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**Artikel:** Reproduction in natural triploid hybrids ( $2n=24$ ) between *Cardamine rivularis* Schur and *C. amara* L.

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**Kapitel:** 3: Results

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## 2. Materials and methods

The material for the present study was collected during 1972-1977. Fixations were made both in the field as well as the experimental greenhouse of the Geobotanical Institute. The flower buds were fixed in acetic alcohol (1:3) with a small addition of ferric acetate and acetocarmine. Micro- and macrosporogenesis as well as the seed development after experimental crosses were mostly studied on microtome sections stained with HEIDENHAIN's heamatoxylin. In addition, some lacto-propionic squashes were investigated. The viability of pollen was tested in acetocarmine smears.

The technique used in experimental crosses was the same as previously described for diploid taxa of the *Cardamine pratensis* group (URBANSKA-WORYTKIEWICZ and LANDOLT 1974) and so were the methods used for the seed germination tests and raising of young plants.

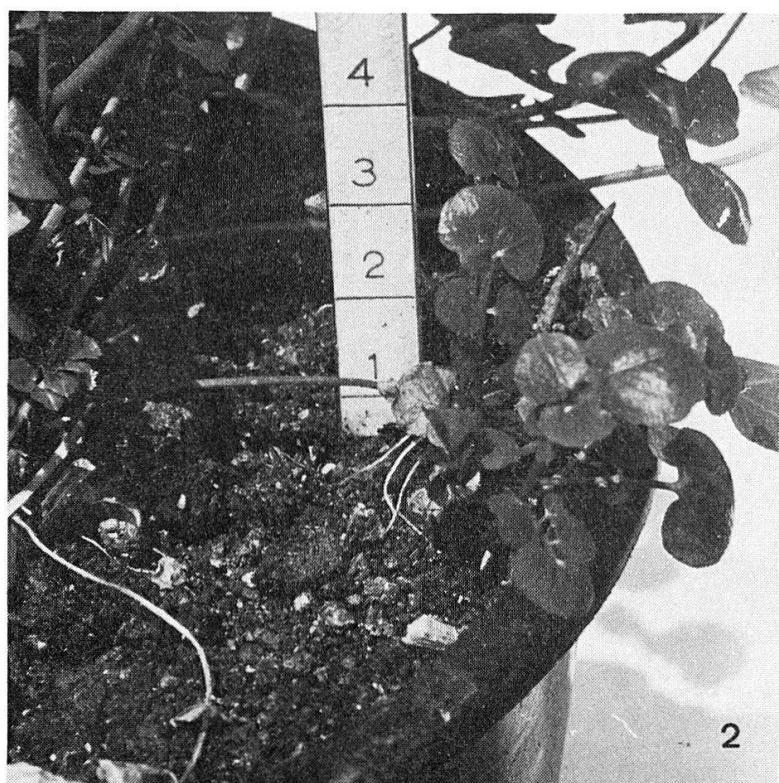
## 3. Results

### 3.1. *Vegetative multiplication*

The triploid hybrids from Urnerboden achieve their vegetative multiplication by various means. New clones are partly formed by stolons; the most characteristic, however, are small daughter plants developing at post-flowering stages. They are observable on flowering shoots, being localized very often at every node (Fig. 3) and sometimes even at the base of inflorescence. Later on, the shoots begin to bend and eventually are laying on the ground; the daughter plants have then an ample opportunity to root in and so 4 - 5 new plants per shoot may appear, separated by a distance of about 5-6 cm from each other. Complete daughter plants develop also at the upper surface of the rosette leaves (Fig. 1); their number may reach

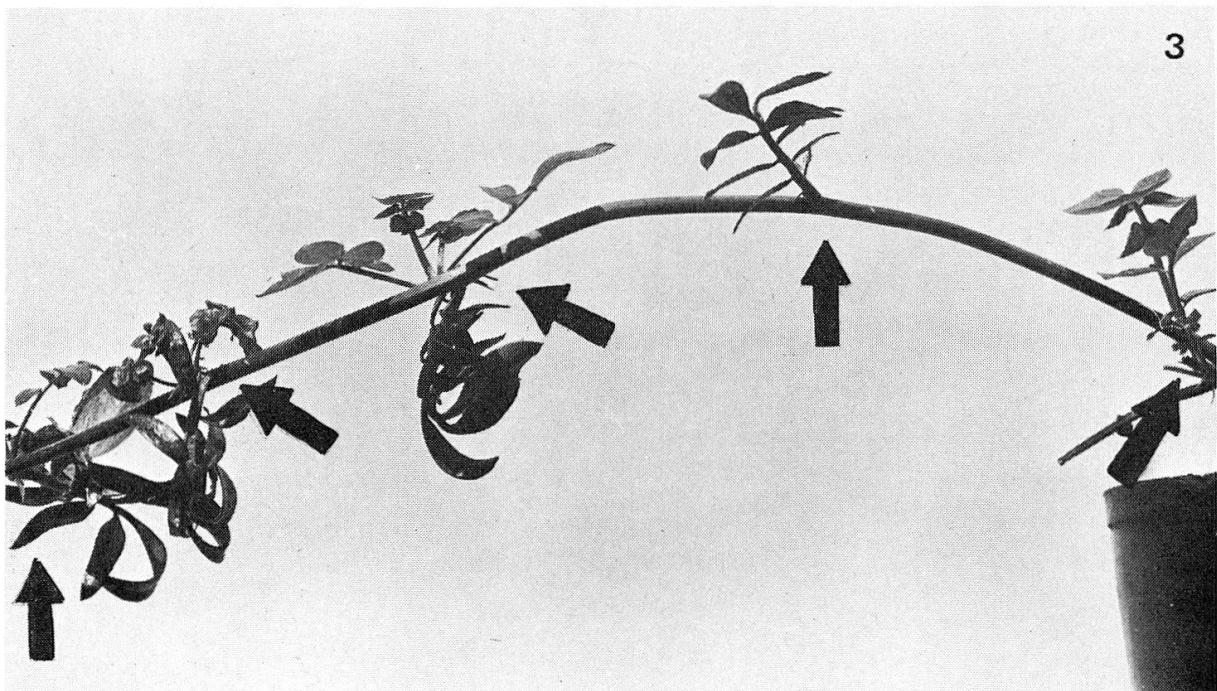


1



2

Figs 1 - 2. Vegetative multiplication of triploid hybrids: daughter plants developing vigorously on the rosette leaves.



