



Satellite-DNA could trace the phylogeny of the genus *Rumex* (*Polygonaceae*)

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INTRODUCTION.-

In a few years, the genus *Rumex* has become an important key to solving some unanswered questions in plant sex-determination systems that still remain puzzling. In fact, *Rumex* constitutes a large group of species in which almost every mating system is present, comprising of hermaphrodite, polygamous, gynodioecious, monoecious and dioecious representatives. Further, regarding dioecy, there are two main types of species: simple sex-chromosome system species (XX/XY) as in *Rumex acetosella*, and complex systems ones (XX/XY₁Y₂), in *R. acetosa*, for example. Thus, this genus constitutes a particular group to test hypothesis concerning sexual systems and sex chromosomes evolution.

In contradiction with the actual classification based on morphological features, we have recently established a new classification of the group based in different molecular markers (nuclear and chloroplastidial), basic chromosome number and sex-chromosome systems (Fig. 1). This proposed new grouping assume three main clades within *Rumex* consistent with the mating system of the species: one clade is composed of hermaphroditic and monoecious species, another clade is composed of polygamous, gynodioecious and incipient dioecious species and the third clade is composed of dioecious species (including both XX/XY and XX/XY₁Y₂ species in different subclades).

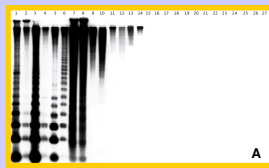


Figure 1.- RAE-180 satellite DNA family. (A) Southern blot showing the presence of this satellite only in dioecious representatives of *Rumex* (B) Location by *in situ* hybridization in *Rumex acetosa* chromosomes. Note the hybridization in two minute autosomal loci as well as the massive presence of this satellite in Y chromosomes, suggesting it would be related to the origin of sex-chromosome systems.

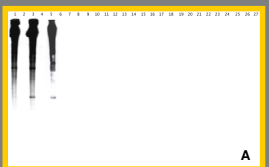
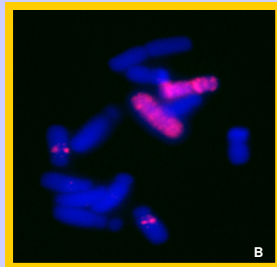
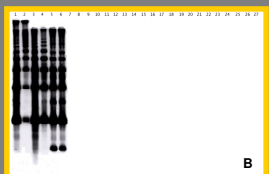


Figure 3.- RAYS1 and RAE-730 satellite DNA families respectively. (A-B) Southern blot showing the presence of these satellites only in dioecious representatives of *Rumex* bearing complex sex-chromosome systems (C-D) Location by *in situ* hybridization showing that RAYS1 is Y linked, being RAE-730 present in two heterochromatic segments.



Note.- Every Southern blot includes following species: (1-2) *R. acetosa* 2n (3-4) *R. papillaris* 2n (5-6) *R. intermedium* 2n (7-8) *R. suffruticosus* 2n (9-10) *R. acetosella* 2n (11-12) *R. hastatulus* (Texas race) 2n (13-14) *R. hastatulus* (North Carolina race) 2n (15) *R. lascephalophorus* (16) *R. induratus* (17) *R. scutatus* (18) *R. madrensis* (19) *R. laxus* (20) *R. roosei* (21-22) *R. sagittatus* 2n (23) *R. conglomeratus* (24) *R. crispus* (25) *R. pulcher* (26) *R. palustris* (27) *R. obtusifolius*.

