Genetica (2009) 135:87–93
DOI 10.1007/s10709-008-9261-y

Molecular cytogenetic characterization of *Rumex papillaris*, a dioecious plant with an XX/XY_1Y_2 sex chromosome system

Rafael Navajas-Pérez · Trude Schwarzacher · Manuel Ruiz Rejón · Manuel A. Garrido-Ramos

Received: 3 December 2007 / Accepted: 14 March 2008 / Published online: 29 March 2008

Abstract *Rumex papillaris* Boiss, & Reut., an Iberian endemic, belongs to the section *Acetosa* of the genus *Rumex* whose main representative is *R. acetosa* L., a species intensively studied in relation to sex-chromosome evolution. Here, we characterize cytogenetically the chromosomal complement of *R. papillaris* in an effort to enhance future comparative genomic approaches and to better our understanding of sex chromosome structure in plants. *Rumex papillaris*, as is common in this group, is a dioecious species characterized by the presence of a multiple sex chromosome system (with females $2n = 12 + XX$ and males $2n = 12 + XY_1Y_2$). Except for the X chromosome both Y chromosomes are the longest in the karyotype and appear heterochromatic due to the accumulation of at least two satellite DNA families, RAE180 and RAYSI. Each chromosome of pair VI has an additional major heterochromatin block at the distal region of the short arm. These supernumerary heterochromatic blocks are occupied by RAE730 satellite DNA family. The Y-related RAE180 family is also present in an additional minor autosomal locus. Our comparative study of the chromosomal organization of the different satellite-DNA sequences in XX/XY and XX/XY_1Y_2 *Rumex* species demonstrates that of active mechanisms of heterochromatin amplification occurred and were accompanied by chromosomal rearrangements giving rise to the multiple XX/XY_1Y_2 chromosome systems observed in *Rumex*. Additionally, Y_1 and Y_2 chromosomes have undergone further rearrangements leading to differential patterns of Y-heterochromatin distribution between *Rumex* species with multiple sex chromosome systems.

Keywords Heterochromatin · In situ hybridization · Satellite DNA · Sex chromosomes · *Rumex papillaris*

Abbreviations

A	Adenosine
bp	Base pair(s)
DAPI	4′,6-diamidino-2-phenylindole
dNTP	Deoxyribonucleoside triphosphate
EDTA	Ethylenediaminetetraacetic acid
h	Hour(s)
ng	Nanogram(s)
ml	Milliliter(s)
mya	Million years ago
PCR	Polymerase chain reaction
SDS	Sodium dodecyl sulfate
SSC	Saline–sodium citrate
T	Thymidine

Introduction

Dioecious species account for 7% of flowering plants (Renner and Ricklefs 1995). Dioecism is commonly accompanied by sex chromosome dimorphism in animals. However, within dioecious plants only a few chromosome-mediated sex-determination systems have been reported

R. Navajas-Pérez (✉)
Plant Genome Mapping Laboratory, University of Georgia, Athens, GA 30602, USA
e-mail: rnavajas@uga.edu

T. Schwarzacher
Department of Biology, University of Leicester, University Road, Leicester LE1 7RH, UK

M. R. Rejón · M. A. Garrido-Ramos
Departamento de Genética, Facultad de Ciencias, Universidad de Granada, Granada 18071, Spain

(Ruiz Rejón 2004). In all cases, the origin of sex chromosome dimorphism in plants seems to be quite recent. To date, the best known systems are the homomorphic sex chromosomes of papaya, evolved 0.5–2.5 mya, as revealed by studying X and Y gene pairs (Yu et al. 2008a, b), and the sex chromosomes of *Silene* that are estimated to have originated about 5–10 mya based on the molecular clock calibration of the chalcone synthase (Chs) and alcohol-dehydrogenase (ADH) nuclear genes in *Brassicaceae* (Koch et al. 2000; Nicolas et al. 2005). Similarly, sex chromosomes in *Rumex* species might have originated at similar time based on the study of nuclear intergenic transcribed rDNA spacers and chloroplast intergenic sequences with dioecy appearing between 15–16 mya (Navajas-Pérez et al. 2005a). Dioecy in *Rumex* is a plesiomorphic condition and dioecious species can be classified onto two different groups: species having a XX/XY sex-chromosome system and those having a XX/XY_1Y_2 system (Navajas-Pérez et al. 2005a; see also Table 1). Since Kihara and Ono (1923) described the sex chromosomes of *Rumex acetosa* (belonging to the section *Acetosa* of the subgenus *Acetosa*), this species has attracted many studies and due to the presence of a complex XX/XY_1Y_2 chromosomal system it is among the most informative ones to investigate the origin and evolution of sex chromosomes in plants (Ruiz Rejón 2004). However, to advance our knowledge of intrinsic features of Y chromosomes architecture, the study of further dioecious species belonging to the same as well as other related sections is needed (see Table 1). We seek to enhance the study of different species of the *Acetosa* section (all XX/XY_1Y_2 and $x = 7$—see Table 1) in order to gather evidence for evolutionary mechanisms and molecular trends that have given rise to sex chromosomes in *Rumex* and ultimately in plants, in general. In particular, we are interested in understanding the mechanisms involved in Y molecular degeneration and evolution. Here we contribute to that aim by studying the chromosomes of *R. papillaris*.

