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Chapter VI

The Genus *Rumex*: A Plant Model to Study Sex-Chromosome Evolution

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ABSTRACT

The origin and evolution of sexual dimorphism and the sex-determining mechanisms are major topics in Evolutionary Biology on which many studies have focused in recent decades. Among flowering plants, the origin of dioecy has resulted from quite recent events, occurring independently in about 7% of the genera. However, only a moderate number of dioecious plant species exhibit chromosome-mediated sex-determination systems. The genus *Rumex* (*Polygonaceae*), with monoecious, gynodioecious, hermaphroditic, and polygamous representatives, and with dioecious species bearing sex chromosomes in different evolutionary stages (homomorphic, XX/XY, XX/XY_1Y_2), has considerably contributed to shed light on this topic.

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Although still patchy, current knowledge on sex-chromosome evolution has greatly benefited from analyses on species of this group, for which the most significant findings are reviewed in this chapter.

INTRODUCTION

The genus *Rumex* has been generally related to human activity since ancient times and properties conferred by chemical compounds –mainly vitamin C and oxalic acid and oxalates [1]- have benefits both for culinary and ethnopharmacological use [2, 3, 4]. One of the first references of *Rumex* can be found in Exodus 12:8: "*And they shall eat the flesh in that night, roast with fire, and unleavened bread; and with bitter herbs they shall eat it*". Also, Pliny mentioned that the army of Julius Caesar would have been cured of scurvy by using the "*Erba britannica*", identified later as *Rumex aquaticus* L. However, raw plants are toxic due to the high content in oxalates, and in fact some *Rumex* species can be used as antifertility or abortive products [5]. In agriculture, some *Rumex* species have been used as feed crops, and continue to have applications as a dietary source of potential bioactive compounds [6], and recently have been proposed as potential choices for Hg phytoremediation of contaminated soils [7].

In science, ever since Kiara and Ono [8] first described the complex system of sex chromosomes in *Rumex acetosa* L., studies on sex determination have nearly monopolized the research on this group of species. Many scientific studies have focused on *Rumex* because of its biological and evolutionary significance in sexual dimorphism (comprising dioecious, gynodioecious, polygamous and hermaphroditic species; [9]). Thus, Löve [10], Löve and Kapoor [11], Smith [12, 13], Degraeve [14, 15, 16, 17] and Wilby & Parker [18] have contributed significantly to general knowledge of cytogenetic features of the group. Works by Navajas-Pérez et al. [19] and Cuñado et al. [20] are outstanding regarding the chromosomal evolution of the group, while those by Ono [21], Löve, [22], Smith, [23], Ainsworth, [24] and Cuñado et al. [20], are essential for understanding the sex-determination mechanisms. Also, Kurita & Kuroki, [25], Shibata et al. [26, 27, 28] and Navajas-Pérez et al. [29, 30] have greatly contributed to the molecular characterization of Y-chromosome heterochromatin.

This chapter highlights the most important findings regarding sex-chromosome origin and evolution.

Molecular Phylogeny and Implications on the Evolution of Dioecy

Around 200 European, American, and Asian species constitute the genus *Rumex*. Meissner [31] and Willkomm [32] classified the group into four different sections: *Rumex* L. and *Platypodium* Willk., both including hermaphroditic or monoecious annual or perennial herbs, and *Acetosa* Miller and *Acetosella* Fourr., with dioecious, gynodioecious, hermaphroditic or polygamous herbs and shrubs. This classification agrees with the most recent phylogenies based on sistematics proposed by Rechinger [9] and López González [33], who considered the four groups at the subgeneric level, and by Löve and Kapoor [11] or Degraeve [14, 15, 16, 17], who considered four genera, renaming *Platypodium* Willk. as *Bucephalophora* Pau.

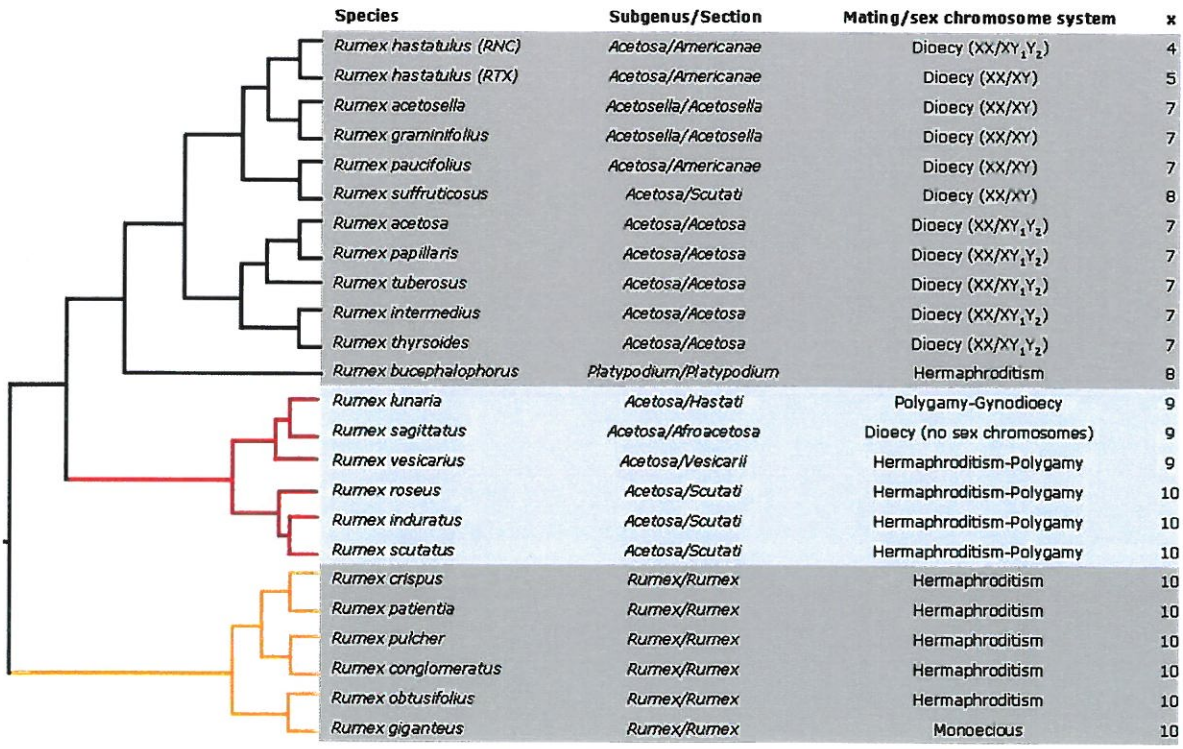
In this chapter we will follow classifications by Rechinger [9] and López González [33]. Thus, according to these authors, the subgenus *Rumex* L. would include the vast majority of species (~75%). These species constitute a highly homogeneous group with a basic chromosome number of x=10. Except for the monoecious *Rumex giganteus* W.T. Aiton, *Rumex skottbergii* Degener & I. Degener and *Rumex albescens* Hillebr., endemic to Hawaii [34], all other representatives of the genus *Rumex* are hermaphroditic. Some ubiquitous species such as *Rumex obtusifolius* L. would have evolved also towards monoecy in Hawaiian populations, so that it is thought that an insular effect might apply in these cases [34, 35, 36].

