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Cytogenetical Relationships between *Rumex tuberosus, R. thyrsiflorus* and *R. acetosa* and Occurrence of Polyploidy among their Hybrids

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Summary. Reciprocal crosses were performed between *Rumex tuberosus* and two other taxa belonging to the *Acetosa* group: *R. acetosa* and *R. thyrsiflorus.* All three taxa are dioecious with the same chromosome numbers, ♀ XX+12A, ♂ XYY+12A, but they differ from one another in the morphology of some autosomes. *R. tuberosus* and *R. thyrsiflorus* are similar in external morphology, while *R. acetosa* differs greatly from the other two taxa in external morphology as well as ecological characteristics.

The F_1 and F_2 hybrids were fully vigorous and viable but their fertility was greatly reduced. Meiotic irregularities were observed in hybrids derived from all crosses. The most remarkable meiotic irregularity was diad formation after first meiotic division. The unreduced gametes derived from diads were responsible for the high frequency of polyploidy observed among the F_2 hybrids. In the cross *R. tuberosus* × *R. acetosa* the whole F_2 generation was tetraploid.

It is concluded from the analysis performed that *R. tuberosus, R. acetosa* and *R. thyrsiflorus* are separate species, closely related to one another but already isolated by genetic and ecological barriers.

Introduction

The *Acetosa* group of *Rumex* consists of four taxa, *R. tuberosus, R. thyrsiflorus, R. acetosa* and *R. arifolius,* all having the diploid chromosome number $2n = 14, 15$. All four taxa are dioecious with the sex chromosome composition ♀ — *XX*, ♂ — *XYY*.

An earlier study of this group by the present author (Świetlińska, 1963) revealed that *R. acetosa* and *R. arifolius* are closely related. They are very similar in external morphology and their hybrids are fully fertile and viable. It was proposed by the author that *R. arifolius* may be considered as a subspecies or ecological race of *R. acetosa. R. thyrsiflorus* is more distinct. It differs from both *R. acetosa* and *R. arifolius* in external morphology, growth form and ecological requirements. Hybrids obtained from crosses between *R. thyrsiflorus* and *R. acetosa* or *R. arifolius* were weak and partially sterile. Among the hybrids polyploidy was a frequent phenomenon (Żuk, 1963, Gajewski et al., 1963, Świetlińska and Żuk, 1965).

R. tuberosus is another species of the *Acetosa* group. In morphology and growth form it clearly resembles *R. thyrsiflorus* but the area of its distribution is higly restricted compared with that of *R. thyrsiflorus.* The present study was undertaken to determine the cytogenetical relationships between *R. tuberosus* and two other species of the group, *R. acetosa* and *R. thyrsiflorus.*

Material and Methods

Seeds of *R. acetosa* and *R. thyrsiflorus* were collected from natural populations growing in Poland. Seeds of *R. tuberosus* were kindly provided by the Botanical Institute in Tbilisi (SU). The parent plants and their hybrids were grown in experimental plots of the Botanical Garden in Warsaw. The crossing procedure was very simple and effective due to the dioecism of the taxa.

Cytological observations were made on temporary squash preparations. Karyotypes were analyzed in root tip metaphases fixed in ethanol acetic acid fixative (3:1) with three hour pretreatment with 0.03% 8-oxyquinoline. Material was stained in aceto-orcein. Material for the study of microsporogenesis was fixed in ethanol acetic acid fixative (3:1) and stained in alcoholic hydrochloric acid carmine, according to Snow (1963).

Results

1. Phenotypic characteristics of the parent taxa and F_1 and F_2 hybrids

All three taxa are perennial. On the experimental plots, *R. acetosa* flowered in May and, as a rule, in the second year of growth. *R. thyrsiflorus* was flowering in its first year of growth, usually from June to August. *R. tuberosus* began flowering in May, but earlier than *R. acetosa,* and most of the plants flowered in the second year.

In leaf morphology *R. tuberosus* closely resembles *R. thyrsiflorus.* In both species the leaves are dark green, thick and narrow. *R. acetosa* has much thinner, shorter and broader leaves than *R. thyrsiflorus* or *R. tuberosus.* All three taxa differ from one another in the type of root system: *R. acetosa* has fibrous roots, not very long and thick; in *R. thyrsiflorus* the roots are much thicker and longer; *R. tuberosus* is characterized by the development of tubers on its roots. The inflorescences of *R. thyrsiflorus* and *R. tuberosus* are compact with many side branches. The inflorescence of *R. acetosa* is much more simple. The seeds are dark brown in all three taxa but in *R. acetosa* they are bigger than in the other two taxa. From a comparison of the main features characteristic for the three taxa, it may be concluded that *R. tuberosus* and *R. thyrsiflorus* resemble each other much more than they do *R. acetosa.*

All F_1 and F_2 hybrids were fully vigorous and viable. The F_1 hybrids were intermediate between their parents in external morphology. In the F_2 generation, segregation of parental traits was noted but variation was clearly of the continuous type as is typical of polygenic inheritance. One of the F_2 populations obtained from the cross between *R. tuberosus* and *R. acetosa* exhibited distinct hybrid vigour. The plants were much taller, with larger and thicker leaves than in the other F_2 hybrids. In the next section, cytological data will be presented to show that all these plants were tetraploid.