Rumex papillaris is an Iberian endemic found in the inner regions of Portugal and Spain with a continental climate (López González 1990). This species although not yet well understood, is probably very closely related to *R. acetosa* (Rechinger 1964). We characterize in this paper for the first

Table 1 List of dioecious species of *Rumex*, indicating their affiliations, basic chromosome number (*n*), sex-chromosome system and first reported reference

Species	*n*	Sex-chromosome system	Reference
Subgenus Acetosa			
Section *Afroacetosa*			
Rumex sagittatus[a] *(SsS)*	9	*No sex chromosomes*	Degraeve (1976)
Section *Acetosa*			
Rumex acetosa	7	(XX/XY_1Y_2)	Kihara and Ono (1923)
Rumex arifolius	7	(XX/XY_1Y_2)	Wilby and Parker (1988b)
Rumex nebroides	7	(XX/XY_1Y_2)	Ainsworth et al. (1999)
Rumex nivalis	7	(XX/XY_1Y_2)	Ainsworth et al. (1999)
Rumex papillaris	7	(XX/XY_1Y_2)	Löve and Kapoor (1967); this paper
Rumex tuberosus	7	(XX/XY_1Y_2)	Ainsworth et al. (1999)
Rumex intermedius	7	(XX/XY_1Y_2)	Ainsworth et al. (1999)
Rumex thyrsoides	7	(XX/XY_1Y_2)	Wilby and Parker (1988b)
Rumex thyrsiflorus	7	(XX/XY_1Y_2)	Wilby and Parker (1988b)
Rumex rothschildianus	7	(XX/XY_1Y_2)	Wilby and Parker (1988b)
Section *Americanae*			
Rumex hastatulus (NCR)	**4**	**(XX/XY_1Y_2)**	**Smith** (1964)
Rumex hastatulus (TXR)	**5**	**(XX/XY)**	**Smith** (1964)
Rumex paucifolius	**7**	**(XX/XY)**	**Smith** (1968)
Section *Scutati*			
Rumex suffruticosus	**8**	**(XX/XY)**	**Navajas-Pérez et al.** (2005a); **Cuñado et al.** (2007)
Subgenus Acetosella			
Rumex acetosella	**7**	**(XX/XY)**	**Löve** (1944)
Rumex graminifolius	**7**	**(XX/XY)**	**Löve** (1944)

Based on Navajas-Pérez et al. (2005a) all dioecious species having sex chromosomes form a monophyletic clade with two differentiated groups: one containing the species of the Acetosa section (XX/XY_1Y_2 species) and one (bold entries) containing the remaining dioecious species (XX/XY species and *R. hastatulus* North Carolina race XX/XY_1Y_2). NCR, North Carolina Race; TXR, Texas Race; SsS, subsection Sagittati.
[a]Indicates that *R. sagittatus* belongs to a different lineage

time the karyotype of *R. papillaris* focusing on Y chromosomes and heterochromatin structure as well as the location of the three satellite DNA families previously described in *R. acetosa*. These satellite families are: RAE180 and RAYSI, major heterochromatin constituents of both Y chromosomes (Shibata, Hizume and Kuroki 1999, 2000a; Navajas-Pérez et al. 2006; Cuñado et al. 2007) and RAE730, that occupies heterochromatic segments present in variable numbers of autosomes (Shibata et al. 2000b). RAE180 is also present in a minor autosomal locus (Cuñado et al. 2007).