The subgenus *Platypodium* (Willk.) Rech. Fil. is monospecific and constituted exclusively by the hermaphroditic, diploid, x=8, *Rumex bucephalophorus* L. [16].

The subgenus *Acetosella* (Meissner) Rech. Fil. includes two species, *Rumex acetosella* L. and *Rumex graminifolius* Rudolph ex Lamb., and several subspecies all dioecious with a basic chromosome number x=7. Populations of *Acetosella* s.l. varies from diploid to octoploid level, including tetraploid, pentaploid or hexaploid intermediates [33, 37]. The presence of an XX/XY sex-chromosome system has been observed for all representatives of this group [38]. It has also been demonstrated that the sex-determining mechanism is mediated by the presence of an active Y chromosome [10].

The subgenus *Acetosa* includes species with a basic chromosome number ranging from x=10 to x=4. The species with a basic chromosome number of x=10

or 9 are mainly hermaphroditic, polygamous or gynodioecious. Except for *Rumex sagittatus,* x=9, dioecious without differentiated sex chromosomes [17] and *Rumex suffruticosus*, x=8, with a heteromorphic XX/XY sex-chromosome system [20], the majority of dioecious representatives of this subgenus are x=7 and are represented by *Rumex acetosa* and its close relatives. These species form a homogeneous group characterized by similar morphological and karyological features, including an XX/XY_1Y_2 sex-chromosome system plus a sex-determination mechanism based on the X/A balance [17, 18, 22, 23, 24]. Notably, in the section *Americanae* the dioecious *Rumex paucifolius* has an XX/XY system [39] and *Rumex hastatulus* has two chromosomal races, one x=5, XX/XY (Texas race -TXR) and the other x=4, XX/XY_1Y_2 (North Carolina race, NCR) [23]. The latter race has an X/A-based sex-determination mechanism and the former a Y-based one (see **Figures 1** and **2**).



Species	Subgenus/Section	Mating/sex chromosome system	x
Rumex hastatulus (RNC)	*Acetosa/Americanae*	Dioecy (XX/XY_1Y_2)	4
Rumex hastatulus (RTX)	*Acetosa/Americanae*	Dioecy (XX/XY)	5
Rumex acetosella	*Acetosella/Acetosella*	Dioecy (XX/XY)	7
Rumex graminifolius	*Acetosella/Acetosella*	Dioecy (XX/XY)	7
Rumex paucifolius	*Acetosa/Americanae*	Dioecy (XX/XY)	7
Rumex suffruticosus	*Acetosa/Scutati*	Dioecy (XX/XY)	8
Rumex acetosa	*Acetosa/Acetosa*	Dioecy (XX/XY_1Y_2)	7
Rumex papillaris	*Acetosa/Acetosa*	Dioecy (XX/XY_1Y_2)	7
Rumex tuberosus	*Acetosa/Acetosa*	Dioecy (XX/XY_1Y_2)	7
Rumex intermedius	*Acetosa/Acetosa*	Dioecy (XX/XY_1Y_2)	7
Rumex thyrsoides	*Acetosa/Acetosa*	Dioecy (XX/XY_1Y_2)	7
Rumex bucephalophorus	*Platypodium/Platypodium*	Hermaphroditism	8
Rumex lunaria	*Acetosa/Hastati*	Polygamy-Gynodioecy	9
Rumex sagittatus	*Acetosa/Afroacetosa*	Dioecy (no sex chromosomes)	9
Rumex vesicarius	*Acetosa/Vesicarii*	Hermaphroditism-Polygamy	9
Rumex roseus	*Acetosa/Scutati*	Hermaphroditism-Polygamy	10
Rumex induratus	*Acetosa/Scutati*	Hermaphroditism-Polygamy	10
Rumex scutatus	*Acetosa/Scutati*	Hermaphroditism-Polygamy	10
Rumex crispus	*Rumex/Rumex*	Hermaphroditism	10
Rumex patientia	*Rumex/Rumex*	Hermaphroditism	10
Rumex pulcher	*Rumex/Rumex*	Hermaphroditism	10
Rumex conglomeratus	*Rumex/Rumex*	Hermaphroditism	10
Rumex obtusifolius	*Rumex/Rumex*	Hermaphroditism	10
Rumex giganteus	*Rumex/Rumex*	Monoecious	10

Figure 1. List of the most representative species of *Rumex*, indicating phylogenetic relationship according to molecular data (left, based on [19]), affiliation based on morphologic classification, mating/sex-chromosome system and basic chromosome number (x).

Polyploidy is rare in X/A-mediated dioecious *Rumex*, while it has been widely observed in Y-mediated ones [40]. This confirms Muller's theory, who proposed that, contrary to Y-mediated systems an X/A balance sex-determining

mechanism will prevent the establishment of dioecious polyploid races in higher animals because of the unbalanced intersexes produced [41].

An assumption of the above classification would imply that dioecy has appeared multiple times through the evolution of *Rumex* directly from hermaphroditic forms. Also, it would support the contention that sex-chromosome evolution would have followed several pathways and that secondary regression to hermaphroditism from dioecious forms would have occurred. Furthermore, this systematic shows no relation between the phylogeny of the group and the evolution of basic chromosome number.

In order to check the veracity of all these assumptions, we performed a phylogenetic analysis in 31 species of the genus by using several molecular markers: from the nuclear genome, the intergenic transcribed spacers (ITS1 and ITS2) between the 18S and the 28S ribosomal genes, and from the chloroplast genome, the intron of trnL gene and the intergenic spacer between this gene and the trnF gene [19]. The analysis considered three criteria: neighbor-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML), implemented by softwares Mega vs2.1 [42] and PAUP* 4.0b10 [43]. In that study, we found no support for the four sub-groups described above. Instead, we found a common origin for all American and Eurasian dioecious *Rumex* species belonging to subgenera *Acetosa* and *Acetosella*, which together with *R. bucephalophorus* of subgenus *Platypodium*, form a well-supported clade (**Figure 1**). This suggests that subgenera *Acetosella* and *Platypodium* might be artificial groups. A second clade includes hermaphroditic, polygamous and gynodioecious species of subgenus *Acetosa*. Also, the dioecious without differentiated sex chromosomes *R. sagittatus,* is included in this latter clade. Finally, a third clade comprises exclusively species belonging to subgenus *Rumex* (**Figure 1**).