Figs 3 - 4. Vegetative multiplication of triploid hybrids: 3. Daughter plants (marked by arrows) developing on a flowering shoot. 4. Densely inhabited sector of the population at Urnerboden: all inflorescences seen in the photograph are those of triploid hybrids.

5-6 per leaf in optimal conditions. Being very vigorous, they root easily (Fig. 2).

The number of daughter plants resulting from vegetative multiplication of a single triploid hybrid is variable, but it might total 35-36 at the end of one season of vegetation.

The amazing vegetative vigour of the hybrids has a marked effect upon the population structure. Firstly, it represents an important mechanism resulting in the development of population; secondly, it undoubtedly contributes to a fast regeneration of adult triploids observed after cutting or grazing. However, the most interesting is that the annual cutting of manured meadows at Urnerboden stimulates rather than inhibits the multiplication of the hybrids, for the fallen pieces of plants bearing the propagules are easily rooting. The greatest density of some hybrid stands was accordingly observed within cut, manured meadows (Fig. 4); for instance, as many as 898 flowering shoots were found in a surface of only  $4\text{ m}^2$  (URBANSKA-WORYTKIEWICZ and LANDOLT 1977). It can be inferred that vegetative multiplication of the 24chromosomal hybrids is directly adaptive in their prevailing biotope.

### 3.2. *Sexual reproduction*

#### 3.2.1. *Microsporogenesis and development of pollen*

The non-dehiscent anthers occurring most frequently in the studied plants were filled with compact, apparently abnormal tissue (Fig. 5), observed already at premeiotic stages.

Out of 337 studied triploids, only five proved to be producing pollen in a proportion higher than 2-3%. Microsporogenesis was studied in all these plants.

The PMCs had rather thick walls; this feature might have accounted for an unsatisfactory staining obtained at earlier stages of the meiotic prophase. The 143 PMCs observed at leptotene-diplotene had a normal appearance; however, the chromosome pairing at diakinesis proved to be variable and incomplete (Table I). PMCs containing eight bivalents and eight uni-

valents were the most frequent (Fig. 6); judging from the size of the particular units, one could assume that the bivalents might be issued from a homogenetic pairing between the 16 chromosomes of *Cardamine rivularis* type, whereas the univalents corresponded to a single genome of *C. amara*. A well-marked asyndesis occurred rather frequently in the studied material (Table 1, Fig. 7); on the other hand, no polyvalents were observed.

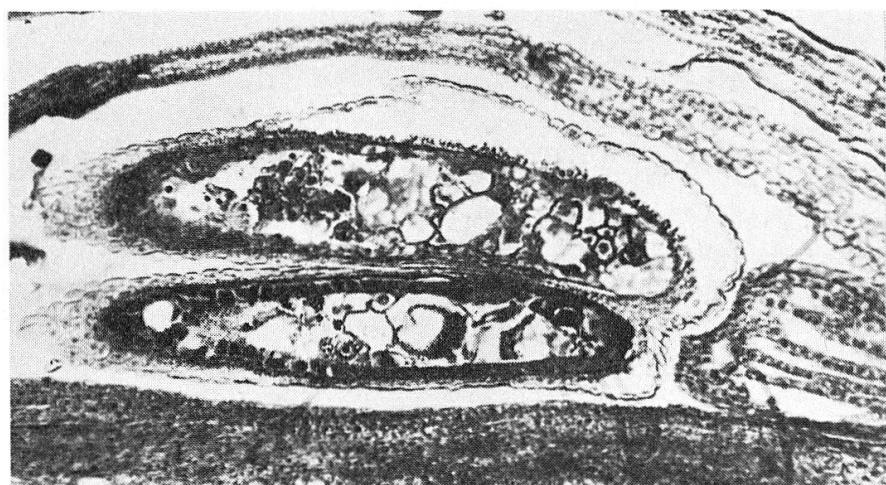
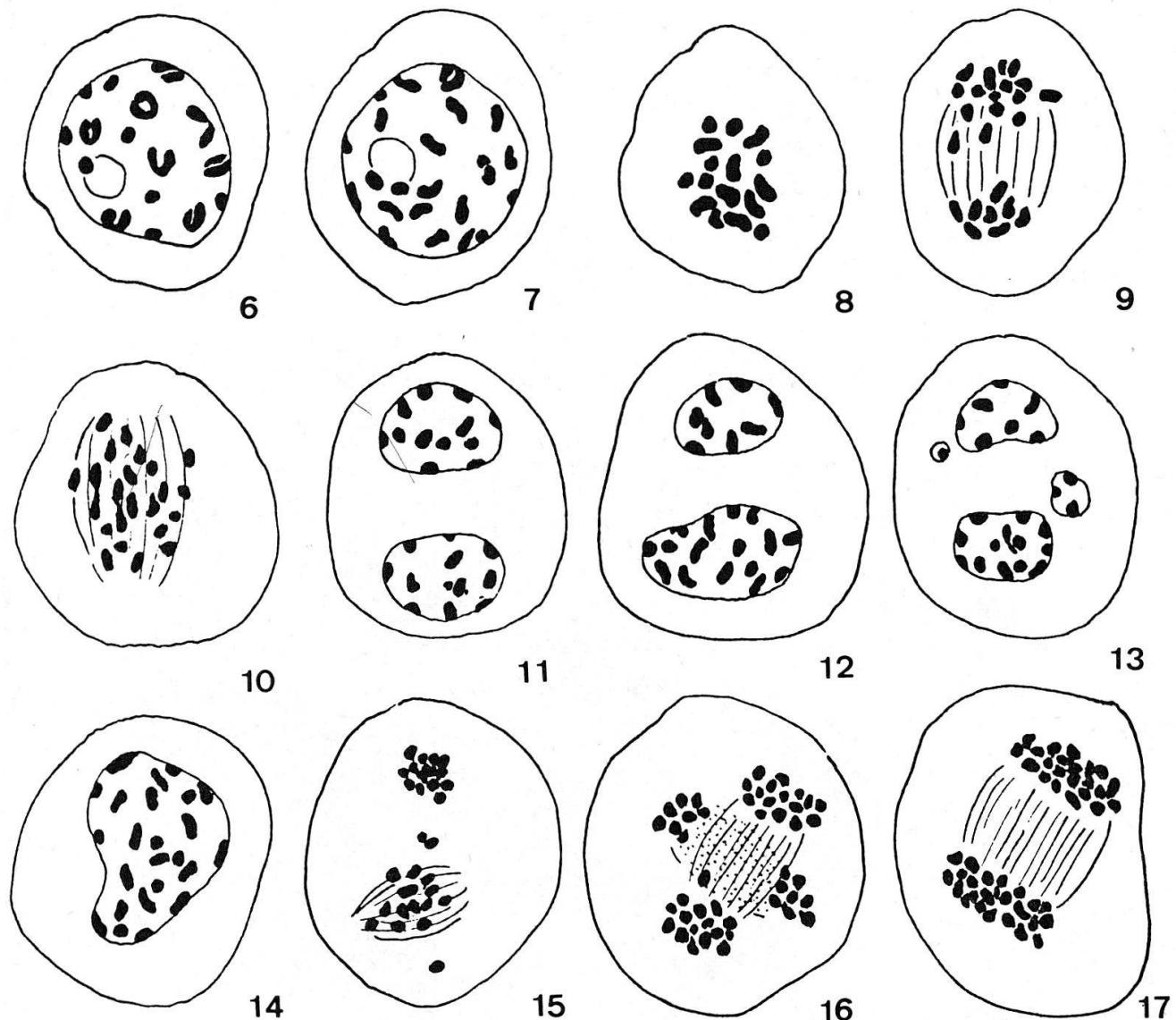