The F_1 hybrids of *R. thyrsiflorus* × *R. tuberosus* flowered during the first year of growth, from the beginning to the end of July as in the mother form. Only 68% of the F_1 hybrids from the reciprocal cross flowered during the first year and much earlier (May to June) than those from the former cross. Therefore, the influence of the mother parent on flowering time was evident in the reciprocal crosses between *R. tuberosus* and *R. thyrsiflorus*. Flowering time in the F_2 hybrids was not studied. F_1 and F_2 populations from the reciprocal crosses between *R. acetosa* and *R. tuberosus* flowered during the second year of growth, in May, as in both parental taxa.

2. *Intercrossing ability of parent taxa and fertility of the hybrids*

In *Rumex*, an exact estimate of the fertility of the female plants is very difficult because of the small size of the seeds and abundant seed setting. In the present paper the number of seeds set was counted after controlled pollination only. The pollen fertility was estimated as the proportion of well stained pollen grains in acetocarmine smears.

Reciprocal crosses were performed between all three taxa. Experimental crossing within each taxon yielded over 1000 seeds for *R. acetosa* and over 2000 seeds for *R. thyrsiflorus* and *R. tuberosus*. Seeds of all three taxa were highly fertile and 70 to nearly 100% germinated. A small quantity of abortive seeds was also observed but usually not more than 5%. Seed setting after intercrossing was greatly reduced. Data concerning seed setting and germination of seeds obtained from the experimentel crosses are given in Table 1. The number of seeds obtained ranged from 250 to 600 and many of them were abortive (up to 60% in some crosses). Seed germination was also greatly reduced, being as low as 20% in the cross *R. thyrsiflorus* × *R. tuberosus*. High seed germination was observed only in the cross *R. acetosa* × *R. tuberosus*; however, in the same cross the number of abortive seeds reached 32%. Thus, it may be concluded that the intercrossing ability of the three taxa is very limited.

Table 1. *Intercrossing ability of R. tuberosus, R. thyrsiflorus and R. acetosa*

cross	No. of seeds set	abortive seeds (per cent)	seed germination (per cent)
R. tuberosus × *thyrsiflorus*	600	25	–
R. tuberosus × *thyrsiflorus*	340	23	58
R. thyrsiflorus × *tuberosus*	250	60	20
R. tuberosus × *acetosa*	400	25	56
R. acetosa × *tuberosus*	250	32	98

The F_1 hybrids obtained from the hybrid seeds were not fully fertile. Observations on seed setting after free pollination revealed that in all F_1 populations female plants did not yield as many seeds as did the parent taxa. Moreover, a few completely sterile plants occurred in all F_1 populations. Table 2

Table 2. *Fertility of F_1 hybrids in crosses of R. tuberosus with R. thyrsiflorus and R. acetosa*

Cross	Pollen stainability		Seed fertility			
	No. of ♂ plant	stainable pollen (per cent)	F_1♀ × F_1♂	No. of seeds set	abortive seeds (per cent)	seed germination (per cent)
R. tuberosus × *thyrsiflorus*	1	75.3	4 × 1	775	50.1	–
	2	62.2	9 × 3	209	64.6	10.0
	3	81.9	5 × 2	862	47.5	64.8
	12	75.6*	10 × 2	343	65.0	35.0
	13	78.5*				
R. thyrsiflorus × *tuberosus*	1	82.2*				
	2	81.6	no pollinations performed			
	3	71.3				
R. tuberosus × *acetosa*	3	32.0	8 × 3	67	38.8	1 seed
	4	78.0	7 × 4	1335	21.4	18.0
	5	0	7 × 5	0	–	–
R. acetosa × *tuberosus*	1	33.0	4 × 1	322	42.0	55.0
	2	79.0	5 × 2	83	50.0	30.0
	3	79.0	5 × 3	350	66.0	36.0
	9	60.0	10 × 9	250	36.0	56.0

* no pollen shedding

shows seed setting after controlled pollination of F_1 hybrids, seed germination and pollen fertility. The data show that seed setting of F_1 hybrids was reduced more or less to the same level as observed in the crosses between the parent taxa. The proportion of abortive seeds was also high and ranged from 21.4 to 66.0 per cent. Seed germination varied from 10.0 to 64.8 per cent for particular combinations of F_1 plants. There were no marked differences in the fertility of F_1 hybrids originating from different crosses. Only the F_1 hybrids *R. tuberosus* × *R. acetosa* were remarkable, because, out of three controlled pollinations performed between individual F_1 plants, only one combination gave a good yield of seeds (1335) while the other two were mainly or completely sterile. No pollinations for F_2 were performed in the F_1 population from the *R. thyrsiflorus* × *R. tuberosus* cross, but it was observed that seed setting in this cross after free pollination was markedly reduced.

