

THE GENUS *RUMEX* AS A STUDY MODEL OF SEX CHROMOSOME EVOLUTION IN PLANTS

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Here, we introduce the genus *Rumex* (Polygonaceae) as a model for the study of the origin and evolution of dioecy and of sex chromosomes in plants. *Rumex* constitutes a large group of species in which almost every mating system is present, including hermaphroditic, polygamous, gynodioecious and dioecious representatives. Furthermore, within dioecious species, there are species with different sex-determination mechanisms and sex-chromosome systems, coexisting dioecious species without sex chromosomes together with species with sex chromosomes. Among these latter, some species have a common sex-chromosome system (XX/XY) and a Y-based sex-determination mechanism while other species have a complex XX/XY₁Y₂ system and a sex determination mechanism based on the balance between the number of Xs chromosomes and the number of sets of autosomes (X/A). Additionally, different species represent several degrees of genetic differentiation between their sex chromosomes. Classical morphological classifications imply that dioecy has appeared several times over the evolution of *Rumex* species directly from a hermaphroditic ancestor; also those classifications imply that there has been no constraint on the evolution of sexual systems and that forward and reverse evolution occur with equal probability. Moreover, recurrent pathways should have occurred in the karyotype evolution of the genus.

Contrary to the predictions of morphological classifications, a new phylogeny based in molecular data (Figure 1; Navajas-Pérez et al., 2005a) suggest a common origin about 15 million years ago for all Eurasian and American dioecious species of *Rumex* with gynodioecy as an intermediate state on the way to dioecy, since a second different lineage could be evolving from hermaphroditism towards dioecy via gynodioecy in Africa. The resulting phylogeny is also consistent with a classification of *Rumex* species according to their basic chromosome number, implying that the evolution of *Rumex* species might have followed a process of chromosomal reduction from x=10 (hermaphroditic species) toward x=7 (dioecious species) through intermediate stages with x=9 and x=8 chromosomes (polygamous and gynodioecious species).

Up to the present, we have focused our research on the analysis of the evolution of sex-chromosome systems. Within dioecious species, there are two monophyletic groups, one composed of species with a XX/XY sex-chromosome system (Y determination) and one composed of XX/XY₁Y₂ (X/A determination) species (Figure 2). Our results support the contention that sex determination based on the X/A balance has evolved secondarily from male-determining Y mechanisms and that multiple sex-chromosome systems, XX/XY₁Y₂, were derived twice from an XX/XY system. The XX/XY species represent an early stage of genetic differentiation between sex chromosomes, contrasting with the advanced state of genetic differentiation between the X and the Y of XX/XY₁Y₂ species (Cuñado et al., 2007).

In addition, the XX/XY₁Y₂ species have undergone a process of Y degeneration accelerated for the accumulation of satellite-DNA sequences (Navajas-Pérez et al., 2005b, 2006) and retrotransposons (Mariotti et al., 2006). This process of degeneration has occurred in a relatively short period of evolutionary time compared with other model systems (12-13 MYA) and it appears to be linked to the rearrangement processes leading to the current complex sex-chromosome system of these species (Figure 2).