Fig. 5. Microtome section of a non-dehiscent anther. (c.) 100x.

Table 1. Chromosome pairing at diakinesis and I metaphase.

Type of association	N of studied PMCs	
	Dia	M I
24 <sub>I</sub>	1	2
1 <sub>II</sub> 22 <sub>I</sub>	2	1
2 <sub>II</sub> 20 <sub>I</sub>	36	6
3 <sub>II</sub> 18 <sub>I</sub>	27	14
4 <sub>II</sub> 16 <sub>I</sub>	3	5
5 <sub>II</sub> 14 <sub>I</sub>	1	6
6 <sub>II</sub> 12 <sub>I</sub>	2	13
7 <sub>II</sub> 10 <sub>I</sub>	-	15
8 <sub>II</sub> 8 <sub>I</sub>	112	147
Total	184	209



Figs 6-17. Microsporogenesis. 6. Diakinesis: 8<sub>II</sub> 8<sub>I</sub>. 7. Irregular diakinesis: 3<sub>II</sub> 18<sub>I</sub>. 8. I metaphase: 8<sub>II</sub> 8<sub>I</sub>. 9. A polarized segregation at I anaphase (8 vs 16). 10. A random distribution of chromosomes at 5 anaphase. 11-14. Interkinesis: 11. A normally reduced PMC (12:12). 12. Polarized nuclei (8+16). 13. Disturbed interkinesis. 14. Restitution nuclei. 15. Irregular II metaphase. 16. II Ind meiotic division in polarized nuclei. 17. II Anaphase in an unreduced PMC. About 1600x.

I metaphase was studied in 209 PMCs. The chromosomes were most frequently arranged in the equatorial plate (Fig. 8), some aberrations being found only in 22 PMCs. The chromosome pairing was comparable to that observed at diakinesis (Table 1); the most frequently occurring configuration was that of eight bivalents and eight univalents, already noticed during our preliminary studies (URBANSKA-WORYTKIEWICZ and LANDOLT 1972).

I anaphase was very diversified. A random distribution of univalents was found in 53 PMCs out of the 116 studied ones (Fig. 10). An apparently normal distribution resulting in the formation of two groups with 12 chromosomes each was observed only in 18 PMCs. On the other hand, a peculiar distribution occurred in 51 PMCs: two unequal groups were formed, one consisting of 16 chromosomes, the other comprising only eight (Fig. 9). The chromosomes gathered into the smaller group were rather uniformly long, whereas the larger group contained a mixture of short chromosomes and longer ones. It seems probable that the distribution of chromosomes in the above described PMCs was of a preferential or polarized type: the 24 chromosomes of the triploid hybrid, corresponding to a genomic constitution RRA (R = genome of *C. rivularis*, A = genome of *C. amara*) were segregated according to R:RA pattern i.e. 16:8.

Observations on I telophase and interkinesis corroborated those concerning I anaphase (Table 2).

Table 2. I telophase and interkinesis.

Type of distribution	N of studied PMCs
random	82
12 + 12 + 0	19
12 + 8 + 4	1
13 + 8 + 3	2
16 + 8 + 0	49
20 + 3 + 1	1
20 + 4	1
22 + 2	4
24 + 0	12
Total	171

On the whole, four types of PMCs were found:

1/ PMCs containing several nuclei of various size (Fig. 13) that apparently resulted from a random distribution of univalents;

2/ PMCs with two nuclei of an unequal size (Fig. 12) formed through a polarized distribution;

3/ unreduced PMCs with restitution nuclei (Fig. 14) and

4/ normally reduced ( $2n=12$ ) PMCs, rather rare in the studied material (Fig. 11).

Some PMCs at interkinesis manifested the first signs of degeneration, their cytoplasm being shrunken and the contents of nuclei often difficult to identify. Such PMCs occurred among the viable ones in nearly all studied loculi.

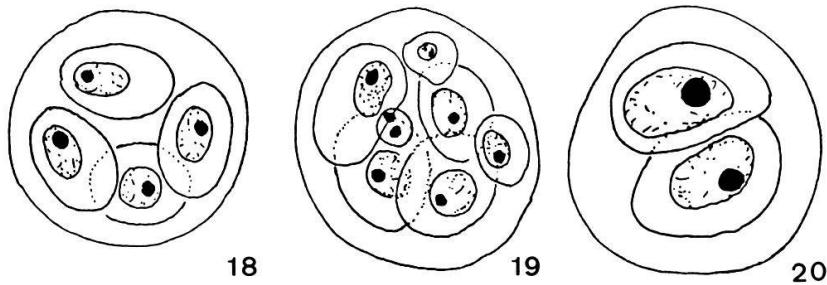
The second meiotic division was frequently very irregular, the chromosomes being scattered along the spindle or eliminated into the cytoplasm (Fig. 15). A regular distribution of chromosomes was observed only in 46 PMCs out of the 114 studied. It is interesting to note that they represented various types: a normal meiosis i.e. four 12chromosomal groups occurred in 6 PMCs; on the other hand, 11 apparently unreduced PMCs contained each only two large groups (Fig. 17). In 28 PMCs that have probably undergone a polarized segregation, groups of eight and sixteen chromosomes, respectively, were found (Fig. 16). In a single PMC, a group of eight chromosomes was observed, whereas another nucleus remained undivided.

