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Experimental hybridization of species in the genus *Rorippa*

Experimentální hybridizace mezi některými druhy rodu *Rorippa*

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Dedicated to the memory of Pavel Tomšovic

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Experimentally produced interspecific hybrids between four Central European species of *Rorippa* (*Brassicaceae*), which are wide-spread in the Czech and Slovak Republics (allogamic *R. amphibia*, *R. austriaca*, *R. sylvestris* and autogamic *R. palustris*), were studied. The hybrid between the allogamic tetraploid species *R. amphibia* and *R. sylvestris* can produce hybrid swarms when they occur sympatrically with the parental species. The most plausible mode of formation of the tetraploid hybrid swarms introgressed by diploid *R. austrica* in nature was confirmed: The chromosome numbers of the offspring resulted from the controlled pollination of the triploid experimental hybrid *R. austriaca* × *R. sylvestris* mostly tended to the tetraploid level. Even healthy tetraploid plants, with high quality pollen, developed in the second generation after open pollination of the experimental triploid *R. amphibia* × *R. austriaca*. Plants with nearly tetraploid or tetraploid chromosome numbers and sufficiently fertile pollen gave rise to fully fertile tetraploid hybrid swarms, even without the presence of tetraploid *R. austriaca*. Failure of most experimental crosses of the autogamous tetraploid *R. palustris* with allogamous species (totally sterile F1 acquired only in combination *R. austriaca* × *R. palustris*) indicated that this species is unlikely to have participated in the formation of hybrid swarms in nature.

Keywords: *Brassicaceae,* chromosome number, PMC meiosis, Czech Republic, interspecific hybridization, ploidy level, *Rorippa*, Slovak Republic

Introduction

Five species of *Rorippa* occur in the Czech and Slovak Republics (Tomšovic 1965, 1969, 1992, Tomšovic & Goliašová 2002): *R. amphibia* (L.) Bess. (2n = 32), *R. austriaca* (Cr.) Rchb. (2n = 16), *R. palustris* (L.) Bess. (2n = 32), *R. sylvestris* (L.) Bess. (2n = 32, very rarely 40, 48) and *R. pyrenaica* (All.) Rchb. (2n = 16). Their identification is often difficult because of intraspecific variation and occurrence of intermediate types, resulting from interspecific hybridization (Tomšovic 1965, 1969, 1992). The following hybrid combinations were identified using morphological characters: Natural polymorphic hybrids of *R. amphibia* × *R. sylvestris* [= *R.* × *anceps* (Wahlenb.) Rchb.] form extensive stands, mainly in disturbed river alluvia, seldom around stagnant water (Tomšovic 1969, 1992, Tomšovic & Goliašová 2002), and do not spread outside the distribution range of *R. amphibia*. The morphological variation of these hybrids includes a whole range of intermediate forms between those of the parent species and their ecological requirements are intermediate as well (Tomšovic 1992). The hybrid *R. amphibia* × *R. austriaca* (= *R.* ×*hungarica* Borbás) occurs where the distributions of the parent species overlap. In the morphology they are very homogenous and in ecological requirements intermediate between those of their parents (Tomšovic 1969, 1992). The natural hybrid swarms of *R. austriaca* × *R. sylvestris* [= *R.* ×*armoracioides* (Tausch) Fuss] have similar ecological requirements to *R. austriaca*, co-occur in the areas of the primary and secondary occurrence of *R. austriaca* and sometimes even further spread as a weed on arable land and along railway tracks. In addition this hybrids occurs on sandy soils in alluvial areas (sandy fields, edges of pine-woods), where the parental species do not occur. The morphology of plants in these hybrid swarms is very variable; the most frequent morphologically defined groups are: 1. leaves entire to deeply dentate, with auricles, fruits ellipsoid to narrowly ellipsoid, 2. leaves pinnatifid to pinnatisect, with auricles, fruits ellipsoid, 3. leaves pinnatisect, without auricles, fruits oblong ellipsoid with long style. The hybrid *R. austriaca* × *R. sylvestris* is probably the most advanced on the way to become a species (Tomšovic 1969, 1992). Following chromosome numbers were detected in natural hybrid swarms: *R. amphibia* × *R. sylvestris* 2n = 32, *R. amphibia* × *R. austriaca* 2n = 32, rarely 24, and *R. austriaca* × *R. sylvestris* 2n = 32, rarely 24. The natural hybrid populations with introgression of *R. austriaca* are interesting karyologically in the predominance of tetraploid individuals, in spite of their parental species being undoubtedly heteroploid (Javůrková-Kratochvílová & Tomšovic 1972).

The breeding systems in the genus *Rorippa* are not uniform. Species of the *R. islandica* group, represented by *R. palustris* in the Czech and Slovak Republics, are autogamic, whereas *R. austriaca*, *R. amphibia* and *R. sylvestris* are allogamic (Jonsell 1968). This trait separates *R. palustris* from the other three above mentioned species. That interspecific hybridization is possible in the genus *Rorippa* was experimentally demonstrated by Jonsell (1968) using plants from northern and western parts of Europe. The occurrence of individual species and their cytotypes in N and W Europe is different from that in Central Europe. *Rorippa islandica* (2n = 16) has an arcto-alpine distribution and diploid *R. amphibia* (2n = 16) occurs only in N and W Europe, and is absent from Central Europe. Furthermore, in Central Europe tetraploid cytotypes of *R. amphibia* and *R. sylvestris* are dominant, with the sporadic occurrence of higher ploidy plants of *R. sylvestris* (Jarolímová 1998), which contrasts with N and W Europe, where tetraploid and hexaploid cytotypes of *R. sylvestris* occur in about the same ratio, and pentaploids, probably a hybrid of the above two species, occur more rarely. The secondary occurrence of *R. austriaca* extends from Central to W Europe, but not northwards. Because of these differences, comparison betweeen Jonsell's study of the genus *Rorippa* (Jonsell 1968) and the results of the hybridization tests made on the plants originating from Central Europe, which are presented here, is possible only in some experimental hybrid combinations: at the tetraploid level *R. amphibia* × *R. sylvestris* and the reverse cross, (*R. amphibia* × *R. sylvestris* natural hybrid) × *R. amphibia,* (*R. amphibia* × *R. sylvestris* natural hybrid) × *R. sylvestris* and the reverse cross, (*R. austriaca* × *R. sylvestris* natural hybrid) × *R. sylvestris*, *R. amphibia* × *R. palustris, R. sylvestris* × *R. palustris* and (*R. amphibia* × *R. sylvestris* natural hybrid) × *R. palustris,* at the heteroploid level *R. austriaca* × *R. sylvestris* and the reverse cross, *R. austriaca* × (*R. austriaca* × *R. sylvestris* natural hybrid).

Introgressive hybridization among *Rorippa* species in Germany was confirmed using molecular methods. Evidence of hybridization and bi-directional introgression between *R. amphibia* and *R. sylvestris* and unidirectional introgression of *R. palustris* into *R. amphibia* is provided by a study of isozymes and noncoding chloroplast DNA (trnL/F spacer) (Bleeker & Hurka 2001). Recently *R. austriaca* rapidly expanded in Germany and formed hybrids with the native *R. sylvestris* (Bleeker 2003). These hybrids (*R.* ×*armoracioides*) are morphologically intermediate or close to *R. sylvestris*. Unidirectional introgression of *R. austriaca* into *R. sylvestris* was detected using chloroplast DNA (trnL intron), whereas the morphologically intermediate hybrid *R.* ×*armoracioides* has the chloroplast DNA of either *R. austriaca* or *R. sylvestris*. Bidirectional introgression between *R. austriaca* and *R. sylvestris* was verified by means of AFLP analysis, hybrids of *R.* ×*armoracioides* have additive AFLP patterns (Bleeker 2003). The invasive hybrid *R.* ×*armoracioides* forms large populations even in absence of its parent species in N Germany, which was observed by Tomšovic also in the Czech and Slovak Republics.

At present, there is a good understanding of self-incompatibility in *Brassica*, a related member of the family *Brassicaceae* (reviewed by Watanabe et al. 2003). The self-incompatibility (SI) system behaves as a single Mendelian locus, designated S-locus, which harbours multiple alleles. Every S-allele consists of a set of genes (SRK, SLG, SCR see below) and is termed a S-haplotype. Self-incompatibility in crucifers is assumed to be a sporophytic system (SSI) triggered by an allele-specific interaction between stigmatic S-locus receptor kinase (SRK) and its pollen ligand, the S-locus cysteine-rich protein (designated SCR or SP11). On the female side, another gene (the first S-locus gene to be isolated) S locus-specific glycoprotein (SLG) enhances the SI recognition reaction [ortholog of the SLG is unknown in *Arabidopsis lyrata* (Kusaba et al. 2001)]. Co-dominance, dominance, incomplete dominance and mutual weakening of the genetic interactions occur between S-haplotypes, and can differ even in stigma and pollen. This may account for the very intricate patterns of SI specifity in the *Brassicaceae*.

