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ORIGINAL PAPER

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A genetic linkage map of the diplosporous chromosomal region in *Taraxacum officinale* (common dandelion; Asteraceae)

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Abstract In this study, we mapped the diplosporous chromosomal region in *Taraxacum officinale*, by using amplified fragment length polymorphism technology (AFLP) in 73 plants from a segregating population. *Taraxacum* serves as a model system to investigate the genetics, ecology, and evolution of apomixis. The genus includes sexual diploid as well as apomictic polyploid, mostly triploid, plants. Apomictic *Taraxacum* is diplosporous, parthenogenetic, and has autonomous endosperm formation. Previous studies have indicated that these three apomixis elements are controlled by more than one locus in *Taraxacum* and that diplospory inherits as a dominant, monogenic trait (*Ddd*; *DIP*). A bulked segregant analysis provided 34 AFLP markers that were linked to *DIP* and were, together with two microsatellite markers, used for mapping the trait. The map length was 18.6 cM and markers were found on both sides of *DIP*, corresponding to 5.9 and 12.7 cM, respectively. None of the markers completely co-segregated with *DIP*. Eight markers were selected for PCR-based marker development, of which two were successfully converted. In contrast to all other mapping studies of apomeiosis to date, our results showed no evidence for suppression of recombination around the *DIP* locus in *Taraxacum*. No obvious evidence for sequence divergence between the *DIP* and non-*DIP* homologous loci was found, and no hemizygosity at the *DIP* locus was detected. These results may indicate that apomixis is relatively recent in *Taraxacum*.

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Introduction

Diplospory refers to one of the types of apomeiosis in gametophytic apomixis (Nogler 1984), the other being apospory. Apomixis *s.s.* is asexual reproduction through seeds, leading to offspring identical to the mother plant (also agamospermy; Nogler 1984; Koltunow 1993). In gametophytic apomixis, the female gametophyte, i.e. the embryo sac, arises from an unreduced embryo sac initial. In diplospory, this initial is an unreduced megaspore mother cell, whereas in apospory, it is a somatic cell of the ovule. Diplospory can be mitotic (*Antennaria*-type; Juel 1900), indicating that the megaspore mother cell does not enter into meiosis at all, or meiotic (*Taraxacum*-type; Juel 1906), referring to a modified, restitutional meiosis. Apart from apomeiosis, apomictic reproduction requires parthenogenetic embryo development and autonomous or pseudogamous endosperm formation, the latter indicating that fertilization of the central cell is crucial.

Apomixis has a potential in plant breeding and seed production, because it allows for the fixation and unlimited propagation of complex and heterozygous genotypes (Vielle-Calzada et al. 1996; Spillane et al. 2001). Apomixis is also of interest in the context of the maintenance of sexual reproduction, questioning the existence of so many sexually reproducing species in spite of disadvantages such as "the cost of males", "the cost of recombination", and "the cost of rarity" (Maynard-Smith 1978; Stearns 1987). For both reasons, strategies have been undertaken to gain insight into the genetic basis of apomixis, including the unravelling of the genetic control of apomixis in natural apomicts (Van Baarlen et al. 1999); interspecific hybridisations between sexual crops and apomictic wild relatives (Savidan 2000); and identification of genes that control elements of apomixis in well-defined sexual species (Grossniklaus 2001). Our interests cover both the evolutionary as well as the breeding aspects of apomixis, using the natural apomict *Taraxacum officinale s.l.* (Wigg.; common dandelion, Asteraceae) as a model system.

The genus *Taraxacum* consists of polyploid, mostly triploid (2*n*=3*x*=*24*) apomictic plants as well as diploid (2*n*=2*x*=*16*) sexual individuals (Richards 1986). Apomixis in *Taraxacum* is diplosporous, with parthenogenetic embryo development and autonomous endosperm formation (Gustafsson 1934). Diplospory is of the *Taraxacum*-type, with the megaspore mother cell entering a meiotic prophase either without, or with only limited chromosome pairing, resulting in a first division restitution nucleus (FDR) (Nogler 1984). Crossing experiments showed that the different apomixis elements are inherited independently in *Taraxacum*, suggesting that apomixis is controlled by more than one locus in this species (Van Dijk et al. 1999). We have found a 1:1 segregation ratio for diplospory versus non-diplospory. The offspring of a sexual diploid maternal plant, and a diplosporous, but non-parthenogenetic tetraploid hybrid, showed a 1:1 segregation ratio for diplospory versus non-diplospory, indicating that diplospory inherits as a dominant, monogenic trait in *Taraxacum* [designated *DIPLOSPOROUS* (*DIP*), Van Dijk and Bakx-Schotman 2003]. This finding is supported by the identification of specific alleles at two microsatellite loci that are linked to diplospory and fit a single dose per locus model in these offspring only (Van Dijk and Bakx-Schotman 2003). Cytological investigations, and a significant linkage between the rDNA locus and the microsatellite alleles that are linked to diplospory, suggest that *DIP* is located on one of the nucleolar organiser region (NOR) chromosomes (Sørensen 1958; Van Dijk and Bakx-Schotman 2003). Although *DIP* is only expressed during female meiosis, it is transferred via reductional and recombinational male meiosis, enabling us to map the trait.

