Analysis of two Different Satellite DNA Subfamilies in the Complex Sex-chromosome System of Rumex acetosa (Polygonaceae)

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INTRODUCTION.-

Rumex acetosa is a dioecious plant species with a complex chromosomal system of sex determination, females having a karyotype composed of 14 chromosomes (2n=12+XX) and males having 15 chromosomes (2n=XY1Y2). *Rumex acetosa* is a classical model in sex determination studies not only for its complex system but also for the heterochromatinization of the Y chromosomes, a unique case reported for plants, which usually have their sex chromosomes in a less advanced stage of differentiation.
Evolutionary processes affecting sex chromosomes in different groups of organisms suggest that progressive suppression of recombination leads to the accumulation of diverse repetitive sequences such as mobile elements and satellite DNAs among other phenomena (Charlesworth et al, 1996). Specifically, to the date, two different satellite DNA families have been reported to be Y-linked in *Rumex acetosa* (Ruiz Rejón et al, 1994; Shibata et al, 1999, 2000).
In the present work, we focused in the evolutionary pathway of one of these Y-specific satellite-DNA families, **RAYSI** (Shibata et al, 1999). We have demonstrated for this family to be comprised of two different subfamilies that show differential patterns of distribution in both Y chromosomes and differences in their sequence.

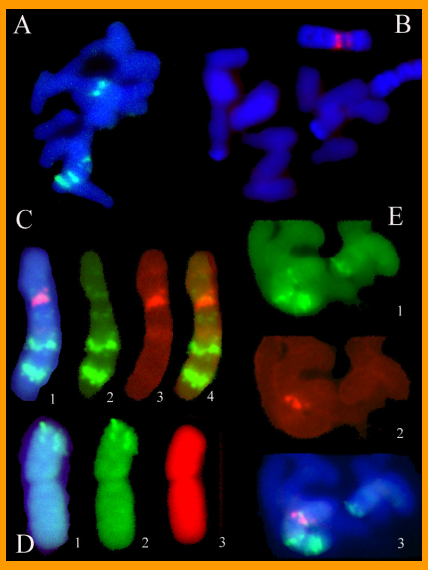

FIGURE 2. *Rumex acetosa* male metaphases showing positive hybridization with RAYSI-J (detected with FITC-green) **(A)** and RAYSI-S (detected with CY3-red) probes **(B)**. The chromosomes were counterstained with DAPI in each case. *In situ* hybridization with both RAYSI subfamilies in the Y1 chromosome **(C)**. Visualized simultaneously **(C.1)**, RAYSI-J subfamily **(C.2)**, RAYSI-S subfamily **(C.3)**, both subfamilies without DAPI counterstaining **(C.3)**. RAYSI sequences location in the Y2 chromosome **(D)**, visualized simultaneously **(D.1)**, RAYSI-J subfamily **(D.2)**, negative hybridization with RAYSI-S probe **(D.3)**. Y1 and Y2 showing the hybridization patterns of the RAYSI subfamilies in a single pro-metaphase **(E)**, RAYSI-J **(E.1)**, RAYSI-J **(E.2)**, visualized simultaneously **(E.3)**.

RESULTS.-

From the genome of *Rumex acetosa*, we have analysed 42 monomers of the RAYSI family, a repetitive-sequence family comprised of 67% AT-rich repeat units between 922 and 932 bp in length.
RAYSI sequences showed relatively high levels of intra-specific variability (11.2%). This degree of sequence variation within this satellite-DNA family is due mainly to the presence of two types (subfamilies) of repeat units. We have named these two RAYSI subfamilies **RAYSI-S** and **RAYSI-J**. Pairwise comparisons between monomeric sequences revealed the presence 91 sites that differentiate them. Each site represents a particular shared mutation among all the sequences of one subfamily, while at the same sites all the sequences of the other subfamily had a different nucleotide. Additionally, an specific 14-bp deletion for RAYSI-S sequences was found between positions 687 and 700. Similarly, RAYSI-J sequences had two deletions of 7 and 9 bp between positions 869 and 875, and 899 and 907, respectively. A phylogenetic analysis of the 42 sequences reveals a neighbour-joining tree with two clades supported by 100% of the bootstrap replicates, one composed by RAYSI-J sequences (23 in total) and the other composed of RAYSI-S sequences (19 in total) (***Figure 1***). The mean interfamilial divergence is 17.3%. However, the mean percentage of differences is 4.4% for RAYSI-S and 5.2% for RAYSI-J.
At the chromosomal level, the FISH technique revealed a different hybridization pattern for each probe (***Figure 2***). RAYSI-J appears at four different loci in the Y1 chromosome and at a single locus in the Y2 chromosome. Thus, hybridization signals were found as two strong and one weak bands on the long arm and as a weak band on the short arm of Y1 chromosome, as well as a band in the telomeric region of the short arm of the Y2 chromosome. The location of RAYSI-S is restricted to the centromeric region of the Y1 chromosome and to two weak bands –these appearing on the short and on the long arms of the same chromosome, the former apparently juxtaposed with a locus of the RAYSI-J subfamily. Meanwhile, the RAYSI-S sequences are absent from the Y2 chromosome.