This paper aims to simulate the mode of formation of hybrid swarms among Central European species of the genus *Rorippa* by experimentally producing hybrids and determining the microsporogenesis and karyotype evolution of the hybrids.

Materials and methods

Plants from natural populations were transplanted to the experimental plot in Průhonice in 1963–1965 and supplemented by a collection in 1967. Only plants with the typical morphological characters of a species were selected and in areas where there were no other species growing. Reciprocal crossbreeding was performed among four species of the genus *Rorippa* in 1967, resulting in six reciprocal crosses (*R. amphibia* × *R. austriaca*, *R. amphibia* × *R. palustris*, *R. amphibia* × *R. sylvestris*, *R. austriaca* × *R. palustris*, *R. austriaca* × *R. sylvestris*, *R. palustris* × *R. sylvestris*). *Rorippa pyrenaica*, which occurs mainly in the Bukovské hory Mts in the E Slovakia was not included in this study.

Every interspecific cross was done at least twice using plants from different localities. The inflorescence used as a female was divested of its flowers and youngest flower buds, leaving the buds close to flowering. After emasculating these buds, the inflorescence was enclosed in a nylon bag. If some of the remaining buds were too young, they were emasculated 1–2 days later. The inflorescence used as a male and for autogamy, was also divested of flowers and flower buds and enclosed in a nylon bag. After two days the emasculated flowers and those with anthers were used for cross pollination, which was repeated over two days. All the flowers of the inflorescence used for autogamy were pollinated repeatedly over two days until their stigmas withered. The pollinated inflorescence was left enclosed in the nylon bag for 1–2 weeks, after which it was removed and fruits left to ripen freely and just before maturity enclosed again to prevent loss of seed. Up to fifty of the seeds were sown in autumn of the same year. In order to explain the origin of the hybrid swarms occurring in nature, F1 plants were over the next three years selfed and backcrossed with both parents, and the triploid F1 generation crossbred with other tetraploid non-parental species.

The morphology (F1), chromosome numbers (F1, F2, B1, "F2" from open pollination), microsporogenesis (P, F1) and pollen fertility (P, F1, F2, B, "F2" from open pollination) of the experimental plants were studied. The material for the cytological analysis was processed as in a previous paper (Javůrková-Kratochvílová & Tomšovic 1972). The origin of the karyotypes of hybrid plants was revealed by studying of PMC meiosis, tetrads, pollen and chromosome counts of the parents and the first and second hybrid generations. Chromosome pairing, crossing-over and orientation of meiotic configurations formed during the first meiotic prophase are crucial for the segregation of the individual chromosomes into the future gametes (Sybenga 1975). Unfortunately, the chromosomes in the genus *Rorippa* are so small that it is impossible to distinguish individual chromosomes or observe the processes occuring at the first meiotic prophase. Most distinctive are the different chromosome configurations in the PMCs, but these descriptive results can not be used to account for the distribution of chromosomes to the gametes. The number and size of cells in the pollen tetrads were evaluated in 100 tetrads per plant. Pollen quality was based on the stainability of the pollen grains. Stained pollen grains were assumed to be viable and the unstained ones, which were usually small and lacked cytoplasm non viable. Plants in which 0–20% of the pollen grains stained had a low fertility and those in which it was 80–100% were fully fertile. Between 20% and 80% of the pollen of the plants with a reproductive disorder was viable. These plants are usually well represented in the population. The pollen grains were stained using glycerol:aceto-carmine 1:1 (Marks 1954). Hundred pollen grains from a single plant were evaluated. The chromosome number and pollen quality were determined also for a few plants raised from the seed of open pollinated spontaneous hybrids. The obvious differences from the data presented in the charts (but not included therein) are described for each hybrid combination. Voucher specimens of the plants studied are deposited in the herbarium of the Institute of Botany, Průhonice (PRA). A list of the plants used along with their origins is given in Appendix 1.

Notes on the features of the parental plants that negatively influenced crossing: *R. austriaca* – in open pollination, many of the siliques did not develop fully, particularly of plants from the ruderal habitats (Tomšovic 1965), this applies to plants no. 1 and 2. In addition, plants of *R. austriaca* were intolerant of being enclosed in a nylon bag and often withered. *Rorippa amphibia* – plant no. 6 was transplanted to the experimental plot in the same year as the cross-breeding was undertaken. During crossing the outstanding feature of this plant was total female sterility even though the microspores were functional. In the following years populations of *R. amphibia* were observed in drainage-ditches of fields and meadows (between the towns of Stará Boleslav and Kostelec nad Labem) where sterile plants predominated over fertile ones. *Rorippa sylvestris* – even though the plants from the ruderal site Modřany (no. 20, 21 and 23) morphologically corresponded to *R. sylvestris*, their offspring, produced by autogamy, segregated in several morphologically distinct groups and the F1 plants of *R. amphibia* × *R. sylvestris* were also morphologically diverse. Thus these plants were affected by introgression by other *Rorippa* species, which reflect on the experimental crossing. Plant no. 22 was transplanted into the experimental plot at the time when the flower buds were being initiated, which inhibited flowering and production of seeds.

Results

The plants of *R. palustris* tested for autogamy were fully fertile. In well developed pods there were 48–68 seeds. Experimentally forced autogamy in allogamic species resulted in only some of the pollinated flowers producing pods, which usually contained reduced seed set. Differences in the ability to produce seeds by autogamy varied both among the species and in individuals of the same species. The best results were achieved with *R. amphibia*, which after experimentally assisted selfing (pollen grains transferred on the stigma) developed 4–18 seeds/pod. Fertility was significantly lower than when free pollination occurred (20–36 seeds/pod) but the inflorescence left in the nylon bag and not assisted in pollination did not produce any seed. Open pollination of the tetraploid *R. sylvestris* resulted in from 16 to 45 seeds per pod. There were big differences among the individual populations. Autogamy resulted in few seeds [1–8 (–16) seeds/pod]; the number varied greatly between individuals and did not depend on whether pollen transfer was experimentally assisted or not. The inhibition of autogamy was probably most marked in *R. austriaca*, in which only a small fraction of selfed flowers produced pods, which contained very little seeds (1–5 seeds/pod). When open pollinated there were 7–20 seeds/pod.

Whether interspecific hybridization occurs in the genus *Rorippa* depends on the mode of sexual reproduction. All three allogamic species hybridized with one another, while crossing autogamic *R. palustris* with allogamic species almost always failed (only *R. austriaca* × *R. palustris* was successful). The following hybrid combinations were obtained:

Rorippa amphibia × *R. sylvestris* and reciprocal crosses

F1 plants: Perennial herbs, with decumbent to ascending stem, 50–70 cm long, stem branching in the upper part, usually with small cavity, seldom full, covered sparsely or densely with unicelular trichomes; the cauline leaves petiolate, elliptical, gradually narrowing towards the apex of stem, the uppermost almost linear, leaf-lamina unequally pinnatilobate-pinnatipartite to pinnatisect, with dentate segments, cauline leaves glabrous, seldom with trichomes on the mid-vein. Petiole often winged, at the base broadened into lobes or auricles; petals golden yellow, 4.0–5.5 mm long, sepals 3–4 mm long, infructescence elongated, pedicels erecto-patent, 4–10 mm long, pods ellipsoid, straight or slightly curved, inclined towards the axis of inflorescence, 4–8 mm long, 1–2 mm wide, style 0.5–1.5 mm long, seeds reddish brown. Great variability in the morphological characters caused probably by male plant of *R. sylvestris* (see the above note on the parents – *R. sylvestris* no. 20) was noted in the K6-population: one plant with a shortened stem, four plants with cauline leaves mainly entire and only serrate at margin, only the lower ones pinnatilobate, one plant with non-petiolate cauline leaves, remaining five plants fitted the above description.

Table 1. – List of the experimentally produced interspecific hybrid combinations of *Rorippa*. AM – *R. amphibia*, AU – *R. austriaca*, PAL – *R. palustris*, SY – *R. sylvestris*. Year when the respective eperiments were conducted is indicated. Hybrid generation: subscript indicates backcross F1 with parental species. Code of female plant: K8/3, K3/2 etc. indicate F1-population/individual. Germination relates to the percentage of seeds from the primary cross that germinated.

