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The allopolyploid origin of *Sedum rupestre* subsp. *rupestre* (*Crassulaceae*)*

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Abstract: In *Sedum rupestre* L. a polyploid series (x = 16) occurs in which aneuploid chromosome numbers and odd levels of ploidy prevail. The most common and widely distributed cytotype, *S. rupestre* subsp. *rupestre*, is 2n = 112. Plants resembling *S. rupestre* subsp. *rupestre* can be obtained by hybridizing the tetraploid cytotypes of *S. forsterianum* SM. (2n = 48) and *S. rupestre* subsp. *erectum* 't HART (2n = 64). Comparison of these artificial hybrids with their parents and a large number of plants of *S. rupestre* subsp. *rupestre* (2n = 112) from nature showed that *S. rupestre* subsp. *rupestre* and the artificial hybrids are morphologically indistinguishable, and intermediate between *S. forsterianum* and *S. rupestre* subsp. *erectum*. Morphologically *S. rupestre* subsp. *rupestre* is closer to subsp. *erectum* than to *S. forsterianum*. Chloroplast DNA restriction patterns of *S. rupestre* subsp. *rupestre*, however, resemble *S. forsterianum* more closely. The combined results of the hybridization experiments, the analysis of the cpDNA restriction patterns, and the morphological variation indicate the allopolyploid origin of *S. rupestre* subsp. *rupestre*.

Morphologically *S. rupestre* L. is an extremely variable species. LINNAEUS (1753, 1755) described it twice, as *S. rupestre* and as *S. reflexum*, respectively ('T HART 1978), and subsequent authors have contributed to the confusion by describing large numbers of segregate and infraspecific taxa (e.g., BOUVET 1882–1883). Furthermore, *S. rupestre* often has been confused with *S. forsterianum* SM. LINNAEUS did not distinguish the two taxa ('T HART & JARVIS 1993), and more recently, FRÖDERSTRÖM (1932) also considered them to be conspecific, though differing at the subspecific level.

Cytologically *S. rupestre* is very variable. The chromosome numbers 2n = 56, 64, 85, 88, 96, 102, 112, 102–119, 140, 153, and 168 have been reported (TOYOHUKU 1935; SOEDA 1944; 'T HART 1972, 1978, 1987; CASTROVIEJO & CALVO 1981; UHL 1982; HÉBERT 1983; LÖVE & LÖVE 1985). The report of 2n = 56 (HÉBERT 1983) refers to a single, only provisionally identified, non-flowering plant found in a population of *S. sediforme* (JACQ.) PAU. Furthermore, 2n = 56 has been reported

* Natural hybrids in *Sedum* (*Crassulaceae*) 4.

for *S. sediforme* by CASTROVIEJO & CALVO (1981). The cytotype 2n = 112 of *S. rupestre* is the most common, and it is widely distributed in central and northwestern Europe ('T HART 1978, HÉBERT 1983). The other cytotypes are rare and have so far only been found in the southern parts of Europe, except for the plants with 2n = 140 and 168 which were reported from Central Europe (HÉBERT 1983).

LINNAEUS described *S. rupestre* from plants that probably originated from northwestern Europe ('T HART & JARVIS 1993). Plants of *S. rupestre* from this region are 2n = 112 and, consequently, the type of *S. rupestre* is considered to be identical with this cytotype ('T HART 1978).

The inflorescences of all cytotypes of *S. rupestre* are reflexed before anthesis, except for the inflorescences of the plants with 2n = 64, which are erect before anthesis. 'T HART (1978) described the latter cytotype as *S. rupestre* subsp. *erectum*. So far, it has only been reported from the karst near Auresina (Italy; prov. Trieste), but it also occurs in adjacent Slovenia ('T HART, unpubl.).

The lowest reliable somatic chromosome number so far reported for *S. rupestre* is 2n = 64. For the six European species that are closely related to *S. rupestre* (usually they are classified in *Sedum* series *Rupestria* BERGER) the basic chromosome numbers are x = 12, 13, 16, and 17 ('T HART 1978); by inference, the basic number of *S. rupestre* has been estimated to be x = 16. CASTROVIEJO & CALVO (1981) and HÉBERT (1983) considered the basic number of *S. rupestre* to be x = 8.