Degeneration of numerous PMCs proceeded further during the second meiotic division, only a few cells remaining viable in some loculi.

Table 3. Tetrad stage.

Type	N of studied PMCs
Normal tetrads	63
Tetrads with microcyte(s)	28
Dyads	16
Dyads with microcyte(s)	7
Triads	1
Polyads	147
Total	262

Tetrad stage was studied in 262 PMCs (Table 3). On the whole, various polyads prevailed in the investigated material (Fig. 19); however, the frequency of given types varied from one loculus to another and apparently normal tetrads predominated in some anthers (Fig. 18). Tetrads with microspores of unequal size, perhaps representing various chromosome numbers were also found. Dyads of unreduced microspores were not very frequent (Fig. 20).



Figs 18 - 20. Tetrad stage. 18. An apparently normal tetrad. 19. Polyad. 20. Dyad of unreduced microspores. (c.) 1600 x.

The viability of microspores at the tetrad stage decreased in some anthers to about 70%. An individual variability was noted in this respect, two triploid hybrids being particularly liable to degeneration (72% and 66%, respectively). Incidentally, some tetrads or polyads were only partly degenerated; it can be assumed that either the degeneration did not proceed simultaneously in all microspores, or more balanced ones might have retained their viability.

The pollen was very irregular, various microcytes being particularly frequent (Fig. 21). Viable, well-stained pollen grains represented two categories: 1/ smaller grains of about 16.5 - 19.5  $\mu\text{m}$  in diameter and 2/ large, apparently unreduced grains (modal value of about 25  $\mu\text{m}$ ); the latter type was much less frequent than the former one (Figs 22 - 23). In spite of size differences occurring between smaller pollen grains, it was not possible to distinguish particular classes.

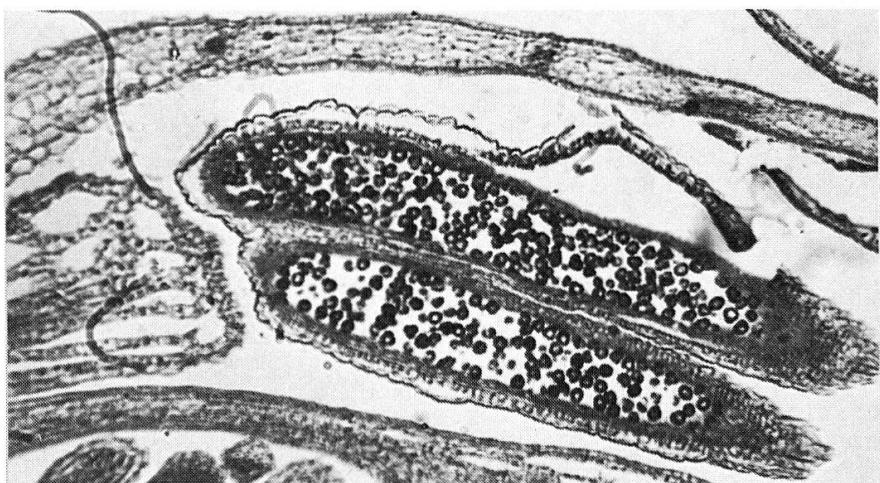
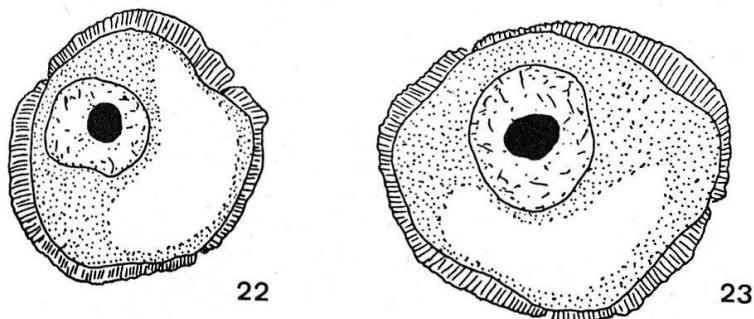


Fig. 21. Microtome section of a dehiscent anther. (c.) 100 x.



Figs 22 - 23. Pollen grains. 22. A smaller pollen grain. 23. An apparently unreduced pollen grain. (c.) 1000 x.

Each of the five studied hybrid plants represented, on the whole, a different level of pollen viability (Table 4); however, the ratio: viable/ degenerated grains varied strikingly even within a single flower of a given individual.

Thus, it seems probable that the triploid *Cardamine* hybrids from Urnerboden produce various categories of the pollen. Numerous aneuploid grains result, on the one hand, from a random segregation of chromosomes at I anaphase. On the other hand, truly reduced i.e. 12chromosomal grains are in fact aneuploid: they contain one genome of *Cardamine rivularis* but only half a genome of *C. amara* (8<sub>R</sub>4<sub>R</sub>). As far as the euploid pollen grains

are concerned, three types may appear:

a/ unreduced grains of a genomic constitution RRA i.e. containing 16 chromosomes of *C. rivularis* and 8 of *C. amara*;

b/ 16chromosomal RA grains containing a single genome of either parent species;

c/ 8chromosomal R grains containing a sole genome of *C. rivularis*.

The two latter types seem to be formed most frequently through a polarized segregation; however, it cannot be excluded that a random distribution of chromosomes may sometimes result in the formation of such pollen grains.

Marked decrease in viability, beginning already at interkinesis and further proceeding at later developmental stages, is probably due to an unbalanced genetical structure of numerous spores that are eliminated.

Table 4. Viability of the pollen at the uninucleate stage.

Code No. of hybrid	N of studied pollen grains		Total
	Apparently viable pollen grains	Degenerated pollen grains	
167/1	109 34.1%	211 65.9%	320
168/24	76 29%	186 71%	262
168/40	128 45.7%	152 54.3%	280
168/350	159 51.3%	153 48.7%	312
168/351	81 26.1%	229 73.9%	310

### 3.2.2. *Macrosporogenesis*

The hybrids have campylotropous tenuinucellate ovules with multicellular archespor. Earlier stages of meiosis occurred non-simultaneously

in at least three EMCs; however, the macrosporogenesis was completed in a sole EMC situated along the long axis of the ovule, directly under the nucellar layer, whereas the lateral EMCs fell behind and eventually degenerated.

Observations on macrosporogenesis were carried out on rather a limited material, only 86 ovules being studied both in the pollen-producing as well as the pollen-sterile hybrids (Table 5).