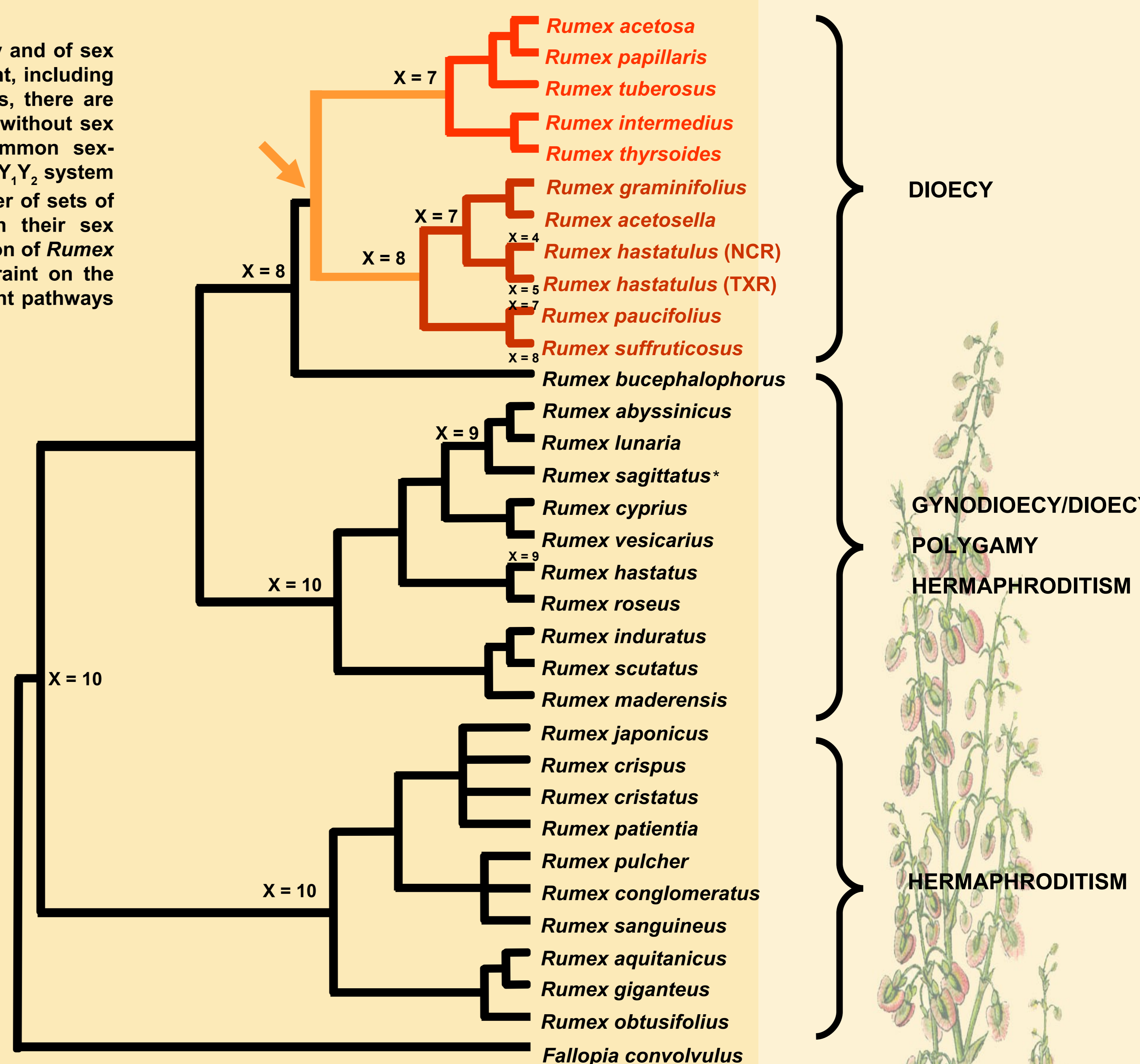


Figure 1. Maximum likelihood tree constructed for *Rumex* species using chloroplastial and nuclear sequences. Correlation between the molecular phylogeny and the evolution of both the basic chromosome numbers (x) and the mating systems is indicated. The arrow mark the origin of dioecy. One clade grouped all Eurasian and American dioecious species with *R. bucephalophorus* –an hermaphroditic basal species. A second clade was comprised of the hermaphroditic, polygamous and gynodioecious species, as well as the African dioecious species, *R. sagittatus**. This last species has no sex chromosomes. The third clade includes strictly hermaphroditic species.