Recent analyses have demonstrated that these satellite-DNA families post-date dioecy in *Rumex* (Navajas-Pérez et al. 2006; Cuñado et al. 2007). Regarding the XX/XY system, RAE180 has been shown to be present in *R. acetosella* L. (Subgenus Acetosella) and *R. suffruticosus* Gay ex Meissner (Subgenus Acetosa, Section Scutati) (Table 1). Regarding XX/XY_1Y_2 systems, in addition to *R. acetosa* all three satellite-DNA families have been found in *R. intermedius, R. papillaris, R. thyrsoides* or *R. tuberosus* (Navajas-Pérez et al. 2006; Cuñado et al. 2007) (Table 1). However, except for *R. acetosa, R. suffruticosus* and *R. acetosella* (Cuñado et al. 2007), no cytogenetical data are available for these type of sequences in other dioecious species. Hence, here we analyze the location and organization of the RAE180, RAYSI and RAE730 satellite-DNA families in *R. papillaris* and discuss their possible role in XX/XY_1Y_2 origin and maintenance.

Materials and methods

Satellite-DNA probe preparation

Repetitive units of three satellite-DNA families previously isolated from *R. papillaris* by Navajas-Pérez et al. (2005a) were used as probes for Southern-blot and in situ hybridizations. Specifically, we used the plasmid inserts of clones RAE180-PAP-16 (RAE180 repeat), RAYSI-PAP-36 (RAYSI) and RAE730-PAP-1 (RAE730). Their DNA sequences are deposited in NCBI/EMBL GenBank under accession numbers AJ580463 (RAE180), AJ580495 (RAYSI) and AJ580477 (RAE730). For probe preparation, satellite-DNA sequences were amplified by PCR using the plasmids as DNA templates and the universal M13 sequencing primers. PCR products were electrophoresed in 1% agarose gels, bands excised and purified using GFX™ PCR DNA and Gel purification Kit (GE Healthcare).

Southern blot hybridization

Probe labelling, hybridization and detection of hybridization for Southern blot procedures was performed using the nonradioactive chemiluminescence method (ECL, GE Healthcare), as described in Cuñado et al. (2007). Hybond N+ nylon filters were hybridized for 12–16 h at 42°C with horseradish peroxidase-labelled probes at 10 ng/ml of ECL hybridization buffer containing 6 M urea, 0.5 M NaCl and 5% blocking agent (GE Healthcare). After hybridization, high-stringency solutions were used to wash the filters, including 6 M urea, 0.1× SSC (saline sodium citrate; 0.3 M NaCl, 0.03 M Na citrate) and 0.4% SDS (sodium dodecyl sulphate) at 42°C for 40 min.

Location of satellite-DNA sequences by fluorescent in situ hybridization (FISH)

Seeds of *R. papillaris* were collected from a population in La Benajara (Sierra de Baza, Granada province, Spain). For FISH, chromosome preparations were made following Schwarzacher and Heslop-Harrison (2000). Probes were labelled with digoxygenin-dUTP or biotin-dUTP (Roche) by random priming according to the specifications of the Random Primer Labelling System (Invitrogen). Labelled probes (25–50 ng) were added to the hybridization mixture (50% formamide, 2× SSC, 20% dextran sulphate, 0.125% SDS and 0.125 mM EDTA–ethylene-diamine-tetra-acetic acid) (see Schwarzacher and Heslop-Harrison 2000). Combined denaturation of the probe and chromosomal DNA was performed at 80°C for 8 min using a Thermo-Hybaid HyPro-20 and re-annealed at 37°C overnight. Stringent washes (20% formamide and 0.1× SSC at 42°C) were made prior to detection. Biotin and digoxygenine probes were detected with Alexa594/streptavidin (Molecular Probes, 0.5 ng/ml) and FITC/anti-digoxygenine (Roche, 1 ng/ml) in 5% (w/v) Bovine Serum Albumin (BSA) in 4× SSC, 0.2% Tween 20 following Schwarzacher and Heslop-Harrison (2000). Preparations were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, 2 μg/ml) and mounted in antifade solution. Preparations were analyzed with a Zeiss Axioplan 2 epifluorescence microscope (Oberkochen, Germany) with suitable filters and photographed with a CCD camera (Optronics, model s97790). Colour figures and overlays were prepared with Adobe Photoshop 7.0 software, using only those processing functions that are applied to all pixels of the image.

Results and discussion

Karyotyping of *Rumex papillaris* and heterochromatin distribution

The spreading technique followed by DAPI staining shows that the mitotic chromosome complement in root tip cells of *R. papillaris* consists of six similarly sized autosomal chromosome pairs: five acrocentric pairs and one

approximately metacentric pair. These were ordered as pairs I to VI on the basis of length and arm ratio (Fig. 1) following the previous description for *R. acetosa* (Wilby and Parker 1988a). Females are $2n = 14$ due to the additional presence of two X chromosomes that are metacentric and larger than the autosomes while males are $2n = 15$ due to the presence of one X chromosome and two submetacentric Y chromosomes (Y_1 and Y_2) next in size between the X chromosome and the autosomes (Fig. 1). Based on classic cytogenetical techniques, the same situation has been described in the remaining species of the *Acetosa* section (Wilby and Parker 1988a, Kuroki et al. 1994; Ainsworth et al. 1999).

