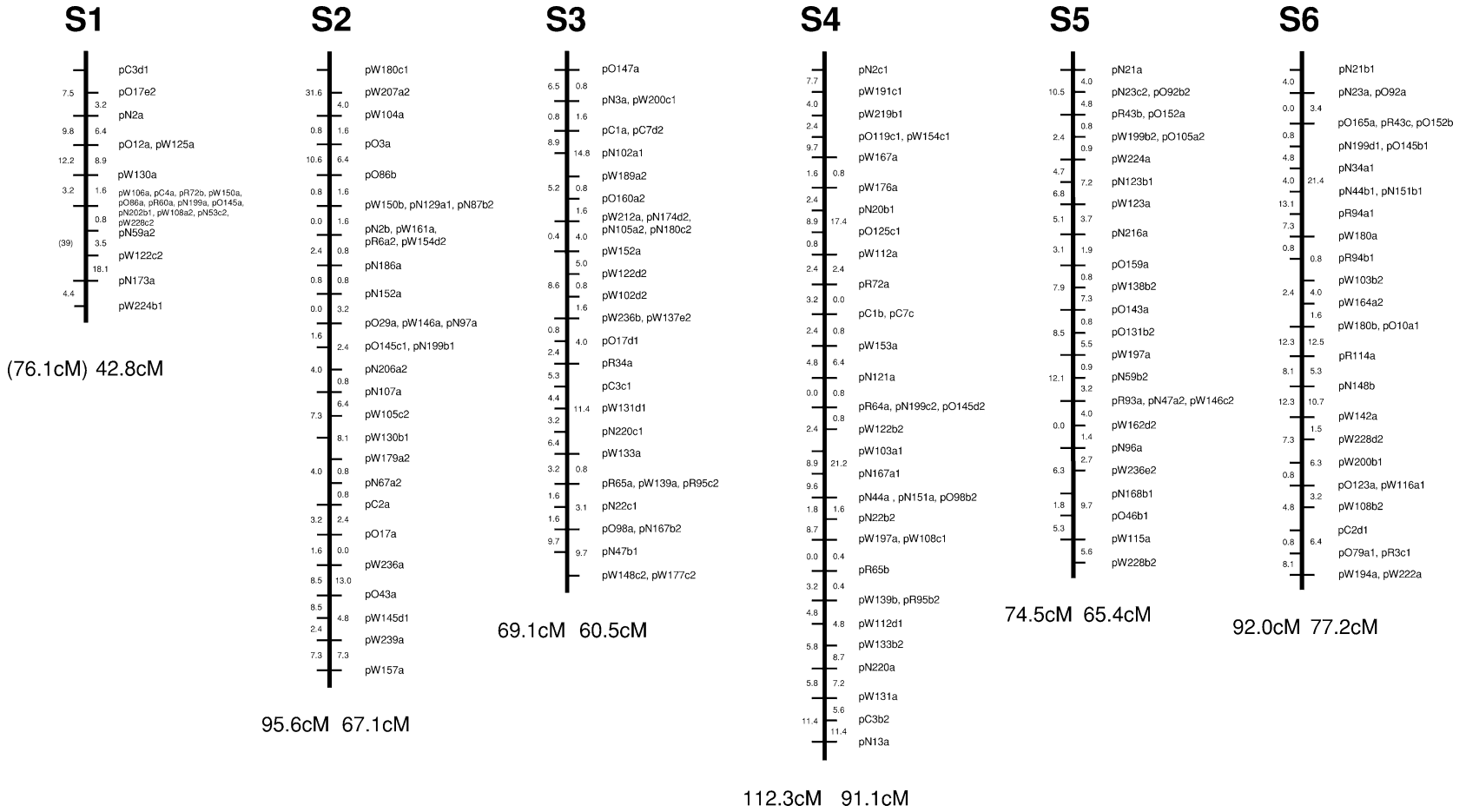



**Fig. 3** Aligned genetic linkage maps of the *S. alba* genome based on the segregation of RFLP-defined loci in two heterozygous parents (P1 and P2). *Vertical lines* represent the 12 aligned linkage groups (*S1– S12*). Loci mapped in the P1 and P2 meioses are indicated by *horizontal bars* to the *left* and to the *right* of the linkage groups, respectively. Locus names are listed on the *right* of each linkage group and consist of the *probe identifier* followed by a *lower case letter* to distinguish different loci detected by the same probe. Locus names *suffixed* by the number *1* were mapped only in the P1 meiosis, while loci mapped only in P2 are indicated by locus names *suffixed* by the number *2*. *Horizontal bars* extending to both sides of the linkage groups indicate loci mapped in both P1 and P2. The sizes of intervals separating adjacent loci are indicated in Kosambi centiMorgans (cM), P1 to the *left*, P2 to the *right*. The size of the interval in *parentheses* in the P1 map of linkage group S1 is given as the percentage recombination (39%). The total length of each linkage group in each map is indicated at the *bottom* of the corresponding linkage group

