Cloning and characterization of dispersed repetitive DNA derived from microdissected sex chromosomes of *Rumex acetosa*

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Abstract: *Rumex acetosa* is characterized by a multiple chromosome system ($2n = 12 + XX$ for females, and $2n = 12 + XY_1Y_2$ for males), in which sex is determined by the ratio between the number of X chromosomes and autosome sets. For a better understanding of the molecular structure and evolution of plant sex chromosomes, we have generated a sex chromosome specific library of *R. acetosa* by microdissection. The screening of this library has allowed us to identify 5 repetitive DNA families that have been characterized in detail. One of these families, DOP-20, has shown no homology with other sequences in databases. Nevertheless, the putative proteins encoded by the other 4 families, DOP-8, DOP-47, DOP-60, and DOP-61, show homology with proteins from different plant retroelements, including polyproteins from Ty3-*gypsy*- and Ty1-*copia*-like long terminal repeat (LTR) retroelements, and reverse transcriptase from non-LTR retroelements. Results indicate that sequences from these 5 families are dispersed throughout the genome of both males and females, but no appreciable accumulation or differentiation of these types of sequences have been found in the Y chromosomes. These repetitive DNA sequences are more conserved in the genome of other dioecious species such as *Rumex papillaris, Rumex intermedius, Rumex thyrsoides, Rumex hastatulus,* and *Rumex suffruticosus*, than in the polygamous, gynodioecious, or hermaphrodite species *Rumex induratus*, *Rumex lunaria, Rumex conglomeratus*, *Rumex crispus*, and *Rumex bucephalophorus*, which supports a single origin of dioecious species in this genus. The implication of these transposable elements in the origin and evolution of the heteromorphic sex chromosomes of *R. acetosa* is discussed.

Key words: Rumex acetosa, sex chromosomes, microdissection, evolution, retroelements.

Résumé : Le *Rumex acetosa* est caractérisé par un système chromosomique multiple ($2n = 12 + XX$ chez les femelles, $2n = 12 + XY_1Y_2$ chez les mâles), où le sexe est déterminé par le ratio entre le nombre de jeux de chromosomes X et d'autosomes. Afin de mieux connaître la structure moléculaire et l'évolution des chromosomes sexuels chez les plantes, les auteurs ont généré une banque génomique spécifique des chromosomes sexuels par microdissection chez le *R. acetosa*. Le criblage de cette banque a permis d'identifier cinq familles d'ADN répétitif qui ont été caractérisées en détail. Une de ces familles, DOP-20, ne montre aucune homologie avec les séquences présentes dans les bases de données. Par contre, les protéines codées par des membres des quatre autres familles (DOP-8, DOP-47, DOP-60 et DOP-61) montrent de l'homologie avec des protéines de divers rétroéléments chez les plantes, incluant les polyprotéines des rétroéléments à LTR Ty3-*gypsy* et Ty1-*copia* ainsi que la transcriptase inverse des rétroéléments sans LTR. Ces résultats indiquent que des séquences appartenant à ces cinq familles sont dispersées à travers tout le génome tant chez les mâles que les femelles. Aucune accumulation ou différenciation appréciable de ces types de séquences n'ont été trouvées chez les chromosomes Y. Ces ADN répétitifs sont davantage conservés dans les génomes d'autres espèces dioïques (*Rumex papillaris*, *Rumex intermedius*, *Rumex thyrsoides*, *Rumex hastatulus* et *Rumex suffructicosus*) qu'elles ne le sont chez les espèces polygames, gynodioïques ou hermaphrodites (*Rumex induratus*, *Rumex lunaria*, *Rumex conglomeratus*, *Rumex crispus* et *Rumex bucephalophorus*). Cette différence supporte l'hypothèse d'une origine unique pour toutes les espèces dioïques au sein du genre *Rumex*. L'implication de ces éléments transposables dans l'origine et l'évolution des chromosomes sexuels hétéromorphes chez le *R. acetosa* est discutée.

Mots clés : Rumex acetosa, chromosomes sexuels, microdissection, évolution, rétroéléments.