In contrast to the current morphological view, this new phylogeny suggests a common origin for all Eurasian and American dioecious species of *Rumex*, with gynodioecy as an intermediate state on the way to dioecy, as the *R. sagittatus* lineage demonstrates. 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 toward x=7 (and finally extending to x=4 in the American lineage) through intermediate stages x=9 and x=8 (**Figure 1**).

The molecular data not only showed that the evolution have followed a main pipeline towards dioecy in the genus *Rumex*, but also support the idea that sex-determining mechanisms based on the balance between the number of X

chromosomes and autosomes (X/A balance) has evolved secondarily from male-determining Y mechanisms, as the American lineage of *R. hastatulus* supports. X/Y sex determination is taxonomically more widely distributed than X/A, especially in groups such as fishes and plants, with poorly developed sex-chromosome systems [44], and thus it is assumed that the X/A balance mechanisms evolved secondarily from male-determining Y-chromosome mechanisms [45]. However, there was no direct evidence supporting the proposal that the X/Y system is indeed older until now.

DECIPHERING THE IMPROBABLE

Although the presence of complex sex-chromosome systems has been reported in other animal [46, 47, 48] and plant species [49], it is a rare occurrence and, to our knowledge, its fragmented distribution through a single genus has rarely been proved before. The new molecular phylogeny we proposed, demonstrates that XX/XY_1Y_2 systems have evolved from XX/XY ones through chromosomal rearrangements. Strikingly, our data also support the contention that the multiple sex-chromosomes systems appeared in *Rumex* twice independently; one in the European lineage, *Acetosa section*, and two in the *Americanae* section, *R. hastatulus* NCR (**Figure 2**).

This scenario agrees with the age estimates of *Rumex* sex chromosomes. Using the mean rate of change in plant nuclear DNA of 0.6% per site per million years [50], rDNA ITS mean distance between clades suggests that dioecy appeared in *Rumex* between 15-16 mya, while the divergence time for the *R. acetosella/R. suffruticosus* XX/XY clade leading to the *Acetosa* XX/XY_1Y_2 clade should be 12-13 mya [19]. The split between the two chromosomal races of *R. hastatulus* leading to the XX/XY_1Y_2 NCR system occurred around 600,000 years ago [51]. It is remarkable that in other plant taxa such as papaya [52, 53], *Fragaria* [54, 55] or *Silene* [56, 57, 58, 59, 60] dioecy has also been demonstrated to be an early event in evolution.

Since all dioecious *Rumex* species appear to have a common origin and that simple systems gave rise to complex ones at different points in evolution, the next question we addressed was whether sex chromosomes of both groups have also a common origin or whether they evolved from different autosomal sources. For that task, we thoroughly analysed the molecular structure of Y chromosomes, focusing on repetitive satellite-DNA sequences.

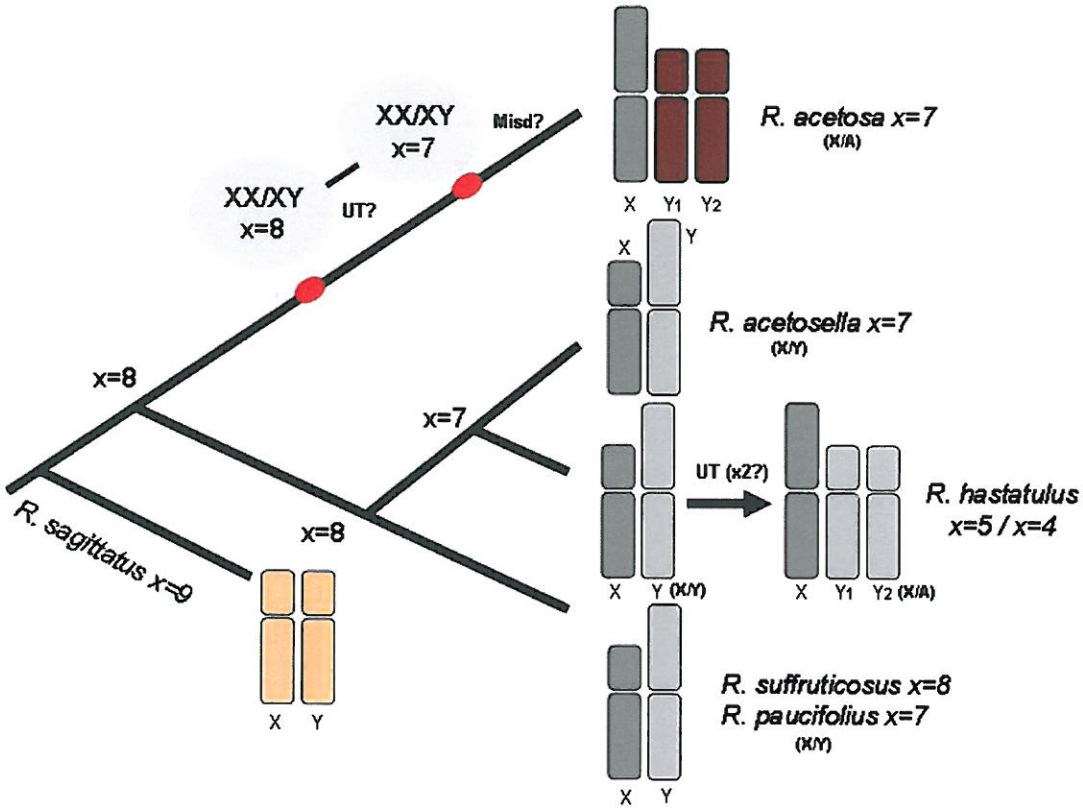


Figure 2. Scheme of sex-chromosome evolution in *Rumex*. *Notes:* there are two possible pathways to explain the origin of complex sex-chromosome systems; by *UT*, unequal translocation from an x=8 ancestor or by *Misd*, misdivision from an x=7 ancestor.

Repetitive DNA and Its Implication in Sex-Chromosome Evolution

The most accepted theories on the origin of sex chromosomes predict that the sexual pair arose form a non-differentiated autosome pair that selectively accumulated the corresponding (male and female) sex-determining genes. To avoid the crossing-over and then the disruption of gender determination, the suppression of recombination can be favoured by methylation or intra-chromosomal rearrangements. In a subsequent stage, the suppression of recombination would spread to surrounding areas and affect the molecular structure of the Y chromosome, which finally would degenerate from the accumulation of repetitive sequences, mainly satellite DNA and transposable elements, mediated by the lack of recombination and Muller's ratchet. A last stage is the dosage compensation in the X chromosome (reviewed in [61, 62]).

