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Sex-linked AFLP markers indicate a pseudoautosomal region in hemp (*Cannabis sativa* L.)

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Abstract In dioecious plants of hemp (*Cannabis sativa* L.), males are regarded as heterogametic XY and females as homogametic XX, although it is difficult to discriminate the X cytologically from the Y. The Y chromosome is somewhat larger than the X. Our aim was to analyse AFLP markers on X and Y, and to use them to gain some insight into the structure of the sex chromosomes. Markers located on the sex chromosomes can be grouped into different classes, depending on the presence or absence of a fragment on the X and/or the Y. They are detected by separately analysing male and female progenies of a single cross. Five markers were found to be located on both chromosomes. A few recombinants were observed for marker pairs of this class in the male progenies. Two completely linked markers located on the Y chromosome in the male parent show a recombination rate of $r = 0.25$ with sex. Recombination must have occurred between the sex chromosomes in the male parent. The recombination analysis led to the conclusion that there is a pseudoautosomal region (PAR) on the sex chromosomes, allowing recombination between the X and the Y chromosome. The other regions of the sex chromosomes show only a few recombination events, for the Y as well as for the X. These results are discussed in comparison to other dioecious plants.

Keywords Hemp · Recombination · Pseudoautosomal region · Sex chromosomes · AFLP

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Introduction

While in higher animals sex dimorphism is normally the case, several modes of sexuality exist in plants. The majority of higher plants have perfect flowers with male and female organs in close proximity. Several plants, among them *Zea mays*, are monoecious with separate male and female flowers on the same plant. Only 3% of the angiosperms and 25% of the gymnosperms are dioecious, with male and female plants (Longo 1994). Examples of dioecious angiosperm plants are *Salix viminalis* and many other *Salicaceae*, *Silene latifolia* (syn. *Melandrium album*), *Spinacea oleracea*, *Urtica dioica*, *Viscum album*, *Mercurialis annua*, *Asparagus officinalis*, *Cannabis sativa* and *Humulus lupulus*. Some are used as crops, and often one sex is preferred, such as the male sex in asparagus (increased vigour) and hemp (fibre quality and quantity) or the female sex in hop (unpollinated flowers for brewing) and in spinach (retarded shooting). In contrast to *Melandrium*, hemp expresses sexual dimorphism. Female hemp plants are more compact than males and, in particular, the inflorescences of females are more squat and leafy.

In hemp, monoecious and other types with mixed sexuality exist as well as the dioecious forms, as is true in many other dioecious plant species (Hoffmann 1961). Such types will not be considered here. So far it has not been known which factors are responsible for sex expression in hemp and at which stages the expression of one sex is suppressed or promoted. Mohan Ram and Nath (1964, cited from Dellaporta and Calderon-Urrea 1993) found that in *C. sativa*, male and female flowers differ radically in morphology and size, and show no evidence of the missing sex. Dellaporta and Calderon Urrea (1993) conclude that in *Cannabis*, sex determination must occur very early in the developing flowers. In *Mercurialis* a similar situation was found, the unisexual flowers being devoid of rudiments of organs of the opposite sex (Durand and Durand 1991, cited from Dellaporta and Calderon-Urrea 1993).

