A satellite DNA evolutionary analysis in the North American endemic dioecious plant *Rumex hastatulus* (Polygonaceae)

M.E. Quesada del Bosque, R. Navajas-Pérez, J.L. Panero, A. Fernández-González, and M.A. Garrido-Ramos

Abstract: We studied the evolution of RAE180 satellite DNA family in the North American endemic dioecious plant *Rumex hastatulus.* In this species, the Texas race is characterized by a single XX/XY sex chromosome system, whereas the North Carolina race has evolved a derived complex XX/XY_1Y_2 sex chromosome system. RAE180 repeats were autosomic and poorly represented (2×10^{-4}% of the genome) with no differences between individuals of different genders or different races of *R. hastatulus*. In fact, the sex chromosomes of the North Carolina race are still euchromatic, and they have not accumulated satellite DNA sequences, which contrasts with that occurring in the rest of dioecious XX/XY_1Y_2 *Rumex* species. In *R. hastatulus*, we detected the existence of three RAE180 subfamilies. Notwithstanding, while in the Texas race the TX1/NC1 subfamily is the most frequent, the TX2/NC2 subfamily is the most abundant in the North Carolina race. Additionally, the third, less represented subfamily (TX3/NC3) appears currently as relict sequences in both genomes. A common feature of RAE180 satellite is the sudden replacement of one sequence variant by another in different species (or populations as in *R. hastatulus* races). Thus, the phylogenetic analysis of RAE180 repeats from six dioecious *Rumex* species supports the "library" hypothesis. According to this hypothesis, we assume that a set of divergent RAE180 variants were present in the ancestral genome of dioecious *Rumex* species, from which novel tandem arrays originated by the amplification of different variants in different lineages. Differential levels of RAE180 satellite DNA amplification in each lineage, at different evolutionary times, and in different chromosomal positions gave rise to differential patterns of sequence evolution.

Résumé : Les auteurs ont étudié l'évolution de l'ADN satellite RAE180 chez le *Rumex hastatulus*, une plante dioïque endémique en Amérique du Nord. Chez cette espèce, la race Texas est caractérisée par un système à un seul chromosome sexuel XX/XY, tandis que la race North Carolina possède un système complexe dérivé de type XX/XY_1Y_2. Les répétitions RAE180 sont autosomiques et peu abondantes (2×10^{-4} % du génome) et ne présentent pas de différences entre individus des deux sexes ou entre les différentes races du *R. hastatulus*. De fait, les chromosomes sexuels de la race North Carolina sont encore euchromatiques et n'ont pas encore accumulé d'ADN satellite, contrairement à ce qui est observé chez les autres espèces dioïques XX/XY_1Y_2 du genre *Rumex*. Chez le *R. hastatulus*, les auteurs ont décelé la présence de trois sous-familles de RAE180. Tandis que chez la race Texas la sous-famille TX1/NC1 est la plus abondante, la sous-famille TX2/NC2 est la plus abondante chez la race North Carolina. De plus, une troisième sous-famille (TX3/NC3), la moins abondante, semble constituer une relique chez ces deux génomes. Le remplacement soudain d'un variant par un autre chez les différentes espèces (ou populations comme les races du *R. hastatulus*) est une caractéristique commune chez le satellite RAE180. Ainsi, l'analyse phylogénétique des répétitions RAE180 identifiées chez six espèces dioïques du genre *Rumex* supporte l'hypothèse de la « bibliothèque ». Selon cette hypothèse, les auteurs postulent qu'une collection de variants distincts de RAE180 était présente dans le génome ancestral des espèces dioïques de *Rumex* et que de nouveaux amas dupliqués en tandem sont apparus suite à une amplification de différents variants au sein des différents lignages. Des degrés différents d'amplification de l'ADN satellite RAE180 au sein de chaque lignage, à différents moments au cours de l'évolution et à différentes positions chromosomiques auraient donné lieu aux différents résultats observés.

Mots-clés : *Rumex hastatulus*, chromosomes sexuels, ADN satellite, évolution concertée, hypothèse de la « bibliothèque ».

[Traduit par la Rédaction]

Received 15 July 2010. Accepted 25 November 2010. Published on the NRC Research Press Web site at genome.nrc.ca on 1 April 2011.

Paper handled by Associate Editor Perry Gustafson.

M.E. Quesada del Bosque, R. Navajas-Pérez, A. Fernández-González, and M.A. Garrido-Ramos. Departamento de Genética, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain.
J.L. Panero. Section of Integrative Biology, 1 University Station, C0930, University of Texas, Austin, TX 78712, USA.

Corresponding author: M.A. Garrido-Ramos (e-mail: mgarrido@ugr.es).

Introduction

American and Eurasian dioecious species of the plant genus *Rumex* form a clade divided in two sister groups, one composed of species with an XX/XY sex chromosome system (*Rumex acetosella* and closely related species such as *Rumex graminifolius* and *Rumex paucifolius*, and *Rumex suffruticosus* and *Rumex hastatulus*) and the other including species with an XX/XY_1Y_2 system (*Rumex acetosa* and its relatives *Rumex papillaris*, *Rumex intermedius*, or *Rumex thyrsoides*) (Navajas-Pérez et al. 2005*a*). Sex chromosomes of the XX/XY species are barely differentiated, whereas the XX/XY_1Y_2 species are in a further stage of differentiation with heteromorphy between sex chromosomes (Cuñado et al. 2007). In addition, the Y chromosomes of XX/XY_1Y_2 species, but not those of XX/XY species, are heterochromatic and have accumulated a set of diverse repetitive sequences (Shibata et al. 1999, 2000; Navajas-Pérez et al. 2006, 2009*a*; Cuñado et al. 2007; Mariotti et al. 2006, 2009).