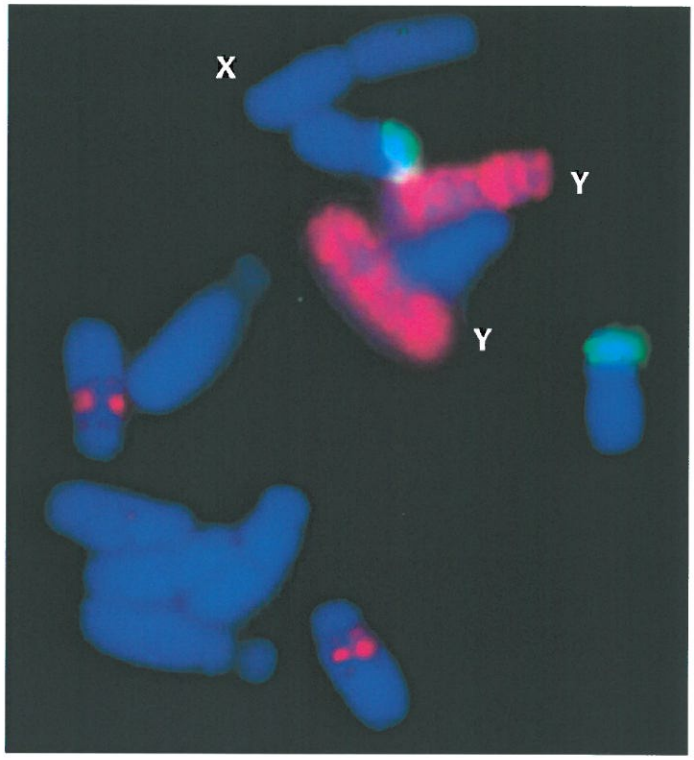


Figure 3. *In situ* hybridization to incomplete root-tip metaphase of *Rumex acetosa* male using as probes RAE180 (red) and RAE730 (green) satellite-DNA sequences.

Repetitive DNA appears to play an important part in the birth and evolution of sex chromosomes. In fact, two satellite-DNA families have been described as being accumulated in the Y chromosomes of several *Rumex* species. On the one hand, RAYSI [26] is a 930-bp repeat satellite exclusively accumulated on both Y chromosomes of XX/XY_1Y_2 species. The same pattern has been found for another two satellite-DNA families, RAYSII and RAYSIII, homologous in sequence with RAYSI [63]. On the other hand, RAE180 [27] is a 180-bp repeat that was demonstrated to be accumulated in a pair of autosomes as well as in the Y chromosomes of *R. acetosa* (**Figure 3**) [20, 27].

We comparatively analysed those families to dissect the molecular structure of sex chromosomes by checking their presence and accumulation in other dioecious species with putative differences in their sex chromosomes. With regard to XX/XY_1Y_2 systems, aside from studying *R. acetosa*, we also studied *R. papillaris, R. intermedius, R. thyrsoides* and *R. tuberosus* (from the section *Acetosa*) and *R. hastatulus* NCR (from the section *Americanae*). With respect to XX/XY systems, we analysed *R. suffruticosus* (from the section *Scutati*) and *R. hastatulus* TXR (from the section *Americanae*). *R. acetosella*, belonging to subgenus *Acetosella* was also analysed (**Figure 1**) [20, 51].

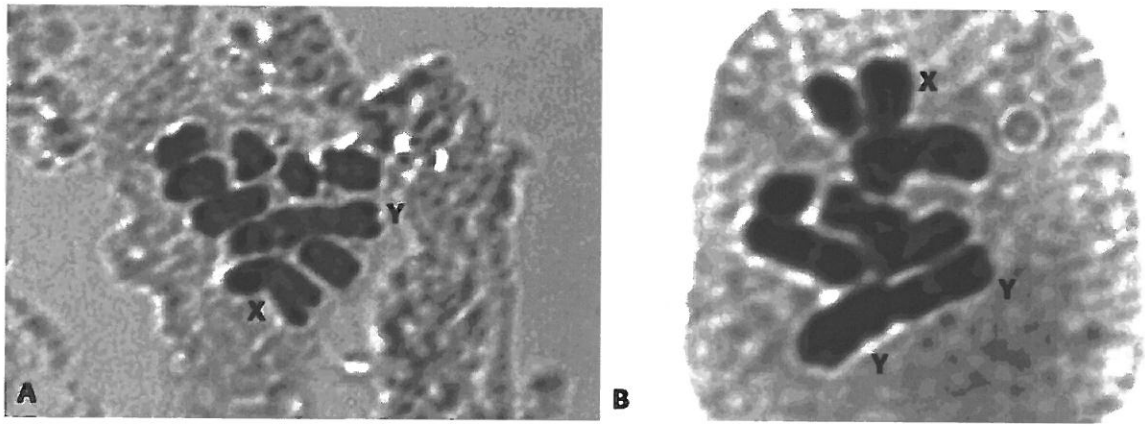


Figure 4. Mitotic chromosomes of *Rumex hastatulus* male; (A) Texas and (B) North Carolina races, showing their sex-chromosome systems.

All species bearing the XX/XY_1Y_2, except for *R. hastatulus* NCR, showed the massive accumulation of RAE180 and RAYSI families in both Y chromosomes that are indeed highly heterochromatic and show massive intra-chromosomal rearrangements (**Figure 3**) [64]. Contrary, the presence of RAYSI in *R. hastatulus* NCR was detected neither by PCR nor by *in situ* hybridization. RAE180 was detected in this species but proved to be very scarcely represented and completely absent from Y chromosomes. In fact, the Y chromosomes of this American endemism remain mostly euchromatic (**Figure 4**) [51]. The XX/XY species showed a similar pattern with the complete absence of RAYSI sequences and the presence of RAE180 in variable amounts but in any case limited to autosomal loci. By DAPI staining *R. hastatulus* TXR and *R. acetosella* Y chromosomes showed no evidence of degeneration, while *R. suffruticosus* exhibited faint signs of molecular differentiation (personal observations).