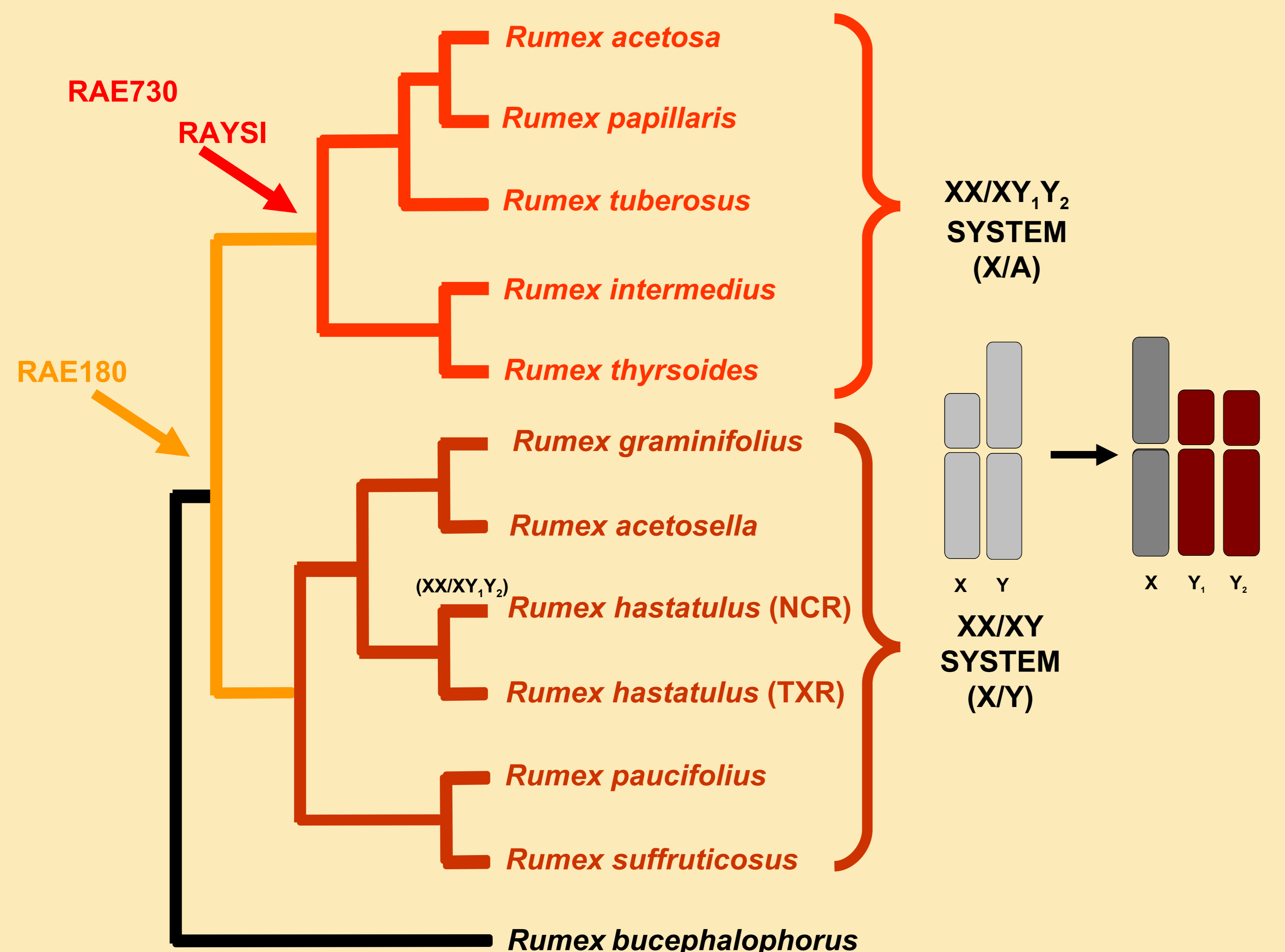


Figure 2. Dioecious species were divided in two subclasses: one composed of the species having a XX/XY sex-chromosome system such as *R. acetosella* and *R. graminifolius* from the subgenus *Acetosella*, and *R. suffruticosus*, *R. paucifolius* and *R. hastatulus* (which includes two allopatric chromosomal races, XX/XY and XX/XY₁Y₂ from the subgenus *Acetosa*); and the second, composed of the rest of dioecious species of the section *Acetosa* of subgenus *Acetosa* with an XX/XY₁Y₂ chromosome system. All dioecious species differ from the rest of *Rumex* species by having the RAE180 satellite-DNA family in their genomes. Within dioecious species, only the XX/XY₁Y₂ species have the RAE730 and the RAYS1 satellite-DNA families.

These satellite-DNA sequences have differentiated patterns of sequence evolution when located in sex chromosomes or in autosomes. Thus, we found slowed rates of sequence homogenization and evolution in the satellite DNAs of XX/XY₁Y₂ species located in the Y-chromosomes (RAYS1 and RAE180) in relation to the autosomal RAE730 satellite DNA (Table 1). 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 (Navajas-Pérez et al. 2005b).