In dioecious plants, species with cytologically dimorphic sex chromosomes can be distinguished from species carrying sex loci on homomorphic chromosomes. A well-investigated plant with dimorphic sex chromosomes and an XY mechanism is *S. latifolia* (Westergaard 1953; Ciupercescu et al. 1990), the male sex being heterogametic. The Y chromosome is larger than the X, which is in sharp contrast to mammals which have a much smaller Y chromosome. In hemp, Sakamoto et al. (1998) also found a small, but significant, increased size of the Y chromosome. The structure of sex chromosomes has been studied in *S. latifolia* by Westergaard (1953). He showed that the X and Y carry a homologous segment comparable with the pseudoautosomal region (PAR) of mammals (Burgoyne 1982; Rappold 1993). Genetically, two genes *M* and *F* are responsible for the sex expression; *MF/mf* plants are male, *mf/mf* plants female. No recombination could be observed between the two loci. *M* is the male-determining factor, *F* the female-suppressing factor, both located in a dominant condition on the Y. But male as well as female plants need at least one X chromosome, since from the anther culture of male plants only haploid female plants with an X, but no male plants carrying a Y, could be developed (Guttmann and Charlesworth 1998). This situation is quite different from *A. officinalis*. In this species, sex determination occurs very late and *Asparagus* shows no cytological dimorphism. The sex is controlled by an XY mechanism located on chromosome 5 (Löptien 1979) with XY as males, but in some cases pistillate organs appear on XY plants allowing them to generate viable YY male progeny (Longo 1994). Antcliff (1980) postulated three alleles, *M*, *H* and *F*, at a single locus for sex determination in *Vitis* with *M* dominant over *H* and *F*, and *H* dominant over *F*. Male types are either *MH* or *MF*, hermaphrodite types *HH* or *HF* and females *FF*. A locus controlling sex in *Vitis* was mapped by Dalbó et al. (2000) on linkage group 14 in a cross between the hermaphrodite cultivar 'Horizon' and Illinois 547-1, a genotype highly resistant to several fungal diseases, but forming only male flowers.

In hemp, markers have been found which are apparent only in male plants, and they co-segregate completely with the male sex. This supports the hypothesis that the male sex is heterogametic with specific markers on the Y chromosome (Mandolino et al. 1998; Flachowsky et al. 2001). However, sex-linked markers showing other patterns are necessary in order to draw conclusions about the structure of the chromosome. This paper is the first report on the analysis of several types of sex-linked AFLP markers giving genetic information of the sex chromosomes in hemp. These results will be compared with those from other dioecious plants.

Materials and methods

Plant materials

The accession Can18, taken from the germplasm collection of *C. sativa* L. at the IPK Gatersleben (Germany), has been used for this study. The progeny, consisting of 47 female and 33 male plants of the cross of a single female plant with a single male plant of this accession, were used for marker analysis. The progenies were grown in the greenhouse and sex was determined several times during flowering by visually scoring the inflorescences. Details have already been described by Flachowsky et al. (2001).

Marker analysis

AFLP analysis was performed for the two parental plants and for the progeny. *Hin*dIII and *Mse*I were chosen as restriction enzymes for AFLP. Analyses were performed on an ALFexpress sequencer (Amersham-Pharmacia, Freiburg). Details are given by Flachowsky et al. (2001). Primer combinations are characterized by the selective nucleotides of the *Hin*dIII-primer (marked with *), the selective nucleotides of the *Mse*I-primer and the time of band appearance (for example: AGA*AAT237). The complete primer designations are given in Tables 1 and 4, else only the selective nucleotides of the *Mse*I-primer and the time of band appearance, sufficient for identification, were noted.

Since the population was an intercross, markers with segregation ratios of 3:1 and 1:1 are expected simultaneously for the dominant AFLP markers, depending on the genotypes of the parents (Schumacher et al. 1997). These segregation ratios correspond to the F_2 situation (both parents heterozygous) and the backcross situation (one parent heterozygous, the other recessive). The mapping program Joinmap 1.3 (Stam 1993) has been used for the calculation of recombination values and the construction of linkage groups. Only markers of linkage groups located on the sex chromosomes are considered in detail. They can be detected by different segregation ratios in male and female progenies, or by linkage to those markers, and can be divided into five possible classes (see Table 1), due to the genotype of the parents and the segregation ratios in the female and male progenies. Sex-linked markers can be detected if the Y chromosome contains a marker not found on the X (class E), or the marker on X is polymorphic with no fragment on the Y (classes A, B and C), or else that the marker is polymorphic on the X and present on the Y (class D). Class B is detected by linkage to A in the male and to D in the female progenies (see below). The marker types of the parents serve as a check. All other genotypes of the parents for sex-linked AFLP markers are not informative, since all progenies show the same phenotype.

Results

Segregation of sex linked markers

Sixteen primer combinations yielded a total of 199 polymorphic AFLP markers, out of which 66 (33.2%) and the male sex were mapped on the sex chromosomes. Most AFLP markers could be grouped in four different classes (Table 1). For all markers the expected parental types could be verified.