There are several satellite DNA families in *Rumex*: RAYSI (Y-specific) and RAE730 (autosomic) appeared only in the XX/XY_1Y_2 species of the genus (Navajas-Pérez et al. 2006, 2009*a*), as well as the RAYSII and RAYSIII families (Mariotti et al. 2009), whereas the RAE180 family is conserved in all dioecious species of *Rumex* (Navajas-Pérez et al. 2005*b*, 2006, 2009*a*, 2009*b*; Cuñado et al. 2007). In the XX/XY species, the RAE180 satellite DNA is located only in a small locus of a pair of autosomes, being poorly represented within their genomes (Cuñado et al. 2007; Navajas-Pérez et al. 2009*a*, 2009*b*). RAE180 repeats are characterized by intraspecific sequence homogeneity and interspecific divergence showing a general pattern of concerted evolution (Navajas-Pérez et al. 2009*b*). In contrast, in the case of XX/XY_1Y_2 species, the RAE180 sequences are present also in a small autosomal locus, but additionally have been massively amplified in the Y chromosomes (Cuñado et al. 2007; Navajas-Pérez et al. 2009*a*, 2009*b*). RAE180 sequences show very high levels of intraspecific variability, mostly ancestral because processes leading to concerted evolution have failed both before and after the diversification of the XX/XY_1Y_2 group. These results were explained on the basis of the absence of recombination in the Y chromosomes, a key factor in the concerted evolution process (Navajas-Pérez et al. 2005*b*, 2009*b*).

Interestingly, the North American endemic *R. hastatulus* resembles the global situation of the genus with two genetically — but not morphologically — differentiated chromosomal races that differ in their sex chromosome systems (Smith 1963, 1969; Navajas-Pérez et al. 2005*a*). The so-called Texas race is characterized by a simple XX/XY system, whereas the North Carolina race has a complex XX/XY_1Y_2 one. Despite its multiple sex chromosome system, according to molecular and cytogenetical data (Navajas-Pérez et al. 2005*a*), the North Carolina race of *R. hastatulus* is classified into the XX/XY group, implying secondary evolution from the XX/XY to the XX/XY_1Y_2 sex chromosome system (Smith 1969). More importantly, the same evolutionary change from one to the other sex chromosome system seems to have occurred independently in the two lineages of *Rumex*, one in the ancestor of the XX/XY_1Y_2 group and the other in *R. hastatulus*, as proposed earlier by Smith (1969) and Degraeve (1976).

In this context, given the parallel origin of the XX/XY_1Y_2 in two different lineages of dioecious species of *Rumex*, we sought to compare the events occurring in sex chromosomes evolution of both lineages and analyze (*i*) the genetic differentiation of the RAE180 satellite DNA between the Texas and North Carolina chromosomal races of *R. hastatulus* and (*ii*) the origin and evolution of this family of repeats, taking into account the existence of differential events of RAE180 satellite DNA amplification in different *Rumex* species. For this, we have compared RAE180 sequences from both *R. hastatulus* races as well as between these *R. hastatulus* repeats and other RAE180 repeats of the rest of dioecious *Rumex* species.

Materials and methods

Materials, DNA extraction, and RAE180 repeats isolation

Female and male plants were collected in the wild in Mason County, Texas (Texas race) and in Cumberland County, North Carolina (North Carolina race). The DNA from leaves of four males and three females from Texas and the DNA from leaves of two males and three females from North Carolina were isolated using DNAeasy mini kit (Qiagen) according to the manufacturer's instructions.

The characterization of RAE180 sequences was carried out separately in the two chromosomal races of *R. hastatulus*. Additionally, RAE180 sequences were also isolated from the genome of *R. intermedius* males (an XX/XY_1Y_2 species closely related to *R. acetosa*). For PCR amplification of RAE180 sequences from each selected individual, we used the primer pair Rae180a and Rae180b, 5′-TCATCGAACTTCATTCAT-3′ and 5′-TATAGTAATATCTCGATC-3′, and PCR procedure as well as purification, cloning, and sequencing of the PCR products were as described in Navajas-Pérez et al. (2009*b*).

A total of 76 repeats from the Texas race (43 from males and 33 from females) and 58 repeats from the North Carolina race (24 from males and 34 from females) were used for sequence analyses. The EMBL accession numbers are FN561567 to FN561625 (North Carolina race) and FN563834 to FN563909 (Texas race). Additionally, eight RAE180 repeats were selected among recombinant clones obtained from *R. intermedius* males (EMBL accession numbers FN564362 to FN564369).