All these observations would support the proposal that: 1) RAE180 and RAYSI satellite-DNA families have significantly contributed to the origin of Eurasian complex sex-chromosomes systems -XX/XY_1Y_2- and their evolution through the accumulation from autosomal loci to Y chromosomes. RAE180 is present in all American and Eurasian dioecious species and absent from the rest of *Rumex* representatives, while RAYSI is limited exclusively to Eurasian XX/XY_1Y_2 species. Thus, RAE180 would mark the origin of dioecy, while RAYSI would pre-date the origin of complex systems in the Eurasian lineage. 2) Both complex systems -Eurasian and American lineages- evolved independently twice, as we determined above, using molecular markers [19]. This was additionally confirmed by studying another satellite-DNA family -RAE730 [28]. This is absent from sex chromosomes, and is restricted to supernumerary

segments of Eurasian XX/XY_1Y_2 species (**Figure 3**), but is not found nor in the American endemic *Rumex* neither in the rest of XX/XY dioecious representatives [29]. Repetitive elements appear to be good makers in this group, being restricted to only closely related species. In this sense, another satellite-DNA family, named RUSI, has been proved to be exclusively represented in *R. scutatus* and *R. induratus*, but not in the rest of species [65](**Figure 1** and 3) On these grounds, although it is the most parsimonious pathway, we cannot affirm that XX/XY Y-chromosomes have the same origin as those from XX/XY_1Y_2 species because we found no sequences shared by both types of sex chromosomes. A fine comparative mapping strategy would be necessary to rule out this aspect.

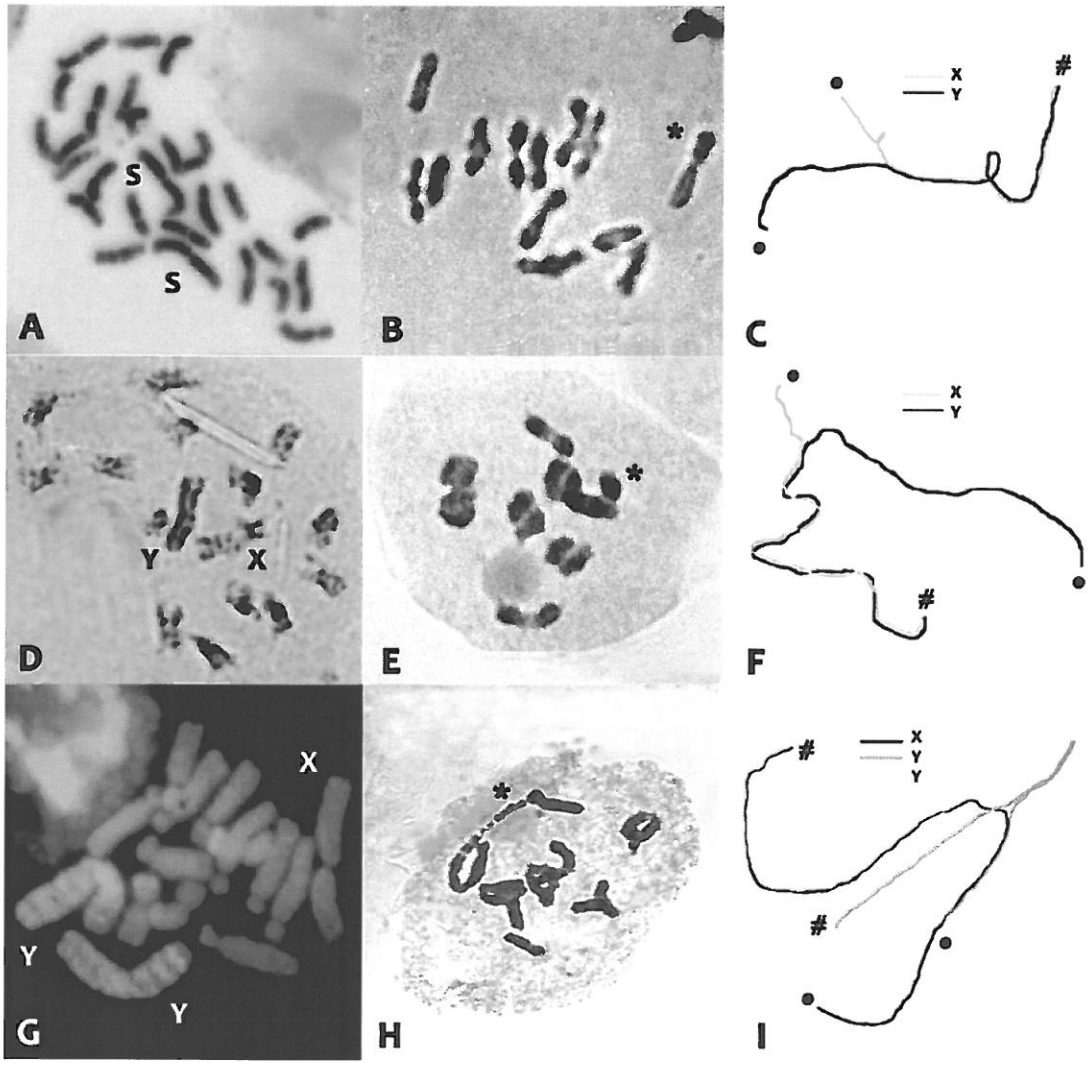


Figure 5. Mitotic and meiotic chromosomes, and sex-chromosome synaptonemal complex ideogram (modified from [20]) of: *Rumex acetosella* (A-C), *Rumex suffruticosus* (D-F) and *Rumex acetosa* (G-I) males. S: indicates sex chromosome, *: sexual bi/trivalent, #: ends of the synaptonemal complex, (black dot) ends of the asynapsed chromosome ends.

TOWARDS SEX-CHROMOSOME DIFFERENTIATION

A combined analysis of both the accumulation of repetitive DNA and the meiotic chromosome features of the most representative species of *Rumex* has demonstrated the existence of sex-chromosomes belonging to three different evolutionary stages in the group [20]. **Figure 5** summarizes the three stages that can be found in *Rumex* sex-chromosomes, namely:

An initial stage would be represented by those species with no signs of degeneration in their Y chromosomes. Thus, *R. acetosella* sex chromosomes consistently formed a monochiasmate heteromorphic bivalent indicating the existence of a pseudoautosomal region between the X and the Y chromosomes; this, however, lacked any contrastable DAPI+ or C+ bands [20] (**Figure 5A-C**). *R. hastatulus* could also be considered within this first stage due to the lack of differentiation in its sex chromosomes. It bears noting that, in this group, *R. hastatulus* TXR (x=5) would have given rise secondarily to *R. hastatulus* NCR (x=4 and XX/XY_1Y_2), most likely by unequal translocation events (**Figure 2** and **4**) [66], without the mediation of molecular degeneration of Y chromosomes. A second stage corresponds to *R. suffruticosus.* This species exhibits some evidence of molecular degeneration in the Y chromosome that stains faintly but positively with DAPI and has a markedly heteromorphic sexual bivalent with an evident area of non-homologous asynapsis [20] (**Figure 5D-F**). A final stage would be represented by *R. acetosa* and their close relatives. This group of species is characterized by a highly differentiated sex-chromosome system XX/XY_1Y_2 in which the Y chromosomes are heteropicnotic and show DAPI+ and C+ bands and are massively degenerated by the accumulation of repetitive sequences (**Figure 3** and **5G-I**) [20, 30, 64, 67]. The cytogenetic differentiation between X and Y chromosomes is also confirmed by the synaptonemal complex analysis. *R. acetosa* males showed six homomorphic bivalents and a sexual trivalent in which each Y chromosome was associated with one of the terminal regions of the X chromosome [20] (**Figure 5G-I**).