Heterochromatic regions were detected by DAPI, a fluorochrome that specifically stains AT-rich regions (see Schwarzacher 2003). Enhanced fluorescence was observed in the two Y chromosomes in the form of heteropycnotic bands present along both arms (see Fig. 1). One additional large supernumerary heterochromatic DAPI positive segment was present at the end of the short arm of each member of chromosomal pair VI, causing these chromosomes to appear metacentric-like (Fig. 1). The same situation was found in all 20 individuals of *R. papillaris* analyzed. The presence of these supernumerary heterochromatic segments has been described in a variable number between one and six in different *R. acetosa* populations in England (Wilby and Parker 1988b) and in Japan (Kuroki 1987). In contrast, we found that in Spanish populations of *R. acetosa* these segments are fixed in chromosomal pair VI in a homozygous condition (Ruiz Rejón et al. 1994). It is likely that a similar situation is occurring in *R. papillaris* and a constant number of two supernumary segments are fixed in the *R. papillaris* population analyzed here. No other significant heterochromatic regions were detected.

Satellite DNA location and organization

Southern-blot hybridization of *Eco*RI restriction enzyme digested DNA revealed a typical ladder pattern consisting of multimeric units with multiples of ~180, ~730 or ~920 bp for RAE180, RAE730 and RAYSI probes, respectively (Fig. 2). No hybridization differences were found between males and females for the RAE730 probe. Similar patterns of hybridization were found for males and females when hybridized with the RAE180 probes. However, the intensity of the signal was significantly higher for males than for females. Remarkably, no RAYSI signal was detected in *R. papillaris* females. These results suggest that the three satellite-DNA families studied are tandemly arrayed in the *R. papillaris* genome and that RAE180 sequences are more represented in males than in females, while RAYSI sequences are exclusive to male individuals.

Next, the chromosomal location of these sequences was determined by fluorescent in situ hybridization (FISH). RAYSI sequences were revealed to be located exclusively on both Y chromosomes of *R. papillaris* (Fig. 3A). The Y_1 has four RAYSI loci, two on the long arm and two on the short arm. However, the RAYSI pattern of the Y_2 is slightly different, with the terminal locus of the short arm being almost disappeared (Fig. 3A). FISH experiments using species-specific RAE180 probe demonstrated the massive presence of this family in both Y chromosomes of *R. papillaris*. Specifically, Y_1 chromosome had five prominent sites, three of them on the long arm, one on the short arm and one at the centromeric/pericentromeric region (Fig. 3B) while chromosome Y_2 showed a strong signal at the pericentromeric region, as well as three additional blocks on the long arm and two on the short arm (Fig. 3B). Other weaker signals were detected along both Y chromosomes (Fig. 3B). Double hybridization using differential staining for RAE180 and RAYSI probes showed that both families occupied differential loci on the Y chromosome (Fig. 3C). RAE180 sequences were also present in an additional punctate autosomal locus (Fig. 4A), while family RAE730 was detected in two heterochromatic supernumerary segments in autosomal pair VI of *R. papillaris* (Fig. 4B). Similarly to what we describe here, Cuñado et al. (2007) found RAE180 to be also present in one autosomal pair in Spanish populations of *R. acetosa*. This situation contrasts with data gathered by Shibata et al. (2000a) who described RAE180 in two autosomal loci in Japanese *R. acetosa* individuals.

Comparative analysis of Y chromosome structure

Sex chromosomes have evolved as a consequence of a rarely recombining region containing the genes involved in

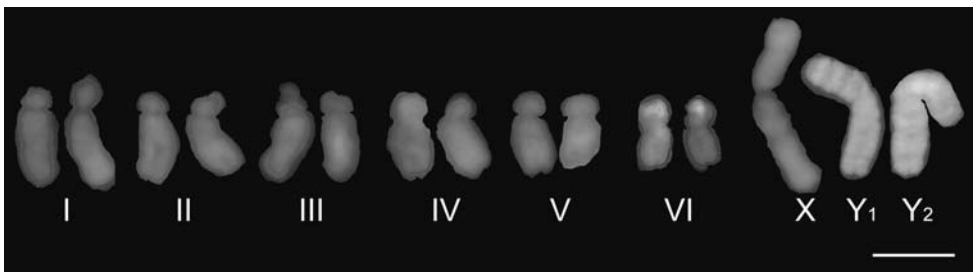

Fig. 1 Karyotype of *Rumex papillaris* stained with DAPI. Note two Y chromosomes and the heterochromatic terminal segments in pair VI are strongly heterochromatic. Bar represents 2.5 μm