Pollen stainability, as may be seen from Table 2, was also reduced but rather less drastically than seed fertility. The amount of well stained pollen grains varied from 32 to 82 per cent, but, in some plants no pollen shedding was observed, and in the cross *R. tuberosus* × *R. acetosa* one plant produced completely abortive pollen.

Observations on the fertility of F_2 hybrids were made only in crosses between *R. tuberosus* and *R. acetosa*. Seed setting after free pollination was greatly reduced both in the F_2 hybrids of *R. tuberosus* × *R. acetosa* and in the reciprocal cross. In the F_2 hybrids of *R. thyrsiflorus* × *R. acetosa* no control pollinations were performed; of the two pollinations performed in the reciprocal cross, one was completely abortive and the second yielded only 50 seeds.

Pollen fertility was estimated for 5 F_2 hybrids from the cross *R. tuberosus* × *R. acetosa*. It varied from 24 to 81 per cent with a mean value of 47 per cent. Pollen fertility for 14 plants from the reciprocal cross varied from 1 to 78 per cent with a mean value of 40 per cent.

3. *Cytological observations on parent taxa and their hybrids*

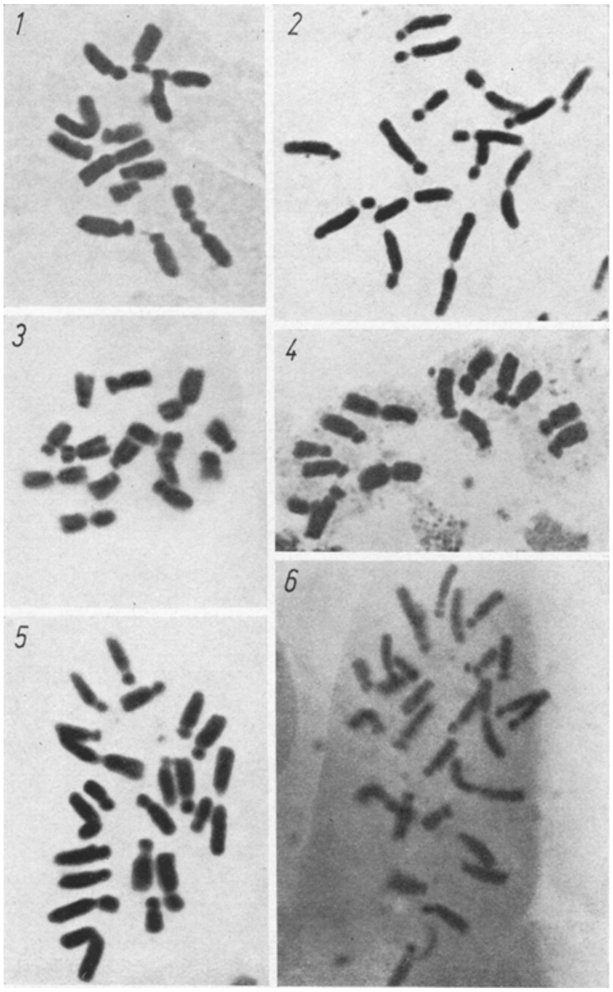
Karyotypes of parent taxa. All the plants examined were normal diploids, with female karyotypes $14\,(X\,X + 12\,A)$ and male karyotypes $15\,(X + Y\,Y + 12\,A)$ where A stands for autosomes. It has already been stated that four types of autosomes occur in *R. thyrsiflorus* (Świetlińska, 1963; Zaborowska, 1969). According to Ono's nomenclature (1935) they are: short acrocentric rod-shaped chromosomes (*i*-type); short almost metacentric chromosomes (*v*-type); submetacentric chromosomes longer than *v*-type (*j*-type); and *i*-type chromosomes but with satellites (*T*-type). Regarding the sex chromosomes, the X chromosomes are metacentric and are the longest in the complement, the Y chromosomes are meta- or submetacentric and shorter than the X chromosomes.