REDUCED RATES OF EVOLUTION IN SEX-CHROMOSOME-LINKED SEQUENCES

Although the role of repetitive sequences has been widely reported in Y-chromosome degeneration (see e.g. [68]), little is known about how this

accumulation occurs or about how the absence of recombination affects the subsequent evolutionary fate of the repetitive sequences in the Y chromosome. In order to understand the evolutionary dynamics of sex chromosomes in *Rumex*, we isolated several monomeric units from three satellite-DNA families, RAYSI, RAE730, and RAE180, in two related species, *R. acetosa* and *R. papillaris*, and comparatively analysed mutation and concerted-evolution rates. RAYSI and RAE730 originated from a common ancestral 120-bp unit through replication cycles [29]. This situation allows us to analyse two sequences which had a common origin but which accumulated in significantly different areas of the genome: RAYSI, in non-recombining Y chromosomes; and RAE730, in autosomes. Also, RAE180 sequences, unrelated to either RAYSI or RAE730, represent a hybrid situation, being accumulated both in autosomes and in Y chromosomes.

This analysis revealed that the evolutionary dynamics of sex chromosomes is characterized by slowed-down rates of evolution. Specifically, sequences accumulated in Y chromosomes (RAYSI and Y-linked RAE180 sequences) have evolved at half the rate and undergo lower rates of sequence evolution and homogenization than do satellite DNAs in autosomes, RAE730 and autosomal RAE180 [29]. It bears mentioning that despite of their common origin, RAYSI and RAE730 display this differential pattern. A subsequent analysis of RAE180 in XX/XY species, where these sequences are exclusively autosomic, showed intra-specific sequence homogeneity and inter-specific divergence that is a general pattern of concerted evolution for these repeats. Contrary to the Y-linked loci of RAE180 in XX/XY_1Y_2 species, where ancestral variability has remained with reduced rates of sequence homogenization and of evolution.

All these data support the contention of the non-recombining nature of Y chromosomes and that the molecular-drive process is significantly affected by mechanisms of non-reciprocal exchange and factors such as location, organization or copy number [69].

CONCLUSIONS

- In contrast to the morphological classification, the phylogeny based on molecular markers (chloroplastidial DNA, rDNA, satellite-DNA, dispersed repetitive DNA) and basic chromosome number evolution, suggests a common origin for all Eurasian and American dioecious

species of *Rumex*, with gynodioecy as an intermediate state on the way towards dioecy.

- The origin of dioecy in *Rumex* has been estimated at around 15-16 mya, and the split of regular and complex sex-chromosome systems at 12-13 mya. This is a new piece of evidence that sex chromosomes in plants are evolutionarily young.
- Due to the presence of species with different mating systems and several levels of genetic differentiation between the sex chromosomes, *Rumex* is a model for analysing the evolution of sex chromosomes in plants and their evolutionary history from early stages.
- Satellite DNA (RAE180 family) pre-dates the origin of dioecy and the appearance of XX/XY_1Y_2 systems (RAYSI and RAE730 families). Also these sequences have played an important role in the molecular degeneration of Y chromosomes in complex systems, contributing to the establishment of heteromorphic sex-chromosome systems.
- Despite of their origin, satellite-DNA sequences that had accumulated on non-recombining Y chromosomes underwent lower rates of sequence evolution and homogenization than did those in the autosomes. This implies that mechanisms of non-reciprocal exchange and factors such as location, organization or copy number of repeats significantly affected molecular-drive process.

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REFERENCES

[1] Duke, J.A. (1983). Medicinal plants of the Bible. Trado-Medic Books, Owerri, NY.

[2] Rivera, D., Obón, C., Inocencio, C., Heinrich, M., Verde, A., Fajardo, J., Llorach, R. (2005). The ethnobotanical study of local Mediterranean food plants as medicinal resources in Southern Spain. *J. Phys. Pharma. 56,* 97-114.

[3] Rivera, D., Obón, C. (1995). The ethnopharmacology of Madeira and Porto Santo Islands, a review. *J Ethnopharmacology* 46, 73-93.

[4] Peña-Chocarro, L. Zapata Peña, L. (1997). Higos, ciruelas y nueces: aportación de la arqueobotánica al estudio del mundo botánico. *Isturitz, 9,* 679-690.

[5] Solomon, T., Largesse, Z., Mekbeb, A., Eyasu, M., Asfaw, D. (2010). Effect of *Rumex steudelii* methanolic root extract on ovarian folliculogenesis and uterine histology in female albino rats. *Afr Health Sci. 10*, 353-61.

[6] Ferreres, F., Ribeiro, V., Izquierdo, A.G., Rodrigues, M.A., Seabra, R.M., Valentão, P. (2006). *Rumex induratus* leaves: interesting dietary source of potential bioactive compounds. *J Agric Food Chem*, 54, 5782-5789.

[7] Moreno-Jiménez, E., Gamarra, R., Carpena-Ruiz, R.O., Millán, R., Peñalosa, J.M., Esteban, E. (2006). Mercury bioaccumulation and phytotoxicity in two wild plant species of Almadén area. *Chemosphere*, 63, 1969-1973.

[8] Kiara, H. & Ono, T. (1925). The sex chromosomes of *Rumex acetosa. Z. Indukt. Abst. U. Vererb. 39*, 1-7.

[9] Rechinger, K.H. Jr. (1964). The genus *Rumex* L. Pp. 82–89 in T. G. Tutin, V. H. Heywood, N. A. Burges, D. H. Valentine, S. M. Walters, and D. A. Webb, eds. *Flora Europaea*, Vol. 1. Cambridge University Press, Cambridge.

[10] Löve, Á. (1944). Cytogenetic studies on *Rumex* subgenus *acetosella. Hereditas* 30, 1-136.

[11] Löve, Á., Kapoor, B. (1967). A chromosome atlas of the collective genus *Rumex. Cytologia* 32:320–342.

[12] Smith, B.W. (1964). The evolving karyotype of *Rumex hastatulus. Evolution* 18:93–104.

[13] Smith, B.W. (1968). Cytogeography and cytotaxonomic relationships of *Rumex paucifolius. Am. J. Bot. 55,* 673-683.