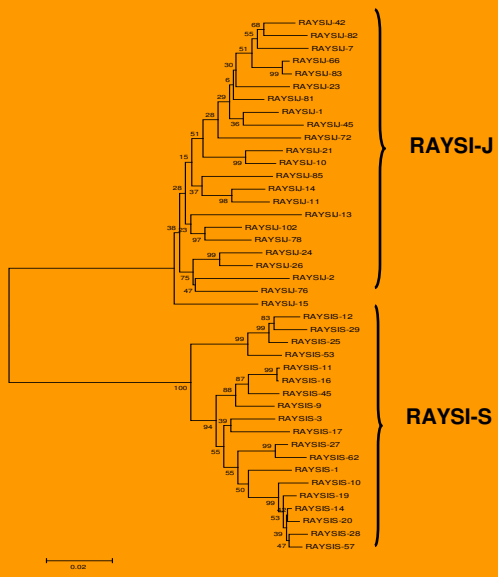

FIGURE 1. Neighbour-joining tree showing the RAYSI sequences in two separate clades (RAYSI-J and RAYSI-S). Numbers at each node indicate the percentage of trees representing the particular node out of 1000 bootstrap replicates. Bootstrap values under 50 are not indicated. In this tree, the labels of the sequences correspond to the first three letters of the species name (ACE) and a number representing the repeat analyzed.

DISCUSION.-

Concerted evolution generally leads to intra-specific sequence identity among the repetitive units of a satellite-DNA family (Ugarkovic and Plohl, 2002). Different molecular mechanisms, such as unequal crossing-over and gene conversion, spread new mutations by horizontal transference throughout the family members at a rate higher than that at which new changes arise (Dover, 1982, 1986; Ugarkovic and Plohl, 2002). However, in some cases this intra-specific homogeneity of a satellite DNA family is not detected within a species since different subfamilies can be found, as in RAYSI sequences. These subfamilies are defined by the presence of several diagnostic sites that unambiguously differentiate the two groups of sequences (Plohl and Ugarkovic, 1994a,b; Pons et al., 2002). Thus, each site has a particular shared mutation among all the sequences of one subfamily, while at the same sites all the sequences of the other subfamily have a different nucleotide. These subfamilies appear to have resulted from the absence of chromosomal exchanges between non-homologous chromosomes. This lack of chromosomal transfer could give rise to chromosome-specific subfamilies (Willard and Waye, 1987a,b; Alexandrov et al., 1988). An extreme case of such a lack of exchange occurs in the non-recombining Y chromosomes.
Y-linked genes follow different patterns of sequence evolution with respect to the genes found in the X chromosome and in the autosomes (Filatov et al., 2000; Filatov et al., 2001) primarily due to their non-recombining nature. The gradual suppression of recombination between the X and Y chromosomes progressively degenerates the Y by the loss of function of most genes (Filatov et al., 2000) and the expansion of tandemly repetitive DNA families (Charlesworth, 1996; Jobling and Tyler-Smith, 2003). After expansion, mechanisms blocking chromosomal exchange (i.e. recombination) negatively influence concerted evolution, as expected for RAYSI sequences of the non-recombining Y chromosomes in the complex XX/XY1Y2 chromosomal system of *R. acetosa*.
Taking into account, that during male meiosis, each Y chromosome pairs with one end of the X chromosome (Ruiz Rejón, 2003), we have demostrated low rates of sequence evolution and sequence homogenization (**See *Poster number P0:08:09 and Abstract 7114***) as well as the appearance of different subfamilies located at different loci within the two Y chromosomes (the present study). These features might be explained, therefore, as a consequence of the lack of recombination between the two Y chromosomes.

REFERENCES.-

Alexandrov, IA, Mitkevich, SP, Yurov, YB (1988). Chromosoma 96:443-453
Charlesworth, B. (1996). Curr Biol 6(2):149-162
Dover, G. (1982). Nature 299:111-117
Dover, G. (1986). Trends Genet 2:159-165
Filatov, DA, Monager, F, Negrutiu, I, Charlesworth, D. (2000). Nature 404:388-390
Filatov, DA, Laporte, V, Vitte, C, Charlesworth, D. (2001). Mol Biol Evol 18(8):1442-54
Jobling, MA, Tyler-Smith, C. (2003). Nat Rev Genet 4:598-612
Plohl, M, Ugarkovic, Đ. (1994). J Mol Evol 39:299-304
Plohl, M, Ugarkovic, Đ. (1994b). J Mol Evol 39: 486-495
Pons, J, Peñalverra, E, Juan, C. (2002). Mol Biol Evol 19(8):1329-1340
Ruiz Rejón, C, Jamilena, M, Garrido-Ramos, MA, Parker, JS, Ruiz Rejón, M. (1994). Heredity 72: 209- 215
Ruiz Rejón, M. (2003). In "Encyclopedia of Plant and Crop Sciences". (Vol. 6):1148-1151 Dekker Agropedia
Shibata, F, Hizume, M, Kuroki, Y. (1999). Chromosoma 108:266-270
Shibata, F, Hizume, M, Kuroki, Y. (2000). Chromosome Res 8:229-236
Ugarkovic, Đ, Plohl, M. (2002). European Molecular Biology Organization Journal 21(22):5955-5959
Willard, HF, Waye, JS. (1987a). J Mol Evol 25:207-214
Willard, HF, Waye, JS. (1987b). Trends Genet 3: 192-198

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