Reduced rates of sequence evolution of Y-linked satellite DNA in *Rumex* (*Polygonaceae*)

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INTRODUCTION.-

Satellite DNAs are tandemly arrayed, highly repetitive DNA sequences of the eukaryotic genomes located in the constitutive heterochromatin (Ugarkovic and Plohl, 2002). The repeats comprising a satellite-DNA family do not evolve independently of one another but rather follow concerted evolution (Dover, 1982; 1986). That is, arrays of non-allelic homologous sequences, homogenized by transfer mechanisms such as unequal crossing-over and gene conversion, evolve as a unit (Dover, 1982; Ugarkovic and Plohl, 2002). Factors affecting concerted evolution include rates of transfer between homologous and non-homologous chromosomes, arrangements of repeats, array sizes, and population structure. Bias in any of these factors can alter the rates of concerted evolution. Thus, for instance, these rates will be reduced by mechanisms impeding chromosomal exchanges (i.e. recombination), as in the non-recombining Y chromosomes.

Specifically, the species under study in this work are *Rumex acetosa* and *Rumex papillaris*, two related *Polygonaceae* species. Both species are dioecious and classical models in sex-determination studies. These species have a complex sex chromosome system, females having a karyotype composed of 14 chromosomes (2n=12+XX) and males having 15 chromosomes (2n=XY1Y2). The interest of this species lies in two main factors. Firstly, during meiosis, the two Y chromosomes pair only with the ends of each X arm. Furthermore, in contrast to other dioecious plant species such as *Silene latifolia*, these are useful for the study of the accumulation of satellite DNAs in the Y chromosomes because the Ys are heterochromatic and rich in satellite-DNA sequences (Ruiz Rejón et al., 1994; Shibata et al., 1999). All data, therefore, suggest that the Ys and the X chromosomes are highly differentiated and that the Y-chromosomes are degenerated, as they are heterochromatic and rich in satellite-DNA sequences (Ruiz Rejón et al., 1994).

To date, three different satellite-DNA families have been described within the genome of *R. acetosa* and *R. papillaris*:
On the one hand, two of them have been related to be present in the Y chromosomes; the **RAE180** family (Shibata et al., 2000a) and **RAYSI** family (Shibata et al., 1999) (***Figure 1A)***. The former is one of the two major types of sequences found within the heterochromatin of the Y-chromosomes (Shibata et al., 2000a). Moreover, two minute additional loci exist in euchromatic regions of the autosome pairs 1 and 4 (Shibata, et al., 2000a). The latter is exclusive of the Y-chromosomes (Shibata et al., 1999). On the other hand, the **RAE730** satellite-DNA family is found in heterochromatic segments of some autosome pairs (Shibata et al., 2000b) (***Figure 1B)***. These supernumerary segments are fixed in homozygosis at the sixth chromosome pair in Spanish populations analyzed (Sierra Nevada and Sierra de Baza, Granada) (Ruiz Rejón et al., 1994).

RESULTS.-

Here, we analyze the rate of concerted evolution of these three satellite-DNA families, for which we compare neighboring sequences (i.e. non-allelic monomers) in tandem arrays. For all three satellite DNAs, we found similar variability levels within individuals and among individuals within a species. ***Table 1*** summarizes the intra-specific and inter-specific variation between monomers of each of the three satellite-DNA families of *Rumex*. In relation to inter-specific divergence, the intra-specific variability of the Y-associated satellite DNAs, RAYSI and RAE180, was much higher than in the autosomic RAE730 sequences, even in the event that RAE730 and RAYSI families share a common origin (***Figure 2***).

Mean genetic distance for RAE730 sequences between *Rumex acetosa* and *Rumex papillaris* proved two-fold to three-fold higher than intra-specific variation. However, the difference between inter-specific and intra-specific distances for RAYSI and RAE180 sequences was slight (0.7%). ***Table 1*** also shows the evolutionary rates for each satellite-DNA family for a divergence time of 2 million years between *Rumex acetosa* and *Rumex papillaris* (Navajas-Pérez et al., non-published data). The rate of sequence change for RAE730 satellite DNA was almost two-fold higher than the rates for the RAYSI and the RAE180 satellite DNAs (***Table 1***).