Class A contains all the markers polymorphic in the X, heterozygous in the female plant and present on the X chromosome of the male parent. A 1:1 segregation ratio is expected in male progenies, while all female progenies will show the fragment as long as there is no recombination with sex in the male parent. Since the female

Table 1 Five possible marker classes on sex chromosomes linked with sex, genotypes of the parents, the expected segregation ratios in female and male progenies and AFLP markers found in hemp. Markers in italics form completely co-segregating groups. Markers in bold showed single recombinants. + fragment present, – fragment absent

Class	Parents				Allelic constitution of progenies								Expected segregation ratios		AFLP markers
	♀		♂		♀				♂				♀	♂	
	X_1	X_2	X_3	Y	X_1	X_3	X_2	X_3	X_1	Y	X_2	Y	+:–	+:–	
A	+	–	+	–	+	+	–	+	+	–	–	–	1:0	1:1	*AGA*AAC262*, *AGA*AGA109*, *ACC*ATC157*, *AGA*ATA490*, **AGA*AAT237, AGA*ACC142, AGA*AGA425**
B	+	–	–	–	+	–	–	–	+	–	–	–	1:1	1:1	*AGA*AGG176*, *AGA*ATA317*, *AGA*GAA154*, **AGA*AAG220, AGA*AAT239, AGA*AAT497, AGA*GAC103, AGG*ATG122**
C	–	–	+	–	–	+	–	+	–	–	–	–	1:0	0:1	–
D	+	–	–	+	+	–	–	–	+	+	–	+	1:1	1:0	*AGA*AAC158*, *AGA*ACA233*, **AGA*AGT96, AGA*ATA176**
E	–	–	–	+	–	–	–	–	–	+	–	+	0:1	1:0	*SEX*, *43 other markers*, **ACC*ATC245**

Table 2 Markers in classes A, B, D and E showing single recombinants with other markers of the same class in female and male progenies

Class	Marker 1	Marker 2	Phenotype of progenies							
			Female				Male			
			++[a]	+ –[a]	– +[a]	– –[a]	++	+ –	– +	– –
A	AAC262	AAT237	47	0	0	0	17	0	1	15
		ACC142	47	0	0	0	17	1	0	15
		AGA425	45	2	0	0	17	0	0	14
	AAT237	ACC142	47	0	0	0	16	1	1	15
		AGA425	45	2	0	0	17	0	0	14
	ACC142	AGA425	45	2	0	0	16	0	1	14
B	AGG176	AAG220	21	1	4	21	11	2	4	14
		AAT239	22	0	0	25	15	0	1	17
		AAT497	20	2	0	25	14	1	0	18
		GAC103	21	0	0	24	15	0	1	17
		ATG122	21	1	0	25	15	0	0	18
	AAG220	AAT239	21	4	1	21	13	3	2	14
		AAT497	20	5	0	22	11	5	2	14
		GAC103	20	4	1	20	13	3	2	14
		ATG122	21	4	0	22	12	4	2	14
	AAT239	AAT497	20	2	0	25	14	2	0	17
		GAC103	21	0	0	24	16	0	0	17
		ATG122	21	1	0	25	15	1	0	17
	AAT497	GAC103	19	0	2	24	14	0	2	17
		ATG122	20	0	1	26	14	0	1	18
	GAC103	ATG122	20	1	0	24	15	1	0	17
D	AAC158	AGT96	20	2	2	23	32	1	0	0
		ATA176	21	1	2	23	30	2	0	0
	AGT96	ATA176	22	0	1	24	30	1	0	1
E	Sex	ATC245	0	0	0	46	32	1	0	0

[a]+ +both markers present, + – only marker 1 present, – + only marker 2 present, – – both markers absent, some individual data could not be determined

parent was heterozygous, coupling and repulsion between different markers of this class is possible, but all eight markers detected were in coupling phase. AAC262, AGA109, ATC157 and ATA490 co-segregated completely (Table 1). Single recombinants were found in pairs with markers AAT237, ACC142 and AGA425 (Table 2). For the co-segregating group only AAC262 is listed (Table 2).