Hybrid	Year	Hybrid generation	Code of female plant	Code of male plant	Number of pollinated flowers	Number of developed pods	Number of seeds	Number of sown seeds	Number of germinated seeds	Number of F1 plants	Code of F1 population	Seed/flower ratio	Seeds per pod	Germination (%)
I. Allogamic species, homoploid level														
AM × SY	1967	F1	6	21	38	0	0	–	–	–				0
		F1	6	17	32	0	0	–	–	–				0
		F1	8	20	24	6[a]	25	25	10	10	K6	–	4.2	40
		F1	7	22	23	23	340	50	4	4	K8	14.8	14.8	8
		F1	21	6	36	35	210	50	1	1	K4	5.8	6.0	2
		F1	17	6	36	34	650	50	7	5	K5	18.1	19.1	14
		F1	20	8	25	24	220	50	1	1	K7	8.8	9.2	2
		F1	22	7	34	32	530	50	0	0	K9	15.6	16.6	0
	1968	$B1_{AM}$	7	K8/3	50	49	b							
		$B1_{SY}$	22	K8/3	61	44	482					9.6	11.0	
		F2	K8/3	K8/3										
	1969	$B1_{AM}$	K8/3	9	21	15	255	50	2			12.1	17.0	
		$B1_{SY}$	K8/3	22	35	23	437	50	5			12.5	19.0	
		F2	K8/3	K8/3				50	33					
	1970	$B1_{AM}$	K8/2	7	44	32[n]	1234					28.1	38.6	
		$B1_{SY}$	K8/2	22	46	26[m]	393					8.5	15.1	
II. Allogamic species, heteroploid level														
AM × AU	1963	F1	AU	AM	?	?	?	?	?	12	K1			
	1967	F1	4	6	38	29	380	50	39	39	K2	10.0	13.1	78
		F1	3	5	37	18	60	50	15	15	K3	1.6	3.3	30
		F1	6	4	44	0	0	0	0	0				0
		F1	5	3	36	30	e							
	1969	$B1_{AU}$	K2/7	3	104		0							
		$B1_{AM}$	K2/7	9	120		0							
		F1 × SY	K2/7	19	193		0							
	1970	$B1_{AU}$	K3/2	3	425		0							
		$B1_{AM}$	K3/2	5	338		106[d6]					0.3		
		F1 × SY	K3/2	18	308		95[d7]					0.3		
AU × SY	1967	F1	3	19	38	28	64	50	16	15	K10	1.7	2.3	32
		F1	1	16	43	40	30[d1]	30	3	2	K12	0.9	0.5	10
		F1	2	22	52	38	21[d2]	21	0	0		0.4	0.6	0
		F1	19	3	39	38	706	50	1	1	K11	18.1	18.6	2
		F1	16	1	33	32	272	50	2	2	K13	8.2	8.5	4
		F1	22	2	31	29	165[d3]	50	2	2	K15	5.6	6.0	4
		F1	23	3	23	23	98	50	3	3	K16	4.3	4.3	6
	1968	$B1_{AU}$	3	K10/6	32		0							
		$B1_{SY}$	19	K10/6	25		0							

Hybrid	Year	Hybrid generation	Code of female plant	Code of male plant	Number of pollinated flowers	Number of developed pods	Number of seeds	Number of sown seeds	Number of germinated seeds	Number of F1 plants	Code of F1 population	Seed/flower ratio	Seeds per pod	Germination (%)
	1969	$B1_{AU}$	K11/1	3	578		10	10	1			0.02		
		$B1_{SY}$	K11/1	19	727		153	153	29			0.21		
		F1 × AM	K11/1	9	459		62	62	7			0.14		
	1970	$B1_{AU}$	K10/9	3	341		20[d4]					0.06		
		$B1_{SY}$	K10/9	18	446		25					0.06		
		F1 × AM	K10/9	7	342		57[d5]					0.20		
III. Autogamic × allogamic species, homoploid level														
AM × PAL	1967	F1	6	11	43	0	0	0	0					
		F1	9	13	22	0	1	1	0			0.05		0
		F1	11	6	35	35	74	50	18	18	K17	2.11	2.11	36
		F1	13	9	45	42	398	50	8	8	K19	8.84	9.48	16
PAL × SY	1967	F1	19	14	32		2	2	0	0		0.06		0
		F1	22	15	26	21	f							
		F1	14	19	38	36	40	40	11	11	K25	1.05	1.11	28
		F1	15	22	34	30	235	50	5	5	K27	6.91	7.83	10
IV. Autogamic × allogamic species, heteroploid level														
AU × PAL	1967	F1	4	12	35	31	50	50	20	7	K20	1.43	1.61	40
		F1	2	10	30	28	20[d8]	20	4	4	K22	0.67	0.71	20
		F1	12	4	35	32	118	50	0	0		3.37	3.69	0
		F1	10	2	37	37	157	50	24	24	K23	4.24	4.24	48

[a] some pods damaged by insects; b – plant withered but seeds seemed sound; [d1] 10 seeds were small; [d2] 20 seeds were small; [d3] 85 seeds were small; [d4] 5 seeds were small; [d5] 10 seeds were small; [d6] 56 seeds were small; [d7] 50 seeds were small; [d8] 5 seeds were small; e – inflorescence destroyed during cultivation; f – small immature seeds, [m] plus 16 underdeveloped pods; [n] plus 12 underdeveloped pods

The reciprocal crosses were mostly successful (Table 1). When the maternal plant was *R. sylvestris,* more seeds but of lower viability were produced than when it was *R. amphibia*. In every case, the amount of seed produced by experimental crossing was lower than by open pollinated parental species. The failure of some experimental hybrid combinations, as illustrated by 22 × 7, was due to the poor growth of the maternal plant, which made the development of viable seed impossible (see the above note on the parents – *R. sylvestris*, no. 22). In addition, when plant no. 6 (*R. amphibia*), affected by female sterility (see the note on parental species – *R. amphibia*), was used, the reciprocal hybrid combinations were not equivalent. No seeds were harvested from the 6 × 21 and 6 × 17 crosses, but the reciprocal 21 × 6 and 17 × 6 crosses (mother plants *R. sylvestris*) produced seed, which developed into F1 plants which were, like the paternal plant, female sterile.

F1 (2n = 32) was fully fertile when open pollinated. Backcrossing and F1 selfing gave results comparable to those obtained when the parental species were crossed; the amount of seed produced was usually less than after free pollination. A break of incompatibility was noted in the hybrids of K8-population (Table 1), which were nearly fully fertile even when selfed, unlike the allogamic parental species. Back-crossed and F2 generation plants were tetraploid and not studied further.

Rorippa amphibia × *R. austriaca* and reciprocal crosses

F1 plants: Perennial herbs, stem 40–60 cm long, slightly ascending or erect, in the upper part branched, hollow, with small cavity, the main stem covered sporadically with trichomes, the lateral branches densely hirsute; cauline leaves sessile, the lower with wide auricles, which gradually change into lobes on the upper leaves, cauline leaves entire, lanceolate to lanceolate-elliptical, the uppermost linear, unequally obtuse dentate, sometimes sinuate at the margin, both sides of leaves with small simple trichomes; petals golden yellow, 3.5–5.5 mm long, sepals 2.5–3.5 mm long, infructescence slightly elongated, pedicels ascending, 7–11 mm long, pods rudimentary, ellipsoidal-spheroid in the direction of pedicel, 1.0–2.5 mm long, 0.5–1.5 mm wide, style 1.1–1.5 mm long, seeds reddish brown.