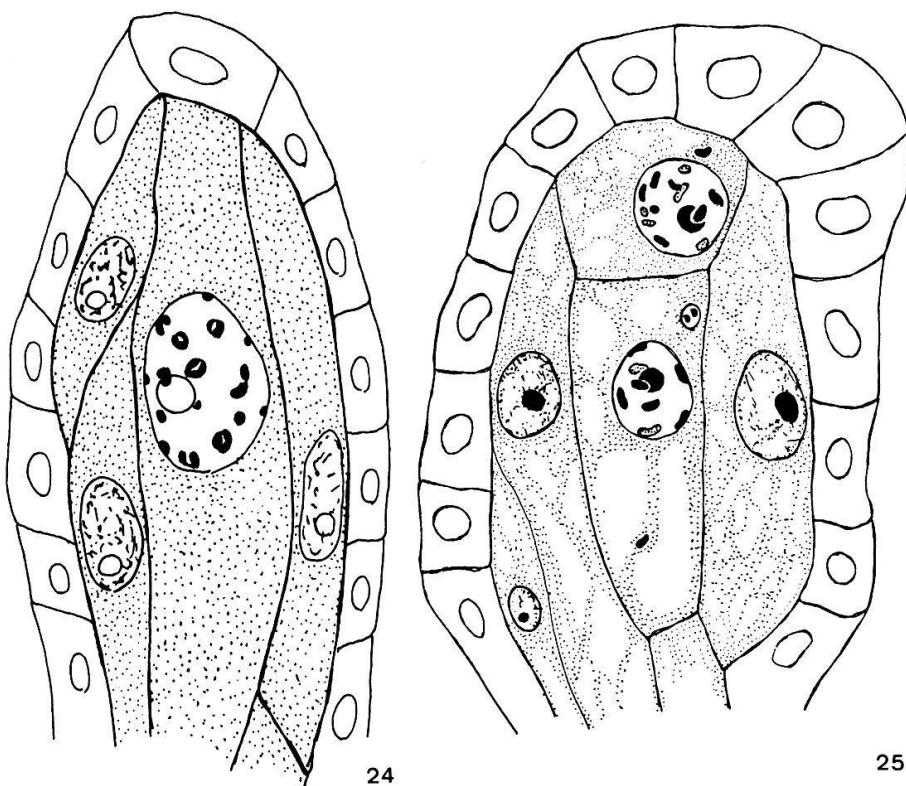
Earlier stages of meiosis were apparently normal; however, at diakinesis, univalents were found in all studied EMCs. Eight bivalents and eight univalents occurred in 9 out of the 11 analysed EMCs (Fig. 24). In the two remaining ones, a pronounced asyndesis was observed.

I metaphase represented a normal aspect, chromosomes being congressed in the equatorial plate. Only in two EMCs was it possible to analyse all associations of chromosomes; they were  $8_{II}8_I$  and  $3_{II}18_I$  respectively.

Table 5. Developmental stages in young ovules.

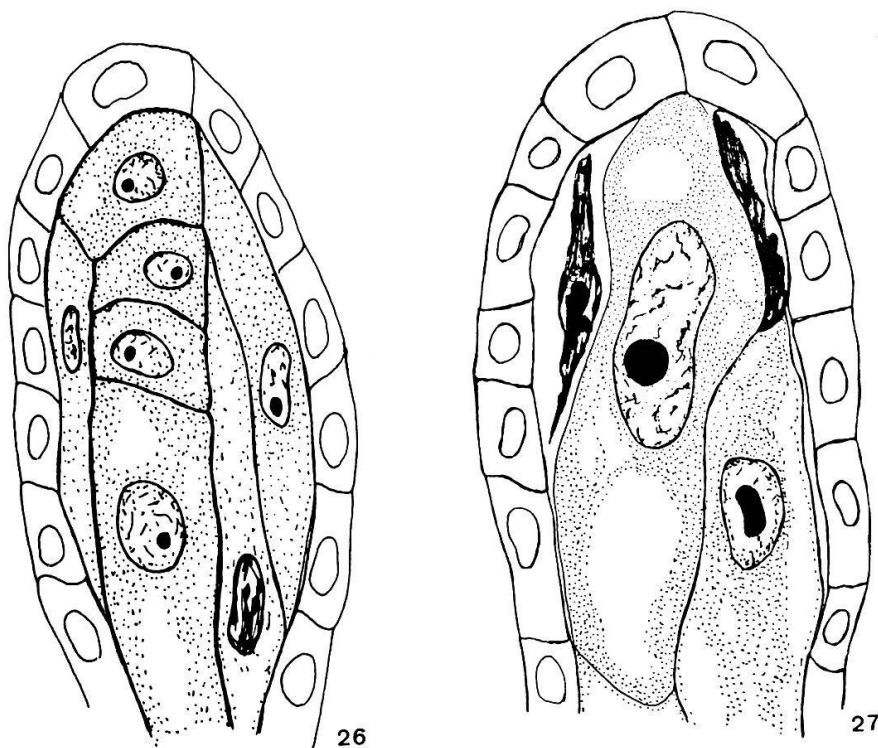
Stage	Number of studied ovules
Leptoten	14
Zygoten	6
Pachyten	16
Diploten	3
Diakinesis	11
I Metaphase	4
I Anaphase	8
I Telophase	-
Interkinesis	4
2nd meiotic division	4
Tetrad	16
Total	86

I anaphase was very irregular: precocious chromosomes as well as lagging ones, some of them being lost in cytoplasm, were observed in most of the studied EMCs. Distribution of chromosomes into two distinct groups was found only once; defective staining rendered impossible a complete analysis, but in one group 16 - 17 chromosomes were counted.



24

25



26

27

Figs 24 - 27. Macrosporogenesis. 24. Diakinesis: 8 II 8 I. 25. Interkinesis: nuclei containing respectively 8 and 12 chromosomes, the remainder lost in cytoplasm. 26. Tetrad of macrospores. 27. Unreduced uninucleate ES.  
(c.) 1600 x.

Out of four ovules studied at interkinesis, one contained a single cell with a large, irregularly shaped nucleus apparently resulting from restitution. In three remaining ovules dyads of macrospores were found, the chalazal macrospore being larger than the micropylar one. In either cell small supernumerary nuclei and/or single chromosomes lost in cytoplasm were observed (Fig. 25). Distribution of chromosomes within main nuclei of the chalazal versus micropylar macrospore of the dyad was most unusual. The following types were found: 8 vs 12 (Fig. 25), 10 vs 9 and 16 vs 6.