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Introduction

Approximately 10% of flowering plants develop unisexual flowers. These plants are characterized by the production of female or male flowers in the same individual (monoecy) or in separate individuals (dioecy). Dioecious plants constitute some 5% of flowering plants, but they are widely distributed in different taxa, being present in 75% of families and in 7% of genera (Renner and Ricklefs 1995). This implies that dioecy is a recent evolutionary event that has originated repetitively in different families of plants. In many dioecious plants, sex is regulated by a number of genes located on homomorphic chromosomes (Vyskot and Hobza 2004). Despite their recent origin, however, there is a reduced number of dioecious species with heteromorphic sex chromosomes, similar to those found in animals. Such is the case of the model dioecious plants *Silene latifolia* and *Rumex acetosa. Silene latifolia* has a sex chromosome system in which females are XX and males XY, representing a group of species in which sex is determined by a dominant Y chromosome (Grant et al. 1994). *Rumex acetosa*, on the other hand, has a complex XX/XY_1Y_2 sex chromosome system, and represents another group of plants in which sex determination is not dependent on the Y chromosomes, but regulated by the ratio between the number of X chromosomes and autosomes (X:A balance) (Ainsworth et al. 1999, 2005).

It has been postulated that heteromorphic sex chromosomes have evolved from a pair of autosomes that have acquired sex-determining genes and suppressed recombination (Charlesworth 2002). The suppression of meiotic recombination is expected to promote the increase of deleterious mutations and the accumulation of repetitive DNA sequences and retroelements, causing the Y chromosome to degenerate and diverge from the X chromosome. In the homomorphic sex chromosomes of papaya, it has been recently observed that around the sex-determining genes, there is a region comprising ~10% of the chromosome in which sex chromosomes showed a severe suppression of recombination, as well as DNA sequence divergence (Liu et al. 2004). This nonrecombining region is also characterized by a lower gene density overall, but a higher density in repeated sequences, including retroelements in the incipient Y chromosome (Liu et al. 2004). As occurs with the human Y chromosome (Skaletsky et al. 2003), this degeneration process is expected to affect almost the whole Y chromosome of plants with heteromorphic sex chromosomes.

The accumulation of repetitive DNA families seems to be a common mechanism associated with differentiation of Y chromosomes in both animals and plants. The accumulation of satellite DNA sequences would explain the heterochromatic nature of many Y chromosomes such as those of humans and *Drosophila*. Moreover, accumulation of retroelements has been found in the non recombining heterochromatic Y chromosomes of *Drosophila melanogaster* and *Drosophila simulans* (Junakovic et al. 1998; Bachtrog 2003). In plants, with a more recent origin of sex chromosomes, it is possible to find different degrees of Y chromosome degeneration and accumulation of repetitive DNA sequences. The sex chromosome system of *R. acetosa* represents an advanced step in sex chromosome evolution, with heterochromatic Y chromosomes enriched in satellite DNA sequences (Ruiz Rejón et al. 1994; Shibata et al. 1999, 2000). On the other hand, the Y chromosomes of *S. latifolia* are euchromatic and although they have accumulated some repetitive sequences in the subtelomeric region, the extension of this degeneration is not yet known (Charlesworth 2002).

Since *R. acetosa* seems to represent one of the most evolved steps in the diversification of X and Y chromosomes (Vyskot and Hobza 2004), it would interesting to test if repeated DNA sequences other than heterochromatic satellite DNAs have been accumulated and diverged in the sex chromosomes. To study this and other molecular aspects, a sex chromosome specific library has been generated by microdissecting X and Y chromosomes from *R. acetosa*. In the present work, we determine the organisation and preferential accumulation of various families of dispersed repetitive DNA families isolated from that library in the genome of males and females of this species. The presence of these repetitive DNA families in the genome of other *Rumex* spp., including different dioecious, hermaphrodite, polygamous, and gynodioecious species, is also analysed.

Materials and methods

Plant material and chromosome preparation

The species of *Rumex* used in this study are summarized in Table 1. They represent dioecious, hermaphrodite, polygamous, and gynodioecious species from different subgenera derived from various natural populations.

Chromosome preparations used for microdissection were obtained from pollen mother cells (PMCs) of immature male inflorescences of *R. acetosa*, previously fixed in 70% ethanol and stored at 4 °C. Fixed male flowers of different sizes were dissected under a stereomicroscope and their individual anthers were squashed in 45% acetic acid. Once a male flower in the appropriate phase of meiosis was identified, the remaining anthers were individually squashed on coverslips, frozen in liquid nitrogen, and the coverslip removed with a razor blade. Air-dried preparations were immediately used for microdissection.

Chomosome microdissection, degenerate oligonucleotide primed PCR, and DNA cloning

Only prophase I or pro-metaphase PMCs in which the sex chromosome trivalent was unambiguously identified were used for microdissection (Fig. 1). The method is described in Jamilena et al. (1995). Trivalents from appropriate cells were collected in sterile PCR tubes with the use of a microneedle driven by the help of an Eppendorf 5171 micromanipulator and a Zeiss inverted phase contrast microscope.