The genetic control of diplospory has thus far been investigated in two other species apart from *Taraxacum*: *Tripsacum dactyloides* (Poaceae; Leblanc et al. 1995; Grimanelli et al. 1998) and *Erigeron annuus* (Asteraceae; Noyes 2000; Noyes and Rieseberg 2000). The former species reproduces via the *Antennaria*-type of diplospory and the latter via a modified *Taraxacum*-type (called *Ixeris*-type; Okabe 1932). In both species, diplospory inherits as a dominant, monogenic trait, as was found for *Taraxacum*. In *T. dactyloides*, the different elements of apomixis inherit as a single locus. In *E. annuus*, diplospory and parthenogenesis are unlinked and inherited independently, whereas parthenogenesis showed also dominant, monogenic inheritance. Mapping results indicated that recombination is suppressed at the locus for diplospory in both *T. dactyloides* and *E. annuus* (Grimanelli et al. 1998; Noyes and Rieseberg 2000). In the latter, no suppression of recombination was observed in the chromosomal region around the gene(s) for parthenogenesis.

Several other studies have dealt with the genetic control of apospory (reviewed by Grossniklaus et al. 2001), e.g. in Poaceae: *Pennisetum squamulatum* (Ozias-Akins et al. 1998), *Paspalum simplex* (Pupilli et al. 2001), and *Poa pratensis* (Matzk et al. 2000; Albertini et al. 2001), and in species of *Hieracium* (Asteraceae; Bicknell et al. 2000). All of these species showed dominant, monogenic inheritance of apospory and, except for *Poa pratensis*, apomixis as a whole to be inherited as a single locus. In *P. pratensis*, evidence was found for two distinct genetic factors that may control apospory and parthenogenesis (Albertini et al. 2001). Mapping studies in *Pennisetum squamulatum* and *Paspalum simplex* indicated that recombination is suppressed at the locus for apomixis (Ozias-Akins et al. 1998; Labombarda et al. 2002). For these two species, also evidence was found for the presence of hemizygous regions linked to apomixis.

In the present study, we genetically mapped the diplosporous chromosomal region in *Taraxacum*. To this end, we performed a bulked segregant analysis (BSA), followed by screening of individual plants, using amplified fragment length polymorphisms (AFLPs). A total of 34 AFLP markers showed linkage to diplospory in the 73 plants analysed. The map length was 18.6 cM and markers were located on both sides of *DIP*. Eight of the AFLP markers were sequenced in order to develop locus-specific PCR markers, and two of them were successfully converted. In contrast to other mapping studies of apomeiotic chromosomal regions to date, our results showed no evidence for suppression of recombination at the *DIP* locus in *Taraxacum*.

Materials and methods

Plant material

A mapping population that segregated at approximately 1:1 for diplospory:non-diplospory was obtained via the crossing scheme shown in Fig. 1 (Tas and Van Dijk 1999; Van Dijk et al. 1999; Van Dijk and Bakx-Schotman 2003). Plants of *Taraxacum officinale* section *Ruderalia*, originating from The Netherlands and France were used. A diplosporous tetraploid plant (PAX; Fig. 1) was constructed that could serve as a donor of meiotically reduced and recombined diploid pollen. The construction of this hybrid was necessary to obtain sufficient offspring to genetically analyse and map *DIP*. PAX originated from a non-apomictic, but diplosporous, triploid hybrid (H6–3; Van Dijk et al. 1999; Fig. 1) and a sexual diploid pollen parent. H6–3 was one of the offspring plants of a cross between an apomictic triploid paternal and a sexual diploid maternal plant (Tas and Van Dijk 1999). Crossing PAX with a sexual diploid maternal plant (S2–125; Fig. 1) resulted in all triploid offspring. These triploids were phenotyped for diplospory versus non-diplospory via pollinations with sexual diploids, followed by analysing the ploidy level of the offspring using flow cytometry (Tas and Van Dijk 1999). All diplosporous plants produced tetraploid offspring only, whereas the non-diplosporous plants produced a range of di-, tri-, and tetraploid offspring. A ‘core population’ of the triploid plants was raised in the greenhouse, of which 73 were used here as the mapping population.