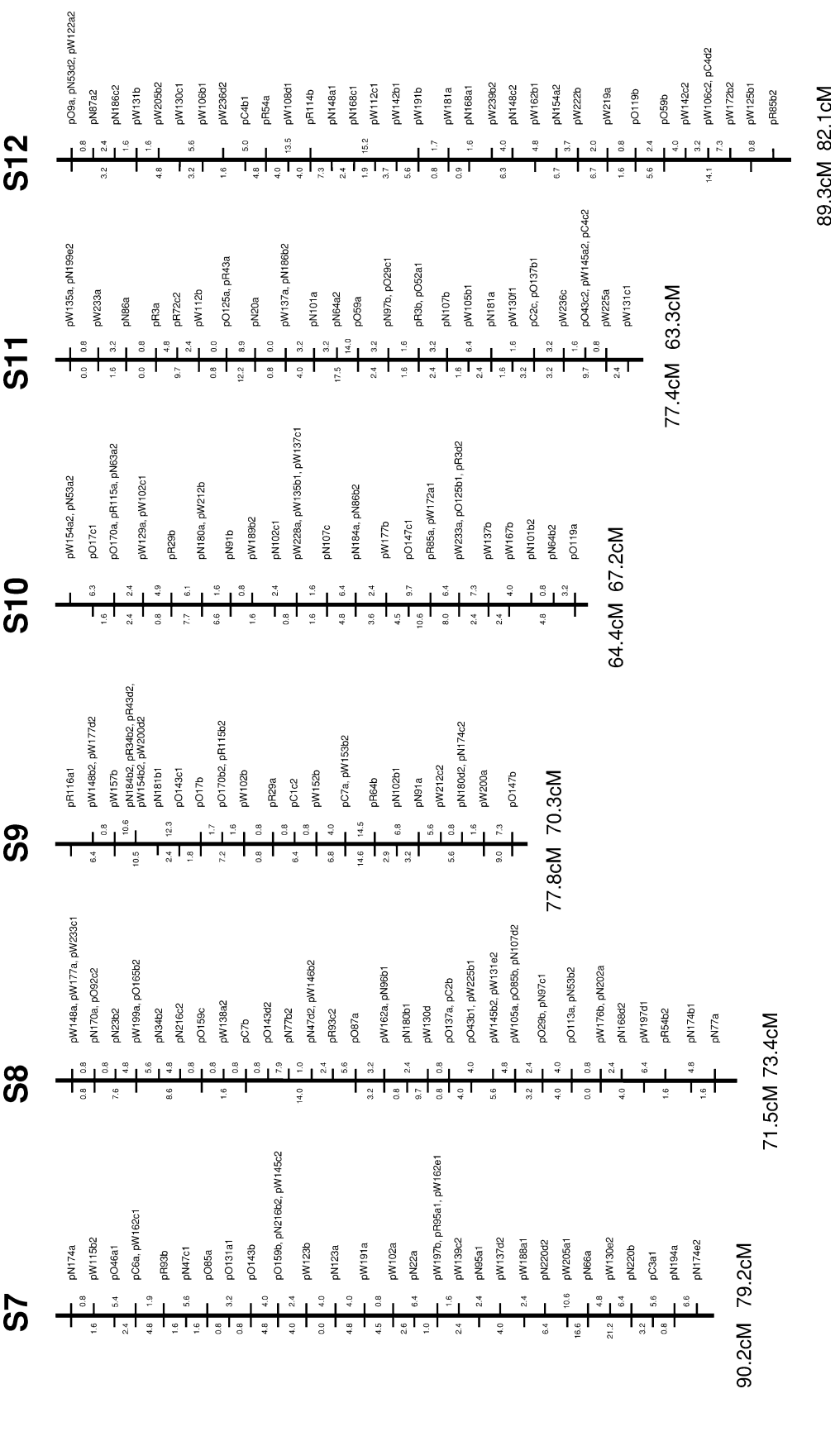



**Fig. 3** (Continued)

regation patterns were mapped to the established linkage groups once their scoring was reversed to reflect the correct linkage phase.