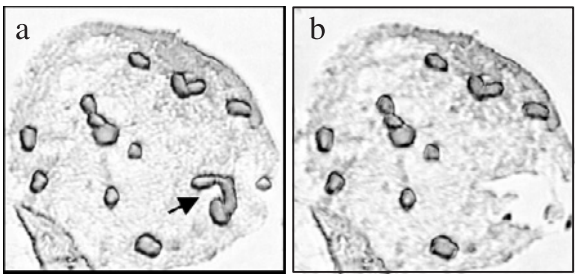
DNA from 3–5 trivalents was amplified by degenerate oligonucleotide primed (DOP)-PCR using the DOP-PCR amplification kit from Boehringer–Mannheim (Mannheim, Germany). To exclude possible contamination, a negative control with no template DNA was performed for each DOP-PCR experiment. The amplified DNA was purified by extraction with chloroform before ligating into linearized pGEMT-Easy vector (Promega, Madison, Wis.), and transformed in *Escherichia coli* DH5α competent cells. The result was a sex chromosome specific library composed of more than 1000 white transformant colonies that were transferred to fresh plates, grown in liquid Luria–Bertani medium and stored at –80 °C for further analysis.

Table 1. Mating systems and location of the populations of the *Rumex* species analysed in this paper.

Species	Mating system (sex chromosomes)	Population
Rumex acetosa	D (XX/XY_1Y_2)	Capileira, Granada (Spain)
Rumex papillaris	D (XX/XY_1Y_2)	La Benajara, S^a Baza, Granada (Spain)
Rumex intermedius	D (XX/XY_1Y_2)	Vollubilis (Morocco)
Rumex thyrsoides	D (XX/XY_1Y_2)	Vollubilis (Morocco)
Rumex hastatulus	D (XX/XY_1Y_2)	Duke University Herbarium, Cumberland County, N.C.
Rumex induratus	P	Padul, Granada (Spain)
Rumex suffruticosus	D (XX/XY)	Pto. Navacerrada, Segovia (Spain)
Rumex lunaria	G	Gáldar, Gran Canaria (Spain)
Rumex acetosella	D (XX/XY)	Capileira, Granada (Spain)
Rumex conglomeratus	H	Atarfe, Granada (Spain)
Rumex crispus	H	Atarfe, Granada (Spain)
Rumex bucephalophorus	H	Padul, Granada (Spain)

Note: D, dioecious; P, polygamous; G, gynodioecious; H, hermaphrodite.

Fig. 1. Chromosome microdissection of sex chromosomes of *Rumex acetosa* from pollen mother cells in meiosis I. (*a*) Metaphase I cell with 6 bivalents and 1 trivalent formed by the 2 Y chromosomes, each attached to an X chromosome telomere (arrowhead). (*b*) The same cell after microdissection of the trivalent.



Characterization of PCR-generated clones

Isolated plasmid DNA from about 200 clones of the sex chromosome specific library was restricted with *Eco*RI to release the inserts, loaded on a gel for electrophoresis, blotted onto nylon membranes, and probed with genomic DNA from male and female plants of *R. acetosa*. Positive clones were further characterized by Southern blot hybridization and DNA sequencing. For Southern blot hybridization, genomic DNA from male and female plants of *R. acetosa*, as well as from other species of *Rumex*, was isolated from the leaves following the protocol of the Plant DNAzol kit (Invitrogen). For probe labeling, hybridization, and detection, the ECL direct labelling and detection system (Amersham) was used following the manufacturer's recommendations.

The cloned DNA fragments were sequenced using the dideoxy sequencing method on an ABI Prism 377 sequencer system from Applied Biosystems (Foster City, Calif.), and the DNA sequences were deposited in the EMBL database under accession numbers DQ003303–DQ003307. Sequence comparison was performed using the BLAST software from the Web site of the National Center for Biotechnology Information (NCBI).

Results

To analyse the structure of the sex chromosomes of *R. acetosa*, we generated a sex-chromosome specific DNA library by microdissection of pollen mother cell nuclei. During meiosis I of pollen mother cells, the 3 sex chromosomes of *R. acetosa* form a trivalent in which both extremes of the X chromosome are attached to one of the Y_1 and Y_2 chromosomes (Fig. 1). We have used this feature to identify and microdissect the 3 sex chromosomes together (Fig. 1). DNA from microdissected chromosomes was randomly amplified by DOP-PCR, resulting in a smear of PCR fragments ranging from about 100 to 1500 bp (data not shown). The amplification product was similar to that found in the positive control (1 ng of genomic DNA from *R. acetosa* as template). No PCR product was obtained in negative DOP-PCRs. Gels with DOP-PCR products were blotted and probed with genomic DNA from *Rumex*. Hybridization signals confirmed that the PCR products from both the positive control and the microdissected sex chromosomes were not the result of a possible contamination, but were *Rumex* DNA (data not shown).