AFLP analysis

Total DNA was isolated from 0.5–0.6 g fresh leaves from individual plants using the hexadecyl-trimethyl-ammonium-bromide (CTAB) procedure described by Rogstad (1992), with minor modifications. We added 1% polyvinylpyrrolidone (PVP-40) to the CTAB buffer, and used a second chloroform extraction, and performed an *RNAse* treatment on the final products, followed by a second ethanol precipitation step. The DNAs were diluted in 500 μl

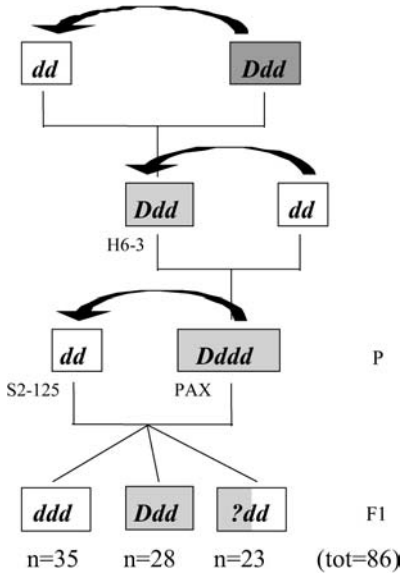

Fig. 1 The crossing scheme used to construct a mapping population for diplospory. Diplospory (*D, shaded*) was introduced into a non-apomictic, triploid hybrid, H6–3, via an apomictic pollen donor (*dark shaded*). H6–3 was used as a maternal plant in a cross with a sexual diploid, resulting in all diplosporous, tetraploid offspring plants. One of these tetraploids, PAX, served as a donor of meiotically reduced and recombined, diploid pollen, in a cross with a sexual diploid maternal plant, S2–125, resulting in triploid offspring. Within this offspring, the segregation ratio of diplospory:non-diplospory was approximately 1:1, indicating that diplospory is a dominant, monogenic trait (*Ddd*). The *half-shaded box* indicates individuals of unknown phenotype

T0.1E (10 mM Tris, 0.1 mM EDTA, pH 8.0) and checked for content and purity by measuring the optical densities at 260 and 280 nm, running on agarose gels, and digesting with *Eco*RI. DNA concentrations ranged between 0.2–1.0 μg/μl and all samples were diluted to 50 ng/μl in T0.1E for AFLP analysis.

AFLP fragments were generated according to the protocol of KeyGene (Wageningen, The Netherlands) version 2.2 described by Vos et al. (1995), with the following modifications. We performed the restriction and ligation simultaneously, using 200 ng DNA. Pre-amplifications were performed by using 5 μl 20x diluted restriction-ligation mixtures, the *Eco*RI+A and *Mse*I+C primers, 0.08 U Super *Taq* polymerase (SphaeroQ, Leiden, The Netherlands) with the supplied buffer, and 36 polymerase chain reaction (PCR) cycles with an annealing temperature of 56ºC. Selective amplifications were performed by using 3 μl 20x diluted pre-amplified products, each of the *Mse*I+C+2 and fluorescent (IRD-700 or -800)-labelled *Eco*RI+A+2 and primer combinations, and 0.04 U Super *Taq* polymerase. Amplification products were analysed on a *LI-COR IR* ^{2} automated sequencer (Westburg, Leusden, The Netherlands) according to the manufacturer's protocol. Between 0.6–0.8 μl of each denatured amplification product/loading dye mixture were loaded onto 5.5% denaturing polyacrylamide gels (Ready-to-use gel matrix KB^{plus}, Westburg) and separated for 2.5 h at 40 W, 1500 V, 25 mA and 55°C.

A BSA (Michelmore et al. 1991) was performed, using a "+"pool that contained pre-amplification products of eight diplosporous plants, and two "-"pools, each containing pre-amplification products of exclusive sets of eight non-diplosporous plants. The pools were diluted and the AFLP reactions performed as described above. A total of 256 *Eco*RI/*Mse*I selective primer combinations were tested. For those primer combinations that showed one or more unique AFLP fragments in the "+" pool, individuals were analysed separately. In case of good co-segregation with *DIP* (>20/24 individuals; chi-square<3.8; P>0.05; df=1) the parents and remaining offspring were analysed for that fragment in order to estimate the genetic distance.

Data analysis and mapping

Gels were interpreted from the digital output and AFLP markers of individual plants were scored manually as dominant markers. Marker names followed the two primers used and the molecular sizes of the amplification products (e.g. E40M62–335), whereas primer numbers referred to their selective nucleotides in alphabetical order (i.e. 31=AAA, 32=AAC etc.).