Aneuploid chromosome numbers and odd levels of ploidy by far outnumber the euploid numbers in the polyploid series of *S. rupestre*. 'T HART (1978) suggested an allopolyploid origin for the cytotype with the chromosome number 2n = 112 as a possible explanation of these dysploid cytotypes. From hybridization experiments with the tetraploid cytotypes of *S. forsterianum* (2n = 48) and *S. rupestre* subsp. *erectum* (2n = 64), which presently are completely allopatric, he obtained hybrids with the chromosome number 2n = 56. Morphologically these hybrids closely resemble *S. rupestre* subsp. *rupestre*, but they are semi-sterile. Doubling of the chromosome number of these hybrids and successive backcrossing and hybridization of the allopolyploid could explain the origin of the plants with the chromosome numbers 2n = 112, 88, and 100 ('T HART 1978). According to HÉBERT (1983) the plants with 2n = 168 and 140 are derived from the allopolyploid (2n = 112), representing an autotriploid and a backcross of the triploid, respectively.

To test the hypothesis that *S. rupestre* subsp. *rupestre* is indeed an old allopolyploid of the aforementioned parentage, experiments were carried out with the artificial hybrids of the tetraploid cytotypes of *S. forsterianum* and *S. rupestre* subsp. *erectum*. In this study the results of hybridization experiments are presented as well as an analysis of the morphological variation and the chloroplast DNA restriction fragment length polymorphisms (cpDNA RFLPs) of these artificial hybrids, their parents, and 144 plants of *S. rupestre* subsp. *rupestre* from nature.

Material and methods

Plant material. The plants used in this study were collected in nature and further cultivated under uniform conditions in the experimental garden of the University of Utrecht. Voucher specimens are deposited at the botanical institute in Utrecht (U). The origin of the plants is presented in Table 1. The table comprises all plants of *S. forsterianum* and *S. rupestre* subsp. *erectum* as well as the plants of *S. rupestre* subsp. *rupestre* that have been used in the hybridization experiments, and the chloroplast DNA studies. A full list of all plants

Table 1. Origins and accession numbers of the plants of *S. forsterianum* SM., *S. rupestre* L. subsp. *erectum* 'T HART, and *S. rupestre* L. subsp. *rupestre* which have been used in the morphological studies (1), the hybridization experiments (2), and the cpDNA studies (3)

Sedum forsterianum SM.
2n = 24: **Spain**: prov. Caceres; Aledanueva de la Vera, 500 m s.m., 29429 (3).
2n = 48: **Portugal**: distr. Bragança: Serra da Coroa, 28 km W of Vinhais, 600 m s.m., 29596 (1); distr. Castelo Branco: vic. of Alcongosta S of Fundao, 15401 (1); distr. Lisboa: Sintra, 6852 (1, 2, 3); Serra de Montejunto 8 km SW of Cercal, 200 m, 29545 (1, 3); distr. Guarda: Serra da Estrela, 750 m s.m., 29567 (1).
2n = 96: **England**: co. Devon: vic. of Lynton, 8141 (3).

Sedum rupestre L. subsp. *erectum* 'T HART
2n = 64: **Italy**: prov. Trieste: karst along the coast S of Auresina, 50–100 m s.m., 16260 (1, 3), 16262 (2), 16263 (1, 3), 16705 (1, 2, 3), 16711 (1).

Sedum rupestre L. subsp. *rupestre*
2n = 88: **Italy**: prov. Pisa: vic. of Volterra, 12391 (2); prov. Siena: near Eremo Agostiniano di Lecceto, 6901 (2). **Spain**: prov. Barcelona: Col de St Elena near Sante Fe, 1100 m s.m., 6879 (2).
2n = 112: **Andorra**: between Port-d'Envalira and Soldeu, 2000 m s.m., 7250 (1, 2, 3). **England**: co. Devon: Dartmoorforest, 8137 (3). **France**: dep. Charente: near le Dognon, E of Gençay, 22961 (1, 2); dep. Hautes Pyrenées: E of Lesponne along the road of Beaudeau, 700 m s.m., 28519 (1, 3); dep. Loire Atlantique: la Censerie N of Ancenis, 7150 (1, 2, 3); dep. Puy de Dôme: Mt Dore, 11226 (1, 3); dep. Vendée: vic. of les Sables d'Olonne (les Gragnes, Sauveterre, St Nicolas de Brem), 13675 (1, 3), 13683 (2); dep. Vienne: 2 km S of Millac, 150–200 m s.m., 22976 (1, 2). **Germany**: Rheinland-Pfalz: near the river Mosel N of Thornich, between Merhing and Neumagen, 22888 (1, 3). **Norway**: prov. Telemark: Dalen, 9997 (1, 3). **Sweden**: Gotland; Torsburgen 15103 (3)

of *S. rupestre* subsp. *rupestre* is available from the senior author on request. Chromosome numbers were determined in root-tip mitoses and hybridization experiments were carried out as described by 'T HART (1978, 1987).