It bears noting that the pattern of RAE180 sequence evolution differs between XX/XY₁Y₂ and XX/XY species (Navajas-Pérez et al. in preparation). Contrary to that found in the XX/XY₁Y₂ species, in the RAE180 autosomal locus of the XX/XY species we found low levels of intraspecific variability and high levels of sequence-divergent homogenization between species with rates of concerted evolution similar to that of other satellite DNAs analysed in autosomal loci of plants.

SATELLITE FAMILY	MEAN INTRA-SPECIFIC DISTANCES (<i>R. acetosa</i> / <i>R. papillaris</i>)	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	22.00x10 ⁹	47	281
RAYS1	0.037/0.042	0.047	11.74x10 ⁹	3	407
RAE180	0.036/0.037	0.045	11.25x10 ⁹	3	74

Table 1. Concerted evolution analysis of each of the three satellite-DNA families of XX/XY₁Y₂ species of *Rumex*. Transitional stages are measured according to Navajas-Pérez et al. 2007.

In fact, the evolutionary history of different satellite-DNA families traces the phylogeny of dioecious species (Figure 2). Thus, we have characterized three satellite-DNA families. Two of them are present in the Y chromosomes of XX/XY₁Y₂ species: the RAE180 family (Figure 3) and the RAYS1 family. The former is one of the two major types of sequences found within the heterochromatin of the Y chromosomes. Moreover, one minute additional locus exists in one of the autosome pairs of their karyotypes. The latter is exclusive of the Y-chromosomes. On the other hand, the RAE730 satellite-DNA family is found in heterochromatic blocks of the sixth chromosome pair of XX/XY₁Y₂ species. The differential pattern of distribution of RAE180 and RAYS1 loci between the Y chromosomes of different XX/XY₁Y₂ species give insights into how Y chromosomal rearrangements have accompanied speciation (Navajas-Pérez et al. in press).

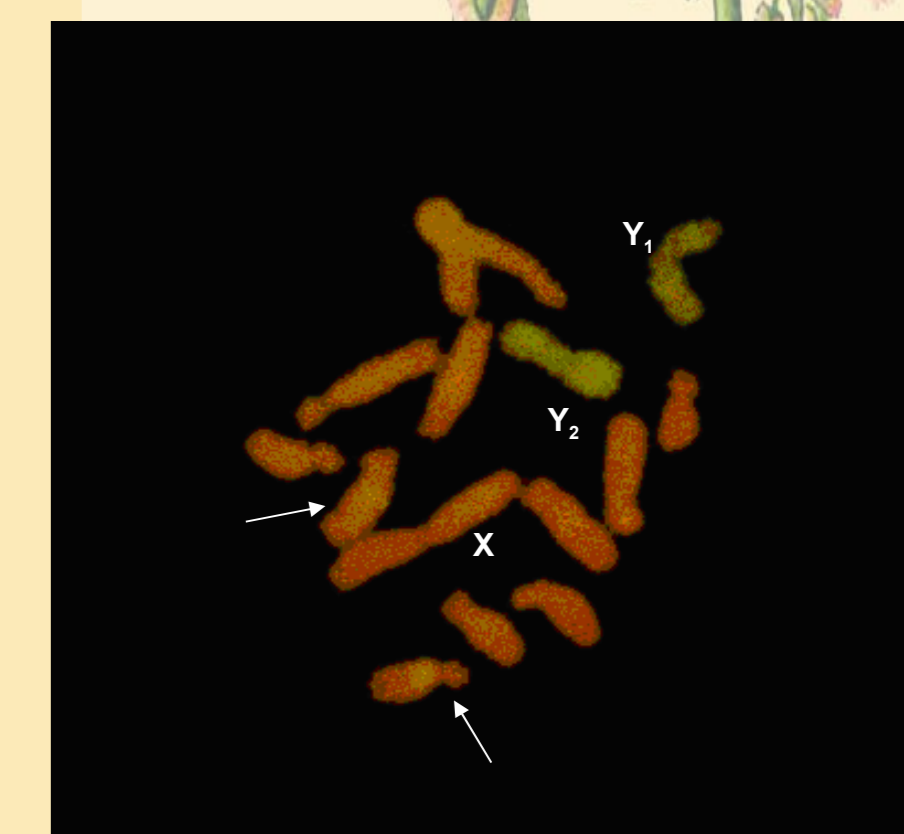


Figure 3. FISH showing that the Y chromosomes of *R. acetosa* species are mostly composed of RAE180 sequences. Arrows indicate an additional punctual RAE180 site present in a pair of autosomes.