This cross probably occurs reciprocally (Table 1) but seed was only obtained from crosses in which *R. austriaca* was the maternal plant. The well developed generative part of the 5 × 3 hybrid combination was destroyed during cultivation and no seeds were produced by the 6 × 4 hybrid combination, of which the female plant was *R. amphibia* (see note on the parents – *R. amphibia*). The fertility of the F1 (2n = 24) plants was conspicuously lower, with only a few seeds produced per pod by open pollinated plants; autogamy was not attempted. In backcross and three-way crosses with *R. sylvestris* F1 plants were used as females. These crosses failed in the K2-population, probably due not only a cytological barrier but also female sterility, an attribute originated from the paternal parent (cf. *R. amphibia* × *R. sylvestris*). In the second F1 population of K3, a small amount of seed was obtained only if the male parent had a higher ploidy level, as is the case in *R. amphibia* or *R. sylvestris*. No seed was available from the experimental backcross in 1969 so no experimental B1-populations were raised. Fortunately offspring from the free pollination of K1-progeny (origin of K1 in Appendix 1) was available. Initially in 1967 there were 30 plants of very different habits, which later decreased in number by natural selection, with after two years only 10 plants surviving, of which three were tetraploids (2n = 32) with relatively highly viable pollen fertility (Table 6). On the basis of the outcome of the experimental back and three-way crosses with both *R. amphibia* × *R. austriaca* and *R. austriaca* × *R. sylvestris*, it is assumed that the offspring of K1-progeny that were open pollinated had either *R. amphibia* or *R. sylvestris* as the male parent, and not *R. austriaca*.

Rorippa austriaca × *R. sylvestris* and reciprocal crosses

F1 plants: Perennial herbs, stem 25–55 cm long, erect, decumbent when fruiting, richly branched in the lower as well as the upper part, solid, covered with small unicellular trichomes; cauline leaves petiolate, leaf-lamina lanceolate or elliptical, usually unequally pinnatilobate-pinnatipartite-pinnatisect, seldom undivided only dentate, leaf segments unequally serrate, petiole of upper leaves sometimes winged, auricles not always developed, the leaves on both sides either sparsely or densely hirsute; petals yellow to golden yellow, 3–5 mm long, sepals 2.0–3.5 mm long, infructescence elongated, pedicels ascending, 2.5–8.0 mm long, pods rudimentary, seldom with one or two seeds, pointing in the direction of pedicel or slightly bent towards the stem, 2.0–4.5 mm long, 0.5–1.5 mm wide, style 1.0 mm long, seeds reddish brown. The progeny of the 3 × 19 cross (K10-population) were numerous and showed higher variability in the shape of the cauline leaves and density of trichomes; habit of some plants was close to that of *R. austriaca* but most plants were similar to *R. sylvestris*.

The cross was successful in both directions (Table 1). More seed was obtained if the maternal plant was *R. sylvestris* (larger pods), but it was less viable. The F1 generation (2n = 24) was characterized by its lower fertility, with seeds only seldom developing when open pollinated. After forced autogamy no seeds developed due mainly to the large amount of aborted microspores. Reciprocal back crosses and crossbreeding with *R. amphibia* did not result in similar amounts of harvested seed. If maternal plant was a pure taxon, no matter if diploid or tetraploid, and the paternal plant an F1 hybrid, no seeds developed due to largely aborted pollen; in the reverse cross (female plant an F1 hybrid), the hybridization with tetraploids was more successful. Controlled backcrossing of an experimental triploid hybrid (*R. sylvestris* × *R. austriaca*) with a diploid species (K11 × *R. austriaca*) yielded much less seed than the backcross and three-way crosses with a tetraploid species (K 11 × *R. amphibia* or *R. sylvestris*). The backcross of K11-progeny with *R. austriaca* resulted in only one plant with chromosome number 2n = 21, which died in early vegetative stage. The offspring of K11-progeny, from the backcross and three-way crosses with tetraploids, had chromosome numbers approaching the tetraploid level (Table 5). Pollen fertility in these plants was very variable (1–45%) but independent of chromosome number. Naturally the chromosome number does not reveal the origin of the chromosomes in the chromosome pool of a hybrid; plants with the same chromosome number can be cytogenetically heterogeneous, which surely reflects the course of meiosis resulting in the variation in pollen quality between individuals.

The chromosome numbers and quality of pollen were recorded for several plants obtained by sowing of seed collected in the field from natural hybrids [V20: 2n = 32–50% of pollen grains stained, V21: 2n = 32–71% of pollen grains stained, V70: 2n = 32–62% of pollen grains stained and V54: 2n = unknown – on average 64% of pollen grains stained (data from 15 plants, range from 8 to 86%) – data for V54 come from files of P. Tomšovic]. Somewhere between 20 and 80% of the pollen of most of these plants stains, which is the range characteristic of hybrid plants with an unbalanced karyotype.

Hybrid combinations with *R. palustris*

If the maternal plant was *R. palustris*, then crosses with the allogamic species *R. amphibia*, *R. austriaca* or *R. sylvestris* resulted in a large or small amount of seed (Table 1), which was identical in size, colour and surface structure with that of *R. palustris*. The plants that grew from these seeds were impossible to distinguish from *R. palustris* in morphology and chromosome number (2n = 32) even when examining crosses at the heteroploid level (*R. palustris* × *R. austriaca*). The plants from seeds of the above hybrid combinations were not real F1 hybrids, but pure *R. palustris*. The only exception to a chromosome number of 2n = 44 was found in one individual among the progeny of the hybrid combination 14 × 19 (K25-population, *R. palustris* × *R. sylvestris*). About 10% of pollen grains were stainable but very irregular in shape and on undergoing autogamy this plant was slightly fertile. Two plants resulting from free pollination of this individual had fewer chromosomes with 2n = 38 (of which two chromosomes were very small) and 37–39.

If the maternal plant was *R. amphibia* or *R. sylvestris*, either no seed developed or they were very small, undeveloped and mostly incapable of germinating. When the maternal plant was *R. austriaca*, fewer seeds were obtained, which gave rise to an F1 generation with a chromosome number of 2n = 24.

Rorippa austrica × *R. palustris*

F1 plants: Annual or biennial herbs, stem 60–80 cm long, erect, lignified at the base, hollow with a small cavity, densely hirsute, richly branched in the lower as well as the upper part; the lower cauline leaves with winged petioles, the upper ones sessile, with narrow base and conspicuous round auricles, parted cauline leaves elliptical to lanceolate, the uppermost nearly linear, the lower pinnatisect, upwards gradually pinnatipartite, pinnatilobate, the uppermost entire, leaf segments unequally dentate, both sides of leaves densely hirsute; petals yellow, 2.5–3.5 mm long, sepals 2.0–2.5 mm long, infructescence elongated, pedicels ascending, 5.0–7.5 mm long, pods rudimentary, ellipsoid, in the direction of pedicels, 1–2 mm long, 0.5–1.5 mm wide, style 0.5 mm long, no seeds develop.

It seems that the sterility of the triploid F1 generation is total; no seeds developed, not even under free pollination. These luxuriant hybrids are unknown in the field but survived for two years in the experimental plot.

Microsporogenesis in the parental species

Meiosis in the diploid *R. austriaca* proceeds normally, eight bivalents form at first metaphase, their disjunction at first anaphase is regular, there are four cells in the pollen tetrads, and the number of well developed pollen grains does not fall below 80%. In tetraploid species meiosis is also normal, at first metaphase, 16 bivalents form and there are four cells in the tetrads. Several irregularities were found in a few cases. In *R. amphibia*, in one PMC two univalents and 15 bivalents formed, at first anaphase rarely 1–2 laggards were observed. In *R. sylvestris*, laggards are more common, but still few in number. Certain anomalies in the development of the pollen tetrads were observed in *R. sylvestris*, in no. 23, it was the frequent occurrence of tetrads with two or three cells in the anthers of some buds, and in no. 21, about 1% of the tetrads had four large and one small cell. Both of these plants (seemingly pure *R. sylvestris*) originate from Modřany. Their hidden hybrid origin was revealed by segregation of morphological characters in the offsprings that resulted from autogamy and from *R. amphibia* × *R. sylvestris* crosss. The quantity of well developed pollen usually does not fall below 80% (Table 4), slightly lower values were found in some plants of *R. amphibia* from Křenek (up to ca 60%) but these plants were different from the plant no. 6 used for crossing experiments. The highest variability, comparable to that in morphological characters, is in *R. sylvestris*, where there are large differences both among populations and individuals in a population. The largest quantity of aborted pollen was observed in plants from the ruderal site in Modřany, where the number of the well developed microspores in single plants was: no. 20: 60%, no. 21: 43% and no. 23 only 29%; the value for no. 23 is not included in Table 4.