Pattern detection methods. Cluster analysis and principal component analysis (PCA) were used to test whether the species, cytotypes, and artificial hybrids are morphologically distinguishable, and to assess the morphological relationships between the taxa. Because of the mixed mode of the characters (multistate and continuous, see Table 3) the values of each character were normalized (maximum value set to 1), and the mean city block distance (BOCK 1974) was used to define the similarity between plants from the normalized characters. Clusters were generated by agglomerative cluster analysis. Because the structure of the dataset was expected to be weak we used WARD's (1963) cluster criterion for its strong pattern filtering capacities (HOGEWEG 1976 b, HOGEWEG & HESPER 1981). The strong dilating properties of WARD's method, however, can cause considerable deformations. Therefore we also used the UPGMA clustering criterion (SNEATH & SOKAL 1973), which has proved to be a useful criterion in systematic and phylogenetic studies (TATENO & al. 1982, NEI & al. 1983). Optimum splitting levels of the dendrogram were calculated using the criterion of HOGEWEG (1976 a). The variation in the dataset has been represented by means of PCA. The components are linear combinations of the original characters and may be seen as new, independent characters.

Chloroplast DNA preparation. Chloroplast DNA (cpDNA) was isolated according to SANDBRINK & al. (1989). Young leaves were ground at 4 °C in a Waring blender with

homogenization buffer (HB) (1.25 M NaCl, 1% PVP, 10 mM 2-ME, 0.1% BSA, 5 mM EDTA, 10 mM Tris-HCl, pH = 8.0) in a 1 : 1 (w : v) ratio. The chloroplast suspension was filtered through one layer of nylon gauze and four layers of Miracloth (Calbiochem). Chloroplasts were pelleted at 1500 × **g**, resuspended in HB, pelleted again at 1500 × **g**, and finally resuspended in a buffer containing 5 mM EDTA, 10 mg/ml proteinase K, and 10 mM Tris-HCl, pH = 8.0 (8 : 1 w : v). An equal amount of lysis buffer (10 mM Tris-HCl, 5 mM EDTA, 4% sarcosyl, 1% SDS, and 10 mg/ml proteinase K) was added. The chloroplast suspension was gently rotated for 30 min to complete lysis. The lysate was centrifuged for 10 min at 3000 × **g** to remove starch and unlysed chloroplasts. An equal amount of 2 × CTAB buffer (2% cetyl triammonium bromide, 1.4 M NaCl, 20 mM 2-ME, 20 mM EDTA, 100 mM Tris-HCl, pH = 8.0) was added. The suspension was incubated for 1 h at 60 °C, and extracted twice with chloroform : isoamylalcohol (24 : 1). DNA was precipitated with isopropanol at − 20 °C. cpDNA was pelleted by centrifugation at 6000 × **g**. Pellets were washed in 76% ethanol, 10 mM ammoniumacetate, dried in a desiccator, and resuspended in TE buffer, pH = 8.0 (MANIATIS & al. 1982). 2–3 microgram of plastid DNA was digested with Bgl II, EcoR I, Hind III, Nco I, Pvu II, and Sca I according to the manufacturers' instructions (Pharmacia). Digested samples were electrophoresed on 0.7% TBE agarose minigels (MANIATIS & al. 1982), photographed, and analysed.

Results

Hybridization. In the hybridization experiments we used the tetraploid cytotypes of *S. forsterianum* (2n = 48) and *S. rupestre* subsp. *erectum* (2n = 64), two cytotypes of *S. rupestre* subsp. *rupestre* (2n = 88, 112), some of the artificial hybrids (2n = 56) of *S. forsterianum* and *S. rupestre* subsp. *erectum*, and the hybrids (2n = 88) of the two cytotypes of *S. rupestre* subsp. *rupestre* (Table 1). The results of the experiments are summarized in Table 2.

Table 2. Crosses between *Sedum forsterianum* SM. (*fors*), *S. rupestre* L. subsp. *rupestre* (*rupe*), and subsp. *erectum* 't HART (*erec*). The figures in each square (combination) subsequently indicate the number of flowers used in the crosses (V when more then hundred flowers were used), the mean number of seeds produced per flower (the figures marked with an asterisk present the total number of seeds obtained), and the somatic chromosome number of the hybrids

F	M	*fors* 48	*fors* × *erec* 56	*erec* × *fors* 56	*erec* 64	*erec* × *rupe* 88	*rupe* 88	*rupe* × *erec* 88	*rupe* 112
forsterianum	48				7: 2: 56				
forsterianum × *erectum*	56		26: 0: − V: 0: −						
erectum × *forsterianum*	56		V: 6*: 112	71: 7*: 112 V: 3*: 112					
erectum	64	11: 4: 56							17: 15: 88
erectum × *rupestre*	88				7: 3: −	59: 2: 132 : 176			6: 16: −
rupestre	88				7: 9: 76		16: 6: 88		11: 43: 100
rupestre × *erectum*	88							37: 1: 132 : 176	
rupestre	112				8: 27: 88				7: 15: 112

The tetraploid cytotypes of *S. forsterianum* and *S. rupestre* subsp. *erectum* can be easily crossed. On average these crosses produced 3 seeds per flower. The F1 hybrids were semi-sterile (pollen fertility averaging less than 40%), but a few seeds could be produced by selfing or crossing of large numbers of flowers (approximately 1 seed from 25 flowers). From the 16 seeds produced in these experiments we obtained 9 mature plants. The F2 hybrids were all 2n = 112. They were semi-fertile with pollen fertilities varying from 50% to 65%.