The 263 loci polymorphic in P1 were grouped into 12 linkage groups spanning 948 cM and one pair (Fig. 3), and the 288 loci polymorphic in P2 were grouped into 12 linkage groups spanning 840 cM with no unlinked loci (Fig. 3). The two parental maps were aligned on the basis of 169 loci polymorphic in both parents, and no disparities in locus order were observed. The integrated map contained 382 loci distributed over 12 linkage groups (Fig. 3). The unlinked couplet in the P1 map was positioned at the bottom of linkage group S1 by reference to a locus shared with the P2 map.

## Distribution of interval sizes

The frequency distribution of the size of genetic intervals flanked by adjacent marker loci can give an indication of whether crossovers are randomly distributed across the genome or localised in certain regions and excluded from other regions. An initial evaluation of the sex-averaged linkage maps derived from meioses in P1 and P2 identified a conspicuous cluster of coincident loci at the same location on linkage groups S1 in both maps (Fig. 3). This cluster might represent a region of exceptionally high marker density but more probably reflects a large segment of chromosome either devoid of recombination or where the products of such recombination are not represented in the mapping population.

The significance of the deviation between the observed and expected cumulative probability distribution of recombination frequency in map intervals flanked by adjacent loci (Fig. 4) was tested in the P1 and P2 maps using the Kolmogorov-Smirnov statistic. Deviation from the random model, where both marker loci and crossovers are distributed randomly across the genome, was not significant in either the P1 or P2 maps when the cluster of coincident markers on S1 was omitted ($P = 0.63$ and 0.10, respectively). However, the deviation of the observed distributions from random distributions increased appreciably in both the P1 and P2 maps when the coincident loci on S1 were included in the analysis, $P = 0.135$ and 0.013, respectively.

## Assaying selected loci in the full populations

In order to rigorously test the effect of sex and genetic background on recombination frequencies, all of the $F_1$ individuals in the reciprocal mapping populations (188 for population A and 194 for population B) were analysed. Seventy-six RFLP probes, which detected a set of 129 dispersed loci, were selected using the initial dense maps to provide full coverage of the P1 and P2 genomes. The segregation patterns for 110 P1 loci and 105 P2 loci were assembled into four separate data sets according to the four classes of gametes. The data from the four sets were assembled into linkage groups fully consistent with those presented in Fig. 3.

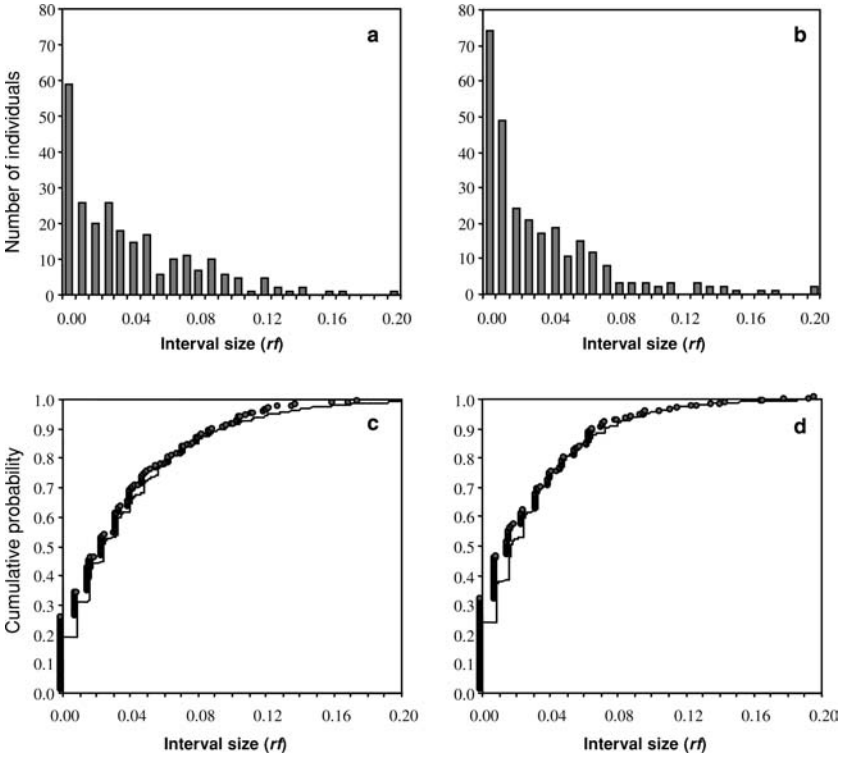



**Fig. 4** **a** and **b** are histograms representing the frequency distribution of interval size for map intervals separated by adjacent loci in the genetic maps of *S. alba*. The interval sizes are measured in recombination frequency (*rf*), and the results for the sex-averaged maps derived from meiosis in P1 (**a** 251 intervals) and P2 (**b** 276 intervals) are represented. **c** and **d** are graphs of the observed (*points*) and expected (*solid lines*) cumulative probability distributions of interval size in the genetic maps derived from P1 and P2, respectively. The expected probability distributions are those that would result from random distributions of both marker loci and crossovers