Sequence analysis

Multiple alignments were performed using ClustalX (Thompson et al. 1997) followed by manual adjustments. Alignments were constructed for the 76 Texas samples, for the 58 North Carolina samples, and for the Texas and North Carolina samples combined. Additionally, multiple alignments were made for a representative set of RAE180 sequences of six *Rumex* species: 16 sequences from *R. hastatulus* (eight from each race) and eight sequences from each of three XX/XY_1Y_2 species (*R. acetosa* and *R. papillaris* — Navajas-Pérez et al. 2005*b*; and *R. intermedius* — this paper) and two XX/XY species (*R. acetosella* and *R. suffruticosus* — Navajas-Pérez et al. 2009*b*). For the characterization of RAE180 sequences from *R. hastatulus*, we determined variation within race (measured as nucleotide diversity, π, the average number of nucleotide differences per site between

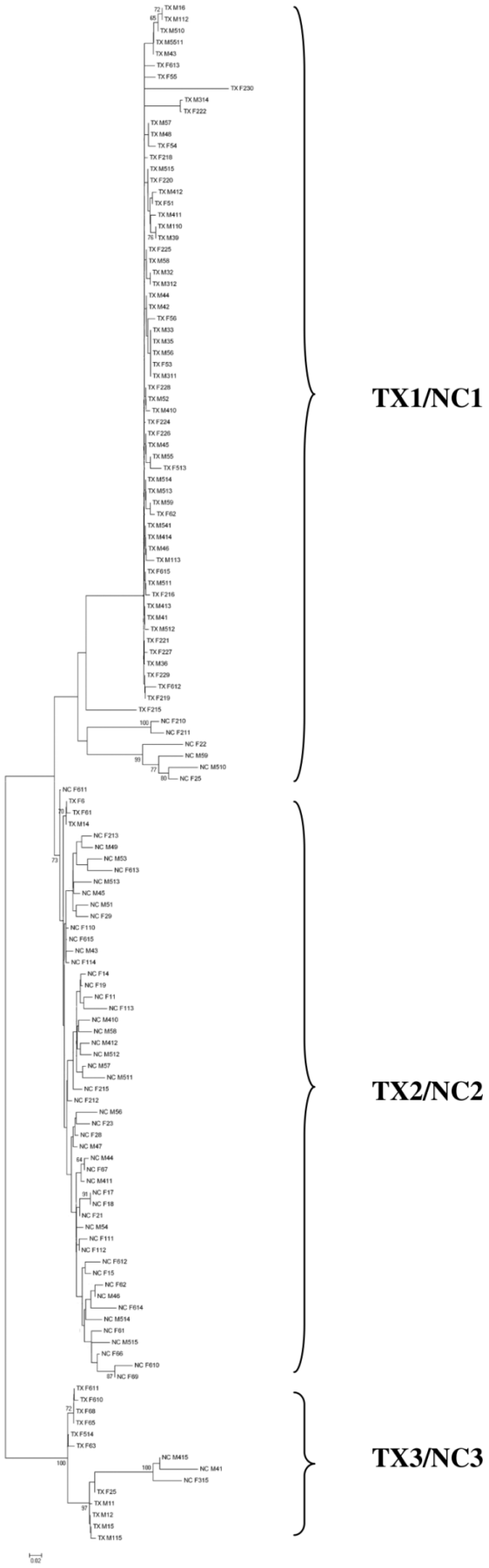


Fig. 1. Bootstrap consensus neighbor-joining tree of RAE180 repeats isolated from both *Rumex hastatulus* chromosomal races. Sequence code: TX, Texas; NC, North Carolina; F,M: female, male; numbers represent the number of the individual followed by the number of the repeat analyzed. To assess internal support for nodes, 1000 bootstrap replicates were performed. Only bootstrap values above 60% are indicated.

two sequences; Nei 1987) and differences between races (measured as the average number of nucleotide substitution per site between races, D_{xy}; Nei 1987) using the software satDNA Analyzer (Navajas-Pérez et al. 2007).

For phylogenetic analyses of RAE180 sequences, we used the aligned data as distance matrix sources. Distances were calculated according to the Kimura gamma distance and trees constructed by the neighbor-joining method in MEGA v4.1 (Tamura et al. 2007). For selection of the distance measuring method, the aligned sequences were subjected to analysis using Modeltest v.3.6 (Posada and Crandall 1998), which performs a hierarchical test of likelihood fits under 56 different models of DNA substitution. To assess internal support for nodes, 1000 bootstrap replicates (Felsenstein 1985) were performed.

Satellite DNA quantification

A quantitative PCR (qPCR) approach, following the procedure described in Navajas-Pérez et al. (2009*b*), was used to quantify relative percentage of RAE180 repeats in different genomes (males and females of the two *R. hastatulus* races analyzed). After relative percent quantification of RAE180 repeats, a rough estimate of the number of RAE180 copies present in *R. hastatulus* males and females for each race was made by assuming a total genome size similar to *R. acetosa* (3234 Mb; Bennett and Leitch 2004), as there are no data for *R. hastatulus* genome size.

Chromosomal analyses

Root tips were obtained from seeds germinated in Petri dishes and then pretreated with 2 mmol/L 8-hydroxiquinoleine for 4 h for metaphase accumulation. They were then fixed in ethanol–glacial acetic acid (3:1) until required. For DAPI staining and for fluorescence in situ hybridization (FISH) experiments, we followed the procedure described in Cuñado et al. (2007). As probes for FISH, we used RAE180 repeats directly amplified by PCR from the genomic DNAs of individuals of both *R. hastatulus* races.