The cytotypes of *S. rupestre* subsp. *rupestre* with 2n = 88 and 112 are fully self-compatible. On average they produced 6 and 15 seeds per flower, respectively. Cytologically as well as morphologically the offspring of these cytotypes was identical to the parents.

The cytotypes of *S. rupestre* subsp. *rupestre* with 2n = 88 and 112 could easily be crossed with each other as well as with the tetraploid *S. rupestre* subsp. *erectum* (2n = 64). The resulting hybrids had the chromosome numbers 2n = 76 (64 × 88), 2n = 88 (64 × 112), and 2n = 100 (88 × 112). All of these hybrids were vigorous and flowered abundantly. The hybrids with 2n = 88 (64 × 112) were fertile or semi-fertile (pollen fertilities 50% to 90%) and selfing of the plants of 3 different crosses produced 2 seeds per flower on average. In contrast to the progeny of the plants with 2n = 88 from nature, the plants of the F2 generation obtained from artificial hybrids (2n = 88) either had the chromosome number 2n = 132 or 2n = 176.

Morphology. The dataset (Table 3) studied with pattern detection methods comprised 5 plants of *S. forsterianum* (2n = 48), 4 plants of *S. rupestre* subsp. *erectum* (2n = 64), a relative excessively large number (144) of plants of *S. rupestre* subsp. *rupestre* (2n = 112) from different parts of the distribution area of this cytotype, and 8 artificial hybrids, 4 with the chromosome number 2n = 56, and 4 with the chromosome number 2n = 112, respectively.

Figure 1 shows a schematic version of the UPGMA dendrogram of the dataset. At the (local) optimal splitting levels (HOGEWEG 1976a) of the dendrogram the following 4 clusters can be distinguished. The largest cluster comprises almost all plants of *S. rupestre* subsp. *rupestre* and also includes the artificial hybrids, though as a separate group. Because the artificial hybrids are highly uniform genetically they are expected to be more similar to each other than to the wild 2n = 112 plants. Subsequently are "chained" to this cluster: (i) a cluster comprising *S. rupestre* subsp. *erectum*, which can be distinguished from all other plants by their erect inflorescences; (ii) an undefined aberrant group of *S. rupestre* subsp. *rupestre*; (iii) finally a cluster comprising *S. forsterianum*, which clearly differs from the other plants.

The results of the UPGMA analysis agree with the dendrogram generated by WARD's criterion (not shown) and the principal component analysis (Fig. 2). In the PCA the artificial hybrids are included in the loose group of plants of *S. rupestre* subsp. *rupestre*. They are intermediate between their parents, though closer to *S. rupestre* subsp. *erectum* than to *S. forsterianum*. The position of the F2 hybrids (2n = 112) in the PCA has shifted towards the centre of the *S. rupestre* subsp. *rupestre* group. The first component of the PCA represents mostly size-related characters, such as the average distance between the central flower and the flower of the first order, the length of the petals, the distance between the insertions of the branches of the inflorescences, and the length of the leaves.

Table 3. Means and standard deviations of the characters used for the analysis of the morphological variation