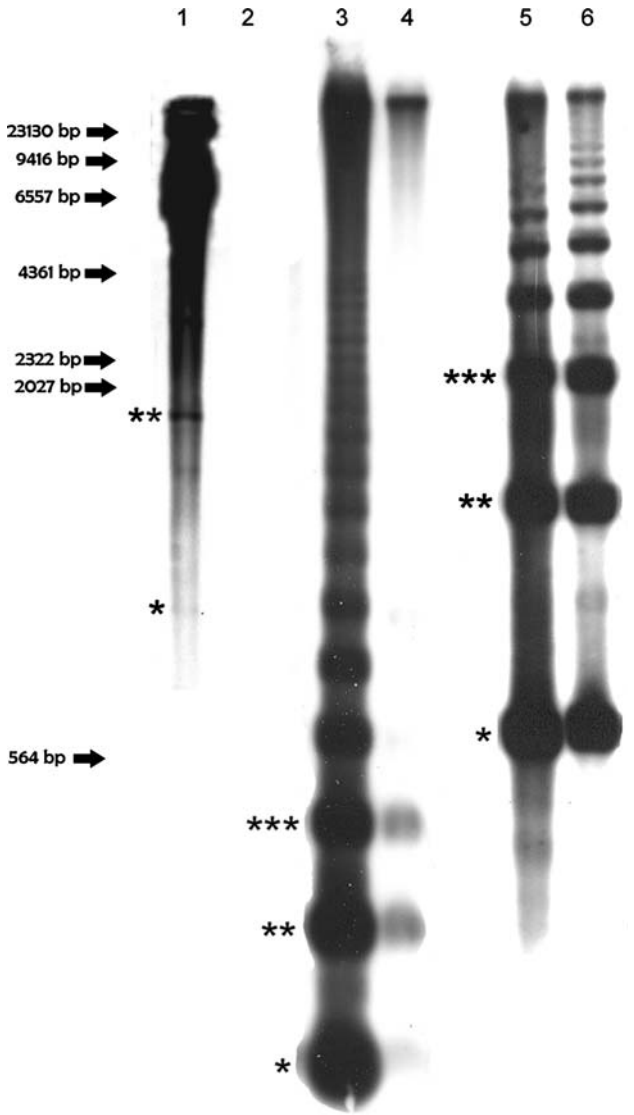

Fig. 2 Southern blot hybridization of *Rumex* total genomic DNA cut with *Eco*RI using the monomeric RAYSI (1, 2) RAE180 (3, 4) and RAE730 (5, 6) satellite DNA sequences as probes. Species: (1, 3, 5) *R. papillaris* male (2, 4, 6) *R. papillaris* female. (*), (**), (***) indicate monomer, dimmer and trimer, respectively

sex determination, followed by Y chromosome degeneration (Charlesworth 1996). The progressive suppression of recombination ultimately leads to the accumulation of diverse repetitive sequences such as mobile elements and satellite DNAs (Steinemann and Steinemann 1997; Skaletsky et al. 2003) that further prevent the recombination between X and Y chromosomes and ensure the maintenance of the dimorphic sex chromosomes. The situation of sex chromosomes in *R. acetosa* and *R. papillaris* with one X and two Y chromosomes, provides an excellent model to study sex chromosome evolution. Both Y chromosomes do not recombine, but form a sexual trivalent with the X chromosome in meiosis (Cuñado et al. 2007). As is often

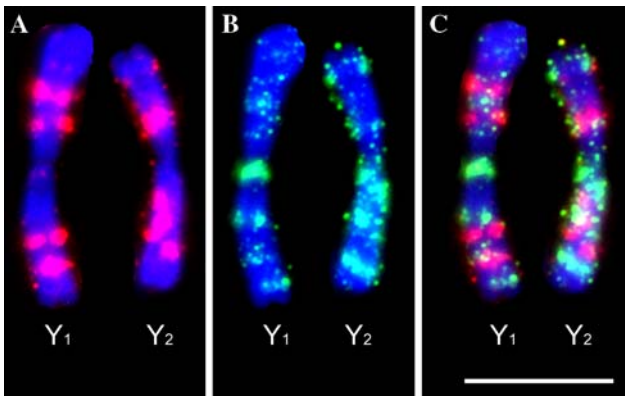

Fig. 3 In situ hybridization to Y_1 and Y_2 chromosomes of male *R. papillaris* with probes RAYSI (**A**—red) RAE180 (**B**—green) and overlay of both signals (**C**). Bar represent 2.5 μm