Results and discussion

Characterization of RAE180 sequences and genetic differentiation between *Rumex hastatulus* chromosomal races

RAE180 satellite DNA represents about 2×10^{-4}% of the genome of *R. hastatulus*, with no differences in amounts between individuals of different genders and different races, which is consistent with an autosomic provenance of these repeats. Assuming that *R. hastatulus* has a similar genome size to *R. acetosa* (3234 Mb; Bennett and Leitch 2004) (there are no genome size data for *R. hastatulus*), this would equate to approximately a few tens of copies of RAE180 repeats.

Fig. 2. Multiple sequence alignment of representative sequences of RAE180 sequences from the two races of *Rumex hastatulus*. Subfamily TX1/NC1: TX_M41 and NC_F22; Subfamily TX2/NC2: TX_M14 and NC_F14; Subfamily TX3/NC3: TX_F65 and NC_M41.

```
TX_M41  TATTGAATTATATCGATCATA-TGTGAAAAA-ATCGGCCCGTTCC-GCCAC--CGATACTCGAGAACGGTCAATATCAT
NC_F22  ..A..G...T.C.........-.T-...G..-.......T...-.-.G.C-AT....T.....A.C.AACA.......C
TX_M14  .....G.....G.........-.T.......-.ATT.GATCG.T.C.A..-AT..........A.....C.........
NC_F14  ...G.G.....G.......AT-AT..G....-.ATT.GATCG.T.C.T.C-AT..........A.C...C.........
TX_F65  .....G.....C.T......TT.TCT.....ACAAT.GTTTG.TTCAAA.AAT.......T..A.....C........-
NC_M41  .....G.....C.T......TG.T.TT....-CAAT.GGTTG.TTCAAA.-AT.......T..A.-.A.C........-

TX_M41  CGAA-TAGTT---AGCAATAGTGAAG----GAAGTTCAATGAAAATAGTCGATATTCACCTATGTCGATGACCGGAACT
NC_F22  TT..-C....-CT.T.G...A.....----......TGG.................G......A..A.......AG...
TX_M14  T...-C.A..-CT...G.....ACGTGAAA.......G.................G.......C...C....A.G...
NC_F14  -...-C.A..CCT...G.....ACGTGAAA.......G.................G.T.....C...C......G...
TX_F65  T...A.....-CT.T.G...A....T----.......GTG.....ATA.T.GA...G..T...A.A..C....AAG...
NC_M41  T...A.....-CT.T.G...A....T----..-....GGG......GA.T.GAT..G..T...A.A..C....A-G...

TX_M41  CGTTTTTGGTTAAATCTTTTTGTATATTTTGATCAA
NC_F22  ..C....C.......TA...................
TX_M14  .A.....CC......G....................
NC_F14  .A.....CC......TC...................
TX_F65  .A.....CT..C...A....GT..............
NC_M41  .A.....CT..CC.A.C...G..TA....CCCC...
```

This is the smallest number of copies found for RAE180 sequences within *Rumex* species since we estimated about 200 and 7000 copies, respectively, in *R. acetosella* and *R. suffruticosus* (XX/XY species) and about one million copies of RAE180 repeats in the XX/XY_1Y_2 species *R. acetosa* (Navajas-Pérez et al. 2009*b*). Therefore, as reported for other *Rumex* species of the XX/XY clade (Cuñado et al. 2007; Navajas-Pérez et al. 2009*b*), RAE180 repeats are autosomic and poorly represented in the genome of *R. hastatulus*. Thus, after analysis with FISH using species-specific RAE180 satellite DNA probes, no hybridization signals were observed in *R. hastatulus* chromosomes, confirming that RAE180 sequences are scarcely represented in this species. No DAPI positive heterochromatic regions were observed in the sex chromosomes or the autosomes of *R. hastatulus* (not shown).

Our data reinforce the hypothesis that although the sex chromosome configurations (XX/XY_1Y_2) of *R. acetosa* and their relatives and those of *R. hastatulus* are similar, they arose independently (Smith 1969; Navajas-Pérez et al. 2005*a*). In addition, despite the convergent origin of the XX/XY_1Y_2 in both lineages, the sex chromosomes of the North Carolina race of *R. hastatulus* are still euchromatic, and they have not accumulated satellite DNA sequences, the main component of heterochromatic regions. A possible explanation could be that the origin of the XX/XY_1Y_2 sex chromosome system of the North Carolina race of *R. hastatulus* is quite recent. In fact, assuming a mean rate of change in plant nuclear DNA of 0.6% per site per million years (Gaut 1998) and using nuclear intergenic transcribed spacer (ITS) mean distance corrected estimates data of Navajas-Pérez et al. (2005*a*), the split between these two races occurred about 600 000 years ago. This contrasts with the origin of the *R. acetosa* clade, which is estimated to be 12–13 million years ago (mya) (Navajas-Pérez et al. 2005*a*).

