ORIGIN AND EVOLUTION OF THE SEX-DETERMINING CHROMOSOMAL SYSTEM OF *RUMEX ACETOSA* USING REPETITIVE DNA SEQUENCES.

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Introduction

The species *Rumex acetosa* (*Polygonaceae*) is characterized by a karyotype in which females are 2n=14 (12 autosomes + XX) while males have an additional chromosome, being 2n=15 (12 autosomes + XY_1Y_2). This complex sex-determining system implies an advanced evolutionary state in plants (Guttman & Charlesworth, 1998). Furthermore, *Rumex acetosa* is a classical example in sex-chromosome research, as it is the only Y-degeneration case reported in plants. Both Y chromosomes appear completely heterochromatinized, given that they have accumulated large amounts of repetitive DNA, and therefore it is deduced that they have a high degree of degeneration (Ruiz Rejón et al., 1994). In view of all these data, here we have analyze three satellite DNA families in the *Rumex acetosa* genome as well as in dioecious *Rumex papillaris* species genome, in order to elucidate the evolutionary pathway that these families follow and shed light about several puzzling aspects of the origin and the evolution of sex chromosomes.

The origin of the three satellite DNA families

In this paper, we characterize three satellite DNA families within the genome of the species *Rumex acetosa* (Figure 1). The RAE180 family has monomeric repeats of 180 bp and it is located at two autosomic loci and as part of constitutive heterochromatin of both Y chromosomes (Ruiz Rejón et al., 1994; Shibata et al., 2000a). The RAYSI family, formed by 930-bp repeats, is located exclusively on Y chromosomes and is therefore a male-specific sequence (Shibata et al., 1999), not appearing in females. The third family, RAE730, is located on heterochromatic supernumerary segments (Shibata et al., 2000b), which are fixed in homozygotic state in the sixth pair of autosome complement in Spanish populations.

The RAE180 family has no significant homology with the two other families. On the other hand, RAYSI and RAE730 are homologous, sharing 55% identity. Furthermore, in parts of the alignment this identity increases to 70%. Additionally, both families RAE180 and RAYSI were found to be formed by sub-repeats of 120 bp (Figure 2). Consecuently, the RAE730 family is made up of 6 of these sub-repeats while the RAYSI family is composed of 8.

The evolutionary scheme, explaining the evolution from the ancestral 120 bp satellite toward the current satellites we propose, is the one in the Figure 3. We have carried out a phylogenetic study of the 120 bp motives. We have found that, between the ancestor 120 bp satellite and the actual RAYSI and RAE730 monomers, an 360 bp intermediate satellite existed. Moreover, we suggest that duplication events originating both of actual satellites occur at different evolutionary times, being the duplication event to form RAYSI, evolutionary more separated in time. We can explain the actual structure of RAYSI monomers by means of additional unequal crossing over phenomena.

We deduce from these data two types of heterochromatin located on both Y-chromosomes. The first type has a common origin with the heterochromatin located on another special region of the genomes, the supernumerary segments. The latter type is located on autosomes and Y-chromosomes as well. However, both types of heterochromatin are not related between them.

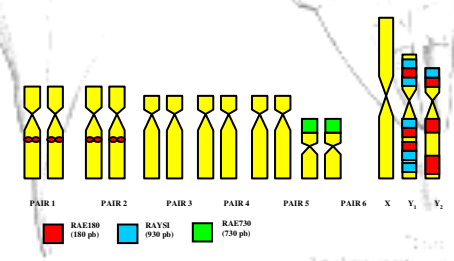

Figure 1.- Chromosomal location of the three satellite DNA families analyzed.

Figure 2.- Alignment of 120-bp sub-repeats of monomeric units of RAE730 and RAYSI.

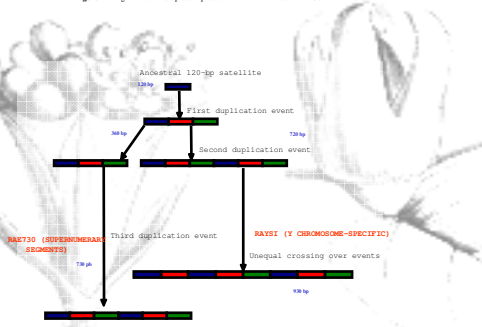

Figure 3.- Evolutionary scheme proposed to explain the evolution of current RAE730 and RAYSI monomers.