Class B can only be identified by linkage analyses with markers of classes A and D, and is discussed later. As for class A, single recombinants were found between markers within this class (Table 2). AGG176 represents the co-

segregating group AGG176, ATA317 and GAA154 (Table 1).

No markers for class C were found. These markers should only be on the X chromosome of the male parent and therefore present in female progenies only. It is the classical case of sex reversal between parents and progenies.

Markers of class D must contain a fragment on one X chromosome in females and the Y chromosome in males, these markers probably indicate a pseudoautosomal region (PAR). They are heterozygous for the female parent. For pairs of markers within class D all male progenies showed both fragments, apart from a few recombinations in pairs with markers AGT96 and ATA176 (Table 2). The linkage phase of the female parent can be analysed in female progenies since the X chromosome of the male parent does not contain the AFLP fragment. The four markers were in coupling. AAC158 and ACA233 co-segregated completely (Table 1), in Table 2 only AAC158 is given.

Many markers of class E are male-specific; they are completely linked with sex. Some have already been described by Flachowsky et al. (2001). One marker (ATC245) showed a single recombinant with sex (Table 2).

Linkage between marker classes

All markers of the classes A, D and E show different segregation ratios in male and female progenies, indicating their location on the sex chromosomes (Table 1). However, their order can only be derived if linkage between marker classes is considered. The results are summarized in Table 3. To simplify discussion, the marker classes are represented by typical markers only. These are, AAC262 for class A, AGG176 for class B, AAC158 for class D and sex for class E. For these markers, the segregation ratios in male and female progenies are given.

(1) Class A with B: the female parent could be in the coupling or repulsion phase, since it is heterozygous for both markers. No recombination was observed between class A markers and sex (class E) in the male parent. Therefore all female progenies must contain the fragment for A, coming from the male parent. The linkage phase of the female parent can be studied in male progenies, since the male parent carried no fragment on the Y chromosome for both markers. These markers were found to be in the repulsion phase.

(2) Class A with D: no recombination between sex (Class E) and A or D, respectively, was found in the male parent. Linkage phases between A and D cannot be analysed since all plants in female progenies show the fragment for A and all plants in the male progenies show the fragment for D.

(3) Class A with E (sex): the female parent is not informative, since E is not present on the X chromosomes. As already mentioned, no recombination between A and

Table 3 Linkage between different marker classes

Class		Marker phenotype		Female progenies				Male progenies				Expected genotype		Information content of parents		
		Female parent	Male parent									Female parent	Male parent	Female parent		Male parent
1	2			++[a]	+–[a]	– +[a]	– –[a]	++	+–	–+	– –	(X_1/X_2)	(X_3/Y)	Female progeny	Male progeny	Both progenies
A	B	++	+–	22	25	0	0	0	18	15	0	+–/–+	+–/– –	n.i.[b]	Repulsion	n.i.
A	D	++	++	22	25	0	0	18	0	15	0	+–/–+	+–/–+	n.i.	n.i.	Repulsion
A	E	+–	++	0	47	0	0	18	0	15	0	+–/– –	+–/–+	n.i.	n.i.	Repulsion
B	D	++	–+	22	0	0	25	15	0	18	0	– –/++	– –/–+	Coupling	n.i.	n.i.
B	E	+–	–+	0	22	0	25	15	0	18	0	– –/+–	– –/–+	n.i.	n.i.	n.i.
D	E	+–	++	0	22	0	25	33	0	0	0	– –/+–	– –/++	n.i.	n.i.	Coupling

[a] ++ markers of both classes present, +– only markers of class 1 present, –+ only markers of class 2 present, – – markers of both classes absent
[b] n.i. not informative

Table 4 Segregation of markers GAA510, AAT330 and AAC109

Marker	Phenotypes of					
	Female parent	Male parent	Female progeny		Male progeny	
			+	–	+	–
AGA*GAA510	–	+	11	36	24	9
AGA*AAT330	–	+	11	36	24	9
AGA*AAC109	–	+	23	23	33	0

E was observed in the male parent, so that all female progenies carried the fragment for A.

(4) Class B with D: no recombination between class D markers and sex was found in the male parent. The linkage phase of the female parent could be analysed in the female progenies, since it is heterozygous for both markers. All markers were found to be linked in the coupling phase.