Given a dominant, monogenic inheritance of *DIP* (*Ddd*, Fig. 1), a presence in simplex (*100*) is expected for AFLP fragments that are in coupling-phase to *DIP*. The AFLP fragments that co-segregated with *DIP* were supposed to be absent from the diploid sexual parent S2–125 (*00*) and present in the tetraploid diplosporous parent PAX (*1000*), as was confirmed by analysing the parental DNAs. Due to the dominant scoring of AFLP markers, the fragments that are present in PAX could, in theory, be present in simplex, duplex, triplex, or quadruplex. This resulted in expected segregation ratios of 1:1 and 5:1 for the presence:absence of AFLP fragments in simplex and duplex, and all presences of fragments in tri- or quadruplex, in the triploid offspring, respectively. The mapping of simplex markers in polyploids is equivalent to back cross (BC) mapping in diploids (Wu et al. 1992). To confirm their presences in simplex, the 1:1 segregation ratios of AFLP fragments that co-segregated with *DIP* were tested with chi-square tests.

Linkage analyses were performed by sorting the data in a binary data matrix in such a way that recombination events were minimised, and by using Joinmap 3.0 (Plant Research International, Wageningen, The Netherlands). For the sorting analysis, only those plants that showed a minimum of two of the markers present or absent, opposed to the other markers considered, were regarded as recombinants and included in the analysis. For the Joinmap analysis, all data was included, the BC population type and Kosambi mapping function were used, and LOD and REC threshold values were set at >1.0 and <0.4, respectively. A final map was drawn on the basis of the Joinmap analysis.

Locus-specific PCR marker development

AFLP amplification products with a minimum length of 200 bp, and located at different loci linked to *DIP*, were selected for sequencing. Three additional selective nucleotides at the *Mse*I-primer sides were determined, and subsequently, the AFLP amplifications were repeated with newly designed *Mse*I+6 and ^{33}P-labelled *Eco*RI+3-primers, using the DNA pools as templates (Brugmans et al. 2003). Fragments were excised from the radioactive gels and re-amplified with the *Mse*I- and *Eco*RI+0 primers of which one or the other was M13-tailed (Brugmans et al. 2003). PCR products were directly sequenced at both ends, using an M13 primer (BaseClear, Leiden, The Netherlands). Sequences were BLASTed in NCBI to identify homologies with sequences from data bases. Locus-specific primer pairs were designed on the basis of the DNA sequences, with the use of the DNAstar software (Lasergene, London, UK; Table 1). Primer pairs were tested by using genomic DNA of the diplosporous and non-diplosporous parents as amplification templates. When PCR products were obtained for both parents, they were analysed for internal polymorphisms with 25 different four base-cutter restriction enzymes (Brugmans et al. 2003), followed by separation on 2% agarose gels.

Table 1 Primer pairs and amplification conditions for PCR markers linked to diplospory

AFLP marker	A-locus	PCR locus	Forward primer (5′→3′)	Reverse primer (5′→3′)	Annealing temp. (°C)	Fragment size (bp)
E41M61–310	A1	S3	GTGACATGTGAGGAGGGTGC	CTGCAACATCTGCTGGCGAC	53	225
E40M60–505	A1	S4	GTCGGCACTCAACTCGCAGC	TGGATTTTGAAATTCCGGTG	53	414
E42M50–440	A3	S7	GAGCGGGTACCTTTAGGGTTGT	GACTTCAATGAAATAATGCGAGAT	53	386
E37M51–495	A3	S8	ACAATTCACGAGGCAACCTG	GTTTCAATCTCCACAGCACG	53	449
		S8b	GTGGCAGGGTGGGAAACTC	CGATCGCCGCCTCCATA	60	348
E35M52–235	A4	S9	TGCTCCCCCTGAGAAGTAGAGTCA	AGAGGCTGCTCCGCAACCAA	53	183
E38M48–215	A4	S10	TTTGTGCTTACCTCGCCATTC	ACAAAATATCAAACACGGAAACG	57	114
		S10b	CCGGTAGTAAGAAGGAAAAT	TCAACATCATAAATAAAACCAATC	57	126
E35M61–515	A6	S11	TTTATTAGAAAATTACTCGGCCAA	TTTATGACACAAAGATAGGGGCAT	53	354
E40M53–450	A9	S14	AGCGACCGAGTTTATGGAGA	CGTGTACAAATTGCCAGGTCTAT	57	c.120

Results

AFLP markers linked to diplospory

A BSA was performed, followed by screening of individual plants, in order to find AFLP markers linked to diplospory (Fig. 2). A total of 256 primer combinations was tested on a "+" pool of eight diplosporous plants, and two "-" pools, each of eight non-diplosporous plants. Each of the primer combinations yielded approximately 25–35 AFLP fragments of length 40–500 bp, resulting in around 7,700 fragments that were considered in this analysis. Forty of the primer combinations showed AFLP fragments that were unique to the "+" pool, and these were used to separately analyse the 24 pool individuals. Twenty-eight of these primer combinations produced one or more fragments that co-segregated with *DIP*, resulting in 34 AFLP markers suitable for mapping the trait.