A sex chromosome specific library of *R. acetosa* was obtained after cloning the DOP-PCR product derived from sex chromosome DNA into the pGEMT-Easy cloning vector. To screen for DNA sequences in the sex chromosomes, the plasmid DNA from approximately 200 randomly selected clones was restricted with *Eco*RI and the released inserts hybridized with genomic DNA from either females or males. Five of the clones, DOP-20, DOP-8, DOP-47, DOP-60, and DOP-61, showed strong hybridization signals with both probes. This clearly indicated that they contained repetitive DNA sequences. These clones were further characterized by DNA sequencing and Southern blot hybridization.

The 5 isolated clones were sequenced and analysed using the BLASTn and BLASTx programs. The length of inserts in DOP-20, DOP-8, DOP-47, DOP-60, and DOP-61 were 430, 517, 389, 426, and 370 bp, respectively. None of the nucleotide sequences of the 5 isolated clones were found to be homologous to each other or to other DNA sequences in the databases. Also, the deduced amino acid sequence of DOP-20 showed no homology with any proteins in the databases. Nevertheless, the deduced protein sequences from the remaining clones did show significant amino acid identity with proteins from plant retroelements (Fig. 2). The complete sequences of DOP-47 and DOP-61 are able to be trans-

Fig. 2. Amino acid similarity of DOP sequences derived from *Rumex acetosa* sex chromosomes to proteins encoded by retroelements in other plant species. Dashes indicate gaps. Identical residues between *Rumex* and other species sequences are shaded in black. (*a*) Alignment of DOP-61 and DOP-47 with Ty3-*gypsy*-like gag–pol polyproteins of LTR centromeric retroelements: CMR of *Zea mays* (accession No. AAM94350; Zhong et al. 2002), RIRE7 of *Oryza sativa* (accession No. AB023335; Kumekawa et al. 2001) and Hv-cereba of *Hordeum vulgare* (accession No. AAK94516; Hudakova et al. 2001). (*b*) Local alignment of DOP-60 with putative polyproteins of Ty1-*copia*-like retroelements in *Oryza sativa* (Os-pol, accession No. AAP46257), and *Arabidopsis thaliana* (At-pol, accession No. AAG60117). (*c*) Local alignment of DOP-8 with a putative non-LTR retroelement reverse transcriptase of rice (Os-RT, accession No. BAA95815). The numbering of the positions of residues in *Rumex* sequences is based on the nucleotide sequences of DOP-61 (370 bp), DOP-47 (389 bp), DOP-60 (426 bp), and DOP-8 (517 bp).

a

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DOP-61       1 ------PTRVRHVVDRQCRISFSIGSYSDSVLCDVLPMDATHLLLGRPWQFDRKAFHDGF 162
DOP-47       1 ----------------------------PTRGSNVVPMNACHILLGRPWKSDRRVFHDGF  96
CMR        526 YHIQWLNNSGKVKVTKLVRINFAIGSYRDVVDCDVVPMDACNILLGRPWQFDSDCMHHGR 585
RIRE7      534 YYIQWLNSSGKVKVTRLVRVHFAIGSYHDSINCDVVPMQACSMLLGRPWQFDKDSLHFGK 593
Hv-cebera  568 YYIQWFNNSGKVKVTRTVRVHFSIATYSDFVDCDVVPMQACSVLLGRPWQFDKNSVHHGR 627

DOP-61     163 LNSYVFVYGGKRVTLLPMTPREILLDHQKRN---------------------------- 258
DOP-47      97 RNRHVFKFKGRRVVLNPMSPTQVAEICEKKK---------------------------- 192
CMR        586 SNQYSLIHHDKKIILLPMSPEAIVRDDVAKATKAKTENNKNIK---------------- 628
RIRE7      594 SNQYSFVHNGKKLVLHPMSPEVILKDELARASKQKNQEHTRSEHLIAANELEKHKKKPTN 653
Hv-cereba  628 TNQYTLVHKDQNITLLPMSPEHIMKDDIARASKAKQDLHKSENQIVAKEFEQHNKPNKSS 687
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b