Table 3. *Karyotypes of R. tuberosus, R. thyrsiflorus and R. acetosa*

Taxon	Frequency of karyotype	Karyotype
R. tuberosus	2	$14\,(2\,X + 9\,i + 2\,j + T)$
	2	$15\,(X + 2\,Y + 9\,i + 2\,j + T)$
	2	$14\,(2\,X + 10\,i + 2\,j)$
	1	$15\,(X + 2\,Y + 10\,i + 2\,j)$
	2	$14\,(2\,X + 9\,i + 3\,j)$
	2	$15\,(X + 2\,Y + 9\,i + 3\,j)$
R. thyrsiflorus	7	$14\,(2\,X + 8\,i + 2\,j + 2\,v)$
	2	$15\,(X + 2\,Y + 8\,i + 2\,j + 2\,v)$
	2	$14\,(2\,X + 7\,i + 2\,j + 2\,v + T)$
	1	$15\,(X + 2\,Y + 7\,i + 2\,j + 2\,y + T)$
	3	$14\,(2\,X + 9\,i + 2\,j + 1\,v)$
	1	$15\,(X + 7\,Y + 9\,i + j + 2\,v)$
	1	$15\,(X + 2\,Y + 8\,i + j + 2\,v + T)$
R. acetosa	2	$14\,(2\,X + 12\,i)$
	1	$14\,(2\,X + 11\,i + T)$

Karyotypes of representatives of the three taxa, with sex chromosome and autosome composition, are given in Table 3. It may be seen that *R. acetosa* representatives had simple karyotypes composed only of the *i* type and *T* type autosomes (Fig. 1). In *R. tuberosus*, besides these two types of autosome, two or three *j* type chromosomes were also observed (Fig. 2). In *R. thyrsiflorus*, all three types of autosomes occurred. The number of *j* type chromosomes in individual plants ranged from one to three, and the number of *v* type autosome from one to two. Fig. 3 represents the karyotype of the male plant $2\,n = 15\,(X + 2\,Y + 8\,i + 2\,j + 2\,v)$.

Karyotypes of F_1 and F_2 hybrids. The karyotypes of 51 F_1 and of 106 F_2 hybrids were established. As can be seen from the data in Table 4, all F_1 hybrids were diploid except for the two plants from the cross *R. thyrsiflorus* × *R. tuberosus* which were triploid. Figs. 4 and 5 show the karyotypes of diploid $14\,(2\,X + 11\,i + 1\,j)$ and triploid $21\,(3\,X + 15\,i + 3\,j)$ F_1 hybrids. In the F_2 of *R. tuberosus* × *R. thyrsiflorus* and *R. acetosa* × *R. tuberosus* a few polyploid plants occurred in addition to the diploids. Four of them were triploids with $2\,n = 22$ and a sex chromosome composition $2\,X + 2\,Y$, and one was tetraploid with $2\,n = 29$ and sex chromosomes $3\,X + 2\,Y$. The most remarkable was the F_2 from the cross *R. tuberosus* × *R. acetosa*. In this F_2 population 20 plants were examined and all of them were tetraploids, (Fig. 6) the most frequent karyotype being $28 = 4\,X + 24\,A$. The whole F_2 population consisted of 35 plants and all of them manifested the vigour characteristic for polyploids. Moreover, cytological examination of microsporogenesis revealed



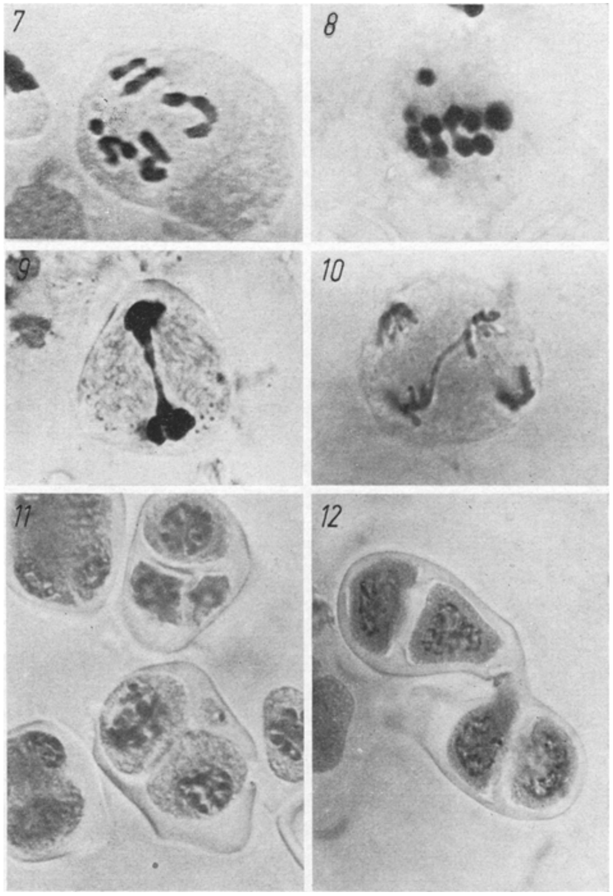
Figs. 1—6. Karyotypes of parent plants and F_1 and F_2 hybrids. × 1250

1. *R. acetosa*: $2n = 14(2X+11i+1T)$
2. *R. tuberosus*: $2n = 15(X+2Y+9i+3j)$
3. *R. thyrsiflorus*: $2n = 15(X+2Y+8i+2j+2v)$
4. F_1 *R. tuberosus* × *R. thyrsiflorus*: $2n = 14(2X+11i+1j)$
5. F_1 *R. thyrsiflorus* × *R. tuberosus*: $2n = 21(3X+15i+3j)$
6. F_2 *R. tuberosus* × *R. acetosa*: $2n = 28(4X+24A)$

that all the male plants in this progeny (14 individuals) were tetraploids. Thus, there is good reason to believe that in the cross *R. tuberosus* × *R. acetosa* the F_2 progeny consisted of tetraploid plants only.