The parent plants and 73 offspring plants were analysed for the 34 AFLP markers as well as the 2 microsatellite markers that showed linkage to *DIP*, and the results are summarised in Table 2. All 36 markers were confirmed to be present in the diplosporous tetraploid parent PAX and absent in the sexual diploid parent S2–125. Each of the markers fitted a 1:1 segregation ratio (chi-square<3.8; *df*=1) in the offspring, sup-

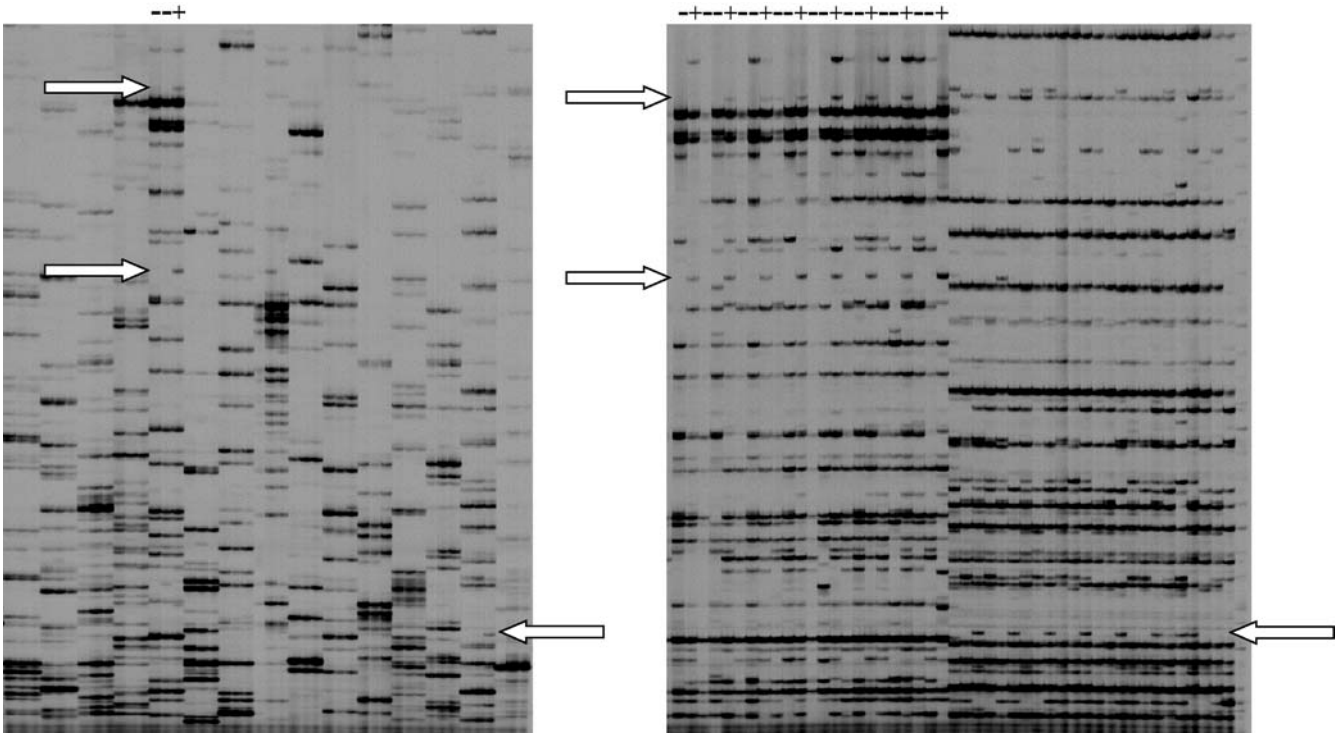

Fig. 2A, B AFLP gels showing the results of primer tests in a bulk segregant analysis, and in the screening of individual plants, in order to find markers linked to diplospory. **A** Two "–" pools, each containing DNAs of eight non-diplosporous plants, and one "+" pool, containing DNAs of eight diplosporous plants, were tested for a total of 256 primer combinations of which 15 are shown here. *Arrows* indicate markers that were candidates for linkage to diplospory. **B** The *lower left* marker showed complete co-segregation with diplosporous individuals, whereas the *upper left* marker as well as the *right* marker were absent in one of the diplosporous and present in one of the non-diplosporous plants

Table 2 Summary of marker distribution among individuals. Numbers in brackets indicate the number of plants (*upper legend*) and markers (*left legend*) with similar distributions. *P* Parent; *(n)D*, (non-)diplosporous; *A* A-locus, with A6 including 2 microsatellite markers. Missing data and plants that showed one marker present or absent opposed to the other markers considered were ignored in the summary given here. The *DIP* phenotype of 18 of the 73 offspring plants was unknown, and is indicated by ?