### Allele frequencies in the four data sets

The allele frequency was calculated at each locus in each of the four data sets to evaluate the degree of deviation from the expected 0.5 transmission frequency for each allele at each locus. The observed frequency distributions for allele frequencies in the four data sets were compared to the expected binomial distributions (Nelson 2000). There was in general a close correspondence between the observed and expected distributions. However, the alleles from grandparent Sel-01 appeared to be slightly favoured in the female gametes of P1, and a set of three adjacent loci at the top of linkage group S6 showed a significant ($P < 0.001$) surplus of the alleles from grandparent Svalöf in the male gametes of P2.

### Effect of sex and genotype on frequency and distribution of recombination

A set of 86 loci that were polymorphic in both P1 and P2, and were approximately evenly distributed across the genome, were selected from the loci assayed in the full populations and used to test for differences in the frequency and distribution of crossovers in the meioses leading to the four classes of gametes (Fig. 5). These selected loci encompassed 90% of the P1 map and 93% of the P2 map. The mean number of crossovers per gamete and the mean number of crossovers per chromatid were used to compare recombination behaviour across the whole genome and in each linkage group, respectively (Table 1).

When the numbers of crossovers per gamete were compared for the four classes of gametes using a chi-square test, differences between male and female meioses were far greater than differences between P1 and P2 meioses. There were significantly greater numbers of crossovers in the male gametes than in the female gametes of both P1 and P2 ($P = 9.4 \times 10^{-5}$ and $3.1 \times 10^{-8}$, respectively; Table 1). Genotype had a considerably smaller, though significant, effect on the frequency of crossovers in P1 than in P2, being higher in both male ($P = 5.5 \times 10^{-3}$) and female ($P = 1.5 \times 10^{-5}$) gametes (Table 1).

The trend of having more crossovers in the male chromatids was very consistent, with 11 out of 12 P1 and all 12 P2 linkage groups showing higher male crossover frequencies (Table 1; Nelson 2000). The trend for higher crossover frequencies in P1 than in P2 was less consistent, but 10 out of 12 female and 8 out of 12 male linkage groups showed higher crossover frequencies in P1 than in P2 (Table 1; Nelson 2000).

Differences in the number of crossovers per interval for each of the 74 chromosome segments flanked by adjacent pairs of marker loci were also investigated (Fig. 5). Corresponding intervals, assayed in the four classes of gametes, were compared and Fisher *P*-values calculated for each pairwise interval comparison. Only two intervals assayed in the male gametes, *pO165a–pW180a* on linkage group S6 ($P = 7 \times 10^{-5}$) and *pW191b-pW222b* on linkage group S12 ($P = 0.009$), and two intervals assayed in the female gametes, *pW197a-pR93a* on S5 ($P = 0.006$) and *pC2c-pW225a* on S11 ($P = 0.006$), showed significant genotype-dependent differences ($P < 0.01$), and in all cases recombination was more frequent in the P1 genetic background. Intervals with significant ($P < 0.01$) male/female differences were much more prevalent, with seven such intervals assayed in the products of P1 and another seven such intervals assayed in the products of P2.

These male/female differences were further analysed in the expanded data sets that included several more terminal loci (Fig. 5): a total of 110 loci assayed in the products of P1 and spanning 99% of the P1 map and 105 loci assayed in the products of P2 and spanning 96% of the P2 map. The vast majority of intervals with male/female differences showed increased recombination in male meioses compared to female meioses. However, one interval on linkage group S12 derived from P1 and one interval on linkage group S2 derived from P2 showed significantly ($P < 0.01$) more recombination in the female gametes than in the male gametes (Fig. 5). A total of six intervals in P1 and ten intervals in P2 showed significantly ($P < 0.01$) increased recombination in the male gametes, and the distribution of these intervals across the genome is represented in Fig. 5. Intervals showing higher male recombination were generally located towards the ends of the linkage groups, and the two intervals showing higher recombination in the female gametes were located near the centre of the linkage groups.