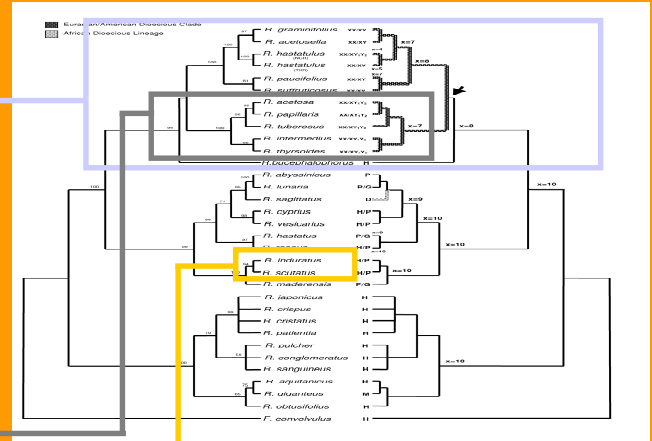


Figure 1.- (From Navajas-Pérez et al., Mol. Biol. Evol. doi:10.1093/molbev/ms1186) Right side: Maximum likelihood tree constructed for *Rumex* species using chloroplastidial and nuclear sequences combined into a single dataset. Numbers at each node indicate bootstrap support values. Left side: Correlation between the molecular phylogeny and the evolution of both the basic chromosome numbers (x) and the mating systems in the genus *Rumex*. Both the Eurasian/American dioecious clade and the African dioecious lineage are marked by thicker branch. The arrow labels the dioecious node. H = hermaphrodite; P = polygamous; G = gynodioecious; M = monoecious; D = dioecious. NCR = North Carolina race of *R. hastatulus*; TXR = Texas race of *R. hastatulus*.

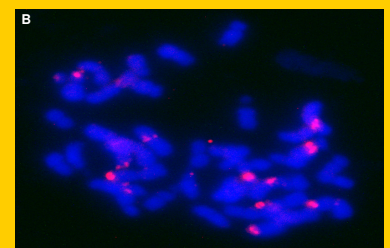
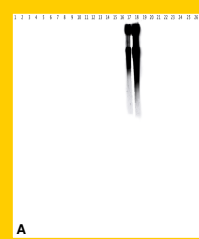


Figure 4.- RUSI satellite DNA family. (A) Southern blot showing the presence of this satellite only in *Rumex scutatus* and *R. induratus* (B) Location by *in situ* hybridization. Note the presence of this satellite in subtelomeric and in some centromeric locations.

RESULTS AND DISCUSSION.-

In *Rumex acetosa*, three different satellite-DNA families have been described so far. Two of them, RAE-180 and RAYS1, constitute the major constituents of the Ys heterochromatin, the other one RAE-730, being specific for autosomal heterochromatic segments. We have used these satellite-DNA sequences as cladistic markers. According to this, we have firstly analysed the presence/absence status by Southern-blot experiments in the rest of species of the genus *Rumex* and secondly we have analysed the location and the sequence evolution of this set of satellites.

So we have found that, firstly, the family RAE-180 characterizes the main group of dioecious species, including both systems of sex determination, and probably being involved in the origin of the mature sex-systems of chromosomes (Fig. 2). Secondly, the families RAYS1 and RAE-730 are present only in those dioecious species bearing complex sex-systems (Fig. 3). This data could suggest that these families are related to the formation of the XX/XY₁Y₂ system. We have not found evidence of the presence of these satellites in other species. Finally, we found a new satellite-DNA family, RUSI, specific for the a small clade belonging to gynodioecious-polygamous group of species, including *R. scutatus* and *R. induratus* (Fig. 4). It is remarkable, that the hermaphrodite-monoecious clade lacks any known satellite-DNA up to the present.

The present study reinforces our new classification and demonstrates the utility of satellite-DNA sequences for cladistic analysis in *Rumex*. In fact, a phylogenetic approach using different monomeric sequences from RAE-180 family, reflects a similar topology for dioecious species than that obtained using other molecular markers (Fig. 5).

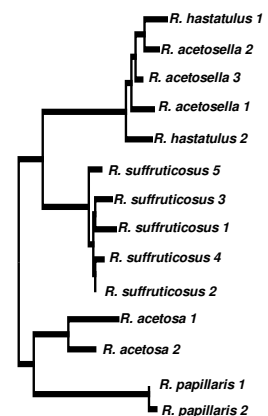


Figure 5.- Neighbour-joining tree using monomeric sequences of RAE-180 satellite DNA family from *R. acetosa*, *R. papillaris*, *R. acetosella*, *R. suffruticosus* and *R. hastatulus*.