Sedum	*forsterianum*		*rupestre* subsp. *erectum*		*rupestre* subsp. *rupestre*		*forsterianum* × *rupestre* subsp. *erectum*			
2n =	48		64		112		56		112	
Number of plants investigated	5		4		144		4		4	
Characters	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Leaves:										
number of orthostiches	14.2	2.8	5	0	6.7	0.8	7.2	0.5	6.7	0.5
length (mm)	7	1.6	7.9	1.3	8.8	1.7	8.2	2	8.7	2
length/width ratio	6.2	2.1	4.1	0.5	4.7	0.7	5.7	0.4	5.1	1.2
cross-section, length/width ratio	1.4	0.1	1.4	0.1	1.5	0.1	1.2	0.1	1.4	0.1
Inflorescence:										
drooping of erect in bud (1–3)	1	0	3	0	1	0.2	1	0	1	0
number of branches 1st order	4.2	1.1	4.2	1.3	3.9	0.6	3.1	0.2	3.5	0.6
length branches 1st order (mm)	6.0	1.4	6	1.7	8.2	1.8	4.3	0.8	6.8	0.8
number of branches 2nd order	7.8	2.2	8.5	2.5	7	1.1	5.7	1	6.2	1
length main axis	1.2	0.4	2.8	1.5	3.2	1.4	1.7	0.3	2.7	0.7
ratio number of bracts/flowers	0.0	0.0	0.9	0.0	1	0.3	0.2	0.1	0.2	0.1
Pedicel length (mm)	1.5	0.5	0.7	0.3	1.2	0.4	0.9	0.3	0.7	0.1
Number of floral parts	6.1	0.4	6.1	0.2	6.5	0.6	6	0	5.9	0.5
Sepals:										
length/width ratio	2.1	0.3	2.7	0.3	2.4	0.4	2.4	0.3	2.3	0.1
number of glandular hairs	0.6	0.9	0	0	4.3	4.3	0.2	0.5	0.1	0.2
Petals:										
colour (1–10, 't HART 1987)	10	0	10	0	9.9	0.3	10	0	9.7	0.5
length (mm)	5.1	1.1	6.6	0.6	7.1	0.6	6	0.4	6.9	1
length/width ratio	3.4	0.7	4.1	0.3	3.8	0.4	4.1	0.6	3.9	0.4
Ratio length petals/sepals	2.6	0.4	2.5	0.2	2.5	0.3	2.3	0.3	2.4	0.2
Ratio length petals/filaments	1.3	0.2	1.2	0.1	1.3	0.1	1.3	0.1	1.3	0.2
Number of basal papillae of the filaments	0	0	40.7	26.6	24.7	17.7	0	0	0	0
Carpels:										
length (mm)	5.3	1.3	6.0	0.8	6.3	0.7	6.1	0.2	7.1	0.7
papillate (0/1)	0	0	0.7	0.3	0.9	0.2	1	0	1	0
Ratio length styles/carpels	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0
Squamae:										
length (mm × 10)	2.6	0.7	4.1	0.5	3.4	0.6	3.4	0.4	4.3	0.8
ratio length/width	0.9	0.2	0.9	0.1	0.9	0.3	0.8	0.2	1	0.3
Begin flowering period (4-day periods, 1–5)	–		–		1.6	0.7	1	0	1	0

cpDNA RFLP. For the study of cpDNA 16 plants were selected, i.e. 1 diploid (2n = 24), 2 tetraploids (2n = 48), and 1 octoploid (2n = 96) of *S. forsterianum*, 3 tetraploids (2n = 64) of *S. rupestre* subsp. *erectum*, and 9 plants of *S. rupestre* subsp. *rupestre* with 2n = 112 (Table 1). With the enzymes used no intraspecific variation could be observed in these three taxa, though not all restriction enzyme/plant combinations were tested.

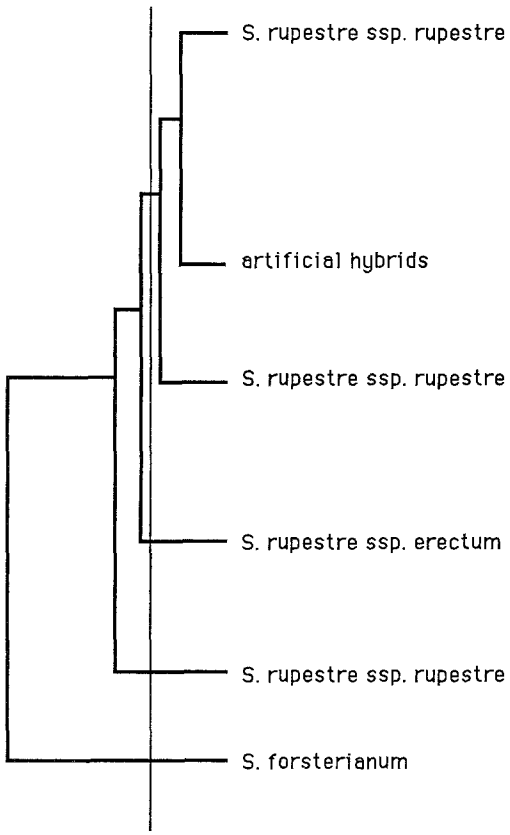

Fig. 1. Schematic version of the UPGMA dendrogram of the *Sedum* taxa investigated. The thin line indicates the optimal splitting level of the dendrogram. At this level four clusters can be distinguished (see text)

The differences in cpDNA restriction fragments between the three taxa are presented as percentages of shared restriction fragments for each enzyme (Table 4). The restriction patterns of *S. forsterianum* and *S. rupestre* subsp. *erectum* differ for the 6 enzymes, i.e. Bgl II, EcoR I, Hind III, Nco I, Pvu II, and Sca I, whereas the patterns of *S. forsterianum* and *S. rupestre* subsp. *rupestre* are identical for 3 enzymes, i.e. Nco I, Pvu II, and Sca I, but different for the other 3 enzymes (see Fig. 3). The two subspecies of *S. rupestre* differ for all 6 enzymes.