Microsporogenesis in F1 hybrids

Tetraploid hybrid combination: *R. amphibia* × *R. sylvestris* and reciprocal crosses

Sixteen bivalents form at first metaphase, irregularities (two univalents) in the pairing of the homologous chromosomes rarely occur, the disjunction of the bivalents at first anaphase is regular, rarely with 1–2 laggards. There are usually four cells in the pollen tetrads (Table 3) and more than 80% of the pollen grains stain (Table 4). The K6-progeny were more variable with one plant with up to 13% of its tetrads with four normal and one small cell and irregular sized pollen of which only 30% stained. In one plant of the K5-progeny more than one third of the pollen tetrads did not develop, even though the chromosomes paired more or less normally and only 5% of the pollen grains were well developed. Three plants had only 60–70% well developed microspores. In both cases sporogenesis in one of the parents was slightly abnormal. The male parent (*R. sylvestris* no. 20, Modřany locality) of K6-progeny produced low grade pollen and only 60% of their microspores were morphologically well developed. The male parent of the K5-progeny was *R. amphibia* no. 6 (Křenek locality) from a population characterized by female sterility and low quality pollen. Both plants, in which 30% and 5% of their pollen grains stained, are not included in Table 4. The quality of the pollen of spontaneously developing offspring of the natural hybrids was evaluated in two cases: in sample V14 there were two plants in which 50 and 51% and in sample V61, one plant 95% of the pollen grains stained.

Triploid hybrid combinations: *R. amphibia* × *R. austriaca* and reciprocal crosses

In the heterotypical metaphase 2–9 univalents, 2–8 bivalents and 1–3 trivalents (Table 2) formed, the three most frequent configurations of chromosomes at first metaphase are $5_{I} + 5_{II} + 3_{III}$ (31%), $6_{I} + 6_{II} + 2_{III}$ (17%), $4_{I} + 4_{II} + 4_{III}$ (12%), the number of laggards at first anaphase varied from 0 to 6. Even though most pollen tetrads had four cells (86%) (Table 3), the pollen grains were mainly degenerate with less than 20% well developed with stainable cytoplasm (Table 4). In one plant of the K2-progeny, the anthers aborted.

Triploid hybrid combinations: *R. austriaca* × *R. sylvestris* and reciprocal crosses

At first metaphase 3–8 univalents, 4–9 bivalents, 0–4 trivalents were formed (Table 2) and the three most often occurring chromosome configurations were $5_{I} + 5_{II} + 3_{III}$ (24%), $4_{I} + 4_{II} + 4_{III}$ (24%), $8_{I} + 5_{II} + 2_{III}$ (14%), at first anaphase chromosome bridges were seen and the number of laggards was 2–8. At second metaphase in one plant of the K10-progeny the following chromosome numbers were recorded: 9 and 14 once, 11 twice, and 12 and 13 thrice. Besides the groups of chromosomes on the equator there were 1–5 chromosomes randomly scattered in the cytoplasm. Pollen tetrads containing four cells prevailed (70%) (Table 3) and 17% contained four normals cells and one small cell. Only a small percentage (up to 10%) of the pollen grains stained (Table 4) and they were often of irregular shape and variable in size. In three individuals among the K10-progeny gametogenesis was disrupted to such extent that the pollen grains did not develop.

Triploid hybrid combinations: *R. austriaca* × *R. palustris*

In contrast to the triploids mentioned above, meiosis in *R. austriaca* × *R. palustris* prevented the development of fertile microspores. Chromosome pairing at first metaphase was very irregular, multivalents often formed (Table 2), 0–7 laggards were recorded at first anaphase and 72% of the pollen tetrads had four cells (Table 3). The pollen grains were generally aborted and only a few irregular sized pollen grains stained, always less than 1% (Table 4).

Table 2. – Configurations at first metaphase of PMC in triploid hybrids. AM – *Rorippa amphibia*, AU – *R. austriaca*, PAL – *R. palustris*, SY – *R. sylvestris*. Number of individual elements configured in a single PMC; I – univalent, II – bivalent, III – trivalent, IV – tetravalent, V – pentavalent.

Configuration			AM × AU		AU × SY		Configuration					AU × PAL	
I	II	III	No. of PMCs	%	No. of PMCs	%	I	II	III	IV	V	No. of PMCs	%
5	2	5	2	3			2	2	6	–	–	3	14.0
2	2	6	2	3			2	3	1	2	1	1	4.5
9	3	3	1	2			3	3	1	3	–	1	4.5
3	3	5	3	5			3	3	2	1	1	1	4.5
7	4	3	2	3	1	5	5	4	2	–	1	1	4.5
4	4	4	7	12	5	24	1	4	2	1	1	2	9.1
8	5	2	2	3	3	14	2	4	2	2	–	2	9.0
5	5	3	18	31	5	24	3	4	3	1	–	2	9.0
6	6	2	10	17	1	5	4	4	4	–	–	1	4.5
3	6	3	3	5	1	5	6	5	1	–	1	1	4.5
4	7	2	4	7	2	10	2	5	1	1	1	1	4.5
7	7	1			2	10	3	5	1	2	–	1	4.5
5	8	1	3	5			4	5	2	1	–	1	4.5
2	8	2	1	2			4	6	–	2	–	1	4.5
6	9	–			1	5	1	6	1	2	–	1	4.5
							3	6	3	–	–	1	4.5
							4	7	2	–	–	1	4.5
Total			58	98	21	102						22	99.5

Table 3. – Cell number in pollen tetrads. AM – *Rorippa amphibia*, AU – *R. austriaca*, PAL – *R. palustris*, SY – *R. sylvestris*. Percentage of the total number of observed tetrads, accounted for by individual combinations of large and small cells, is given for each hybrid combination.

Number of large + small cells in tetrads	AM × SY		AM × AU		AU × SY		AU × PAL	
	No. of tetrads	%	No. of tetrads	%	No. of tetrads	%	No. of tetrads	%
2+0	1	0.1	11	1.8	22	3.1	5	1.2
2+1			4	0.7	4	0.6	2	0.5
2+2	1	0.1			1	0.1	1	0.2
2+3			1	0.2				
3+0	1	0.1	4	0.7	4	0.6	2	0.5
3+1	1	0.1	1	0.2	1	0.1	2	0.5
3+2			3	0.5				
3+3			1	0.2				
3+4			1	0.2				
4+0	980	98.0	517	86.2	493	70.4	292	73.0
4+1	15	1.5	46	7.4	122	17.4	79	19.8
4+2	1	0.1	8	1.3	42	7.0	14	3.5
4+3			2	3.3	5	0.7	2	0.5
4+4					2	0.3		
5+0			1	0.2	4	0.6	1	0.2
Total	1000		600		700		400	

Table 4. Pollen quality of parents and F1 expressed as a percentage of pollen grains that stained. AM – *Rorippa amphibia*, AU – *R. austriaca*, PAL – *R. palustris*, SY – *R. sylvestris*. See Table 1 for population codes.

Species	Code of parents or F1 hybrid populations	Number of plants	Percentage of stained pollen grains		
			Range	Mean	Female + male parent
AU	1, 2, 3, 4	4	82–98	87	
AM	5, 6, 7, 8, 9	5	78–95	88	
PAL	10, 11, 12, 13, 14, 15	6	86–99	94	
SY	16, 17, 18, 19, 20, 21, 22, 23	7	43–93	77	
AM × SY	K6 = 8 × 20	9	87–92	90	88+60
	K8 = 7 × 22	4	81–89	86	80+92
	K5 = 17 × 6	4	64–83	70	85 + 78
	K7 = 20 × 8	1	90	90	60+88
AU × AM	K1	1	1	1	?
	K2 = 4 × 6	1	0	0	98+78
	K3 = 3 × 5	4	2–18	8	82+90
AU × SY	K10 = 3 × 19	11	0–10	3	82+88
	K12 = 1 × 16	3	5–6	6	86+93
	K13 = 16 × 1	2	1–2	2	93+86
	K15 = 22 × 2	1	3	3	92+83
AU× PAL	K20 = 4 × 12	7	0–3	1	98+98
	K22 = 2 × 10	4	0–2	1	83+88
PAL × SY (2n = 44)	K25 = 14 × 19	1	10	10	99+88

Discussion

Autogamy/allogamy separated *R. palustris* from *R. austriaca*, *R. amphibia* and *R. sylvestris*. Self-incompatibility is incomplete in these three allogamic species, partial selfing occurred in *R. amphibia*, but was rare in diploid *R. austriaca*. *Rorippa sylvestris*, a mostly vegetatively reproducing species, showed variability in its mode of mating, both when open pollinated and selfed. It often happens that vegetatively propagated populations of *R. sylvestris* arising from one clone are sterile due to the absence of convenient gametes and/or disorders in gametogenesis. Intensive study of the S-locus in two wild crucifer species, *Brasicca campestris* and *Arabidopsis lyrata*, indicates that partial self-compatibility is attributable to the weakening of SI in some S-alleles or S-allele combinations (Mable et al. 2003). Furthermore, the ability of the stigma to reject its own pollen is regulated developmentally and is manifested only in mature buds and young flowers, in which the amount of SRK is at the maximum level. The amount of SRK decreases in old flowers, results in the weakening of SI (Nasrallah et al. 2002). We can expect something similar to SSI in the genus *Rorippa*.