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DOP-60      19  VEKKNIELTRCKNDDQIADIFTKVLAREPFV 111
Os-pol    1294  VDRQEVKLEFCRTDEQLADIFTKALSKEKFV 1324
At-pol    1273  VSKKDVQLEYVKTHDQVADIFTKPLKREDFI 1303
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c

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DOP-8       125 SLEALEAGFRLPLSGLGVALSRHLCVTPGQLSPESWRFITAHAQRSQELGRMPTLEDFR- 302
Os-RT       301 YARALEAGMRLPLHPFACELLRHLGVAPSQITPNGWRVVAGFLLLSHHAAAPPSLAVFRR 360
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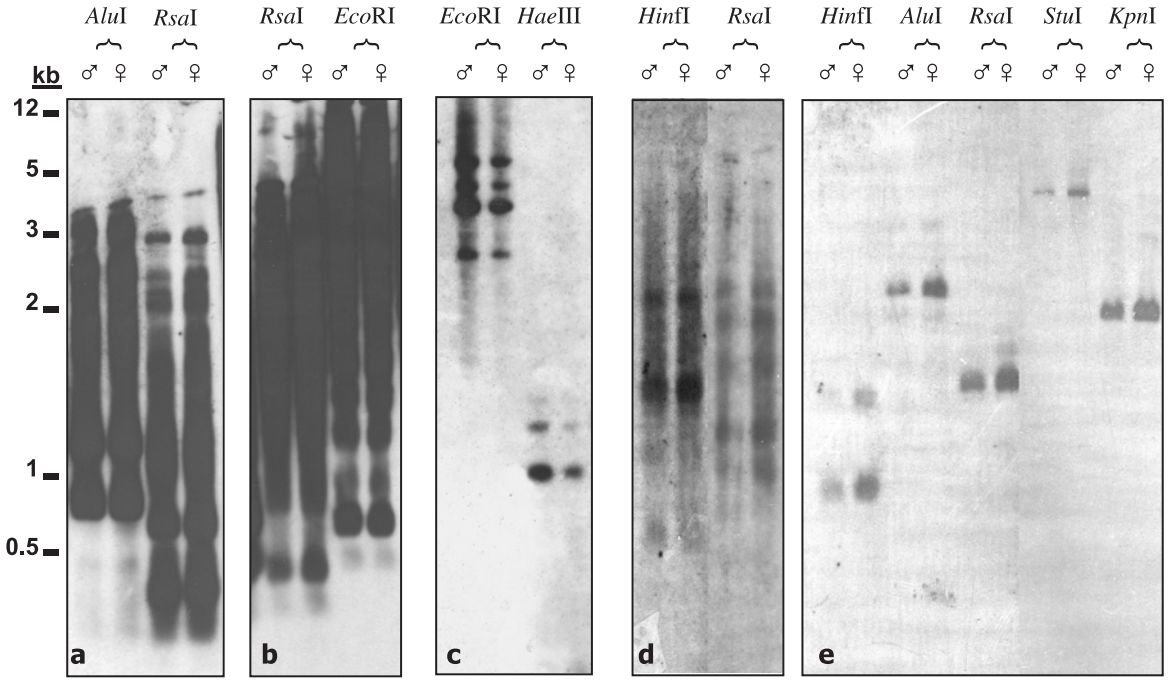
lated into continuous amino acid sequences with no stop codon and share 56% identity in their amino-terminal half (residues 29–75 in DOP-61, and 7–52 in DOP-47; Fig. 2A). In this same region, the deduced amino acid sequences of DOP-61 and DOP-47 show up to 63% identity with polyproteins of different Ty3-*gypsy*-like long terminal repeat (LTR) retroelements, especially with those that are specifically located in cereal centromeres (Fig. 2A). In contrast, the deduced amino acid sequences of DOP-60 and DOP-8 were discontinuous and contained various stop codons, but small fragments showed homology with plant retroelement proteins. A short fragment of the putative protein encoded by DOP-60 (bases 19–111) shared 51% and 45% identity with gag–pol polyproteins of Ty1-*copia*-like LTR retroelements in *Arabidopsis thaliana* (accession No. AAG60117) and *Oryza sativa* (accession No. AAP46257), respectively (Fig. 2B), as well as with other Ty1-*copia*-like polyproteins from these and other plant species. The deduced amino acid sequence from a 177 bp fragment of DOP-8 (bases 125–302), on the other hand, showed 42% similarity with a putative reverse transcriptase from an *O. sativa* non-LTR retroelement (accession No. BAA95815) (Fig. 2C).