The maternal inheritance of cpDNA was tested in two hybrids, of reciprocal crosses between *S. forsterianum* (2n = 48) and *S. rupestre* subsp. *erectum* (2n = 64), as well as in 1 allopolyploid hybrid (2n = 112), with Nco I, Pvu II, and Sca I. The cpDNA restriction patterns of the hybrids as well as the allopolyploid plant were completely identical with the patterns of their maternal parent.

Discussion

The production of unreduced gametes is the most common cause of polyploidy in plants, and in general is to a large extent under genetic control (HARLAN & DEWET 1975, DEN NIJS & PELOQUIN 1977). The frequent occurrence of unreduced gametes in hybrids, on the other hand, is probably due to genetic imbalance. In crossing experiments artificial *Sedum* hybrids often produce allo-triploid or -tetraploid offsprings, and allopolyploid plants have also been found in natural hybrid populations (UHL 1976; 'T HART 1978, 1987; FAVARGER & WELTER 1979). Our hy-

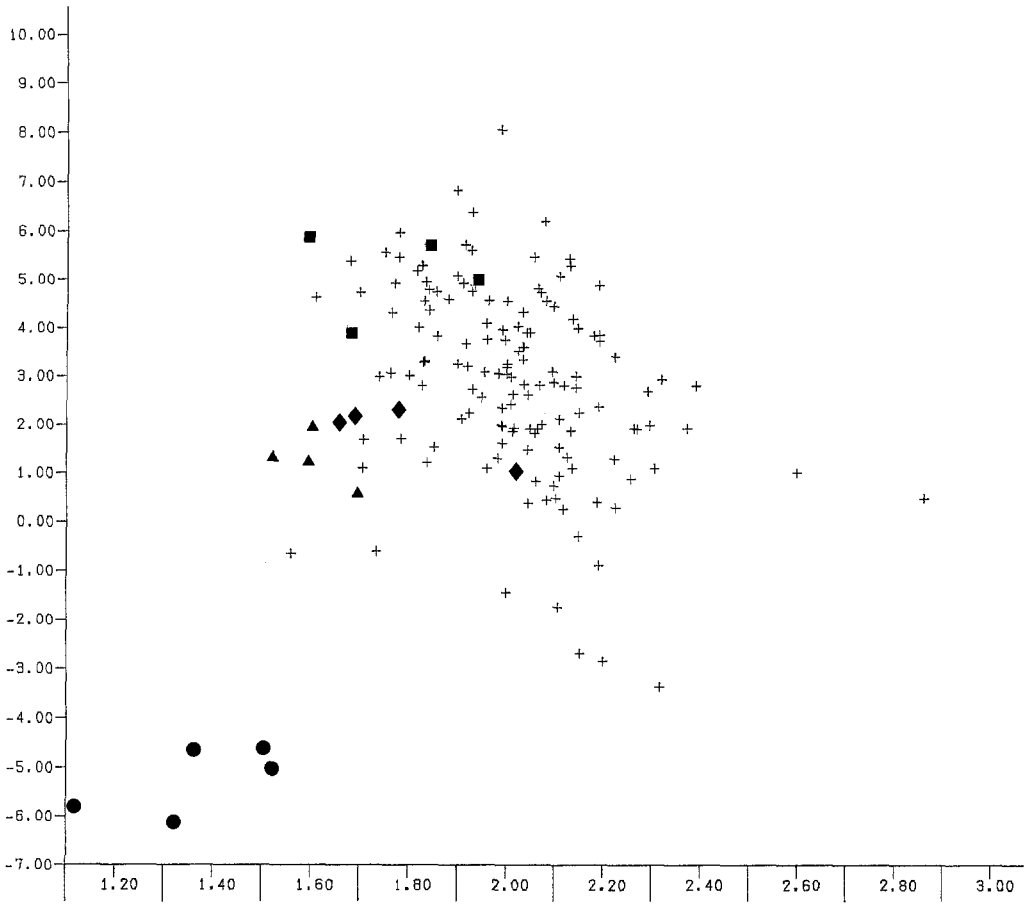

Fig. 2. Scatter diagram on the first two principal components (representing 24% of the total variation). The horizontal axis represents the first component, the vertical axis the second component. ● *Sedum forsterianum* SM., ■ *S. rupestre* L. subsp. *erectum* 't HART, + *S. rupestre* subsp. *rupestre*, Artificial hybrids: ▲ F1 (2n = 56), ◆ F2 (2n = 112)

bridization experiments (Table 2) show that an allotetraploid plant with the chromosome number 2n = 112 can be produced indeed from hybrids between the tetraploid cytotypes of *S. forsterianum* (2n = 48) and *S. rupestre* subsp. *erectum* (2n = 64).