Sequence analysis of RAE180 repeats demonstrated the existence of three satellite DNA subfamilies in both races of *R. hastatulus*: TX1, TX2, and TX3 in the Texas race and NC1, NC2, and NC3 in the North Carolina race. Each RAE180 subfamily was characterized by a set of diagnostic sites, each one representing a particular mutation shared by all the sequences of the subfamily, while at the same sites all the sequences of the other subfamilies had a different nucleotide (see Supplementary data,[1] Fig. S1). In both races, divergence between subfamilies was higher than variation within subfamilies (Tables S1 and S2), and phylogenetic analysis grouped together sequences by subfamily provenance (Figs. S2 and S3).

Table 1. Divergence between RAE180 sequences from both chromosomal races of *Rumex hastatulus*.

	Divergence
Between orthologous sequences	
TX1/NC1	0.252
TX2/NC2	0.050
TX3/NC3	0.179
Between most common sequences for each race	
TX1/NC2	0.190

Orthology of RAE180 subfamily types was determined based on shared clade membership on the combined-race phylogenetic tree (Fig. 1) and the comparison between individual race trees (Figs. S2 and S3) and the combined species tree. In the combined phylogenetic tree of RAE180 sequences, three groups of orthologous repeats were evident: TX1/NC1, TX2/NC2, and TX3/NC3 (Fig. 1). Sequences from different races shared diagnostic sites when they belonged to the same orthologous subfamily (Fig. 2). Genetic distances between the Texas and North Carolina races (Table 1) were highest for subfamily 1 (0.252) and lowest for subfamily 2 (0.050), with that of subfamily 3 well above the median (0.179).

However, the abundance of each RAE180 subfamily is different between races. In the Texas race, the TX1 subfamily was the most abundant (82% of the sequenced repeats), whereas only 4% of the RAE180 sequences belonged to the TX2 subfamily, and 14% belonged to the TX3 subfamily. In the North Carolina race, the NC2 subfamily was found to be the most abundant with 85% of the sequenced repeats. An average of 10% of the RAE180 sequences belonged to the NC1 subfamily, whereas 5% belonged to the NC3 subfamily.

[1]Supplementary data are available with the article at www.nrcresearchpress.com/gen

Fig. 3. (A) Bootstrap consensus neighbor-joining tree of RAE180 sequences from different dioecious species of the genus *Rumex*: RAC, *R. acetosa*; PAP, *R. papillaris*; INT, *R. intermedius*; ACE, *R. acetosella*; TX, *R. hastatulus*, Texas race; NC, *R. hastatulus*, North Carolina race. Only bootstrap values above 60% are indicated. (B) Phylogenetic relationships between dioecious *Rumex* species, according to Navajas-Pérez et al. (2005*a*).

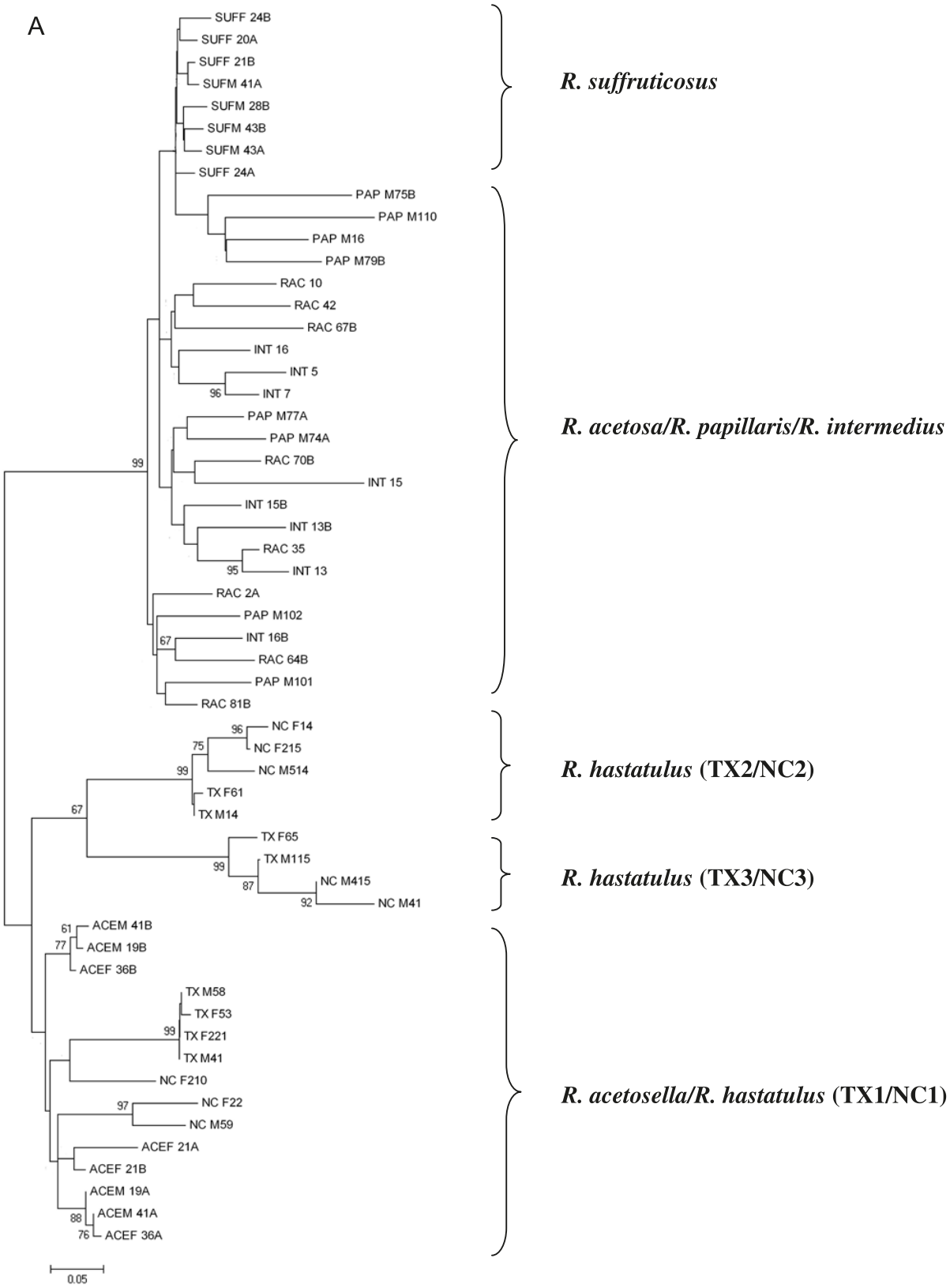
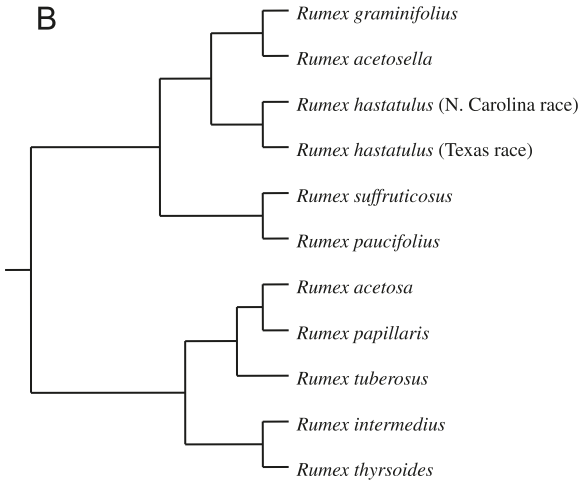