		P1 n*D*	P2 *D*	F1											
				D (1)	n*D* (1)	n*D* (1)	n*D* (1)	? (1)	n*D* (1)	*D* (1)	*D* (1)	*D* (1)	n*D* (1)	n*D* (1)	n*D* (32)	*D* (30)
A1	(7)	0	1	0	0	0	0	0	0	1	1	1	1	1	0	1
A2	(6)	0	1	0	0	0	0	0	0	1	1	1	1	0	0	1
A3	(3)	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1
DIP	-	0	1	1	0	0	0	?	0	1	1	1	0	0	0,?	1,?
A4	(3)	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1
A5	(1)	0	1	1	1	0	0	0	0	1	1	0	0	0	0	1
A6	(8+2)	0	1	1	1	1	0	0	0	1	0	0	0	0	0	1
A7	(1)	0	1	1	1	1	1	0	0	1	0	0	0	0	0	1
A8	(3)	0	1	1	1	1	1	1	0	0	0	0	0	0	0	1
A9	(2)	0	1	1	1	1	1	1	1	0	0	0	0	0	0	1

porting their presence in simplex in PAX. Eleven of the offspring plants were regarded as recombinants by showing a minimum of 2 of the 36 markers present or absent, opposite to the other markers considered. Another 8 plants showed one marker present or absent, opposite to the other 35 markers analysed, but these were ignored in the summary given in Table 2, since these scores may be artificial rather than recombination events.

Linkage analyses were performed by sorting the data of the 11 recombinant plants (see former paragraph) as well as by analysing all data using Joinmap. In the sorting analysis, the 36 markers clustered into nine loci of between 1 and 10 markers (A1 to A9; Table 2). These loci could be mapped in such a way that a maximum of only one recombination event per plant was necessary, giving the most parsimonious distribution possible of the markers over the *DIP* chromosomal region (Table 2). The results showed a maximum of two recombinations (2.7 cM; Table 2) between subsequently loci, i.e., between A5-A6 and A7-A8. The total map length was 15.2 cM. In the Joinmap analysis, also the plants that showed a single marker present or absent, opposite to the other 35 markers analysed, were considered, resulting in a map of length 18.6 cM (Fig. 3). The marker distribution was largely similar to that of the sorting analysis (indicated in Fig. 3). No markers were found to completely co-segregate with *DIP*. Markers were located on both sides of *DIP*: 16 on one side and 20 on the other, corresponding to 5.9 cM and 12.7 cM, respectively.

Of the 73 plants analysed, 29 were meiotic, 26 were diplosporous, and 18 were of unknown phenotype due to a shortage of flowers for progeny tests. From the AFLP data, the phenotypes of these 18 plants could be deduced: 10 were meiotic and 8 diplosporous. This would result in a total of 39 meiotic and 34 diplosporic plants in the mapping population, confirming the 1:1 segregation ratio of diplosporous:non-diplosporous (chi-square=0.34; df=1).

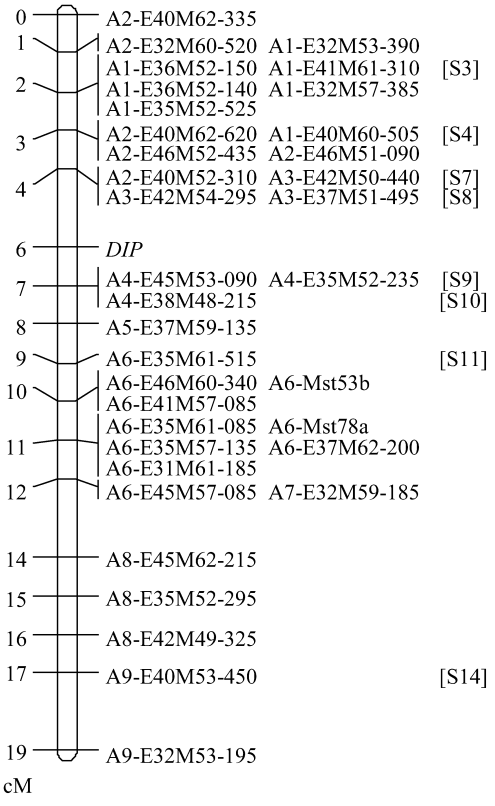

Fig. 3 Map of the *DIP* chromosomal region of *Taraxacum*. The map is based on a Joinmap analysis of 34 AFLP and 2 microsatellite markers in 73 plants of a segregating population. The results according to the sorting anlysis are indicated (A1 to A9; Table 2). Numbers in *brackets* denote the markers selected for PCR marker development (see Table 1). Total map length is 18.6 cM