## Discussion

The *S. alba* populations analysed in this paper provided a rare opportunity to assay recombination behaviour accurately across a whole plant genome. The high levels of marker polymorphism observed in both the P1 and P2 parents allowed the generation of extensive, dense maps of the *S. alba* genome from which a representative set of spaced markers was selected. The $F_1$ populations showed balanced allele frequencies, indicating that very little selection had been imposed on the gametes or zygotes sampled in these populations. With the exception of the central region of S1, an analysis of the distribution of interval sizes in the dense genetic maps based on meiosis in P1 and P2 (Fig. 4) revealed patterns consistent with random distributions of both markers and sex-averaged crossovers. This result suggested that all chromosomes were able to pair regularly at meiosis, except for chromosome corresponding to linkage group S1. Presumably the *S. alba* chromosomes contain recombination 'hotspots', such as those observed in other species (Schnable et al. 1998), and experience position effects

along the telomere-centromere axis (Jones et al. 2002), but these effects must be relatively mild in this species. This background of random behaviour was the ideal environment for detecting subtle differences in the frequency and distribution of crossovers controlled by either sex or genotype.

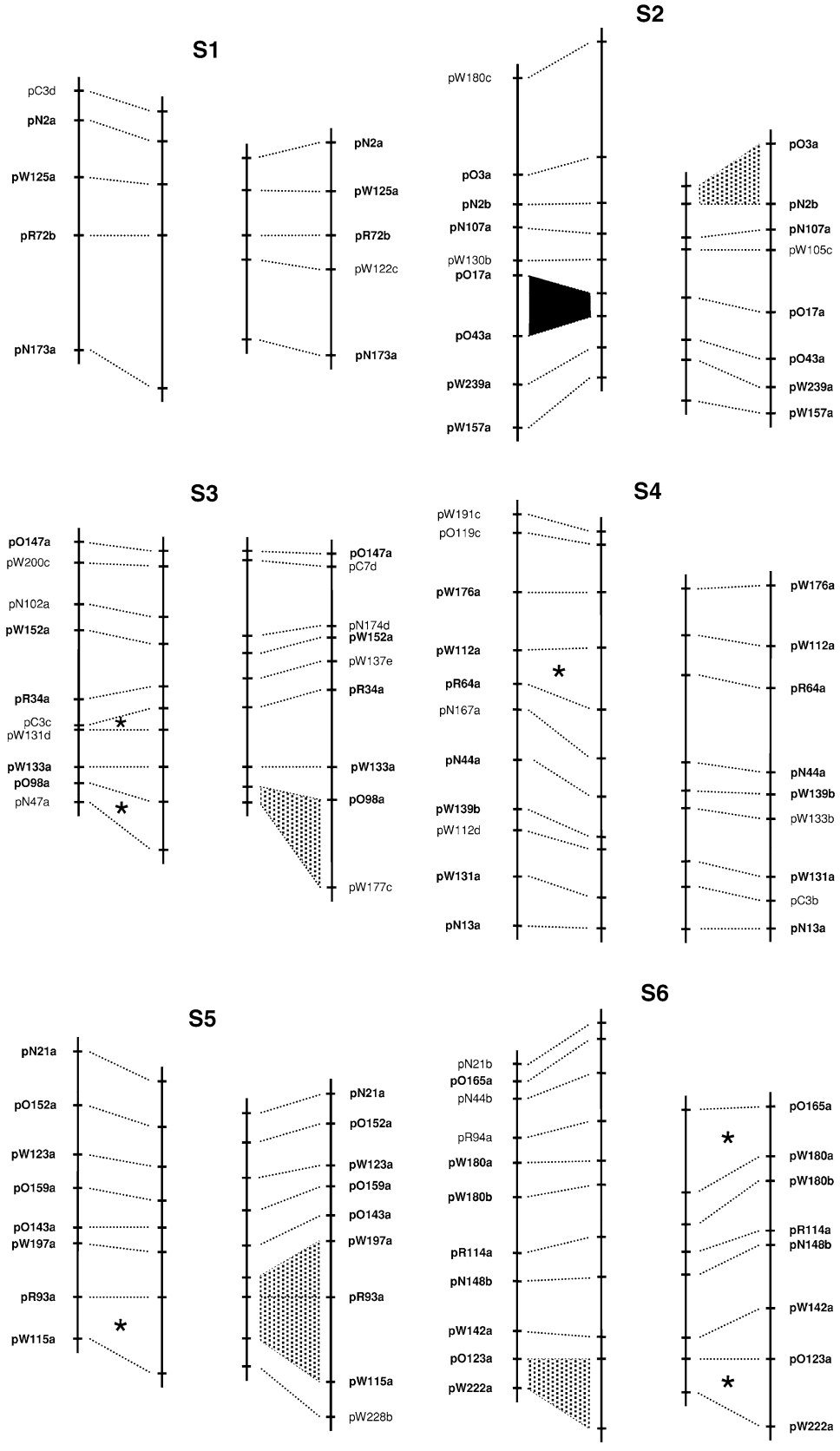
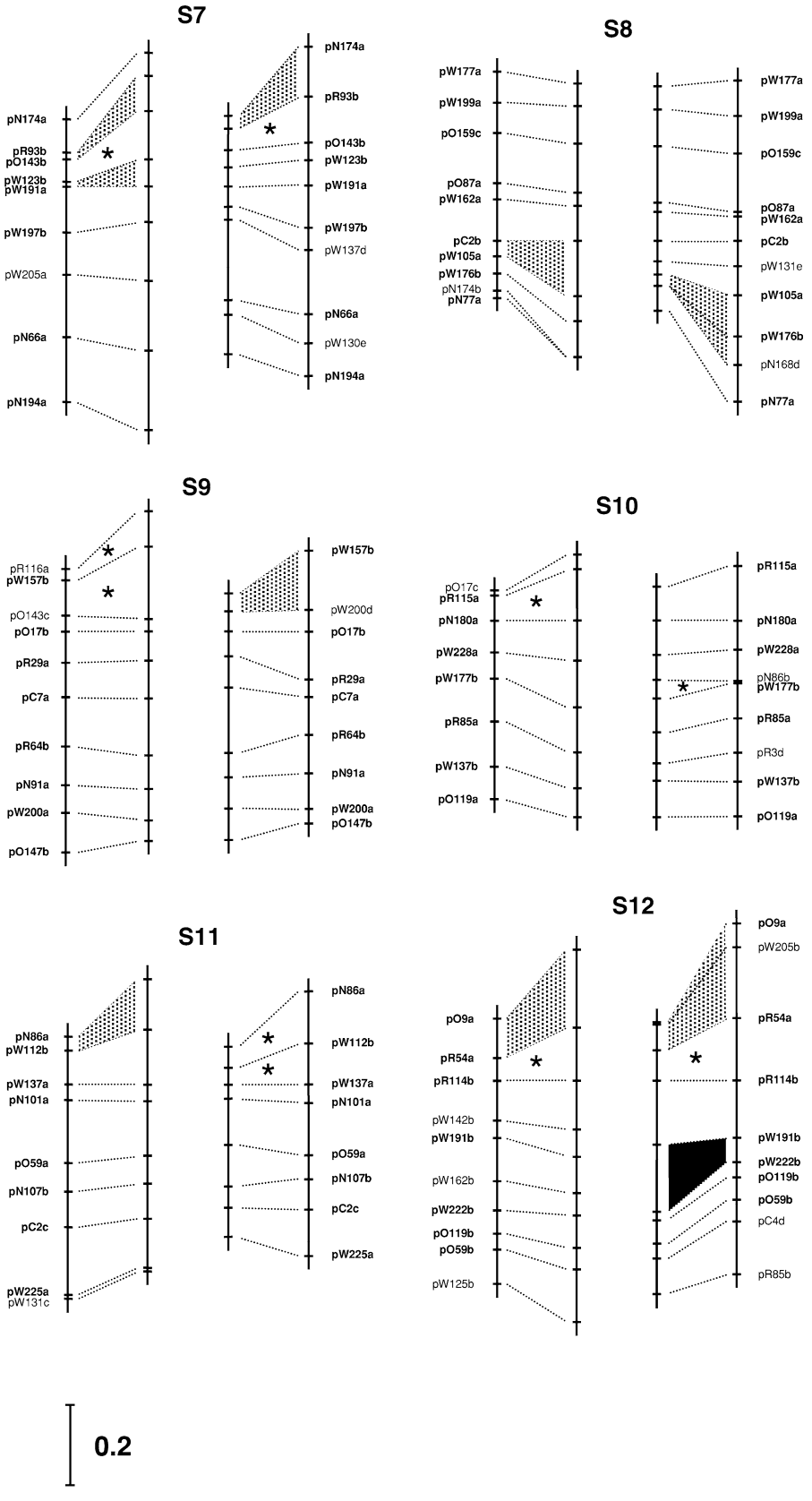