The hybridization experiments showed that all three allogamic species can be crossed to produce hybrids, which blurs the borders between taxa. Two small experimental "hybrid swarms" developed from the seeds sown in 1969, one from crossing *R. amphibia* × *R. sylvestris* and the other from crossing *R. austriaca* × *R. sylvestris*. A third "hybrid swarm" resulting from crossing *R. amphibia* × *R. austriaca* consisted of offspring that resulted from free pollination of F1 individuals (K1 – *R. amphibia* × *R. austriaca*), because no seed was harvested from the experimental crossings in 1969.

Table 5. – Chromosome numbers and percentage of pollen grains that stained in back crosses and three-way crosses of *Rorippa austriaca* × *R. sylvestris*. AM – *R. amphibia*, AU – *R. austriaca*, PAL – *R. palustris*, SY – *R. sylvestris*. See Table 1 for sample codes.

Plant no.	2n	Estimated chromosome no.	% of stained pollen grains
(SY × AU) × AU – samples K11/1 × 3			
1	21	13	–[1]
(SY × AU) × AM[2] – samples K11/1 × 9			
1	28	12	25
2	29	13	17
3	28	12	35
4	29	13	28
5	31	15	48
(SY × AU) × SY[3] – samples K11/1 × 19			
1	29	13	
2	28	12	25
3	ca 31	15	
4	30	14	17
5	30	14	44
7	29	13	
8	28	14	1
9	31	15	4
10	31	15	7
12	ca 28	12	
13	30–32	14–16	
14	ca 30	14	45
16	31	15	
17	28	12	12
18	(28)–30	(12)–14	
19	30	14	21
20	31	15	25
29			4

[1] died in vegetative stage
[2] range of 2n: 28–31; % of stained pollen grains: mean 30.6, range 17–78
[3] range of 2n: 28–31; % of stained pollen grains: mean 18.6, range 1–45

Table 6 – Chromosome numbers and percentage of pollen grains that stained of the progeny (labelled V99) of open pollinated experimental F1 hybrids *Rorippa amphibia* × *R. austriaca* (K1 population).

Individual	1	2	3	4	5	6	7	8	9	10
2n	32	32	29	32	30	(29)–30	28		29	30
% of stained pollen grains	76	84	0	65	51	51	18	54	24	57

Further evidence of the ability of both allogamic tetraploid species (*R. amphibia* and *R. sylvestris*) to form hybrid swarms was provided by the hybridization tests carried out by Jonsell (1968) on the same hybrid combinations, which had a similar outcome. The genetic barrier between them is very weak and probably the same as that between individuals within populations of a species and the two species are isolated by phytogeographical and mainly ecological barriers. Worthy of mention is the breakdown of SI in the nearly self-compatible F1 progeny of K8, which resulted from the genetic interaction between *R. amphibia* and *R. sylvestris* S-haplotypes. The shift from outcrossing to self-fertilization is caused by inactivation of one or both self-recognition genes (SRK and SCR) involved in the mating system (Nasrallah 2002). In the self-incompatibility response, SCR interacts with its cognate SRK, and starts signalling pathways resulting in self-pollen rejection; any defect in this process results in the failure of SI. It is possible that pollen of the recessive genotype may elude the S haplotype-specific stigmatic surveillance mediated by SRK and the subsequent binding of the SCR-SRK does not occur and SI fails.

Using noncoding chloroplast DNA and isozyme analyses, hybridization and bidirectional introgression between *R. amphibia* and *R. sylvestris* were detected in plants growing along the river Elbe in Germany. Several plants of *R.* × *anceps* from the same area were shown to be highly polymorphic hybrids of the putative parents *R. amphibia* and *R. sylvestris* (Bleeker & Hurka 2001).

Primary triploid hybrids are rarely found among plants in sympatric populations of allogamic species (Javůrková-Kratochvílová & Tomšovic 1972). For most hybrids a tetraploid chromosome number 2n = 32 is reported (Jonsell 1968, Javůrková-Kratochvílová & Tomšovic 1972), even though the hybrids showed undoubted vestiges of introgression of genes from *R. austriaca* (2n = 16). There are several possible explanations of this phenomenon. Based on observations and experiments, Jonsell (1968) suggests three possible origins of the hybrid combination *R. austriaca* × *R. sylvestris*. They are: hybridization of diploid *R. austriaca* with hexaploid *R. sylvestris*; fusion of a non-reduced gamete from a diploid *R. austriaca* with tetraploid *R. sylvestris*; and finally the occurrence and crossing of a tetraploid cytotype of *R. austriaca* with the tetraploid *R. sylvestris*. Jonsell (1968) suggests the first is unlikely because he failed to produce the hybrid experimentally and the morphology of natural hybrids between *R. austriaca* × *R. sylvestris* does not support this suggestion. For Central Europe, where the hexaploid *R. sylvestris* has a sporadic occurrence (Jarolímová 1998), it is not possible. The second and third possible origins are speculative. Jonsell's finding that plants of the hexaploid *R. sylvestris* can produce a small amount of giant pentacolpate pollen supports the suggestion that non-reduced gametes can occur in the genus *Rorippa*. Nevertheless the formation of non-reduced gametes is likely to be an anomalous event and nothing indicates that it occurs in diploid *R. austriaca*, in which microsporogenesis is almost without irregularities. As in *R. amphibia* or *R. sylvestris*, the existence of another ploidy level in *R. austriaca* is likely and the existence of a tetraploid taxon would be the simplest explanation for the occurrence of the tetraploid hybrids with *R. austriaca*. However, no tetraploid cytotype of *R. austriaca* has been recorded, not even where hybrid swarms with *R. austriaca* occur in Central Europe. It is therefore necessary to look for the origin of natural hybrid swarms *R. amphibia* × *R. austriaca* and *R. austriaca* × *R. sylvestris*, consisting of mainly tetraploid and fertile hybrid plants, in the primary triploid hybrids and their offspring.

From the experimental crossing of heteroploid allogamic species, a triploid generation arises in which the cytological barrier is very vigorously manifested, nevertheless these triploids produce a small amount of seed. Pollen fertility of triploid hybrid plants was so poor that they were probably only rarely the male parent. Thus triploids serve mainly as females and the crossing occurs only in those natural populations, in which orthoploid plants with good pollen quality dominate. The offspring from consecutive crosses, beginning with tetraploids, have aneuploid chromosome numbers tending to tetraploid and usually better pollen quality than the parental triploids. Among the offspring ("F2") of the experimental triploid *R. amphibia* × *R. austriaca*, which resulted from free pollination followed by natural selection, there were vital tetraploid plants with high quality pollen. This demonstrates that the most likely origin of hybrid swarms are heteroploid parents giving rise to tetraploid hybrid swarms: but this is dependent on the initial development of triploid plants. If there are enough tetraploid male plants, then nearly or even fully tetraploid individuals will start to appear in the next hybrid generation. Subsequently after backcrossing with tetraploid parents the number of tetraploids increase, undoubtedly some of them will produce some high quality pollen. These plants will be able to produce tetraploid hybrids, which are the basis of tetraploid hybrid swarms in to which *R. austriaca* genes spread in the absence of tetraploid *R. austriaca*.

The most vigorous of the above combinations are hybrid swarms of *R. austriaca* × *R. sylvestris*, in which the F1 are more vigorous than those of *R. austriaca* × *R. amphibia*. A combination of features of both parents, such as the vegetative propagation of *R. sylvestris*, efficient root system of *R. austriaca* and their ecological requirements favour offspring of this hybrid combination. Also the flexible genome of *R. sylvestris* affects the viability of hybrids with this species, which in spite of a slightly abnormal meiosis due to an imbalance in the chromosome set are able to produce some viable gametes. The occurrence of the aneuploid hybrid plants in the field has not been confirmed, probably because there are very few and they are less viable (cf. Jonsell 1968) compared to the triploids, which are sterile. At the heteroploid level (diploid × tetraploid) Jonsell (1968) attempted unsuccessfully to experimentally cross diploid *R. austriaca* with tetraploid *R. sylvestris*. The failure of the *R. austriaca* × *R. sylvestris* crosses could be due to the low fertility of *R. austriaca* caused by the nature of the environment of the secondary habitats in N Europe. The results of the experimental hybridization of species of Central European yellow cress support Jonsell's (1968) finding for the same hybrid combinations of allogamic species. The differences are only in the amount of seed produced, which depends on the type and number of the S-alleles present in the parents. AFLP analysis provided evidence of bidirectional introgression (unidirectional using chloroplast DNA: *R. austriaca* into *R. sylvestris*) between *R. austriaca* and *R. sylvestris*, which is not in accord with the results obtained in the crossing experiments, which indicate introgression of *R. austriaca* into *R. sylvestris*. Introgression of tetraploid *R. sylvestris* into diploid *R. austriaca* could occur by multiple repeated backcrossing of the primary triploid hybrids or their offspring with diploid *R. austriaca*, resulting in fully fertile diploid *R. austriaca*. It is very difficult to imagine *R. austriaca* with such a rebuilt diploid chromosome pool.