(5) Class B with E (sex): neither parent was informative since the female parent did not contain a fragment for E, nor did the male parent for B.

(6) Classes D with E (sex): the female parent is not informative, and no recombination occurred in the male parent, as has already been mentioned, so that all male progenies carried the fragment for D.

Arrangement of classes within the sex chromosomes

The results presented so far can now be used to formulate a hypothesis on the structure of the sex chromosomes. A few recombinants were found within marker classes A, B, D and E (Table 2). However, the number of recombinants was too small to allow mapping within classes. The markers printed in bold (Table 1) are not included in the following discussion, so that recombination within classes need not be regarded.

The female parent was heterozygous for classes A, B and D (Table 1). A and B were in repulsion phase, B and D in coupling phase. Therefore the linkage phase between A and D, though not directly determined, was in repulsion phase. The male parent showed a fragment on X only for A and on Y only for D and E, indicating repulsion between A and D as well as between A and E, but coupling between D and E (Table 3). No recombination was found. In both parents A, B and D formed a linkage group with almost no recombination, but with a fragment common to X and Y in class D.

In the male parent, class B markers could not be linked since they were not informative. No recombination was observed between markers of the other classes.

Further markers on the sex chromosomes

The markers described above showed the typical sex behaviour, i.e. almost no recombination, although markers of class D have to be present on both sex chromosomes to fit the segregation ratios. Besides these, three

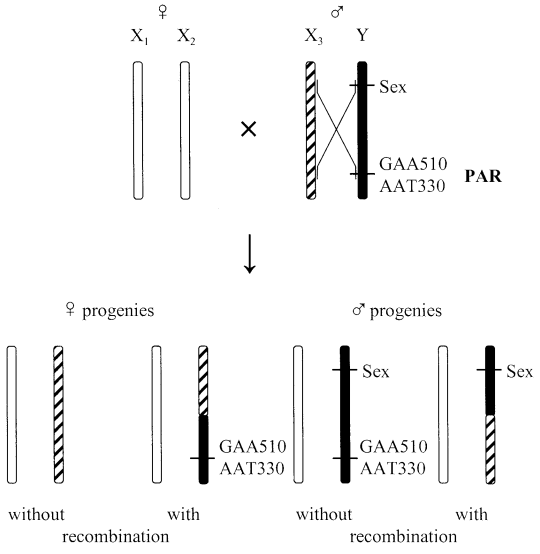

Fig. 1 Schematic graph of recombination between the sex chromosomes. Recombination was observed between GAA510 and AAT330 and sex, indicating a pseudoautosomal region (PAR)

AFLP markers were found which have to be located on the sex chromosomes, showing deviations in the segregation ratios. Two of these markers, AAT330 and GAA510, are co-segregating; the third marker is AAC109. The situation for these markers in the parents as well as in the male and female progenies is described in Table 4. Without the information of the parents, marker AAC109 would belong to class D, due to its segregation in the female progeny only, but the female parent lacks that specific fragment (Table 4) in contrast to class D markers (Table 1).

Markers GAA510 and AAT330 showed reciprocal differences in the segregation ratios in the male and female progenies. This observation indicates recombination between these markers and the sex locus, i.e. recombination between the X and the Y chromosome of the male parent (Fig. 1). These markers are in coupling phase with sex in the male parent.

Table 5 shows the relationships between the four marker classes of sex-linked markers and the markers GAA510 and AAC109. The results for AAT330 are identical to those for GAA510. The female parent is not informative (no fragment). The observed frequencies of all progenies can be explained only by a recombination rate of $r = 0.25$ between GAA510 and other sex-linked markers. Remarkable differences in the segregation ratio

Table 5 Segregation of marker classes of sex chromosomes with markers GAA510 and AAC109