the case in plants, the *Rumex* sex chromosomes are the largest in the complement (Fig. 1). They are strongly heteropycnotic and harbor many satellite-DNA and retrotransposable sequences (Figs. 1 and 3; Shibata et al. 1999, 2000a; Navajas-Pérez et al. 2005b, 2006; Mariotti et al. 2006; Cuñado et al. 2007). While not only supporting the general trend of evolution of plant sex chromosomes through a significant increase in DNA, *R. acetosa* and *R. papillaris* represent the more advanced stage in plant sex chromosome evolution.

According to our previous analysis (Navajas-Pérez et al. 2005a), the multiple sex chromosome system XX/XY_1Y_2 evolved from an XX/XY system in *Rumex*. The presence of RAE180 exclusively in an autosomal locus in XX/XY species and in a minor locus in XX/XY_1Y_2 species (Cuñado et al. 2007 and this study) as well as the common origin of autosomal RAE730 and Y-specific RAYSI sequences, that are postulated to have evolved from an ancestral 120-bp repeat (Navajas-Pérez et al. 2005b) suggest an autosomal origin of Y-linked satellite DNAs in *Rumex*. It implies that Y degeneration in the derived complex XX/XY_1Y_2 system was accelerated by massive heterochromatinization (Lengerova and Vyskot 2001; Mosiolek et al. 2005) with the accumulation of RAYSI and RAE180 sequences (Shibata et al. 2000a; Cuñado et al. 2007; this paper) and that this process has been accompanied by chromosomal rearrangements giving rise to the multiple Y chromosomes.

Subsequently, we found in this paper (Figs. 3–5), Y_1 and Y_2 chromosomes are undergoing further rearrangements leading to differential patterns of Y-heterochromatin distribution between *Rumex* species with multiple sex chromosome systems. While autosomal satellite-DNA sequences in *R. papillaris* resemble the organization previously found in *R. acetosa*—that is, a minor locus of RAE180 in an autosomal pair and two heterochromatic segments in pair VI occupied by RAE730 sequences (see

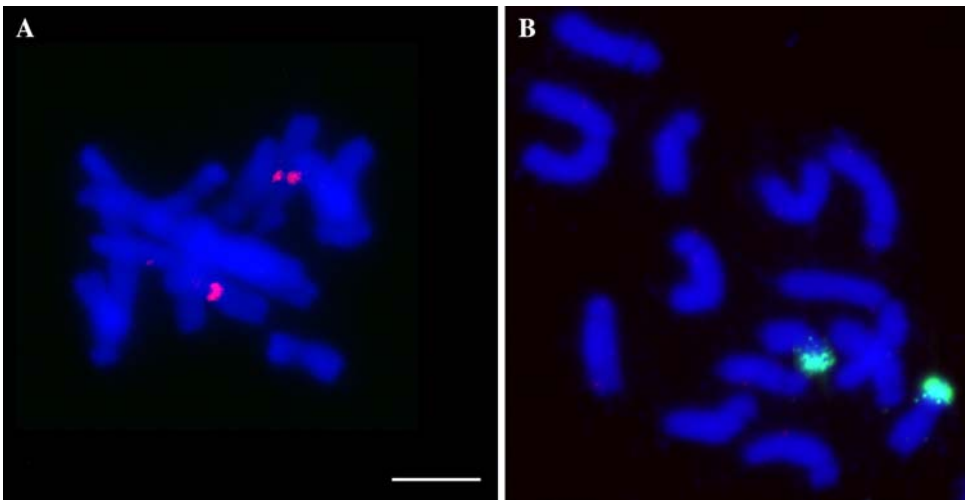

Fig. 4 In situ hybridization to root tip metaphase chromosomes of female *R. papillaris* ($2n = 14$) using as probes RAE180 (**A**) and RAE730 (**B**) sequences. Bar represents 2.5 μm