The genomic organisation of the isolated repetitive DNA families was determined by Southern analysis (Fig. 3). When used as probes, the 5 isolated sequences produced a hybridization pattern characteristic of dispersed repeated DNA sequences. On genomic DNA digested with different enzymes, DOP-8, DOP-20, DOP-47, and DOP-60 probes hybridized to a smear of DNA fragments, but also to a number of discrete bands, suggesting that these sequences represent different dispersed, repeated, DNA families with a high level of sequence divergence among repeating units. Moreover, with most of the restriction enzyme digestions, the DOP-61 probe produced only one or two hybridizing bands. This would indicate that the sequences of the repeating units of this family are more conserved. That hybridization pattern may also suggest that the DOP-61 family could be clustered in a reduced number of genomic sites. However, in *Rumex* genomic DNA digested with *Eco*RI and *Hin*dIII, two enzymes having no recognition site in the DOP-61 sequence, this probe produced hybridization signals consisting of a very faint smear of DNA fragments (data not shown), which indicates that this repeated family is also dispersed in the genome of this species. The intensity of the hybridization signals produced by the different probes (Fig. 3) also indicates that DOP-20 and DOP-8 are abundant repetitive elements in the genome of *R. acetosa*, while the copy number of DOP-60, DOP-47, and especially DOP-61 is much lower. The hybridization patterns produced by the 5 repeated sequences when hybridized to male and female genomic DNAs restricted with the same enzyme was identical, with no more intense hybridization signal in the male genome (Fig. 3). Moreover, none of the 10 restriction enzymes used were able to reveal a male-specific band with any of the probes (Fig. 3). These results clearly indicate that these sequences have not been preferentially accumulated in

Fig. 3. Genomic organisation of sex chromosome derived DOP-sequences in male and female genomes of *Rumex acetosa*. DNA from male and female plants was digested with different enzymes, separated on a 1% agarose gel, and hybridized with labelled DOP-8 (*a*), DOP-20 (*b*), DOP-47 (*c*), DOP-60 (*d*), and DOP-61 (*e*). Note that none of the probes revealed enhanced hybridization signals or any specific polymorphism in male genomes.



the Y chromosomes of *R. acetosa*, but are similarly distributed in male and female genomes.

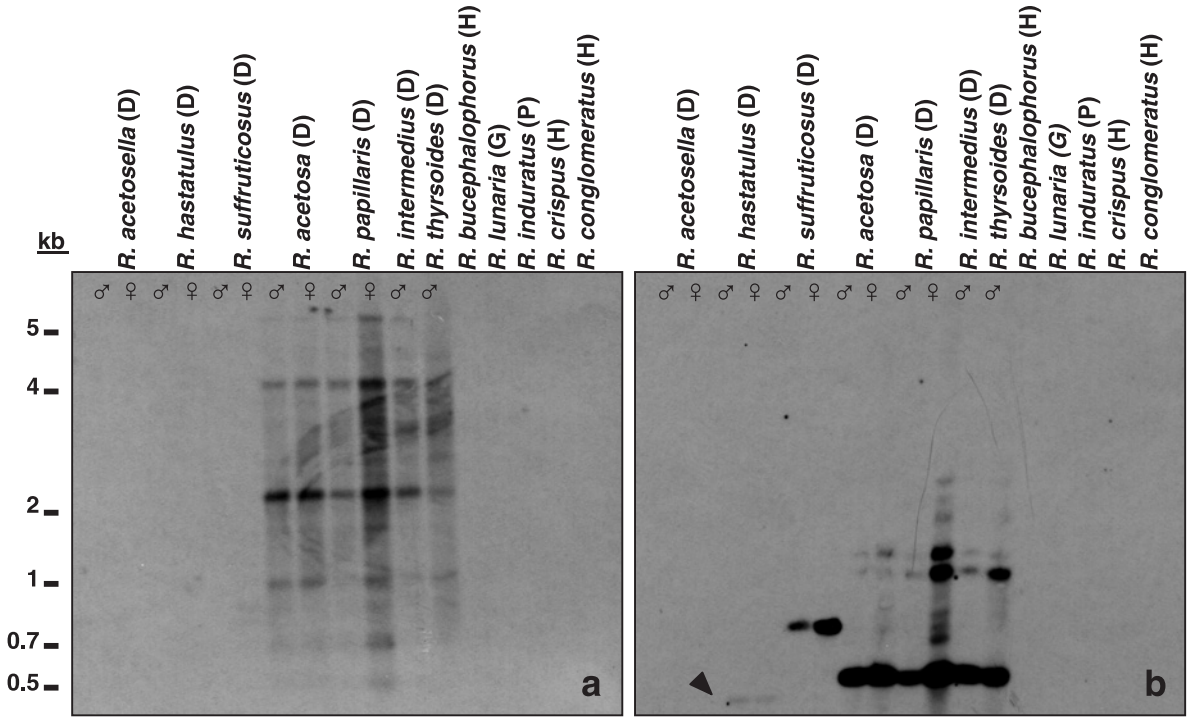
To assess the evolutionary conservation of the cloned repeated sequences, we investigated their presence in the genome of dioecious, polygamous, and hermaphrodite species of *Rumex* by Southern blot hybridization (Fig. 4). DOP-20, DOP-8, and DOP-60 gave almost the same hybridization patterns with male and female genomes of the dioecious species *Rumex acetosa*, *Rumex papillaris*, *Rumex intermedius*, and *Rumex thyrsoides* (Fig. 4*a*). Nevertheless, they did not hybridize with genomic DNA from other dioecious species such as *Rumex hastatulus*, *Rumex suffruticosus*, or *Rumex acetosella,* nor with the polygamous or gynodioecious species *Rumex induratus* and *Rumex lunaria*, or with the DNA from hermaphroditic species such as *Rumex bucephalophorus*, *Rumex crispus,* and *Rumex conglumeratus*. On the other hand, DOP-61 and DOP-47 were detected in the dioecious species *R. papillaris*, *R. intermedius*, *R. thyrsoides*, *R. suffruticosus* and *R. hastatulus*, although they gave no hybridization signal with other dioecious species such as *R. acetosella*, nor did they appear to hybridize with polygamous or gynodioecious species (*R. induratus* and *R. lunaria*), or hermaphroditic species (*R. conglumeratus*, *R. crispus*, and *R. bucephalophorus*) (Fig. 4*b*).