[14] Degraeve, N. (1980). Contribution à l'étude cytotaxonomique des *Rumex*. III Le genre *Acetosella* Fourr. *Genetica, 54,* 29-34.

[15] Degraeve N. (1975a). Contribution à *l'étude* cytotaxonomique des *Rumex* . I. Le genre *Rumex* L. *sensu stricto. Caryologia, 28,* 187-201.

[16] Degraeve N. (1975b). Contribution à *l'étude* cytotaxonomique des *Rumex* . II. Le genre *Bucephalophora* Pau. *Caryologia, 28*, 203-206.

[17] Degraeve, N. (1976). Contribution à l'étude cytotaxonomique des *Rumex*. IV Le genre *Acetosa* Mill. *La Cellule, 71,* 231-240.

[18] Wilby, A.S., Parker, J.S. (1988). Recurrent patterns of chromosome variation in a species group. *Heredity, 61,* 55–62.

[19] Navajas-Pérez, R., de la Herrán, R., López-González, G., Jamilena, M., Lozano, R., Ruiz-Rejón, C., Ruiz-Rejón, M., Garrido-Ramos, M.A. (2005a). The evolution of reproductive systems and sex-determining mechanisms within *Rumex* (polygonaceae) inferred from nuclear and chloroplastidial sequence data. *Mol BiolEvol., 22,* 1929-39.

[20] Cuñado, N., Navajas-Pérez, R., de la Herrán, R., Ruiz Rejón, C., Ruiz Rejón, M., Santos, J.L., and Garrido-Ramos, M.A. (2007). The evolution of sex chromosomes in the genus *Rumex* (Polygonaceae): identification of a new species with heteromorphic sex chromosomes. *Chromosome Res, 15,* 825-832.

[21] Ono, T. (1935). Chromosomen und Sexualität von *Rumex acetosa. Sci Rep Tohoku Imp Univ IV, 10,* 41–210.

[22] Löve, Á. (1957). Sex determination in *Rumex. Proc Genet SocCan, 2,* 31-36.

[23] Smith, B.W. (1969). Evolution of sex-determining mechanisms in *Rumex. Chromosomes Today, 2,* 172-182.

[24] Ainsworth, C.C., Lu, J., Winfield, M., Parker, J.S. (1999). Sex determination by X: autosome dosage: *Rumex acetosa* (sorrel). In: Ainsworth CC, ed. *Sex Determination in Plants.* Oxford: BIOS Scientific Publishers, pp. 124-136.

[25] Kurita, M. & Kuroki, Y. (1970). Y-chromosome and heterochromatin in *Rumex acetosa. Jpn. J. Genet. 45,* 255-260.

[26] Shibata, F., Hizume, M., Kurori, Y. (1999). Chromosome painting of Y chromosomes and isolation of a Y chromosome-specific repetitive sequence in the dioecious plant *Rumex acetosa. Chromosoma, 108,* 266-270.

[27] Shibata, F., Hizume, M., Kurori, Y. (2000a). Differentiation and the polymorphic nature of the Y chromosomes revealed by repetitive sequences in the dioecious plant, *Rumex acetosa. Chromosome Res., 8,* 229-236.

[28] Shibata, F., Hizume, M., Kurori, Y. (2000b). Molecular cytogenetic analysis of supernumerary heterochromatic segments in *Rumex acetosa. Genome, 43,* 391-397.

[29] Navajas-Pérez, R., de la Herrán, R., Jamilena, M., Lozano, R., Ruiz Rejón, C., Ruiz Rejón, M., Garrido-Ramos, M.A. (2005b). Reduced rates of sequence evolution of Y-linked satellite DNA in *Rumex* (Polygonaceae). *J. Mol. Evol., 60,* 391–399.

[30] Navajas-Pérez, R., Schwarzacher, T., de la Herrán, R., Ruiz Rejón, C., Ruiz Rejón, M., Garrido-Ramos, M.A. (2006). The origin and evolution of the variability in a Y-specific satellite-DNA of *Rumex acetosa* and its relatives. *Gene, 368*, 61-71.

[31] Meissner, K.F. (1856). *Rumex*. In: Candolle, A.P.D. (Ed.), Prodromus. Sumptibus Victoris Masson, Parisiis, pp. 41-74.

[32] Willkomm, H.M. (1862). *Polygoneae*. In: Willkomm, H.M., Lange, J. (Eds.), Prodromus Florae Hispanicae. Sumtibus E. Schweizerbart (E. Koch), Stuttgartiae, pp. 279-292.

[33] López González, G. 1990. Género *Rumex* L. Pp. 595–634 in S. Castroviejo, M. Laínz, G. López González, P. Montserrat, F. Muñoz Garmendia, J. Paiva, L. Villar, eds. *Flora Iberica*, Vol. II. CSIC, Real Jardín Botánico de Madrid, Madrid, Spain.

[34] Wagner, W.L., Shannon, R., Herbst, D.R. (1997). Contributions to the flora of Hawaii. VI. *Bishop Mus. Occas. Pap., 48,* 51-65.

[35] Mosyakin, S.L. & Wagner, W.L. (1998). Notes to two alien taxa of *Rumex* L. (*Polygonaceae*) naturalized in the Hawaiian Islands. *Bishop Museum Occasional Papers, 55*, 39-44.

[36] Wagner, W.L., Herbst, D.R., Sohmer. S.H. (1999). *Manual of the flowering plants of Hawaii,* Volume 2. Revised edition. Bishop Museum Special Publication 97. University of Hawaii and Bishop Museum Press, Honolulu, Hawaii.

[37] Singh, R.B. (1968). A dioecious polyploid in *Rumex acetosella. J. Hered. 59,* 168-170.

[38] Singh, R.B. (1964*). Cytogenetic studies in Rumex acetosella and its hybrids with Rumex hastatulus with special consideration of sex determination.* Doctoral Thesis, University of North Carolina.

[39] Löve, Á.& Sarkar, N. (1956). Cytotaxonomy and sex determination of *Rumex paucifolius. Can. J. Bot. 34*, 261-268.

[40] Σμιτη, B.Ω. (1955). Sex chromosomes and natural polyploidy in dioecious *Rumex. J Hered, 46,* 226-232

[41] Muller, H. J. (1925). Why polyploidy is rarer in animals than in plants? *Am. Nat. 59,* 346-353.

[42] Kumar, S., K. Tamura, I. B. Jacobsen, and M. Nei. (2001*). MEGA 2: molecular evolutionary genetics analysis software.* Arizona State University, Tempe.

[43] Swofford, D.L. 1998. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Mass.

[44] Charlesworth, B. (1996). The evolution of chromosomal sex determination and dosage compensation. *Curr Biol, 6,* 149-62.