SATELLITE	EVOLUT. CHANGE RATIO	INTERSPEC. DIST.	INTRASP. DIST. (acet/papil)	FIXED SITES	TRANSITION STAGES
RAYSI (Y chromosomes)	$13x10^{-9}$	0.053	0.049/0.045	6	176
RAE180 (Y chromosomes)	$13x10^{-9}$	0.051	0.044/0.044	0	41
RAE730 (supernumerary s.)	$22x10^{-9}$	0.088	0.044/0.027	26	100

Table 1.- Data from evolutionary analysis of the three satellite DNA families.

The evolution of the satellites

In the present work we have analyze not only the three DNA satellite families cited above but we study the presence of these in a representative group of rumices species too, these belonging to the 4 subgenera in which the genera *Rumex* is divided. The only two *Rumex* species analyzed having the three satellite DNA families are the two dioecious *Rumex acetosa* and *Rumex papillaris* relatives. Both *Rumex acetosa* and *Rumex papillaris* are classified into the *Acetosa* section of the subgenus *Acetosa*. These two representatives as well as the other *Acetosa* species group form a robust taxa, at the molecular and the morphological levels. All of these species possess a complex sex-determining system. Taking into account that the distance for ITS sequences between these two species is 0.024, and assuming a evolutionary-change rate of 0.6% per site per million of years (Gaut, 1998) we have calculated a divergence time of 2 m.y.a. between the two. The fact that we had two closely related species sharing three satellite DNA families and that these three families were located in sex chromosomes (Y-chromosomes) as well as in autosomes prompted us to analyze whether the evolutionary rates of the two types of satellites are different.

It is well known that satellite DNA is characterized by concerted and rapid evolution. This kind of evolution depends on several factors, but they are fundamentally subordinated to homogenization processes- that is, recombination events (Ugarkovic & Plohl, 2002). Therefore, we investigated the evolutionary process of satellite DNA in chromosomes with no recombination (as in Y-chromosomes).

A comparative analysis between the satellite DNA sequences in Y-chromosomes and satellite DNA sequences in autosomes revealed that the absence of recombination causes slow evolutionary-change and concerted-evolution rates (Table 1). However, there is a remaining homogenization rate that, in absence of recombination, must be related to intra-chromosomal gene conversion. It would not be surprising for this gene-conversion phenomena to have led to sub-families formation. In our case, we found two sub-families within *Rumex acetosa* RAYSI family: RAYSI-S and RAYSI-J. About 70 diagnostic sites are fixed in one or the other sub-family. Moreover, exclusive sub-family deletion sites in both RAYSI-J and RAYSI-S families exist. We tested this data by means not only of molecular but also by cytogenetic methods as well. We carried out fluorescent *in situ* hybridization (FISH) experiments (Figure 4) that demonstrated the existence of two loci-separated sub-families of RAYSI, suggesting that these Y chromosome evolved separately and do not recombine between themselves.

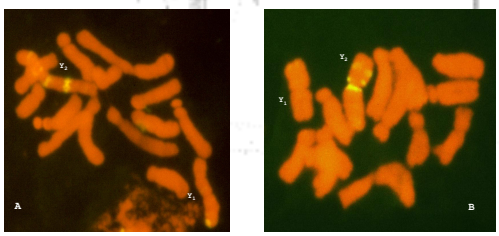

Figure 4.- Chromosomal location of the RAYSI-S sub-family, located on several Y_2 chromosome clusters and on the Y_2 chromosome distal region (**4a**). Localization of the RAYSI-J sub-family, located exclusively on Y_1 chromosome (**4b**).

References

Gaut, B. S. (1998). Evolutionary Biology 30:93-120.

Guttman, D. S. & Charlesworth, D. (1998). Nature. 393:1009-1014.

Ruiz Rejón, C., Jamilena, M., Garrido Ramos, M., Parker, J. S. & Ruiz Rejón, M. (1994). Heredity 72:209-215.

Shibata, F., Hizume, M. & Kuroi, Y. (1999). Chromosoma 108:266-270.

Shibata, F., Hizume, M. & Kuroi, Y. (2000a). Chromosome research 8:229-236.

Shibata, F., Hizume, M. & Kuroi, Y. (2000b). Genome 43:391-397.

Ugarkovic, D. & Plohl, M. (2002). EMBO 21(22):5955-5959.