Marker class 1	Marker class 2	Phenotype		Female progenies				Male progenies				Expected genotype			
		Female parent	Male parent	++[a]	+–[a]	–+[a]	– –[a]	++	+–	–+	– –	Female parent X_1/X_2		Male parent X_3/Y	
A	GAA510	+–	++	11	36	0	0	13	5	11	4	+–/– –		+–/–+	
B	GAA510	+–	–+	7	15	4	21	11	4	13	5	– –/+–		– –/–+	
D	GAA510	+–	++	7	15	4	21	24	9	0	0	– –/+–		– –/++	
E	GAA510	– –	++	0	0	11	36	24	9	0	0	– –/– –		– –/++	
												X_1/X_2	a_1/a_2*	X_3/Y	a_3/a_4*
A	AAC109	+–	++	23	23	0	0	18	0	15	0	+–/– –	–/–	+–/–+	+/–
B	AAC109	+–	–+	11	11	12	12	15	0	18	0	– –/+–	–/–	– –/–+	+/–
D	AAC109	+–	++	11	11	12	12	33	0	0	0	– –/+–	–/–	– –/++	+/–
E	AAC109	– –	++	0	0	23	23	33	0	0	0	– –/– –	–/–	– –/++	+/–

[a] ++ markers of both classes present, +– only markers of class 1 present, –+ only markers of class 2 present, – –markers of both classes absent

Marker AAC109 absent (–/–) on the autosomal chromosome pair "a_1/a_2" in the female parent and heterozygous present (+/–) on the autosomal chromosome pair "a_3/a_4" in the male parent

between male and female progenies were also found for marker AAC109. The fragment present in the female progeny must be inherited from the male parent. The 1:1 segregation ratio indicates that marker AAC109 is not linked to sex; on the other hand, all male progenies expressed this fragment, indicating complete linkage with sex (Table 4).

These findings can be explained if this marker represents two different loci: one locus on the Y chromosome linked completely in coupling to sex, and a second locus being located on a sex chromosome with free segregation or on an autosomal chromosome. The male parent has to be heterozygous for the second locus (Table 5, on chromosome "a_3"), whereas the female parent shows no fragment. The expected 1:1 segregation can be proved in the female progeny only, because the locus on the Y is inherited with the Y chromosome in all male progenies additionally to "a_3". The relationships between the four marker classes of sex-linked markers and AAC109 support the hypothesis of two loci for this marker (Table 5).

Discussion

In the present case information was available for parents and offspring only. A complete linkage analysis would have required three consecutive generations, but the information on the grandparents was missing. Conclusions were drawn from the observed segregation ratios and the marker analysis of the parents. In dioecious plant species such as hemp, sex-linked markers can be detected if segregation ratios in male and female progenies differ. The main conclusions were:

(1) There was recombination between X and Y in the male parent between sex and markers AAT330 and GAA510, respectively, so that in this region homology between X and Y is assumed, indicating a pseudoautosomal region. Single recombinants between sex and ATC245 (class E), and AGT96 and ATA176 (class D), were also observed (Table 1).
(2) Markers were detected showing a fragment in one of the X chromosomes of the female parent and the Y chromosome of the male parent (class D), indicating partial homology between the Y chromosome and the X chromosome.
(3) Markers AAT330 and GAA510 are not Y chromosome-specific, though the fragments must have been on the Y chromosome of the male parent before meiosis. Due to recombination they can be transferred to the X chromosome.

Sex-linked RAPD markers, one on X and one on Y, were detected by Harvey et al. (1997) in *Actinidia chinensis*, a species closely related to the kiwi. They observed the expected inversion of sex in the progenies, when the X-linked marker is present only in the male parent. They also observed rare recombination events. However, the small number of plants did not allow them to analyse this in more detail. No inversion for a female-linked RAPD marker was observed by Hormaza et al. (1994) in *Pistacia vera*. They analysed the parents and the progenies, and found a specific RAPD marker, but solely in females. No explanation was given by the authors, but the easiest explanation would be that in this case the female was heterogametic as in some polyploid *Fragaria* species (Bauer and Gruber 1962) and the Aves.

The literature clearly distinguishes dimorphic sex chromosomes determining sex and homomorphic sex chromosomes with sex-controlling genes. The classical example for dimorphism is *S. latifolia* (Westergaard 1958). In hemp, no clear answer is possible, although Sakamoto et al. (1998) found differences in the morphology of the sex chromosomes; X was submetacentric and Y was subtelocentric with a satellite in the terminal part of the short arm and a small, but significant, difference in size, the Y chromosome being extended in length. It is possible that our class E is part of this extension.