Discussion

Five repetitive DNA sequences were isolated from the microdissection of sex chromosomes of *R. acetosa*, and all appeared to be dispersed in the genome of males and females of this species. Although this might indicate that the sex chromosomes of *R. acetosa* are enriched in dispersed repeated sequences, it is more likely that these types of repeated sequences are preferentially amplified by the DOP-PCR method that we have used for the amplification of microdissected chromosomes. Other studies have demonstrated that a high proportion of both Y_1 and Y_2 chromosomes of *R. acetosa* are composed of tandem repeated sequences or satellite DNAs. A satellite DNA composed of tandem repeats of about 180 bp is preferentially located in the 2 Y chromosomes of *R. acetosa* (Shibata et al. 2000). RAYSI satellite DNA was found to be specifically located in the DAPI positive bands of both Y chromosomes (Shibata et al. 1999). The fact that we were not able to detect these tandem repeated sequences in our sex chromosome specific library indicates that these sequences are not good templates for degenerate primers in DOP-PCRs, a fact that has been previously-observed by other authors (Hobza et al. 2004), and therefore that the enrichment in dispersed repeated DNA in our sex chromosome specific library is caused by a preferential amplification of this type of repeats by DOP-PCR.

Sequence analysis has demonstrated that, with the exception of DOP-20, the isolated repeated DNAs are homologous to plant retroelements. As shown in Fig. 2, short regions of DOP-8 and DOP-60 are homologous at the amino-acid level with reverse transcriptase of plant non-LTR retrotransposons, and Ty1-*copia*-like polyproteins of LTR retroelements, respectively. A higher homology was found between DOP-47 and DOP-61 and Ty3-*gypsy*-like polyproteins of LTR retroelements. No homology was detected among the isolated *R. acetosa* retroelements at the DNA level. Therefore, although DOP-47 and DOP-61 share a high percentage sequence identity at the amino-acid level (Fig. 2), the fact that they show no homology at the DNA level suggests that they

Fig. 4. Distribution of DOP-sequences in the genome of dioecious (D), polygamous (P), gynodioecious (G), and hermaphrodite (H) species of *Rumex*. DNA from different *Rumex* species was digested with *Rsa*I and probed with labelled DOP-20 (*a*) and DOP-47 (*b*). Arrowhead points to the faint hybridization bands in female and male genomes of *Rumex hastatulus*.



represent 2 divergent families of LTR retrotransposon. Our data indicate that the most abundant dispersed repeated families, represented by DOP-20, the non-LTR retroelement DOP-8 and even the Ty1-*copia*-like sequence DOP-60, have higher sequence divergence than that found in the Ty3-*gypsy*-like families DOP-47 and DOP-61, which have an intermediate number of repeats. Thus, the dispersion and amplification of retroelements in the genome of *R. acetosa* has been accompanied by a process of DNA sequence degeneration. DOP-20, DOP-8, and DOP-60 therefore represent more ancient and degenerated retroelements in the genome of *Rumex*. On the other hand, given the evolutionary conservation of DOP-47 and DOP-61 *Rumex* species, it is likely that the sequence conservation found for these two Ty3-*gypsy*-like families indicates an evolutionary constraint owing to a possible functionality of these repeated DNAs. In fact, we have found that DOP-47 and DOP-61 are particularly homologous to the Ty3-*gypsy*-like LTR retroelements specifically located in the centromeres of grasses (Miller et al. 1998; Cheng et al. 2002; Zhong et al. 2002).