PCR-based polymorphic markers

Eight AFLP markers, located at different loci linked to *DIP* (indicated in brackets in Fig. 3) were selected for sequencing in order to develop PCR-based polymorphic markers. None of the sequences showed homologies >25 bp with sequences from published databases. For all

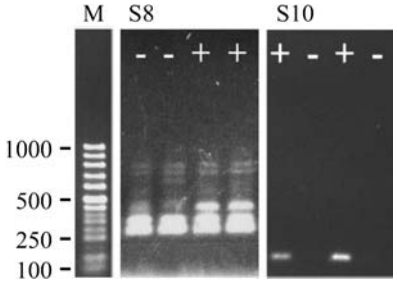

Fig. 4 Agarose gels showing the amplification products of the two markers that were successfully converted into dominant SCARs (see also Table 1; Fig. 3). "+" denotes the diplosporous parent and a pool of 6 diplosporous individuals, respectively; "–" denotes the non-diplosporous parent and a pool of 6 non-diplosporous individuals, and *M* indicates a size-marker (bp)

eight markers, locus-specific primer pairs (Table 1) amplified PCR products of the lengths expected. Longer and shorter PCR products in addition to those expected were obtained for S3 and S8, respectively. Two of the eight markers, S8 and S10, were successfully converted into dominant sequence characterised amplified regions (SCARs; Paran and Michelmore 1993; Fig. 4). Both SCARs showed complete co-segregation with the AFLP markers from which they were derived in an analysis of the mapping population. However, under non-optimal PCR conditions they sometimes generated weak products for non-diplosporous individuals. The second primer pairs at S8 and S10 (Table 1) also amplified single PCR products for the diplosporous individuals, but also occasionally gave weak products for non-diplosporous plants. This indicated sequence divergence at the primer sites rather than hemizygosity at S8 and S10. For the 6 markers that were not converted, the diplosporous and non-diplosporous individuals showed amplification products of the same length with no restriction site polymorphisms for the 25 enzymes tested.

Discussion

No evidence for suppression of recombination at the *DIP* chromosomal region

In the present study, a first molecular map of the diplosporous chromosomal region in *Taraxacum* is presented (Fig. 3). This map is derived from 73 plants of a segregating population and is based on 34 AFLP and 2 microsatellite markers (Table 2). A relatively large number of recombinant plants (11 out of 73; Table 2) showed a more or less equal distribution of markers over the *DIP* chromosomal region (Fig. 3), and no markers were found to completely co-segregate with diplospory. This is in striking contrast to the results found in the other mapping studies of apomixis to date (reviewed in Grossniklaus et al. 2001), that have all shown a cluster of molecular markers strictly linked to the apomeiotic trait and an additional one or two markers at a much larger distance, e.g. 12–18 cM. These studies include another AFLP mapping study of diplospory in the Asteraceae (*Erigeron*; Noyes and Rieseberg 2000), one study of diplospory in *Tripsacum* (Poaceae; Grimanelli et al. 1998) based on restriction fragment length polymorphisms (RFLPs), and several studies of apospory, all concerning grasses, and based on AFLPs (*Brachiaria*, Pessino et al. 1998; *Paspalum* Labombarda et al. 2002), random amplified polymorphic DNAs (RAPDs) (*Pennisetum*, Ozias-Akins et al. 1998) or RFLPs (*Cenchrus*, Gustine et al. 1997; *Brachiaria*, Pessino et al. 1997). The strict linkage of molecular markers to apomeiosis found in these studies was interpreted as suppression of recombination around the apomeiotic loci. In some cases, this was confirmed by comparative mapping in sexual relatives, e.g. markers from maize (Grimanelli et al. 1998; Pessino et al. 1998) and rice (Pupilli et al. 2001; Labombarda et al. 2002) that completely co-segregated with apomeiosis in the apomicts, covered 15–40 cM on the chromosomal segments of their sexual relatives. The general occurrence of suppression of recombination at the apomeiotic loci suggested that this was independent from the type of apomeiosis: diplospory or apospory, its presence in dicots or monocots, and the marker systems used. However, our results contradict this generality, by showing no evidence for suppression of recombination at the *DIP* locus in *Taraxacum*.