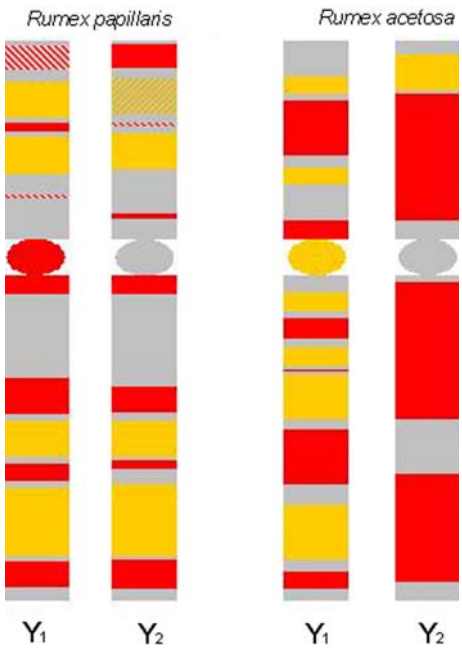

Fig. 5 Comparative scheme of distribution of RAYSI (in orange) and RAE180 (in red) sequences in Y_1 and Y_2 chromosomes of *R. papillaris* and *R. acetosa*. Data for *R. acetosa* based on Navajas-Pérez et al. (2006) and Cuñado et al. (2007). Lines indicate weaker signal intensities

Fig. 4; Shibata et al. 2000b; Cuñado et al. 2007), Y-linked satellite DNAs exhibit significantly different chromosomal distribution (Fig. 5). In *R. acetosa*, the chromosome Y_1 bears several RAYSI bands in centromeric and pericentromeric regions in addition to the four loci detected in *R. papillaris* (Fig. 5). Also, RAE180 sequences form more prominent bands in the Y_1 chromosome of *R. acetosa* compared to *R. papillaris*. RAYSI and RAE180 sequences have a similar distribution pattern in both Y chromosomes, with slight differences in the case of *R. papillaris*. This contrasts with the distribution in *R. acetosa* where only one RAYSI locus is present in the terminal region of the short arm, and RAE180 is widespread in the major part of the rest of Y_2 heterochromatin (Fig. 5).

These distinct and different patterns of satellite DNA distribution of the Y chromosomes of *R. acetosa* and *R. papillaris* support the hypothesis that these two taxa, although related, are different species (Rechinger 1964), but also demonstrate that heterochromatin of the Ys, in contrast to the autosomal regions, is the subject of rapid evolutionary amplification and rearrangement mechanisms. Similarly, in the male-specific Y (MSY) region of the papaya Y chromosome, inversions, deletions, insertions, duplications and translocations have been recently described (Yu et al. 2008a, b). In conclusion, the analysis of RAE180/RAYSI and RAE730 patterns is useful to clarify the evolution of the complex sex chromosome system of *Rumex* species and the mechanisms involved and support satellite-DNA based techniques in general as powerful markers for these types of analysis.

Acknowledgements We thank Dr John Bailey, University of Leicester, for helpful discussions and checking the English of the manuscript. This work was supported by grant CGL2006-00444/BOS awarded by the Ministerio de Educación y Ciencia, Spain. We are deeply indebted to the Parque Natural de la Sierra de Baza in Granada (Spain), for kindly providing the material analyzed in this paper. R.N.P. is a Fulbright postdoctoral scholar supported by grant FU-2006-0675 of Spanish M.E.C.

References

Ainsworth CC, Lu J, Winfield M et al (1999) Sex determination by X: autosome dosage: *Rumex acetosa* (sorrel). In: Ainsworth CC (ed) Sex determination in plants. BIOS Scientific Publishers Limited, pp 124–136

Charlesworth B (1996) The evolution of chromosomal sex determination and dosage compensation. Curr Biol 6:149–162

Cuñado N, Navajas-Pérez R, de la Herrán R et al (2007) The evolution of sex chromosomes in the genus *Rumex*