**Fig. 5** The distribution of male/female differences in recombination frequency across the *S. alba* genome. Each linkage group (*S1–S12*) is represented by four *vertical lines* that indicate (from *left* to *right*) the recombination frequency assayed in P1 female gametes, P1 male gametes, P2 female gametes and P2 male gametes, respectively. Loci mapped in all four types of gamete are shown in *bold*. Interval sizes (expressed as recombination fractions) are drawn to scale. Intervals with significantly increased male recombination ($P < 0.01$) are indicated by *stippled boxes*, and intervals with significantly increased female recombination ($P < 0.01$) are indicated by *solid boxes*. Intervals with less significant male/female differences ($0.01 < P < 0.05$) are identified with an *asterisk*

**Fig. 5** (Continued)




The experimental design also added to the sensitivity and confidence with which sex-based and genotype-based effects on recombination behaviour could be detected. A large number of gametes (764 in all) were assayed genome-wide. This sample, divided almost equally into four classes based on sex and genotype,

**Table 1** Numbers of crossovers[a] observed in chromatids derived from four classes of meioses detected using 86 dispersed loci

| Linkage group | P1 female | | P1 male | | P2 female | | P2 male | |
|---|---|---|---|---|---|---|---|---|
| | Crossovers | Chromatids | Crossovers | Chromatids | Crossovers | Chromatids | Crossovers | Chromatids |
| S1 | 99 | 179 | 110 | 191 | 80 | 189 | 93 | 183 |
| S2 | 78 | 118 | 57 | 125 | 66 | 125 | 80 | 118 |
| S3 | 105 | 183 | 115 | 190 | 104 | 190 | 106 | 182 |
| S4 | 142 | 182 | 152 | 193 | 155 | 191 | 149 | 182 |
| S5 | 126 | 182 | 135 | 190 | 103 | 192 | 126 | 184 |
| S6 | 131 | 184 | 181 | 193 | 127 | 193 | 129 | 184 |
| S7 | 127 | 184 | 176 | 192 | 113 | 193 | 146 | 184 |
| S8 | 105 | 183 | 133 | 192 | 104 | 189 | 143 | 183 |
| S9 | 125 | 183 | 149 | 190 | 122 | 192 | 128 | 184 |
| S10 | 94 | 184 | 120 | 191 | 110 | 191 | 116 | 184 |
| S11 | 116 | 181 | 138 | 191 | 92 | 192 | 118 | 183 |
| S12 | 107 | 184 | 152 | 191 | 104 | 186 | 130 | 184 |
| Total | 1,355 | 2,127 | 1,618 | 2,229 | 1,280 | 2,223 | 1,464 | 2,135 |

[a]Crossovers were counted in only those chromatids for which there were genotype data for both terminal loci

provided the raw material for precise statistical testing. The $F_1$ populations, produced from reciprocal crosses between two genetically distinct and highly heterozygous plants in the same experiment, provided internal controls for factors that influence recombination frequency, such as temperature and age (Zetka and Rose 1990), recombination 'hotspots' (Schnable et al. 1998) and position effects (Jones et al. 2002).

The recombination frequencies measured in the male gametes of *S. alba* were significantly higher than those measured in the female gametes in both parents. This trend was observed in 11 out of the 12 *S. alba* linkage groups (Table 1). The biological significance of such increased male recombination frequencies is unclear given the varied patterns of male/female differences in recombination frequency observed in a range of plant and animal species (Benito et al. 1996; Rivard et al. 1996; Kelly et al. 1997, Broman et al. 1998; Groenen et al. 1998). Presumably there is no overwhelming biological imperative to have higher recombination in either male or female gametes.

In *S. alba*, the elevated level of recombination in male gametes was not uniformly distributed across the genome but rather localised towards one end in each of 10 of the 12 linkage groups (Fig. 5). This pattern of higher male recombination frequencies near the ends of linkage groups was also observed in *Brassica nigra* (Lagercrantz and Lydiate 1995), a close relative of *S. alba* (Warwick and Black 1991). A comparison of the *S. alba* and *B. nigra* linkage maps may show whether or not particular RFLP marker-defined intervals are associated with enhanced male recombination frequencies in both species, or whether it is only the location of the intervals on the chromosomes that are associated with sex-differences in recombination.

Few other investigations have had sufficient resolution to detect male/female differences in the distribution of crossovers, but there is a strong tendency for male recombination to be relatively higher towards the ends of chromosomes, while female recombination is relatively higher towards the middle of chromosomes. Even in species where female recombination frequencies are predominantly higher, such as in human (Broman et al. 1998) and rainbow trout (Sakamoto et al. 2000), the ends of chromosomes often show enhanced male recombination.