The XX/XY₁Y₂ species share with the XX/XY species the RAE180 satellite-DNA family but, notably, these sequences are not abundantly amplified in the XX/XY species and they are located exclusively at one autosomal locus (Figure 4).

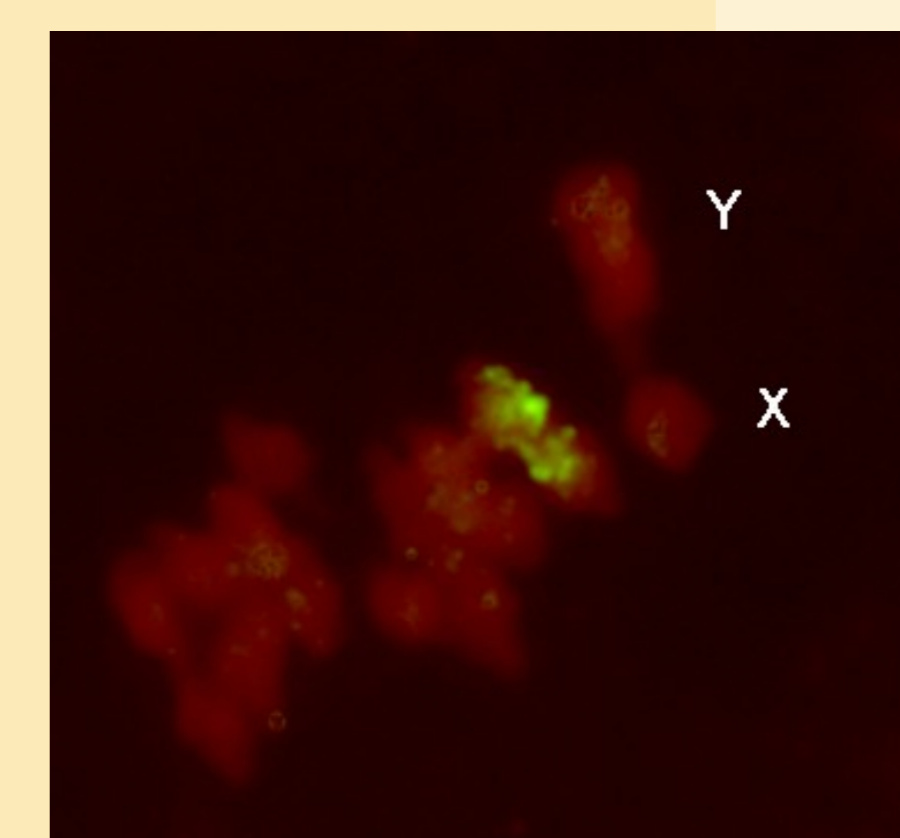


Figure 4. FISH showing that RAE180 sequences are located in a pair of autosomes in *R. suffruticosus*.

CONCLUDING REMARKS AND FUTURE PROSPECTS

In summary, according to the research to date and the open perspectives derived of such research, we believe that the genus *Rumex* constitutes an excellent study model for topics in evolutionary biology, such as: chromosome evolution, mating-systems evolution, sex-determination mechanisms evolution and sex-chromosomes evolution.

Thus, we propose this genus for further research in these topics. Within the genus, two representative species (*R. acetosa* and *R. suffruticosus*) are remarkable as models for such experiments seeking to find genes involved in the determination of the individual gender both in XX/XY systems (*R. suffruticosus*) and in XX/XY₁Y₂ systems (*R. acetosa*). In fact, our group is concentrating now on this aspect, pursuing several strategies such as those looking for between sexes differential AFLPs or differentially expressed genes. In this latter case, we are proceeding both by the subtractive hybridization technique between cDNAs of male and female flower and by the RT-PCR technique. Sex-linked markers and sex-determining genes can open new insights also into the evolutionary view of sex determination in plants.

References

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