One reason for the different results found in these other studies as compared to *Taraxacum* may be that in the other studies the mapping populations originated from wide crosses. For instance, interspecific crosses were used in *Erigeron* (Noyes and Rieseberg 2000), *Tripsacum*, (Grimanelli et al. 1998), and *Pennisetum* (Ozias-Akins et al. 1998), whereas in our study, *Taraxacum* plants were used from within the same section. If suppression of recombination is indeed the result of diverged genomes, this is expected to be the case for the entire genome and not only for the apomixis specific region(s). However, this was not found for *Erigeron* in which suppression of recombination was only detected around the locus for diplospory. For *Tripsacum* and *Pennisetum*, no entire map was generated, but comparative mapping and hemizygosity suggested that wide crosses did not explain suppression of recombination in these species, respectively. Additional studies are necessary to confirm the generality of suppression of recombination at the apomeiotic loci. For *Taraxacum*, a comparative map in a sexual cross is needed to confirm the results, and further fine-mapping of the trait may resolve whether the absence of suppression of recombination also holds for regions closer to *DIP*. An advantage of the occurrence of recombination around the *DIP* locus in *Taraxacum* is that it enables us to perform map-based cloning for diplospory.

The *DIP* chromosomal region lacks obvious sequence divergence

The occurrence of recombination between the *DIP* and non-*DIP* homologous loci, and the detection of only a few polymorphisms in the AFLP marker regions analysed, indicate that there is relatively little sequence divergence at the *DIP* locus in *Taraxacum*. This is also supported by preliminary results from Southern blots using S8 and S9 as probes that showed similar hybridisation patterns for the diplosporous and non-diplosporous parental DNAs. No evidence was found for hemizygosity at the *DIP* locus, as was reported for the loci controlling apospory in *Pennisetum* (Ozias-Akins et al. 1998) and *Paspalum* (Labombarda et al. 2002). Sequence divergence has been suggested to be an evolutionary side-product of long-term asexual reproduction, due to the absence of recombination (Birky 1996, Welch and Meselson 2000). Also, asexuals are expected to accumulate slightly deleterious mutations in small populations due to chance (Muller 1964), and inefficient purging in infinite populations (Kondrashov 1982). Both sequence divergence and deleterious mutations are expected to accumulate around the apomixis genes, because closely linked chromosomal regions have a long asexual history, spanning multiple clonal generations (Van Dijk 2003). In contrast, in chromosomal regions that are not linked to apomixis genes, sequence divergence and deleterious mutation accumulation may decrease as a result of repeated back-crosses with sexual relatives.

Apomixis is almost completely absent in diploid species, and evidence has been found in several species for segregation distortion of apomixis loci (Grossniklaus et al. 2001) or lethal post-zygotic mechanisms (Bicknell et al. 2000). This can be explained by the presence of deleterious mutations around the loci for apomixis. In *Taraxacum*, no apomictic diploids are known, and microsatellite alleles that are linked to *DIP* showed severe segregation distortion in haploid pollen grains, but not in diploid ones (P.J. Van Dijk, unpublished), which may be explained by the expression of recessive lethality. Although expected, the present study shows no obvious evidence for sequence divergence between the *DIP* and non-*DIP* homologous loci in *Taraxacum*. One possible explanation for this is that apomixis is of relatively recent age in *Taraxacum*. Comparative sequencing of DNA stretches at different loci linked to *DIP* will be performed to gain more insight into the extent of sequence divergence at the *DIP* locus in *Taraxacum*. In addition, further fine-mapping of *DIP* may shed light on the accumulation of mutations closer to *DIP*.

The location of *DIP* in the *Taraxacum* genome

The summary of results found in this study indicate that *DIP* is not located in an recombinationally suppressed region or a unique DNA segment. This excludes heterochromatin-rich regions such as the centro- or telomeres, and DNA segments that are inverted, translocated or introgressed from another species. The fact that markers were found on both sides of *DIP* also suggests that *DIP* is not located near a recombinationally suppressed region or on the distal part of a chromosome. According to a previous study, *DIP* co-segregates with one of the NOR-chromosomes in *Taraxacum* (Van Dijk and Bakx-Schotman 2003). These chromosomes are particularly heterochromatin-rich and the arms that carry the satellites undergo no or only minor recombination. According to the present results, *DIP* is then likely to be located on the arm that does not carry the satellite. A more precise localisation of *DIP* should be investigated by physical mapping of the trait.

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