In addition, we found that within the RAE180 repeat units approximately 49% of the sites represented shared polymorphisms between *R. acetosa* and *R. papillaris*. However, we detected only three nearly fixed differences (1.5% of the sites) between these two species and a 38% of polymorphic transitional stages. These data contrast with those found for the RAE730 sequences. In this case, 4% of nucleotide sites represented shared polymorphic sites, while 6.5% were fixed differences between *R. acetosa* and *R. papillaris* and 38% were transition differentiation stages.

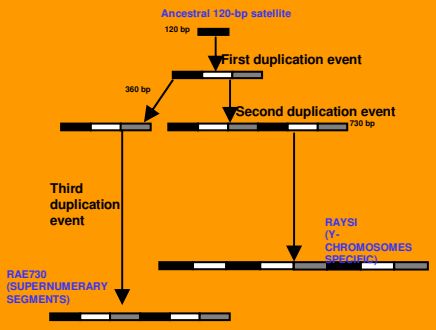
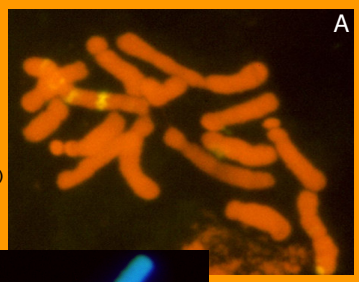


Figure 2.- Evolutionary scheme proposed to explain the evolution of current RAYSI and RAE730 monomers.



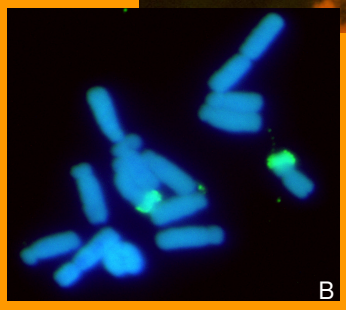


Figure 1.- *In situ* hybridization showing RAYSI family location **(1A)** Location of RAE730 family **(1B)**

SATELLITE FAMILY	*MEAN INTRA-SPECIFIC DISTANCES (R. acetosa/R. papillaris)*	*MEAN INTER-SPECIFIC DISTANCES*	*EVOLUTIONARY RATES*	*FIXED DIFFERENCES BETWEEN SPECIES (Transition stages 4+5)*	*TRANSITIONAL DIFFERENCES BETWEEN SPECIES (Transition stages 2+3)*
RAE730	0.046/0.029	0.088	22.00×10^{-9}	47	281
RAYSI-J	0.037/0.042	0.047	11.74×10^{-9}	3	407
RAE180	0.036/0.037	0.045	11.25×10^{-9}	3	74

Table 1. Analysis of the three satellite DNA families found in *Rumex acetosa* and *R. papillaris* after excluding inter-specific shared polymorphic sites. See text for details.

CONCLUSIONS.-

1.- We found low rates of sequence homogenization and evolution in the satellite DNAs located at the Y-chromosomes (RAYSI and RAE180) in relation to the autosomic RAE730 satellite DNA.
2.- These data support the idea that there is no recombination between the Y-chromosomes in *Rumex* and that this situation strongly influences the evolutionary pathways of satellite DNAs in sex chromosomes. In fact, the first observation would be a consequence of this lack of recombination.

REFERENCES.-

Dover, G. (1982). Nature 299:111-117
Dover, G. (1986). Trends Genet 2:159-165
Ruiz Rejón, C., M. Jamilena, M. A. Garrido-Ramos, J. S. Parker and M. Ruiz Rejón. (1994). Heredity 72: 209- 215.
Shibata, F., Hizume, M., Kuroi, Y. (1999). Chromosoma 108:266-270
Shibata, F., Hizume, M., Kuroi, Y. (2000a). Chromosome Res 8:229-236
Shibata, F., Hizume, M., Kuroi, Y. (2000b). Genome 43:391-397
Ugarkovic, D. & Plohl, M. (2002). European Molecular Biology Organization Journal 21(22):5955-5959

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