Interspecific hybridization between autogamic *R. palustris* and any of the allogamic species nearly always failed (only *R. austriaca* × *R. palustris* was successful). If *R. palustris* was pollinated by any allogamic species, none true hybrid was obtained. The birth of offspring identical to *R. palustris* indicates that the prevention of self-pollination was only partially successful or apogamy took place. If *R. palustris* was used as the male and the maternal plants were the tetraploid allogamic species (*R. amphibia*, *R. sylvestris*) none offspring were produced, only crossing at the heteroploid level (diploid female *R. austriaca* with tetraploid male *R. palustris*) resulted in triploid offspring. Low compatibility of the genotypes of the autogamic and allogamic species of *Rorippa* was confirmed by the failure of most experimental hybrid combinations and/or the development of multivalents in the course of meiosis and the subsequent total sterility of the triploid hybrid (*R. austriaca* × *R. palustris*). With regard to the crossability of autogamic and allogamic species, Jonsell (1968) reports similar results. He obtained no hybrids from experimental crosses at the heteroploid level (*R. amphibia* 2n = 16 × *R. palustris* – 3 crossing combinations) but was successful at the tetraploid level using three crosses of *R. amphibia* (2n = 32) × *R. palustris*. In plants from one of the crosses he observed laggards at first anaphase (he does not mention first metaphase), n = 16 at second metaphase and only 15% of the pollen grains stained. In plants of another cross it was even lower, only 3%. Other hybrid combinations were unsuccessful as either the seeds did not germinate [natural hybrid (*R. amphibia* × *sylvestris*) × *R. palustris* – 2 crosses] or development ceased at an early stage or germinated but resulting plant died (*R. sylvestris* × *R. palustris* – 9 crosses). Natural hybrids were also recorded. Howard (1947) in Great Britain noted the occurrence of the triploid perennial hybrid *R. amphibia* × *R. palustris*, which propagated vegetatively and produced aborted pollen and no seeds. Bleeker & Hurka (2001) reported one triploid individual *R.* × *erythrocaulis* (= *R. amphibia* × *R. palustris*) raising from seed collected from a diploid *R. amphibia* population near Cologne. Isozyme analysis of this triploid showed an additive allozyme banding pattern of *R. palustris* and of diploid *R. amphibia*. At the tetraploid level, unidirectional introgression of *R. palustris* into *R. amphibia* was confirmed by using cpDNA and isozyme analyses of several plants growing along drainage ditches in NW Germany (Bleeker & Hurka 2001). Implicit in the above is that the mutual relationship between the genotypes of *R. palustris* and *R. amphibia* is much greater than between those of *R. palustris* and *R. sylvestris.* Undoubtedly hybridization of autogamic *R. palustris* and allogamic species occurs less frequently than hybridization among allogamic species and if it happens *R. palustris* is always the male parent. Progeny of crosses at the heteroploid level (diploid × tetraploid) is almost certainly restricted to F1, introgression of *R. palustris* into allogamic *Rorippa* populations occurs via crosses at the homoploid level. Introgressive hybridization of tetraploids was confirmed both experimentally and by molecular methods.

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Souhrn

Jednou z častých příčin potíží při taxonomickém vymezení středoevropských druhů rodu *Rorippa* je předpokládaná interspecifická hybridizace jejímž výsledkem je vytváření hybridních rojů v oblastech společného výskytu. Schopnost resp. neschopnost mezidruhové hybridizace byla ověřena hybridizačními pokusy se čtyřmi druhy rodu *Rorippa* vyskytujícími se na území České a Slovenské republiky. Do pokusů byly zahrnuty dva alogamické tetraploidní druhy *R. amphibia* a *R. sylvestris*, jeden alogamický diploidní druh *R. austriaca* a jeden autogamický tetraploidní druh *R. palustris*. Výsledky mezidruhové hybridizace jsou následující:

Rorippa amphibia × *R. sylvestris*: Plně plodná tetraploidní F1 s pravidelnou meiozí a dobrou kvalitou mikrospor, dala vznik F2 generaci a zpětným křížencům s oběma rodiči. Schopnost obou druhů vyvářet hybridní roje v sympatrických populacích tím byla potvrzena, slabá genetická bariéra mezi druhy je patrně stejná jako mezi jedinci téhož druhu uvnitř populace.

Rorippa amphibia × *R. austriaca* a *R. sylvestris* × *R. austriaca*: Experimentálně získaní primární hybridi jsou triploidní s nepravidelným průběhem meioze, špatnou kvalitou pylových zrn a razantně sníženou plodností. Kontrolovaným sprášením F1 s tetraploidními *R. amphibia* a *R. sylvestris* vzniká potomstvo s chromozomovým počtem blížícím se k tetraploidní úrovni (lépe se uplatňují EMC s vyšším počtem chromozomů) a se zvyšující se kvalitou mikrospor. V potomstvu z volného sprášení F1 byly zaznamenány dokonce i rostliny s tetraploidním po-

čtem chromozomů a dobrou kvalitou pylových zrn. Takovéto hybridní rostliny se mohou křížit s dalšími tetraploidy a jsou patrně rozhodující pro vytváření tetraploidních hybridních rojů s introgresí *R. austriaca* nalézaných v přírodě; jejich vznik tedy není podmíněn výskytem tetraploidního cytotypu *R. austriaca*.

Rorippa palustris × *R. amphibia*, *R. palustris* × *R. sylvestris* a *R. palustris* × *R. austriaca*: Hybridizační pokusy prokázaly jen omezenou schopnost mezidruhové hybridizace autogamní *R. palustris* s alogamními druhy způsobenou zřejmě malou slučitelností genotypů obou skupin. Výsledky reciprokého křížení se liší. Je-li mateřským individuem *R. palustris*, vzniká potomstvo totožné s *R. palustris* morfologicky i karyologicky, tedy nikoli F1. Pokud byli mateřskými rostlinami alogamní tetraploidi, hybridizace nebyla úspěšná. Tam, kde byla mateřskou rostlinou *R. austriaca*, vznikli triploidní luxuriantní F1 hybridi s výrazně narušenou meiozí a naprosto sterilní. Výsledky hybridizačních pokusů nasvědčují tomu, že *R. palustris* má na vzniku přirozených hybridních rojů patrně jen malý podíl, a to jako samčí rodič; triploidní hybridní jedinci jsou omezeni asi jen na Fl generaci.

Na způsob dědičnosti některých morfologických znaků typických pro jednotlivé taxony bylo možno usuzovat z habitu experimentálních hybridních rostlin. Intermediární charakter mají dutost/plnost a křehkost/tuhost lodyhy, délka korunních plátků a délka plodů, dominantní je výskyt drobných jednoduchých trichomů na lodyze a lodyžních listech (typické pro *R. austriaca*), dominantní je rovněž řapíkatost a dělenost lodyžních listů (typické pro *R. sylvestris*) a vytváření oušek (typické pro *R. austriaca*) nebo laloků (typické pro *R. amphibia*) na bázi lodyžních listů.

References

Bleeker W. (2003): Hybridization and *Rorippa austriaca* (*Brassicaceae*) invasion in Germany. – Mol. Ecol. 12: 1831–1841.

Bleeker W. & Hurka H. (2001): Introgressive hybridization in *Rorippa* (*Brassicaceae*): gene flow and its consequences in natural and anthropogenic habitats. – Mol. Ecol. 10: 2013–2022.

Howard H. V. (1947): Chromosome numbers of British species of the genus *Rorippa* Scop. (part of the genus *Nasturtium* R. Br.). – Nature 159: 66–67.