The second meiotic division was not always simultaneous in both cells of the dyad and the chalazal macrospore usually was the first to undergo the divisions. Several lagging chromosomes were observed; as a result, small supernumerary nuclei occurred in some tetrads.

All studied tetrads were linear (Fig. 26). In some ovules, macrospores with nuclei of an unequal size were noted. Vacuolation of the cytoplasm in the chalazal macrospore took place rather early and subsequent development of the young uninucleate ES progressed simultaneously with degeneration of the sister macrospore. A single ovule contained the developing ES with a large, probably unreduced nucleus (Fig. 27).

The above presented results suggest that mechanisms involved in the development of ovules in the 24chromosomal hybrids are very similar to those operating during the microsporogenesis (see pp. 47-54). As a result, three balanced types of macrospores i.e. R, RA and RRA may occur in addition to various aneuploid ones. Low frequency of viable embryo sacs can be attributed to their selective development.

### 3.2.3. *Remarks on the seed setting after open pollinations*

Field observations on the seed setting after open pollinations have not yet been completed in triploid hybrids, the subject being investigated in a long-term program. For this reason, only preliminary data are available.

Numerous visiting insects were noticed on expanded hybrid flowers; it is interesting to note that not only the pollen producing plants but also those with non-dehiscent anthers were visited. 87 hybrids were tested for the presence of the pollen on stigmas; various-sized grains, some of

them producing pollen tubes, were found in all studied flowers. Variable amount of pollen was observed: some stigmas were literally loaded with pollen, whereas the others apparently received only a few grains.

The 24chromosomal hybrids manifested a pronounced seed sterility, only five-six seeds developing in a single flowering shoot. Compared to the number of flowers within inflorescence and, accordingly, to the number of ovules, the seed output reached on average 0.8% - 1.1%. It should be emphasized that developing seeds were observed also in the male-sterile plants predominating at Urnerboden. One might assume that very low seed fertility of the triploid hybrids might be partly compensated by the great number of flowering shoots; detailed investigations on the population level are, however, indispensable for more precise estimations.

A few hybrid plants with developing seeds were transferred from Urnerboden to the experimental greenhouse of the Geobotanical Institute, where the seed setting was completed. A germination test was then carried out on 21 seeds.

Seeds resulting from open pollinations in the wild were of various sizes, some being very small; each of them nevertheless contained fully developed embryo, visible under a semi-transparent seed-coat. Seeds remained dormant on the moist blotting paper for about two months. It is very interesting to note that all seeds gave rise to seedlings that were rather vigorous, the first leaf and 1 - 2 roots appearing seven days after the beginning of germination. The hypocotyle of 3-day-old seedlings was tinged with anthocyan and this shade appeared rather soon at the base of the leaves, whereas cotyledons remained most frequently bright-green. As in the other taxa of *Cardamine*, a heteroblastic development was noted also in the hybrid seedlings; the first three-four leaves were simple, the later appearing ones being compound. The chromosome number studied in all 21 seedlings was invariably the same as that of their mother plants i.e.  $2n=24$  with a genomic constitution RRA.

It may be inferred that seed sterility in triploid plants is conditioned by strong selective factors. First of all, an elimination of numerous micro- and macrospores undoubtedly results in a greatly reduced gamete pool. Secondly, there might be a selection occurring at the stigma after

the pollination. Finally, it is conceivable that some zygotic combinations are eliminated. All these mechanisms add up to a very limited seed production; on the other hand, seeds that sustain the pressures of selection apparently are vigorous. It seems therefore, that sexual reproduction of hybrids may contribute to the development of the population. Curiously enough, no aneuploid segregates occurred in the offspring of the 24chromosomal plants, only replicas of the mother plants having been found. Further investigations are continued.

### 3.3. *Experimental crosses*

The aim of experimental crosses was twofold: a/ verification of the functional gametes in the 24chromosomal hybrids and b/ study on genetic isolation barrier(s) occurring between hybrids and their parent species. The following experiments were carried out:

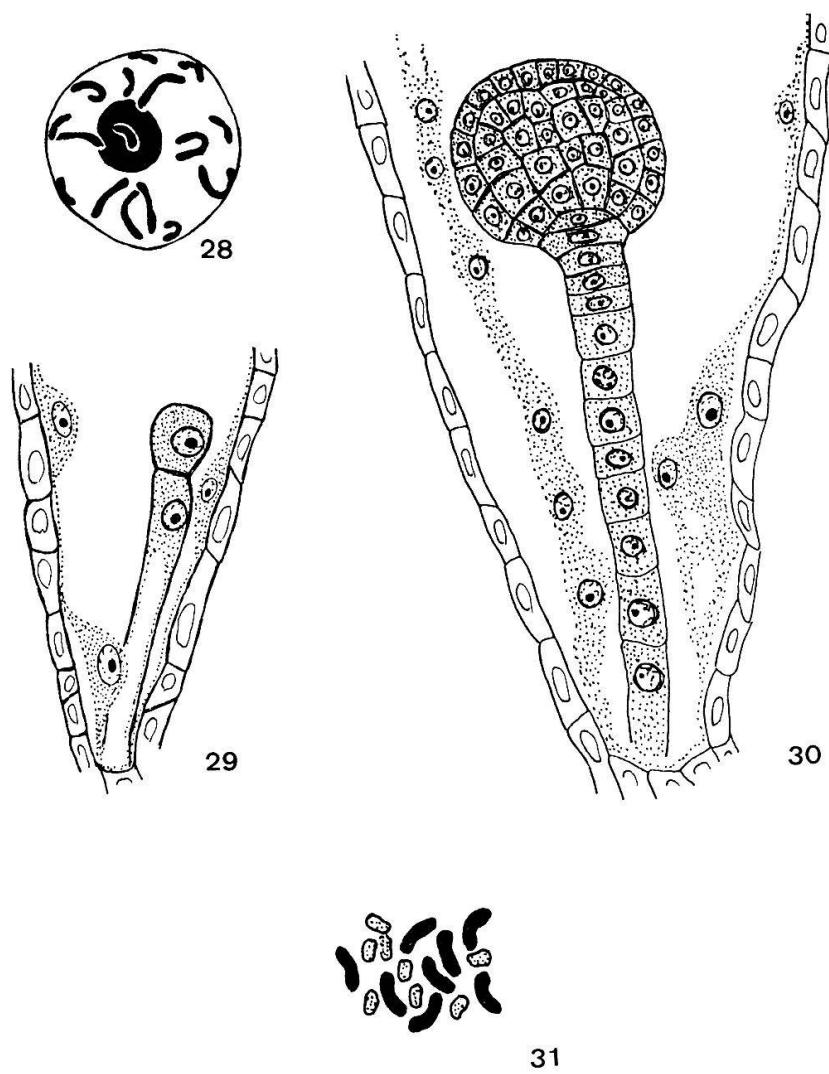
- 1/ crosses between *Cardamine rivularis* and *C. amara*
- 2/ outcrosses and selfings within the triploid hybrids
- 3/ backcrosses to both parent species
- 4/ crosses between various backcross plants
- 5/ crosses between natural triploids and some backcross progeny
- 6/ crosses between *Cardamine rivularis* and some backcross plants.