Morphologically the artificial hybrids of the tetraploid cytotypes of *S. forsterianum* and *S. rupestre* subsp. *erectum* (both the F1's and the F2's) are closer to *S. rupestre* subsp. *erectum* than to *S. forsterianum*. In the PCA (Fig. 2) the F2 hybrids (2n = 112) have shifted towards the centre of the group of *S. rupestre* subsp. *rupestre* (2n = 112). The characters of the first axis of the PCA may be directly associated with the chromosome number, as they are all size related, which could explain why doubling of the number of chromosomes makes the hybrids more similar to the plants with the chromosome number 2n = 112 from nature (see also Table 3).

The results of the cpDNA RFLP analysis contrast sharply with the results of the morphological studies (Table 4). With respect to cpDNA variation *S. forsterianum, S. rupestre* subsp. *rupestre*, and *S. rupestre* subsp. *erectum* are distinct

Table 4. The coefficients of similarity (F) of cpDNA of the three *Sedum* taxa indicated by their shared restriction fragments. [F = 2Nxy/(Nx + Ny); Nei & Li 1979]

Taxon	Bgl II			EcoR I			Hind III		
	fors	*rupe*	*erec*	*fors*	*rupe*	*erec*	*fors*	*rupe*	*erec*
S. forsterianum	1	0.92	0.88	1	0.93	0.86	1	0.97	0.88
rupestre subsp. *rupestre*		1	0.92		1	0.84		1	0.88
subsp. *erectum*			1			1			1
	Nco I			Pvu II			Sca I		
	fors	*rupe*	*erec*	*fors*	*rupe*	*erec*	*fors*	*rupe*	*erec*
S. forsterianum	1	1	0.90	1	1	0.82	1	1	0.80
rupestre subsp. *rupestre*		1	0.90		1	0.82		1	0.80
subsp. *erectum*			1			1			1
	total								
	fors	*rupe*	*erec*						
S. forsterianum	1	0.96	0.87						
rupestre subsp. *rupestre*		1	0.87						
subsp. *erectum*			1						

lineages. *S. rupestre* subsp. *rupestre*, however, is more closely related to *S. forsterianum* than to *S. rupestre* subsp. *erectum*, though morphologically it differs significantly from subsp. *erectum* only in the position of the inflorescence before flowering. Considering the relationships of these three taxa at the cpDNA level, in combination with their cytological and morphological variation and the results of the hybridization experiments, a hybrid origin of *S. rupestre* subsp. *rupestre* is most likely (Fig. 4).

The hybridization experiments indicate that the crosses between *S. forsterianum* and *S. rupestre* subsp. *erectum* can equally easily occur in both directions. The reciprocal hybrids are equally vigorous and flower abundantly. Although both hybrids are semi-sterile, they are perennial and theoretically have equal chances to produce a fertile allopolyploid offspring. Therefore one would expect to find two types of cpDNA in natural *S. rupestre* subsp. *rupestre*, similar to the variation in allopolyploid taxa of *Tragopogon* (Soltis & Soltis 1989) and *Glycine* (Doyle & al. 1990). The restriction patterns of the nine plants of *S. rupestre* subsp. *rupestre* are completely identical, though they originated from different parts of the distribution area of this taxon (Table 1). The uniformity of the restriction patterns of the plants of *S. rupestre* subsp. *rupestre* indicates that they either descended from one single hybrid or from several different hybrids that all had *S. forsterianum* as maternal parent (polytope origin). If we assume that *S. forsterianum* and *S. rupestre* subsp. *erectum* had equal chances to serve as the maternal parent, the uniform cpDNA restriction patterns indicate some kind of selection. This selection might