In all crosses, F_1 and F_2 hybrids had different combinations of *i*-, *j*- and *v*-type autosomes. In crosses between *R. tuberosus* and *R. thyrsiflorus*, all three types of autosome occurred but the number of individual types of autosomes varied from plant to plant. In hybrids between *R. tuberosus* and *R. acetosa*, no *v* type chromosomes were observed because this type of autosome was lacking in both parental species.

Microsporogenesis in hybrid plants. Microsporogenesis was investigated in F_1 hybrids derived from all crosses. F_2 hybrids were investigated only in the reciprocal crosses between *R. tuberosus* and *R. acetosa*. Table 5 gives the frequency of the different types of irregularity observed in reciprocal F_1 hybrids between *R. tuberosus* and *R. thyrsiflorus*. Eight hybrid plants were examined and all were found to have disturbed meiosis in a proportion of the PMC's. Univalents in MI (Fig. 8) were observed in 6.7 per cent of PMC's in F_1 of *R. tuberosus* × *R. thyrsiflorus* and in 8.1 per cent in the reciprocal cross. The univalents were considered to be the result of desynapsis, because six bivalents and the sex chromosome trivalent (*X*-*Y*-*Y*) were regularly formed at diakinesis (Fig. 7). In some PMC's in AI lagging chromosomes were observed but a much more frequent irregularity was the occurrence of bridges and fragments (Fig. 9) — 12 per cent of PMC's in the F_1 of *R. tuberosus* × *R. thyrsiflorus* and 9.7 per cent in the reciprocal cross. Bridges and frag-



Figs. 7—12. Microsporogenesis in hybrid plants. × 750

7. Diakinesis in F_1 *R. acetosa* × *R. tuberosus* (note 6 bivalents and sex chromosome trivalents *Y*-*X*-*Y*)
8. Univalent in MI of F_1 hybrid *R. tuberosus* × *R. thyrsiflorus*
9. Bridge and fragment in AI of F_1 hybrid *R. tuberosus* × *R. thyrsiflorus*
10. Chromatin bridge in AII of F_1 hybrid *R. tuberosus* × *R. acetosa*
11. Diad and irregular triad in F_1 hybrid *R. tuberosus* × *R. thyrsiflorus*
12. Diads in F_2 hybrid *R. tuberosus* × *R. acetosa*

Table 4. *Chromosome numbers in F_1 and F_2 hybrids and karyotypes of some polyploid plants*

Cross	hybrid generation	No. of plants examined	Frequency of diploids	triploids	tetraploids	Karyotypes of F_2 hybrids
R. tuberosus × *thyrsiflorus*	F_1	18	18	—	—	
	F_2 (4 × 1)	10	10	—	—	
	F_2 (9 × 3)	9	8	1	—	22 ($2X + 2Y + 14i + 3j + v$)
	F_2 (5 × 2)	31	28	2	1	22 ($2X + 2Y + 10i + 6j + 2v$)
						22 ($2X + 2Y + 13i + 3j + 2v$)
						29 ($3X + 2Y + 17i + 4j + 2v$)
R. thyrsiflorus × *tuberosus*	F_1	21	19	2	—	21 ($3X + 15i + 3j$)
R. tuberosus × *acetosa*	F_1	8	8	—	—	
	F_2 (8 × 3)	1	—	—	1	28 ($4X + 24i$)
	F_2 (7 × 4)	19	—	—	19	28 ($4X + 22i + 2j$)
						28 ($4X + 20i + 2j + 2T$)
R. acetosa × *tuberosus*	F_1	6	6	—	—	
	F_2 (4 × 1)	18	18	—	—	
	F_2 (5 × 3)	9	8	1	—	22 ($2X + 2Y + 18A$)
	F_2 (5 × 2)	9	9	—	—	

ments (Fig. 10) also occurred in AII, but not as frequently as in AI. The most remarkable irregularity observed in F_1 hybrids was the formation of diads instead of tetrads (Fig. 11) and the failure of second meiotic division in some PMC's. In such PMC's, as soon as the first meiotic division was completed, a cell wall was formed dividing the PMC into two daughter cells, diads with 7 ($X + 6A$) and 8 ($YY + 6A$) chromosomes. In diad nuclei the chromosomes undergo homeotypic division but this is not followed by cytokinesis. Consequently, unreduced pollen grains were formed with a chromosome constitution 14 ($XX + 12A$) and 16 ($YYYY + 12A$).