Javůrková-Kratochvílová V. & Tomšovic P. (1972): Chromosome study of the genus *Rorippa* Scop. em. Reichenb. in Czechoslovakia. – Preslia 44: 140–156.

Jarolímová V. (1998): Chromosome counts of *Rorippa sylvestris* in the Czech Republic. – Preslia 70: 69–71.

Jonsell B. (1968): Studies in the North-West European species of *Rorippa* s. str. – Symb. Bot. Uppsal. 19/2: 1–222.

Kusaba M., Dwyer K., Hendershot J., Vrebalov J., Nasrallah J. B. & Nasrallah M. E. (2001): Self-incompatibility in the genus *Arabidopsis*: characterization of the S locus in the outcrossing *A. lyrata* and its autogamous relative *A. thaliana*. – Plant Cell 13: 627–643.

Mable K. B., Schierup M. H. & Charlesworth D. (2003): Estimating the number, frequency, and dominance of S-alleles in a natural population of *Arabidopsis lyrata* (*Brassicaceae*) with sporophytic control of self-incompatibility. – Heredity 90: 422–431.

Marks G. E. (1954): An acetocarmine glycerol jell for use in pollen fertility counts. – Stain Technol. 29: 277.

Nasrallah J. B. (2002): Recognition and rejection of self in plant reproduction. – Science 296: 305–308.

Nasrallah M. E., Liu P. & Nasrallah J. B. (2002): Generation of self-incompatible *Arabidopsis thaliana* by transfer of two S locus genes from *A. lyrata*. – Science 297: 247–249.

Sybenga J. (1975): Meiotic configurations. – Springer-Verlag, Berlin etc. [251 pp.]

Tomšovic P. (1965): Taxonomický rozbor československých druhů rodu *Rorippa* Scop. em. Reichenb. – Thesis, Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice.

Tomšovic P. (1969): Nejdůležitější výsledky revize československých rukví (*Rorippa* Scop. em. Reichenbach). – Preslia 41: 21–38.

Tomšovic P. (1992): *Rorippa* Scop. – rukev. – In: Hejný S. & Slavík B. (eds), Květena České republiky 3: 76–86, Academia, Praha.

Tomšovic P. & Goliašová K. (2002): *Rorippa* Scop. – In: Goliašová K. & Šípošová H. (eds.), Flóra Slovenska V/4: 280–308, Veda, Bratislava.

Watanabe M., Takayama S., Isogai A. & Hinata K. (2003): Recent progresses on self-incompatibility research in *Brassica* species. – Breeding Science 53: 199–208.

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Appendix 1. – List of plants used for crossbreeding. * chromosome numbers are published in Javůrková-Kratochvílová & Tomšovic (1972). Label of cultivation: T – plants transplanted from the field to experimental plots, V – plants raised from seeds collected in the field, A – plants produced by autogamy of transplanted plants, K – experimental hybrids.

Plant no.	Brief locality	Full locality	Label of cultivation
R. austriaca – 2n = 16 *			
1	Hodkovičky	Czech Republic, C. Bohemia, Praha-Hodkovičky, right bank of the river Vltava near the railway-bridge, coll. P. Tomšovic 1962	T11/1, A6
2	Hodkovičky	ditto, another individual	T11/3, A5
3	Lednice	Czech Republic, S. Moravia, town of Lednice, dam of the third small pond, S of the Prostřední rybník fishpond, coll. P. Tomšovic 1962	V9/4, A8
4	Ústí-Horní Bečva	Czech Republic, E. Moravia, bank of the river Horní Bečva by the bridge near the village of Ústí u Vsetína, coll. P. Tomšovic 1962	T21/2, A7
R. amphibia – 2n = 32 *			
5	Budapest	Hungary, island of Gödisziget near Alsógöd, N of the city of Budapest, coll. P. Tomšovic 1962	T23a/1, A1
6	Stará Boleslav	Czech Republic, C. Bohemia, drainage ditch in the field W of the village of Křenek near the town of Stará Boleslav, coll. V. Kratochvílová 1967	T40/1
7	Lanžhot	Czech Republic, S. Moravia, detached river arm of the river Morava in meadows E of the village of Lanžhot, coll. P. Tomšovic 1962	T6/1
8	Moravský Svatý Ján	Slovak Republic, SW. Slovakia, detached river arm “Kalifáš” of the river Morava near the village of Moravský Sv. Ján S of the village of Kúty, coll. P. Tomšovic 1962	T3/2, A2
9	Staré Město	Czech Republic, S. Moravia, right bank of the river Morava in the village of Staré Město near the town of Uherské Hradiště, coll. P. Tomšovic 1962	T19/2, A3
R. palustris – 2n = 32 *			
10	Drahonice	Czech Republic, S. Bohemia, a small fish-pond near the road NE of the village of Drahonice (SSW of the town of Písek), coll. P. Tomšovic and V. Kratochvílová 1967	T55/1, A24
11	Modřany-potok	Czech Republic, C. Bohemia, Praha-Modřany, at the mouth of the brook Modřanský potok, on the right bank of the river Vltava, coll. V. Kratochvílová 1967	T38/1, A23
12	Průhonice	Czech Republic, C. Bohemia, Černý rybník pond N of the village of Průhonice SE of Praha, coll. V. Kratochvílová 1967	T36/1, A20
13	Průhonice	ditto, another individual	T36/2, A21
14	Průhonice	ditto, another individual	T36/3, A22
15	Strunkovice nad Volyňkou	Czech Republic, S. Bohemia, deposits along right bank of the river Volyňka, near the railway station, in the village of Strunkovice nad Volyňkou, coll. P. Tomšovic and V. Kratochvílová 1967	T56/1, A25
R. sylvestris – 2n = 32 *			
16	Křenek-Labe	Czech Republic, C. Bohemia, field on the right bank of the river Labe in the village of Křenek, near the town of Stará Boleslav, coll. V. Kratochvílová 1967	T41/1, A12
17	Křenek-Labe	ditto, another individual	T41/3, A14
18	Křenek	Czech Republic, C. Bohemia, weed in the garden of house No. 48 in the village of Křenek near the town Stará Boleslav, coll. V. Kratochvílová 1967	T42/2, A15
19	Křenek	ditto, another individual	T42/4, A16
20	Modřany	Czech Republic, C. Bohemia, Praha-Modřany, right bank of the river Vltava at the sugar factory, coll. V. Kratochvílová 1967	T37/7, A9

21	Modřany	ditto, another individual	T37/10, A11
23	Modřany	ditto, another individual	T37/8, A10
22	Strunkovice nad Blanicí	Czech Republic, S. Bohemia, left bank of the river Blanice near the bridge in the village Strunkovice nad Blanicí, coll. P. Tomšovic & V. Kratochvílová 1967	T52/1, A17, A18
R. austriaca × *R. amphibia* – experimental F1			
		Experimental F1 hybrids of *R. austriaca* × *R. amphibia* produced by Dr. Švéřepová in the Botanical garden in Prague (origin of parents – *R. amphibia*: Czech Republic, E. Bohemia, left bank of the river Orlice near village of Krňovice, *R. austriaca*: Czech Republic, NW Bohemia, left bank of the river Labe near village of Mlékojedy). Twelve of these hybrid plants were grown in the experimental plot at Průhonice in 1965, the seed that resulted from open pollination (labeled V99) was cultivated the next year. In 1967 30 hybrid plants, which originated from the primary hybrid combination *R. amphibia* × *R. austriaca* were grown.	K1
R. amphibia × *R. sylvestris*			
	Krňovice-Běleč	Czech Republic, E. Bohemia, left bank of the river Orlice, between the villages of Krňovice and Běleč, coll. Tomšovic P. 1962	V14
	Staré Město	Czech Republic, S. Moravia, village of Staré Město near the town of Uherské Hradiště, right bank of the river Morava, coll. Tomšovic P. 1962	V61
R. austriaca × *R. sylvestris*			
	Lovosice	Czech Republic, NW Bohemia, left bank of the river Labe in the town of Lovosice, coll. Tomšovic P. 1961	V20
	Trojský ostrov	Czech Republic, C. Bohemia, Praha–Trojský ostrov, river Vltava, S part of Trojský ostrov island near the turf, coll.Tomšovic P. 1961	V21
	Tiszafüred	Hungary, left bank of the river Tisza by the bridge near the village of Tiszafüred, coll.Tomšovic P. 1963	V54
	Troja	“F2” from open pollinated plants in a natural locality: Czech Republic, C. Bohemia, Praha-Troja, right bank of the river Vltava near the Zoological Park	V70