The implication of retroelements in the evolution and degeneration of the Y chromosomes of dioecious plants is a controversial topic. Sex chromosomes in dioecious plant species appear to have evolved from a pair of autosomes in related hermaphroditic ancestors, an event that has occurred many times in the evolution of plants (Renner and Ricklefs 1995; Guttman and Charlesworth 1998). Theories of sex chromosome evolution postulated that reduction of meiotic recombination between X and Y chromosomes is accompanied by a degeneration of Y chromosomes, a process that could be driven by the accumulation of deleterious mutations and transposable elements (Charlesworth and Charlesworth 2000). The accumulation of transposable elements in the non-recombining regions of *Drosophila* Y chromosomes seems to have played an important role in their degeneration (Steinemann and Steinemann 1998; Bachtrog 2003). The genomic approach used to characterize the primitive Y chromosome of papaya has recently demonstrated that the male-specific region of the Y chromosome (MSY) is not only characterized by a lower gene density, but also by a higher density in inverted repeats and retroelements (Liu et al. 2004). Non-LTR retrotransposons have also been found to accumulate in the terminal region of the long arm of the *Cannabis sativa* Y chromosome (Sakamoto et al. 2000). Nevertheless, this is not a common situation for plant Y chromosomes. Therefore, the different families of retrotransposons isolated from the genome of *S. latifolia* are not specifically accumulated in the sex chromosomes but are equally distributed in autosomes and sex chromosomes (Matsunaga et al. 2002; Obara et al. 2002). In the same way, no retroelement has been detected in a 35 kb long fragment from the Y chromosome of the liverwort *Marchantia polymorpha*; rather, it is mostly composed of a mosaic structure of repeats within repeats (Okada et al. 2001). Although we have been able to isolate different types of retroelements from the sex chromosomes of *R. acetosa*, our data exclude a preferential accumulation of these sequences in the Y chromosome. *Rumex acetosa* Y chromosomes are, however, quite differentiated from X chromosomes, as they are heterochromatic and have accumulated satellite DNA sequences (Ruiz Rejón et al. 1994; Lengerova and Vyskot 2001; Shibata et al. 1999, 2000). It is likely that, as in the Y chromosome of *M. polymorpha*, the differentiation of *R. acetosa* sex chromosomes is not driven by the accumulation of transposable elements

in the Ys, but instead by an amplification mechanism that has resulted in the accumulation of various related families of satellite DNA.

The hybridization patterns shown by *R. acetosa* dispersed repeated sequences in the genome of different dioecious and hermaphrodite species of *Rumex* support the phylogenetic tree recently obtained by our research group using nuclear and chloroplast sequence data (Navajas-Pérez et al. 2005). Our phylogenetic data suggest a common origin for all dioecious species of the genus *Rumex*, which can be subdivided into 2 groups. One group is composed of species such as *R. acetosa, R. papillaris*, *R. intermedius*, and *R. thyrsoides* with an XX/XY_1Y_2 multiple sex chromosome system. The second group comprises species such as *R. suffruticosus* and *R. acetosella*, having an XX/XY system, together with *R. hastatulus,* a species with 2 chromosomal races (the Texas race, with a XX/XY system, and the North Carolina race, with a XX/XY_1Y_2 system; Smith 1969). In accordance with this, our results in this paper demonstrate a high degree of sequence conservation for DOP-20, DOP-8, and DOP-60 sequences among the closely related dioecious species *R. papillaris*, *R. intermedius*, and *R. thyrsoides*, since all of these dispersed repeated sequences gave a similar strong positive hybridization with the genome of these species. In addition, the sequences of the DOP-47 and DOP-61 Ty3-*gypsy*-like LTR retroelements of *R. acetosa* were conserved not only in *R. papillaris*, *R. intermedius*, and *R. thyrsoides*, but we also found less intense hybridization signals in the other dioecious species *R. hastatulus* and *R. suffruticosus*.

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