Fig. 3 (*concluded*).



Concomitantly, phylogenetic analysis between RAE180 sequences of both *R. hastatulus* races show two major clades (Fig. 1), each corresponding to the Texas race and the North Carolina race. This result is due to the fact that more than 80% of the repeats for each race belong to two different subfamilies: subfamily 1 (TX1) in the case of Texas race and subfamily 2 (NC2) in the case of North Carolina race, with a value of genetic differentiation between TX1 and NC2 of 19% (Table 1). The replacement of one sequence variant by another in different species (or populations as in *R. hastatulus* races) is a common feature of satellite DNA as a consequence of the high dynamism of satellite DNA evolution, as discussed below. In addition, comparisons of orthologous sequences of both chromosomal races show a remarkable level of genetic differentiation (Table 1). RAE180 satellite DNA genetic differentiation between the two chromosomal races of *R. hastatulus* is comparable to that found for the RAE730 satellite DNA of other *Rumex* species (Navajas-Pérez et al. 2005*b*) and the *Eco*RI satellite DNA of sparids (de la Herrán et al. 2001). The RAE180 satellite DNA is thus a valuable taxonomic marker for the genetic identification of individuals from different races.

RAE180 origin and evolution

According to the "library" hypothesis (Fry and Salser 1977; Meštrović et al. 1998, 2006; Mravinac et al. 2002), related taxa share a library of different conserved satellite DNA sequences (different satellite DNA families but also monomer variants or subfamilies of a satellite DNA family), which may be differentially amplified in each taxon with the subsequent replacement of one sequence variant by another in different species (or populations). In *R. hastatulus*, we have found support for the library hypothesis. The presence of the three RAE180 subfamilies in the two populations analyzed suggests that the existence of this variation preceded the isolation and differentiation of the two *R. hastatulus* chromosomal races. Even though the number of repeated sequences is still low (less than 100 according to our data), there is a differential amplification process in each race of this species for the different RAE180 subfamilies, in agreement with the library hypothesis. In addition, our understanding of the molecular evolution of RAE180 sequences in *R. hastatulus* has helped us understand the evolution of these repeats in other dioecious species of the genus *Rumex*.

The phylogenetic tree of RAE180 sequences isolated from dioecious species of the genus *Rumex* (Fig. 3A) is not congruent with the phylogeny of these species based on other markers (Fig. 3B). Using nuclear ITSs and chloroplast intergenic sequences, we have recently demonstrated that the genus *Rumex* contains three major clades (Navajas-Pérez et al. 2005*a*): a basal clade composed of strictly hermaphroditic species; a second clade composed of hermaphroditic, polygamous, and gynodioecious species of the genus; and a third clade including all the American and Eurasian dioecious species of *Rumex*. The third clade contains two subclades: (Fig. 3B) one containing *R. hastatulus*, labeled therein as the XX/XY group (*R. hastatulus*, *R. acetosella*, *R. graminifolius*, *R. paucifolius*, and *R. suffruticosus*) and the other composed of *R. acetosa* and relatives (*R. papillaris, R. intermedius*, or *R. thyrsoides*) and characterized by having an XX/XY_1Y_2 system (Degraeve 1976; Wilby and Parker 1988). According to Navajas-Pérez et al. (2005*a*), the dioecious clade of the genus *Rumex* split from the rest of other *Rumex* species 15–16 mya, whereas the two dioecious groups split approximately 12–13 mya.