(Polygonaceae): identification of a new species with heteromorphic sex chromosomes. Chromosome Res 15:825–833
Degraeve N (1976) Contribution à l'étude cytotaxonomique des Rumex. IV Le genre *Acetosa* Mill. Cellule 71:231–240
Kihara H, Ono T (1923) Cytological studies on *Rumex* L. I. Chromosomes of *Rumex acetosa* L. Bot Mag Tokyo 37:84–90
Koch M, Haubold AB, Mitchell-Olds T (2000) Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, Arabis, and related genera (*Brassicaceae*). Mol Biol Evol 17:1483–1498
Kuroki Y (1987) Karyotype variation and heterochromatin in *Rumex acetosa*. Plant Chromosome Res 1987. Nishiki Print Co, Ltd, Hiroshima, Japan, pp 227–230
Kuroki Y, Yokohama A, Iwatsubo Y (1994) Fluorescent chromosome banding in *Rumex montanus* (Polygonaceae). Kromosomo 74:2591–2597
Lengerova M, Vyskot B (2001) Sex chromatin and nucleolar analyses in *Rumex acetosa* L. Protoplasma 217:147–153
López González G (1990) Género *Rumex* L. In: Castroviejo S, Laínz M, López González G, Montserrat P, Muñoz Garmendia F, Paiva J, Villar L (eds) Flora Iberica, vol 2. CSIC, Real Jardín Botánico de Madrid, Madrid, Spain, pp 595–634
Löve Á (1944) Cytogenetic studies on *Rumex* subgenus *acetosella*. Hereditas 30:1–136
Löve Á, Kapoor B (1967) A chromosome atlas of the collective genus *Rumex*. Cytologia 32:320–342
Mariotti B, Navajas-Pérez R, Lozano R et al (2006) Cloning and characterisation of dispersed repetitive DNA derived from microdissected sex chromosomes of *Rumex acetosa*. Genome 49:114–121
Mosiolek M, Pasierbek P, Malarz J et al (2005) *Rumex acetosa* Y chromosomes: constitutive or facultative heterochromatin? Folia Histochem Cytobiol 43:161–167
Navajas-Pérez R, de la Herrán R, López González G et al (2005a) The evolution of reproductive systems and sex-determining mechanisms within *Rumex* (Polygonaceae) inferred from nuclear and chloroplastidial sequence data. Mol Biol Evol 22:1929–1939
Navajas-Pérez R, de la Herrán R, Jamilena M et al (2005b) Reduced rates of sequence evolution of Y-linked satellite DNA in *Rumex* (Polygonaceae). J Mol Evol 60:391–399
Navajas-Pérez R, Schwarzacher T, de la Herrán R et al (2006) The origin and evolution of the variability in a Y-specific satellite-DNA of *Rumex acetosa* and its relatives. Gene 368:61–71
Nicolas M, Marais G, Hykelova V et al (2005) A gradual process of recombination restriction in the evolutionary history of the sex chromosomes in dioecious plants. PLoS Biol 3:47–56
Rechinger KH Jr (1964) The genus *Rumex* L. In: Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM, Webb DA (eds) Flora Europaea, vol 1. Cambridge University Press, Cambridge, UK, pp 82–89
Renner SS, Ricklefs RE (1995) Dioecy and its correlates in the flowering plants. Am J Bot 82:596–606
Ruiz Rejón M (2004) Sex chromosomes in plants. In: Goodman RM (ed) Encyclopedia of plant and crop sciences, vol 4. Dekker Agropedia, Marcel Dekker, New York, pp 1148–1151
Ruiz Rejón C, Jamilena M, Garrido-Ramos MA et al (1994) Cytogenetic and molecular analysis of the multiple sex chromosome system of *Rumex acetosa*. Heredity 72:209–215
Schwarzacher T (2003) DNA, chromosomes and in situ hybridization. Genome 46:953–962
Schwarzacher T, Heslop-Harrison JS (2000) Practical in situ hybridization. BIOS Scientific Publishers Limited, Oxford, UK
Shibata F, Hizume M, Kuroki Y (1999) Chromosome painting of Y chromosomes and isolation of a Y chromosome-specific repetitive sequence in the dioecious plant *Rumex acetosa*. Chromosoma 108:266–270
Shibata F, Hizume M, Kuroki Y (2000a) Differentiation and the polymorphic nature of the Y chromosomes revealed by repetitive sequences in the dioecious plant, *Rumex acetosa*. Chromosome Res 8:229–236
Shibata F, Hizume M, Kuroki Y (2000b) Molecular cytogenetic analysis of supernumerary heterochromatic segments in *Rumex acetosa*. Genome 43:391–397
Smith BW (1964) The evolving karyotype of *Rumex hastatulus*. Evolution 18:93–104
Smith BW (1968) Cytogeography and cytotaxonomic relationships of *Rumex paucifolius*. Am J Bot 55:673–683
Skaletsky H, Kuroda-Kawaguchi T, Minx PJ et al (2003) The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 423:825–837
Steinemann M, Steinemann S (1997) The enigma of the Y chromosome degeneration: TRAM, a novel retrotransposon is preferentially located on the neo-Y chromosome of *Drosophila miranda*. Genetics 145:261–266
Wilby AS, Parker JS (1988a) The supernumerary segment systems of *Rumex acetosa*. Heredity 60:109–117
Wilby AS, Parker JS (1988b) Recurrent patterns of chromosome variation in a species group. Heredity 61:55–62
Yu Q, Hou S, Feltus FA et al (2008a) Low X/Y divergence in four pairs of papaya sex-linked genes. Plant J 53:124–132
Yu Q, Navajas-Pérez R, Tong E et al (2008b) Recent origin of dioecious and gynodioecious Y chromosomes in papaya. Trop Plant Biol. doi: 10.1007/s12042-007-9005-7