It is tempting to speculate that it is the interaction between the biochemical and cytological machinery of meiosis and the contrasting physical and developmental environments experienced in the male meiotic cells compared to female meiotic cells that give rise to the differences in the distribution of crossovers revealed by a range of investigations. Chromosomes associate with their homologues and with the nuclear envelope at their telomeres (Rockmill and Roeder 1998; Trelles-Sticken et al. 2000), and it is conceivable that the complexes that give rise to chiasmata, and therefore crossovers, load onto the chromosomes at these structures. The tendency for male recombination to be relatively more frequent towards the ends of chromosomes, while female recombination is relatively more frequent towards the middle of chromosomes might then be a result of these complexes having insufficient time to migrate to the middle of chromosomes in generally faster male meiosis. Telomere loading and a fixed migration rate might also explain the shortage of recombination in the middle of the extremely large chromosomes of wheat (Delaney et al. 1995; Hohmann et al. 1995) and even the absence of crossovers in the male gametes of Drosophila (Morgan 1912, 1914).

Sex-specific differences in the frequency and distribution of crossovers clearly affect the efficiency of breeding programmes. For example, linkage blocks at the ends of chromosomes will more often be broken in male gametes and will more often be conserved in female gametes. Provided that the specific sex differences in recombination attributed to particular species (usually on the basis of a very small sample) are universally true for that species and provided that the chromosomal locations of key genes are known, this information could usefully be taken into account when designing breeding strategies such as gene introgression through back-

crossing. This present study also demonstrated that genotype significantly influences recombination frequencies of both male and female gametes of *S. alba*, with P1 gametes showing significantly higher recombination frequencies than P2 gametes and that these differences appeared to be evenly distributed across the genome. Again, this information could be useful in a breeding context where a genotype known to have relatively high meiotic recombination frequencies could be used as the recurrent parent in an introgressive backcrossing programme.

The integrated genetic map of *S. alba*, based on meiosis in each of two plants (P1 and P2) and constructed using loci detected by *Brassica* RFLP probes, is the first genetic map of this species. The map consists of 12 extensive linkage groups with no unlinked loci, and these linkage groups probably correspond to the 12 chromosome pairs of *S. alba* (Bennett et al. 1982). The map will provide a robust framework for future genetic studies in *S. alba* and help in the development of genotyping assays for marker-assisted selection in white mustard breeding. *Brassica* RFLP probes worked xtremely well in *S. alba*, with 95% of the *Brassica* DNA fragments hybridising strongly to low copy-number sequences in the *S. alba* genome. This was due to the close evolutionary relationship between *S. alba* and the *Brassica* A and C genomes (Warwick and Black 1991) and to the prior selection for RFLP probes that represent low copy-number, genic regions of the *Brassica* genome (Sharpe et al. 1995). Because the new map of *S. alba* was constructed using RFLP probes already used extensively to produce genetic maps of *Brassica* species (Lagercrantz and Lydiate 1995; Parkin et al. 1995; Sharpe et al. 1995; Bohuon et al. 1996), it will be possible to generate comparative maps relating the *S. alba* genome to other *Brassica* genomes. This comparative analysis will not only help to elucidate the evolutionary history of the Brassicaceae family but will also assist in the intergenomic transfer of useful genes between *Sinapis* and *Brassica* species.

In contrast to the complete absence of marker clustering that was characteristic for 11 of the 12 *S. alba* linkage groups, linkage group S1 contained a very pronounced cluster of markers at its centre (Fig. 3). This cluster was present in both the P1 and P2 maps and contained 12 of the 22 loci on S1. Given the extraordinary lack of recombination observed between these marker loci compared to the otherwise random distribution of marker loci in the rest of the *S. alba* genome, it is likely that the cluster represented a region of S1 devoid of recombination or a region where the products of such recombination were not recovered in the mapping populations, rather than a region with a very high marker density. The most likely explanation for the unusual behaviour of S1 is that it exists in two forms with one form carrying a pericentric inversion with respect to the other form. This explanation assumes that both P1 and P2 were inversion heterozygotes. This hypothesis predicts that progeny derived from meiosis in individuals with a pair of S1 chromosomes carrying the same phase of the inversion would represent normal, high levels of recombination in the region of S1 represented by the cluster of markers in the P1 and P2 maps. This prediction will be tested by crossing Sel-01 (Fig. 1) to both *Sabre* and Svalöf and then developing separate segregating populations derived from both new parents. If our hypothesis is correct, then in one segregating population, the S1 markers would again cosegregate (where that parent was also an inversion heterozygote), whereas in the other segregating population the S1 markers would be resolved (where the parent would have the same form of the chromosome corresponding to S1).

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