Experimental pollinations were performed in two consecutive years 1973 - 1974, except for backcrosses run in four series from 1973 to 1976. The triploid hybrids used in crosses represented both pollen-producing and pollen-sterile types. *Cardamine amara* used in experiments originated from Urnerboden only, whereas *C. rivularis* was selected from several stations.

#### 3.3.1. *Crosses between Cardamine rivularis (2n=16) and C. amara (2n=16)*

On the whole, 206 flowers were pollinated; however, only a limited number of fertilized embryo sacs was found within the control material fixed at random by five and twelve days after pollination, respectively (Table 6). A slightly higher percentage of fertilized ESs was observed in crosses with *Cardamine rivularis* used as a female parent. Fertilized ESs from both

reciprocal crosses, fixed by five days after pollinations contained most frequently the zygote and a few endosperm nuclei; the first mitosis in the zygote was once observed and a two-celled embryo was found in a single ovule (Fig. 29). By 12 days after pollinations, all embryos were multi-cellular (Fig. 30). Conforming to expectation, a diploid chromosome number  $2n=16$  was found in some cells of the suspensor, the chromosomes corresponding respectively to *C. rivularis* and *C. amara* (Fig. 28). It should be noted that the development of the endosperm was apparently slow and seemed defective in older embryo sacs.



Figs 28 - 31. Experimental crosses between *Cardamine rivularis* and *C. amara*.  
 28. A prophase in the suspensor:  $2n=16$  ( $8\frac{8}{8}$ ). (c.) 2000 x. 29 - 30. Young embryos. (c.) 1000 x. 31. Root-tip metaphase of the experimental hybrid:  
 $2n=16$  ( $8\frac{8}{8}$ ). (c.) 2000 x.

Table 6. Frequency of fertilized ESs resulting from experimental crosses

Cross*	Time lapse	Number of siliques	Total number of ovules	Number of fertilized ovules
<i>C. rivularis</i>				
x	5 days	6	168	19
<i>C. amara</i>				11.3%
<i>C. rivularis</i>				
x	12 days	5	110	21
<i>C. amara</i>				19.1%
-----				
<i>C. amara</i>				
x	5 days	5	173	12
<i>C. rivularis</i>				6.9%
<i>C. amara</i>				
x	12 days	5	156	16
<i>C. rivularis</i>				10.3%

\* female parent listed first unless otherwise mentioned.

The seed output was very limited (Table 7). It is interesting to note that crosses with *C. rivularis* used as female parent yielded very few seeds but nearly all of them were sound-looking; on the other hand, numerous seeds developed in the reciprocal cross-direction, but most of them were empty.

Table 7. Experimental crosses between *Cardamine rivularis* and *C. amara*

Cross type	Number of pollinated flowers	Number of seeds*	Number of seedlings	2n	Genomic constitution
<i>C. rivularis</i>					
x	65	15 (14)	3	16	RA
<i>C. amara</i>					
-----					
<i>C. amara</i>					
x	120	243 (6)	1	16	RA
<i>C. rivularis</i>					

\* number of sound-looking seeds given in brackets.

No germination occurred in normal conditions; however, some seedlings were obtained after the removal of the testa. Four of those developed into young plants, whereas the others died after eight days.

The development of rosettes was completed in about five months. The cytological control proved that the plants indeed were hybrids, their chromosome number being diploid ( $2n=16$ ) and their genomic constitution RA (Fig. 31). They persisted in form of rosettes for two years yet never flowered and eventually perished.

The fragmentary morphological description given below may serve for any of the four diploid hybrids, for they are all alike. No stolons were present; thin rhizomes was often very short. Rosette leaves resembled those of *Cardamine rivularis* i.e. consisted of numerous small, orbiculate or obovate leaflets, the lateral ones diminishing gradually in size towards the base of the petiole. On the other hand, sparse hairs occurring at the leaf margin were longer than those of *C. rivularis* (0.2 mm versus 0.02 - 0.04 mm) and virtually represented the only character corresponding to *C. amara*.

It can be concluded from the above presented data that the incompatibility occurring between *Cardamine rivularis* and *C. amara* manifests itself not only at pre-mating stages but also after fertilization. Both seed development as well as the growth of young hybrid plants seem to be affected; sterility of the hybrids may represent a further aspect of this phenomenon.

*C. rivularis* and *C. amara* often occur side by side within the subalpine zone (URBANSKA-WORYTKIEWICZ and LANDOLT 1972, URBANSKA-WORYTKIEWICZ and LANDOLT unpubl.). The flowering time of the two species overlaps, at least partly. It may be inferred that the incompatibility represents the main barrier to hybridization between these two taxa. The present results give thus an explanation to the unsuccessful attempts of LÖVKVIST (1956, 1957).

### 3.3.2. Selfings and cross-pollinations in triploid hybrids

Disturbances in micro- and macrosporogenesis observed in the 24chromosomal plants apparently lead to a pronounced sterility both in anthers as well as the ovules. The results of selfings and cross-pollinations further

confirm the study on meiosis; they also corroborate our preliminary observations on natural seed setting (see pp. 57-59).

On the whole, very few seeds were obtained (Table 8). No seeds developed from selfings; this result suggests that the limited sexual reproduction of the triploid plants is based on allogamy. Further studies are needed to decide whether the allogamy is predominant or complete, but the hybrids correspond generally in this respect both to their parent species as well as to various other taxa of the genus *Cardamine* (LÖVKVIST 1956, URBANSKA-WORYTKIEWICZ and LANDOLT 1974, URBANSKA-WORYTKIEWICZ unpubl.).