As may be seen from Table 5, diads were more frequent in the cross *R. tuberosus* × *R. thyrsiflorus*, occurring in 32.2 per cent of the PMC's of one plant. It is evident that diad formation was responsible for the occurrence of polyploid plants among F_2 hybrids. It may be assumed that triploid F_2 hybrids with karyotype 22 ($2X + 2Y + 18A$) (Table 4) originated from the unreduced gametes 14 ($XX + 12A$) and the normal gametes 8 ($YY + 6A$). The tetraploids 28 ($4X + 24A$) were obviously derived from two unreduced gametes with the same chromosome constitution 14 ($2X + 12A$), produced by the male and female parents as a result of the failure of the second meiotic division. Another tetraploid presented in Table 4, with the chromosome constitution 29 ($3X + 2Y + 24A$), was obviously of different origin. This tetraploid probably originated from the gametes 14 ($2X + 12A$) produced by the female parent and 15 ($X + 2Y + 12A$) produced by the male plant. Taking into account the sex chromosome composition $X + 2Y$, the gamete 15 ($X + 2Y + 12A$) could not have resulted from the failure of the second meiotic division. It is assumed that such gametes resulted from division of the restitution nucleus. In diploid male plants, restitution nuclei resulting from the failure of the first meiotic division would have 15 chromosomes ($X + 2Y + 12A$), and subsequent division would lead to the formation of two gametes each containing 15 chromosomes ($X + 2Y + 12A$).

Table 5. *Meiotic irregularities in PMC's of reciprocal F_1 hybrids between R. tuberosus and R. thyrsiflorus (200 cells were scored for each meiotic stage)*

Cross	*R. tuberosus* × *thyrsiflorus*		*R. thyrsiflorus* × *tuberosus*	
Meiotic irregularities	range for 5 plants (per cent)	average (per cent)	range for 3 plants (per cent)	average (per cent)
Univalents in MI	2.8 – 8.8	6.7	5.0 – 10.5	8.1
Laggards in AI	0.5 – 2.3	1.6	2.2 – 3.8	2.9
Bridges and fragments in AI	7.3 – 18.6	12.0	8.3 – 14.0	9.7
Bridges and fragments in AII	0 – 3.5	2.7	3.3 – 8.9	5.9
Diads	2.5 – 32.2	12.7	2.0 – 10.3	5.6

In reciprocal hybrids between *R. tuberosus* and *R. acetosa*, meiotic irregularities of the same type as those described for reciprocal crosses of *R. tuberosus* × *R. acetosa* were observed and more or less with the same frequency. The only significant difference was in the frequency of diad formation. In the F_1 of *R. tuberosus* × *R. acetosa*, two male plants were examined and two of them produced only unreduced gametes. The pollen grains derived from diads were almost twice as large as those produced by the parent plants. Macrosporogenesis in F_1 hybrids was not investigated but the fact that in the cross *R. tuberosus* × *R. acetosa* the whole F_2 progeny consisted of tetraploid plants suggests that unreduced gametes were also produced by female F_1 hybrids. Moreover, investigation of microsporogenesis in tetraploid F_2 progeny revealed that unreduced gametes were also formed in some F_2 plants. Three groups of male plants could be distinguished among this F_2 progeny:

the first consisted of plants producing regular tetrads; in the second group diads were formed after I meiotic division (Fig. 12); in the third group both diads and tetrads were observed. However, it was noticed that in some plants diad nuclei undergo some kind of second meiotic division which is usually highly irregular and, as a result, triads, pentads and irregular tetrads with micronuclei were formed.

The fact that the phenomenon of diad formation after first meiotic division was transmitted from F_1 to F_2 hybrids strongly suggests genotypic control for this type of meiotic irregularity. As the problem seems to be of more general interest, the detailed analysis of meiosis in hybrids of *R. tuberosus* × *R. acetosa* is in progress in our laboratory and the results will be published in a separate paper.

Discussion

According to data available in the Flora of USSR (Komarov, 1936), the distributions of *R. tuberosus, R. thyrsiflorus* and *R. acetosa* overlap, at least partly, *R. thyrsiflorus* and *R. acetosa* are distributed throughout the lowlands of almost the whole continent of Euro-Asia and North America. *R. tuberosus* is not so widespread, and its distribution is restricted to central and western Asia and South-East Europe. Although geographical isolation of the three taxa is out of the question, it is still possible that ecological, seasonal or other barriers may be operating to prevent intercrossing of the taxa in their natural habitats. The ecological and seasonal barriers have been well documented for *R. thyrsiflorus* and *R. acetosa* (Świetlińska, 1963). The two taxa have different ecological requirements but even when growing in the same neighbourhood their intercrossing is greatly restricted by different flowering times.