However, most RAE180 sequences did not group by taxonomic affinity but rather by repeat type (Fig. 3). Most, but not all, sequences grouped according to the sex chromosome system. The exception in both cases was the eight sequences of *R. suffruticosus* that formed a monophyletic clade derived within a statistically well-supported clade containing all XX/XY_1Y_2 species (*R. acetosa*, *R. papillaris*, and *R. intermedius*) sequences. TX2/NC2 sequences and TX3/NC3 sequences each formed well-supported monophyletic clades also. However, TX1/NC1 sequences did not constitute a monophyletic clade and appear more closely related to *R. acetosella* sequences than to either TX2/NC2 or TX23/NC3, but without significant bootstrap support. Therefore, this tree supports again the library hypothesis for RAE180 satellite DNA evolution in *Rumex*.

Based on our phylogenetic analyses, we assume that the ancestral genome of dioecious *Rumex* species contained a set of divergent RAE180 variants from which novel tandem arrays originated by the amplification of different variants in different lineages. The TX1/NC1 variant, predominant in the Texas race of *R. hastatulus*, is closely related to the RAE180 sequences of *R. acetosella*, appearing then as an older type with respect to the TX2/NC2 variant. The derived TX2/NC2 variant is replacing the TX1/NC1 type in the North Carolina race of *R. hastatulus*. In any case, the RAE180 family is autosomic and poorly represented in these two species (Navajas-Pérez et al. 2009*b*; this paper). In addition, there is a species-specific variant slightly amplified in one pair of autosomes of *R. suffruticosus* (Cuñado et al. 2007; Navajas-Pérez et al. 2009*b*). In this species, RAE180 repeats are highly homogenized (Navajas-Pérez et al. 2009*b*; this paper). Finally, there is a sequence variant related to that of *R. suffruticosus* (Fig. 3) and shared by the three XX/XY_1Y_2 species (*R. acetosa*, *R. papillaris*, and *R. intermedius*). The evolution of RAE180 repeats in the XX/XY_1Y_2 lineage is unusual because we have found high levels of intraspecific variability in these species and slowed rates of sequence evolution and

homogenization (Navajas-Pérez et al. 2005*b*, 2009*b*; see also Fig. 3). Most of the high intraspecific variability found in each of these species would be ancestral variability, and the nonrecombining nature of the Y chromosomes would explain how processes leading to concerted evolution have failed both before and after the diversification of XX/XY_1Y_2 species (Navajas-Pérez et al. 2005*b*, 2009*b*; this paper).

In summary, the study of the RAE180 satellite DNA in *Rumex* reveals a complex evolutionary pattern where variability can remain by reduced action of molecular mechanisms of genomic turnover, thus leading to sequence variants persisting as a "library" (Meštrović et al. 1998, 2006; Mravinac et al. 2002). From this library, the amplification of particular variants could give rise later to new enlarged arrays in different lineages. Differential levels of RAE180 satellite DNA amplification in different lineages, at different evolutionary times, and in different chromosomal places gave rise to differential patterns of sequence evolution in which the onset of concerted evolution depended on factors such as location, organization, and copy number of the repeats (Navajas-Pérez et al. 2009*b* and references herein; this paper).

Acknowledgements

This research was supported by grant CGL2006-00444/BOS of the Ministerio of Ciencia e Innovación (Spain) and Fondo Europeo de Desarrollo Regional (FEDER, EU).

References

Bennett, M.D., and Leitch, I.J. 2004. Plant DNA C-values database Release 3.0.

Cuñado, N., Navajas-Pérez, R., de la Herrán, R., Ruiz Rejón, C., Ruiz Rejón, M., Santos, J.L., and Garrido-Ramos, M.A. 2007. The evolution of sex chromosomes in the genus *Rumex* (Polygonaceae): identification of a new species with heteromorphic sex chromosomes. Chromosome Res. **15**(7): 825–833. doi:10.1007/s10577-007-1166-6. PMID:17899410.

de la Herrán, R., Rejon, C.R., Rejon, M.R., and Garrido-Ramos, M. A. 2001. The molecular phylogeny of the Sparidae (Pisces, Perciformes) based on two satellite DNA families. Heredity, **87**(6): 691–697. doi:10.1046/j.1365-2540.2001.00967.x. PMID: 11903564.

Degraeve, N. 1976. Contribution à l'étude cytotaxonomique des *Rumex*. IV Le genre *Acetosa* Mill. Cellule, **71**: 231–240.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, **39**(4): 783–791. doi:10.2307/2408678.

Fry, K., and Salser, W. 1977. Nucleotide sequences of HS-α satellite DNA from kangaroo rat *Dipodomys ordii* and characterization of similar sequences in other rodents. Cell, **12**(4): 1069–1084. doi:10.1016/0092-8674(77)90170-2. PMID:597857.

Gaut, B.S. 1998. Molecular clocks and nucleotide substitution rates in higher plants. Evol. Biol. **30**: 93–120.