A. chinensis as well as *P. vera* are regarded as homomorphic with sex-controlling genes only. A similar situation is found in *A. officinalis* (Rick and Hanna 1943; Löptien 1979) and *Vitis* (Antcliff 1980), and probably also in *Atriplex garrettii* (Ruas et al. 1998). Sex loci could be integrated into linkage maps of asparagus (Spada et al. 1998) and grapevine (Dalbó et al. 2000). Seefelder et al. (2000) presented a short map for both sex chromosomes in the hop. Male-specific segments were sequenced in *A. garrettii* (Ruas et al. 1998) and *S. latifolia* (Guttmann and Charlesworth 1998; Delichère et al. 1999).

Recombination between molecular markers mapped on sex chromosomes has been reported for *Silene dioica* by Di Stilio et al. (1998). In a progeny of 33 plants from a cross between two plants they found five recombinants between a marker on the X and another on the male-specific segment on the Y chromosome of the male parent. The situation in hemp is different since we observed recombination between markers with fragments on the Y (class E). In our case, recombination of the markers AAT330 and GAA510 with other marker classes could also be detected; namely with class A, where the fragment is on one X chromosome of the female parent and on the X chromosome of the male parent, as well as with class D, where the fragment is on one X chromosome of the female parent and on the Y of the male parent. A complete linkage analysis requires three consecutive generations. Di Stilio et al. (1998) concluded that the markers present on X and Y simultaneously indicate a homologous region of the sex chromosomes, a so-called pseudoautosomal region (PAR). Such a segment has already been described by Westergaard (1953) for the sex chromosomes of *S. latifolia*. PARs are known in mammals, including humans (Burgoyne 1982). In humans, two PARs have been described on the short (X_p/Y_p) and the long (X_q/Y_q) arm of the sex chromosomes (Rappold 1993; Crow 2000). In humans non-combining regions on the Y chromosome (NRY) contain regions similar to counterparts on the X chromosome, though recombination is inhibited (Tilford et al. 2001). The situation in hemp is as follows. Single recombinant genotypes were found for marker pairs within the same class (Table 2) and between classes. Classes D and E allowed detection of recombination between the X and Y, since the male parent carried the fragment on the Y chromosome. Male progenies without the fragment were recombinants. A pairing of the hemp sex chromosomes at meiotic prophase I of pollen mother cells was reported by Sakamoto et al. (2000). Their cytological observations promote the molecular results obtained.

Apparently there is some similarity between *S. latifolia* and *C. sativa*: an increased length of the Y chromosome (Westergaard 1953, Sakamoto et al. 1998), although this is not so clear in hemp and is questioned by other authors (Westergaard 1958; Durrand and Durrand 1990), as is the existence of a pseudoautosomal region. But there are differences, too. In hemp, monoecious forms also exist and sex-linked secondary characters are found. According to Dellaporta and Calderon-Urrea (1993) flower differentiation starts earlier in *C. sativa* and resembles more the situation in *M. annua*.

As already mentioned, single recombinants were detected within classes A, B and D. In larger populations it will be possible to map these loci within the main classes and to compare the recombination rates in X and Y. This will give more insight into the fine structure of sex chromosomes in hemp.

The results obtained for marker AAC109 can be explained only if the male parent carried two loci for this marker. Two explanations are possible. It is most likely that two different fragments with the same size were amplified with the respective primer combination. One fragment segregating completely with sex and an independent fragment on an autosomal chromosome of the male parent. A second explanation may be that the locus is duplicated, again one locus on the Y chromosome segregating completely with sex and the other on a sex chromosome with free segregation or on an autosomal chromosome. Li et al. (2000) reported that microsatellites detected two different loci, but the two loci were completely linked. The situation that a specific AFLP fragment represents not only one but two independent loci may explain distorted segregation ratios for markers of mapping populations.

For future analysis of the hemp sex chromosomes we will attempt to convert some important markers, e.g. GAA510, into SCAR markers to facilitate the application of markers and we plan to visualize specific regions on the sex chromosomes by FISH.

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