R. tuberosus was not observed by the authors in its natural habitat but information provided by the Flora of the USSR suggests that its ecological requirements are different from those of *R. thyrsiflorus* and *R. acetosa. R. tuberosus* is known to occupy stony soils on the slopes of hills, while both *R. acetosa* and *R. thyrsiflorus* are typical lowland taxa. Moreover, the distinct differences in the root type of the three taxa seem to be the result of adaptation to different living conditions. *R. tuberosus* and *R. thyrsiflorus*, according to observations on the experimental plots, do not flower at the same time, thus the flowering time may be another barrier preventing intercrossing of the two taxa.

The results of crossing reported in the present paper reveal that under experimental conditions hybrids between *R. tuberosus* and *R. thyrsiflorus* or *R. acetosa* can be easily obtained and they are fully vigorous. However, the seed setting after intercrossing was highly reduced and this may be interpreted as an incompatibility barrier between *R. tuberosus* and the two other taxa. Again, the high proportion of abortive seeds and reduced germination rate of hybrid seeds suggests the presence of barriers partly separating *R. tuberosus* from *R. acetosa* and *R. thyrsiflorus*. Another barrier separating *R. tuberosus* from the other two taxa is the reduced fertility of their hybrids. The symptoms of hybrid sterility are low seed setting, the production of abortive seeds, and decreased pollen stainability and seed germination in the F_1 and F_2 generations.

The investigation of microsporogenesis in F_1 hybrids revealed meiotic irregularities suggesting that the chromosomes of *R. tuberosus* are not completely homologous with those of *R. thyrsiflorus* and *R. acetosa*. However, univalents in first metaphase and bridges with acentric fragments in first anaphase occurred no more frequently than in some 12 per cent of PMC's of F_1 hybrids and, therefore, the lack of homology between chromosomes could not be the main cause of nonstainable pollen observed in the hybrids. It may be concluded that there is also genic control of pollen and seed fertility in the hybrids. It is especially evident in the case of some F_1 hybrids between *R. tuberosus* and *R. thyrsiflorus* in which no pollen shedding was observed in spite of quite good pollen stainability. Again, low seed setting and seed germination and the production of abortive seeds by the hybrids suggest the presence of several genetic factors preventing fertilization or resulting in death of the hybrid zygotes.

The most interesting meiotic irregularity observed in the hybrids was diad formation after first meiotic division. This resulted in the production of unreduced gametes by the male plants and probably by the female plants as well. This may be concluded from the fact that the F_2 progeny derived from diploid F_1 hybrids of the *R. tuberosus* × *R. acetosa* cross consisted of tetraploid plants only.

Although polyploidy has been involved in the evolution of many plant genera, the occurrence of polyploidy in the *Acetosa* group of *Rumex* is of no evolutionary significance. This is no doubt because of the dioecism and the mechanism of sex determination operating in this group. It has been well established that sex in this group depends on the balance between the X chromosome determining female sex and the autosomes carrying male determining factors; Y chromosomes are completely inactive in sex determination (Ono, 1935; Żuk, 1963, 1969, 1970). In diploid male plants sex chromosomes conjugate forming a trivalent Y-X-Y, which ensures the undisturbed segregation of the sex chromosomes $X - Y\ Y$ to the gametes. In contrast, in tetraploids with a sex chromosome constitution $XXYYYY$, the conjugation and segregation of sex chromosomes is highly disturbed (Świetlińska and Żuk, 1965). The resulting gametes have unbalanced combinations of autosomes and sex chromosomes which, on being introduced into the zygotes, cause intersexuality and high sterility of the offspring.

The situation is completely different in the section *Acetosella* of the genus *Rumex*. In this group, with a sex determination system of the X/Y type, there is no significant obstacle to the establishment of polyploid lines. In contrast to the *Acetosa* group, which contains diploid taxa only, in the subgenus *Acetosella* tetra-, hexa- and octoploids are also known (Löve, 1944).

Polyploid F_2 and F_3 hybrids have also been obtained in crosses between *R. acetosa* and *R. thyrsiflorus* (Świetlińska, 1960; 1963; Świetlińska and Żuk, 1965). The process of diad formation was essentially the same as that observed in the hybrids reported in the present paper. However, diad formation was never observed by the authors in the parent taxa, the occurrence of two triploids in the F_1 of *R. thyrsiflorus* × *R. tuberosus* suggests that unreduced gametes were sporadically produced by one of the parent plants. The two triploids had the same karyotype, $XXX + 18\,A$ and could have resulted from the failure of either the first or the second meiotic divisions. Nevertheless, there is no doubt that the failure of the second meiotic division and diad formation is much more frequent in F_1 hybrids obtained from the intercrossing of specimens belonging to different taxa. It may be concluded, therefore, that some combinations of genic factors, introduced to the hybrid zygote by representatives of different taxa, are responsible for diad formation and subsequent polyploidy of the next generation.