Mariotti, B., Navajas-Pérez, R., Lozano, R., Parker, J.S., de la Herrán, R., Rejón, C.R., 2006. Cloning and characterization of dispersed repetitive DNA derived from microdissected sex chromosomes of *Rumex acetosa*. Genome, **49**(2): 114–121. doi:10.1139/G05-089. PMID:16498461.

Mariotti, B., Manzano, S., Kejnovský, E., Vyskot, B., and Jamilena, M. 2009. Accumulation of Y-specific satellite DNAs during the evolution of *Rumex acetosa* sex chromosomes. Mol. Genet. Genomics, **281**(3): 249–259. doi:10.1007/s00438-008-0405-7. PMID:19085011.

Meštrović, N., Plohl, M., Mravinac, B., and Ugarković, Đ. 1998. Evolution of satellite DNAs from the genus *Palorus* — experimental evidence for the "library" hypothesis. Mol. Biol. Evol. **15**(8): 1062–1068. PMID:9718733.

Meštrović, N., Castagnone-Sereno, P., and Plohl, M. 2006. Interplay of selective pressure and stochastic events directs evolution of the MEL172 satellite DNA library in root-knot nematodes. Mol. Biol. Evol. **23**(12): 2316–2325. doi:10.1093/molbev/msl119. PMID: 16982821.

Mravinac, B., Plohl, M., Meštrović, N., and Ugarković, Đ. 2002. Sequence of PRAT satellite DNA "frozen" in some Coleopteran species. J. Mol. Evol. **54**(6): 774–783. doi:10.1007/s0023901-0079-9. PMID:12029359.

Navajas-Pérez, R., de la Herrán, R., López González, G., Jamilena, M., Lozano, R., Ruiz Rejón, C., 2005*a*. The evolution of reproductive systems and sex-determining mechanisms within *Rumex* (Polygonaceae) inferred from nuclear and chloroplastidial sequence data. Mol. Biol. Evol. **22**(9): 1929–1939. doi:10.1093/molbev/msi186. PMID:15944442.

Navajas-Pérez, R., de la Herrán, R., Jamilena, M., Lozano, R., Rejón, C.R., Rejón, M.R., and Garrido-Ramos, M.A. 2005*b*. Reduced rates of sequence evolution of Y-linked satellite DNA in *Rumex* (Polygonaceae). J. Mol. Evol. **60**(3): 391–399. doi:10.1007/s00239-004-0199-0. PMID:15871049.

Navajas-Pérez, R., Schwarzacher, T., de la Herrán, R., Ruiz Rejón, C., Ruiz Rejón, M., and Garrido-Ramos, M.A. 2006. The origin and evolution of the variability in a Y-specific satellite-DNA of *Rumex acetosa* and its relatives. Gene, **368**: 61–71. doi:10.1016/j.gene.2005.10.013. PMID:16324803.

Navajas-Pérez, R., Rubio-Escudero, C., Aznarte, J.L., Rejón, M.R., and Garrido-Ramos, M.A. 2007. SatDNA Analyzer: a computing tool for satellite-DNA evolutionary analysis. Bioinformatics, **23**(6): 767–768. doi:10.1093/bioinformatics/btm005. PMID: 17242030.

Navajas-Pérez, R., Schwarzacher, T., Rejón, M.R., and Garrido-Ramos, M.A. 2009*a*. Molecular cytogenetic characterization of *Rumex papillaris*, a dioecious plant with an XX/XY_1Y_2 sex chromosome system. Genetica, **135**(1): 87–93. doi:10.1007/s10709-008-9261-y. PMID:18373205.

Navajas-Pérez, R., Quesada del Bosque, M.E., and Garrido-Ramos, M.A. 2009*b*. Effect of location, organization, and repeat-copy number in satellite-DNA evolution. Mol. Genet. Genomics, **282**(4): 395–406. doi:10.1007/s00438-009-0472-4. PMID:19653004.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Posada, D., and Crandall, K.A. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics, **14**(9): 817–818. doi:10.1093/bioinformatics/14.9.817. PMID:9918953.

Shibata, F., Hizume, M., and 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**(4): 266–270. doi:10.1007/s004120050377. PMID:10460415.

Shibata, F., Hizume, M., and Kuroki, Y. 2000. Differentiation and the polymorphic nature of the Y chromosomes revealed by repetitive sequences in the dioecious plant, *Rumex acetosa*. Chromosome Res. **8**(3): 229–236. doi:10.1023/A:1009252913344. PMID: 10841050.

Smith, B.W. 1963. The mechanism of sex determination in *Rumex hastatulus*. Genetics, **48**(10): 1265–1288. PMID:17248184.

Smith, B.W. 1969. Evolution of sex-determining mechanisms in *Rumex*. Chromosomes Today, **2**: 172–182.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software

version 4.0. Mol. Biol. Evol. **24**(8): 1596–1599. doi:10.1093/molbev/msm092. PMID:17488738.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **25**(24): 4876–4882. doi:10.1093/nar/25.24.4876.

Wilby, A.S., and Parker, J.S. 1988. Recurrent patterns of chromosome variation in a species group. Heredity, **61**(1): 55–62. doi:10.1038/hdy.1988.90.