[45] Westergaard, M. (1958).The mechanism of sex determination in dioecious flowering plants. *Adv. Genet., 9,* 217-281.

[46] Toder, R., O'Neill, R.J., Wienberg, J., O'Brien, P.C., Voullaire, L., Marshall-Graves, J.A. (1997). Comparative chromosome painting between two marsupials: origins of an XX/XY_1Y_2 sex chromosome system. *Mamm Genome, 8,* 418-22.

[47] Centofante, L., Bertollo, L.A., Moreira-Filho, O. (2006) Cytogenetic characterization and description of an XX/XY_1Y_2 sex chromosome system in catfish *Harttia carvalhoi* (*Siluriformes, Loricariidae*). *Cytogenet Genome Res.* 112:320-4.

[48] de Oliveira, R.R., Feldberg, E., dos Anjos, M.B., Zuanon, J. (2008). Occurrence of multiple sexual chromosomes (XX/XY_1Y_2 and $Z_1Z_1Z_2Z_2/Z_1Z_2W_1W_2$) in catfishes of the genus *Ancistrus* (*Siluriformes: Loricariidae*) from the Amazon basin. *Genetica*, 134, 243-9.

[49] Grabowska-Joachimiak, A., Mosiolek, M., Lech, A., Góralski, G. (2011). C-Banding/DAPI and *in situ* hybridization reflect karyotype structure and sex chromosome differentiation in *Humulus japonicus* Siebold & Zucc. *Cytogenet Genome Res., 132,* 203-11.

[50] Gaut, B.S. (1998). Molecular clocks and nucleotide substitution rates in higher plants. *Evol.Biol, 30,* 93-120.

[51] Quesada del Bosque, E., Navajas-Pérez, R., Panero, J.L., Fernández-González, A., Garrido-Ramos, M.A. (2011). A satellite-DNA evolutionary analysis in the North American endemic dioecious plant *Rumex hastatulus* (*Polygonaceae*). *Genome, in press.*

[52] Yu, Q., Hou, S., Feltus, F.A., Jones, M.R., Murray, J., Veatch, O., Lemke, C., Saw, J.H., Moore, R.C., Thimmapuram, J., Liu, L., Moore, P.H., Alam, M., Jiang, J., Paterson, A.H., Ming, R. (2008a). Low X/Y divergence in four pairs of papaya sex-liked genes. *Plant J., 53,* 124–132

[53] Yu, Q., Navajas-Perez, R., Tong, E., Robertson, J., Moore, P.H., et al. (2008b). Recent Origin of Dioecious and Gynodioecious Y Chromosomes in Papaya. *Tropical Plant Biology, 1,* 49-57.

[54] Ashman, T.L., Spigler, R.B., Goldberg, M.T., Govindarajulu, R. (2011) *Fragaria*: a polyploid lineage for understanding sex chromosome evolution.

In: Navajas-Pérez, R. (ed), *New Insights on Sex Plant Chromosomes*, Nova Publishers, Hauppauge NY.

[55] Goldberg, M.T., Spigler, R.B., Ashman, T.L. (2010). Comparative genetic mapping points to different sex chromosomes in sibling species of wild strawberry (*Fragaria*). *Genetics, 186,* 1425-33.

[56] Filatov, D. (2011). How much do we know about evolution of sex chromosomes in plants? In: Navajas-Pérez, R. (ed), *New Insights on Sex Plant Chromosomes,* Nova Publishers, Hauppauge NY.

[57] Nicolas, M., Marais, G., Hykelova, V., Janousek, B., Laporte, V., Vyskot, B., Mouchiroud, D., Negrutiu, I., Charlesworth, D., Moneger, F. (2005). A gradual process of recombination restriction in the evolutionary history of the sex chromosomes in dioecious plants. *PLoS Biol., 3,* 47-56.

[58] Guttman, D.S., Charlesworth D. (1998). An X-linked gene with a degenerate Y-linked homologue in a dioecious plant. *Nature, 393,* 1009–1014.

[59] Desfeux, C., Lejeune B. (1996). Systematics of Euromediterranean *Silene* (*Caryophyllaceae*): evidence from a phylogenetic analysis using ITS sequences. C. *R. Acad. Sci. III 319,* 351–358.

[60] Desfeux, C., Maurice S., Henry J.P., Lejeune B., Gouyon P.H.. (1996). Evolution of reproductive systems in the genus *Silene. Proc. R Soc. Lond. B 263,* 409–414.

[61] Ruiz Rejón, M. (2004).*Sex chromosomes in plants. In: Encyclopedia of Plant and Crop Sciences* (Vol 6): Dekker Agropedia (6 vols), Marcel Dekker Inc., New York, pp. 1148-1151.

[62] Charlesworth, D. (2002). Plant sex determination and sex chromosomes. *Heredity* 88:94–101.

[63] Mariotti, B., Manzano, S., Kejnovský, E., Vyskot, B., and Jamilena, M. (2009). Accumulation of Y-specific satellite DNAs during the evolution of *Rumex acetosa* sex chromosomes. *Mol Genet Genomics, 281,* 249-59.

[64] Navajas-Pérez, R., Schwarzacher, T., Ruiz Rejón, M., Garrido-Ramos, M.A. (2009a). Molecular cytogenetic characterization of *Rumex papillaris*, a dioecious plant with an XX/XY_1Y_2 sex chromosome system. *Genetica, 135,* 87-93.

[65] Navajas-Pérez, R., Schwarzacher, T., Ruiz Rejón, M., Garrido-Ramos, M.A. (2009b). Characterization of RUSI, a telomere-associated satellite-DNA, in the genus *Rumex* (*Polygonaceae*). *Cytogenetic and Genome Research, 124,* 81-89.

[66] Smith, B.W. (1963). The mechanism of sex determination in *Rumex hastatulus*. *Genetics, 48*, 1265-1288.

[67] Ruiz Rejón, C., Jamilena, M., Garrido-Ramos, M.A., Parker, J.S., Ruiz Rejón, M. (1994). Cytogenetic and molecular analysis of the multiple sex chromosome system of *Rumex acetosa*. *Heredity*, *72,* 209-215.

[68] Kazama, Y., Matsunaga, S. (2011). The role of repetitive sequences in the evolution of plant sex chromosomes. In: Navajas-Pérez, R. (ed), *New Insights on Sex Plant Chromosomes*, Nova Publishers, Hauppauge NY.

[69] Navajas-Pérez, R., Quesada del Bosque, M.E., Garrido-Ramos, M.A. (2009c). Effect of location, organization, and repeat-copy number in satellite-DNA evolution. *Mol. Genet. Genomics, 282,* 395-406.