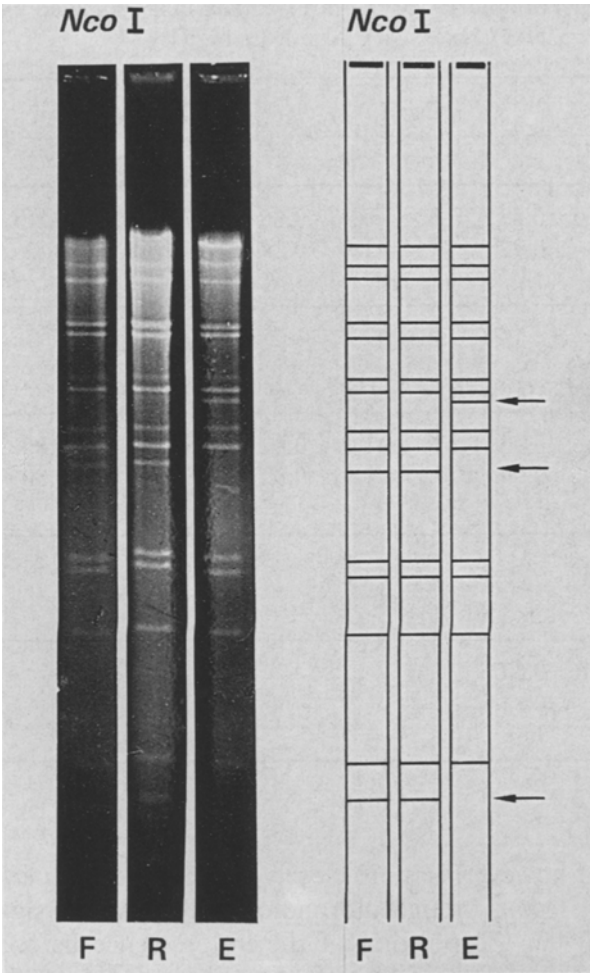

Fig. 3. Nco I digested cpDNA of *Sedum forsterianum* SM., no. 6852 (F), *S. rupestre* L. subsp. *rupestre*, no. 7150 (R), and *S. rupestre* L. subsp. *erectum* 't HART, no. 16260 (E)

have been either biological, due to a preference for one of the crosses in nature (in contrast to the results of the crossing experiments), or result from extermination of a large part of the original population of allopolyploids, due to changes in the environment (e.g., ice ages).

The current geographical distribution of the taxa supports the latter of these evolutionary pathways. At present the tetraploid cytotypes of *S. forsterianum* and *S. rupestre* subsp. *erectum* are completely allopatric ('T HART 1978), whereas they must have been sympatric at the time *S. rupestre* subsp. *rupestre* evolved. The area of the tetraploid cytotype of *S. rupestre* subsp. *erectum* has apparently been reduced to the karst near Auresina during the ice ages. The distribution of the cytotypes of *S. forsterianum* ('T HART 1978, HÉBERT 1983) in combination with their uniform cpDNA restriction patterns indicate that the polyploids have evolved, and spread to their present territories quite recently, probably also as a result of the ice ages. Similarly the original area of *S. rupestre* subsp. *rupestre* may have been reduced to a single population or a few small ones somewhere in southern Europe. From this refugium *S. rupestre* subsp. *rupestre* might then have spread over Central and northern Europe as far as Britain, Central Scandinavia and eastern europe.

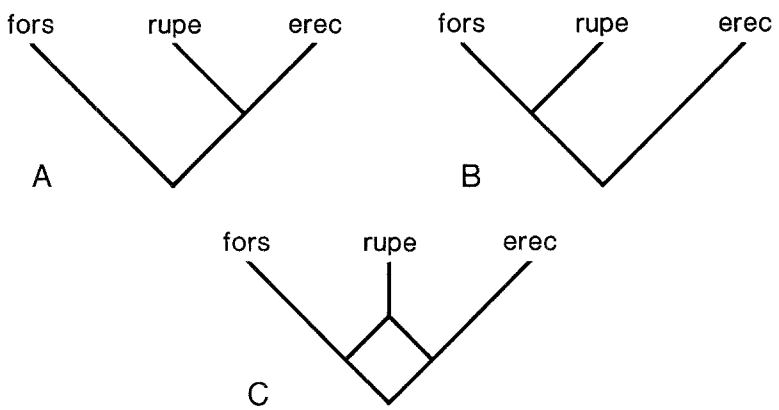

Fig. 4. Schematic representation of the relationships between *Sedum forsterianum* SM. (*fors*), *S. rupestre* L. subsp. *erectum* 'T HART (*erec*), and *S. rupestre* L. subsp. *rupestre* (*rupe*). *A* Morphological relationships. *B* Relationships based on the cpDNA restriction fragment analysis. *C* Origin of *S. rupestre* subsp. *rupestre* as inferred from hybridization experiments, and the morphological and cpDNA variation

In conclusion our results show that *S. rupestre* subsp. *rupestre* is intermediate between *S. forsterianum* and *S. rupestre* subsp. *erectum*. Morphologically *S. rupestre* subsp. *rupestre* is closer to subsp. *erectum* (Fig. 4 A), but in regard to its cpDNA restriction patterns it resembles *S. forsterianum* more closely (Fig. 4 B). The cytological data and the results of our hybridization experiments in combination with the results of the morphological studies and the analysis of the cpDNA RFLPs clearly indicate the allopolyploid origin of *S. rupestre* subsp. *rupestre* (Fig. 4 C).

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