As mentioned above, the hybrids between *R. tuberosus* and *R. thyrsiflorus* or *R. acetosa* were fully vigorous. The situation was different in crosses between *R. thyrsiflorus* and *R. acetosa* or its subspecies *R. arifolius*, for in such crosses weakness of the hybrids was a frequent phenomenon (Świetlińska, 1963). It may be concluded therefore that there are closer relationships between *R. tuberosus* and *R. thyrsiflorus* or *R. acetosa* than between *R. thyrsiflorus* and *R. acetosa*. The closeness of the relationship between *R. tuberosus* and *R. thyrsiflorus* is also supported by the fact that the two taxa are very similar in external morphology. On the other hand, *R. acetosa* is distinctly different in external morphology from both *R. tuberosus* and *R. thyrsiflorus*.

In view of the data now available, *R. tuberosus*, *R. thyrsiflorus* and *R. acetosa* can be considered to be separate species, closely related to each other but already isolated by internal (genetical) and external (ecological and seasonal) barriers.

It seems that evolution within the *Acetosa* group proceeded by adaptation to different ecological environments. The process of adaptation was paralleled by the differentiation of karyotypes (autosomal polymorphism) and morphological and physiological traits. The autosomal polymorphism is especially evident within *R. thyrsiflorus* which contains median and submedian autosomes as well as rod-shaped, acrocentric autosomes. As found by Żuk (1969), the median and submedian autosomes in *R. thyrsiflorus* are partly heterochromatic and late replicating. It may be interpreted from thus that evolution of the karyotype in this species proceeds by translocations and heterochromatization of some segments of the autosomes. It may be worthwhile to point out that *R. thyrsiflorus*, which has the most highly differentiated karyotype, is known as the most effective coloniser of new habitats (Löve and Löve, 1957). Also the occurrence of B chromosomes reported for this species (Zaborowska, 1969), which are considered by many authors as being of adaptive value (Hansen, 1969; Moss, 1969), makes it possible to consider *R. thyrsiflorus* as the most rapidly differentiating species of the group.

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References

1. Gajewski, W., Świetlińska, Z., Żuk, J.: Relationship between biosystematic and formal taxonomy of the *Rumex Acetosa* group. Regnum Vegetabile **27**, 16—24 (1963). — 2. Hansen, G. P.: B-chromosome-stimulated crossing over in maize. Genetics **63**, 601—609 (1969). — 3. Komarov, W. A.: Flora USSR. T. IV, Leningrad 1936. — 4. Löve, A.: Cytogenetic studies on *Rumex* subgenus *Acetosella*. Hereditas **30**, 1—36 (1944). — 5. Löve, D., Löve, A.: *Rumex thyrsiflorus* new to North America. Rhodora **59**, 1—5 (1957). — 6. Moss, J. P.: The adaptive significance of B-chromosomes in rye. Chromosomes Today Vol. 1 (1969). — 7. Ono, T.: Chromosomen and Sexualität von *Rumex acetosa*. Sci. Rep. Tohoku Univ. **10**, 41—210 (1935). — 8. Snow, R.: Alcoholic hydrochloric acid-carmine as a stain for chromosomes in squash preparation. Stain Technol. **38**, 9—13 (1963). — 9. Świetlińska, Z.: Spontaneous polyploidization in *Rumex* hybrids. Acta Soc. Bot. Pol. **29**, 79—98 (1960). — 10. Świetlińska, Z.: Cytogenetic relationships among *Rumex acetosa*, *Rumex arifolius* and *Rumex thyrsiflorus*. Acta Soc. Bot. Pol. **32**, 215—273 (1963). — 11. Świetlińska, Z., Żuk, J.: Further observations on spontaneous polyploidization in *Rumex* hybrids. Acta Soc. Bot. Pol. **34**, 439—450 (1965). — 12. Zaborowska, D.: Autosomal polymorphism in *Rumex thyrsiflorus*. Acta Soc. Bot. Pol. **38**, 115—124 (1969). — 13. Żuk, J.: An investigation on polyploidy and sex determination within the Genus *Rumex*. Acta Soc. Bot. Pol. **32**, 5—67 (1963). — 14. Żuk, J.: Analysis of Y chromosome heterochromatin in *Rumex thyrsiflorus*. Chromosoma (Berl.) **27**, 338—355 (1969a). — 15. Żuk, J.: An autoradiographic study of the chromosomes of *Rumex thyrsiflorus* with special regard to the sex chromosomes. Chromosomes Today Vol. II, 183—188 (1969b). — 16. Żuk, J.: Functions of Y chromosomes in *Rumex thyrsiflorus*. Theor. Appl. Gen. **40**, 124—129 (1970).

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