Table 8. Selfings and cross-pollinations in natural triploid hybrids.

Pollination type	Number of pollinated flowers	Number of seeds	Number of seedlings	2n	Genomic constitution
Selfing	38	-	-	-	-
Cross-pollination	33	11	4 1	24 32	RRA RRRA

Out of five seedlings obtained from cross-pollinations, four had the same chromosome number ( $2n=24$ ), genomic constitution (RRA) and general morphology as their parents. They all were male-sterile with non-deshiscent anthers. The appearance of these plants does not offer any positive information about gametic types functioning in the triploids, for they might have developed either through an union of the two reduced (i.e. 12chromosomal) gametes or result from a combination of two polarized ones, respectively carrying 8(R) and 16(RA) chromosomes. On the other hand, the 32chromosomal plant comprising three genomes of *C. rivularis* and a single genome of *C. amara* does prove the occurrence of 8chromosomal polarized gamete (R) as well as the unreduced one (RRA).

### 3.3.3. Backcrosses

Reciprocal backcrosses to both parent species were performed on 232 flowers (Table 9). Attempts to cross the triploid hybrids with *Cardamine*

*amara* remained unsuccessful; on the other hand, backcrosses to *C. rivularis* yielded some seeds that subsequently gave rise to young plants.

Table 9. Backcrosses to both parent species

Cross type	Number of pollinated flowers	Number of seeds	Number of seedlings
Triploid hybrid x <i>C. rivularis</i>	61	32	24
<i>C. rivularis</i> x triploid hybrid	39	11	3
Triploid hybrid x <i>C. amara</i>	67	-	-
<i>C. amara</i> x triploid hybrid	65	-	-

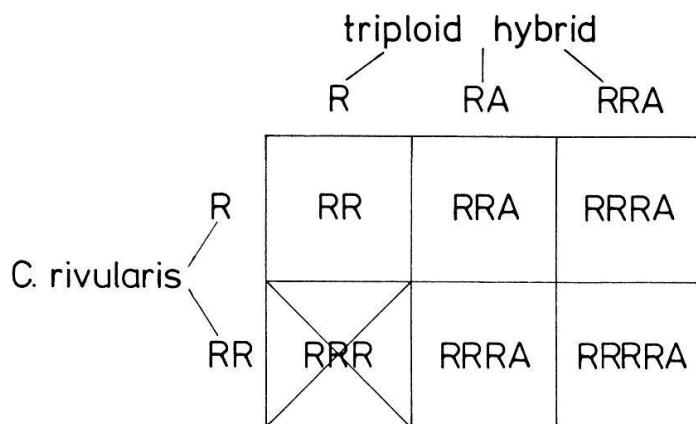
Thus, incompatibility barriers isolating the triploid hybrid from *Cardamine amara* apparently are not complete as far as the second parent i.e. *C. rivularis* is concerned. However, the backcrosses do not follow the pattern of the Mendelian segregation (Fig. 32).

The backcross plants revealed an interesting diversity in their morphology, chromosome number as well as the genomic constitution (Fig. 33 - 40, Table 10). Their most amazing feature, however, was that all of them were euploid viz. diploid ( $2n=16$ , RR), triploid ( $2n=24$ , RRA), tetraploid ( $2n=32$ , RRRA) and pentaploid ( $2n=40$ , RRRRA).

The further experimental evidence has therefore been obtained as to the functioning of some particular gamete types in the triploid hybrids. It should be stressed that out of five theoretically possible combinations, four have been realized (Fig. 32); the backcrosses revealed as well that unreduced gametes of *Cardamine rivularis* may sporadically be functional.

Table 10. Backcross types

Backcross	2n	Genomic constitution	Number of plants	Pollen fertility
<i>C. rivularis</i> x triploid hybrid	16	RR	3	12-18%
Triploid hybrid x <i>C. rivularis</i>	16 24 32 40	RR RRA RRRA RRRR	15 5 2 2	6-16% (28%) nil nil 52%



R = genome of *C. rivularis*

A = genome of *C. amara*

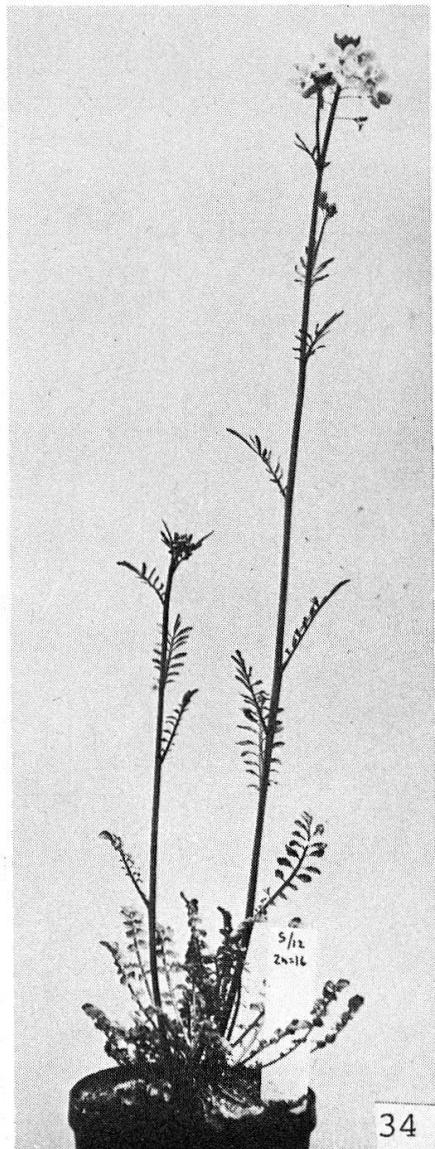
Fig. 32. Behaviour of triploid hybrids in backcrosses to *C. rivularis*.

It seems probable that the functioning of gametes in some backcross combinations is influenced either by certation or some other selective mechanisms. The 8chromosomal gametes (R) of the hybrid not only appeared to be most successful competitors on the diploid stigmas of *C. rivularis*, but were apparently favourized in the opposite backcross direction, comparable patterns occurring in the experiments performed in four consecutive seasons 1973 - 1976.

Morphological description of backcross plants



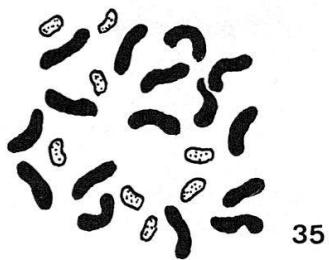
33



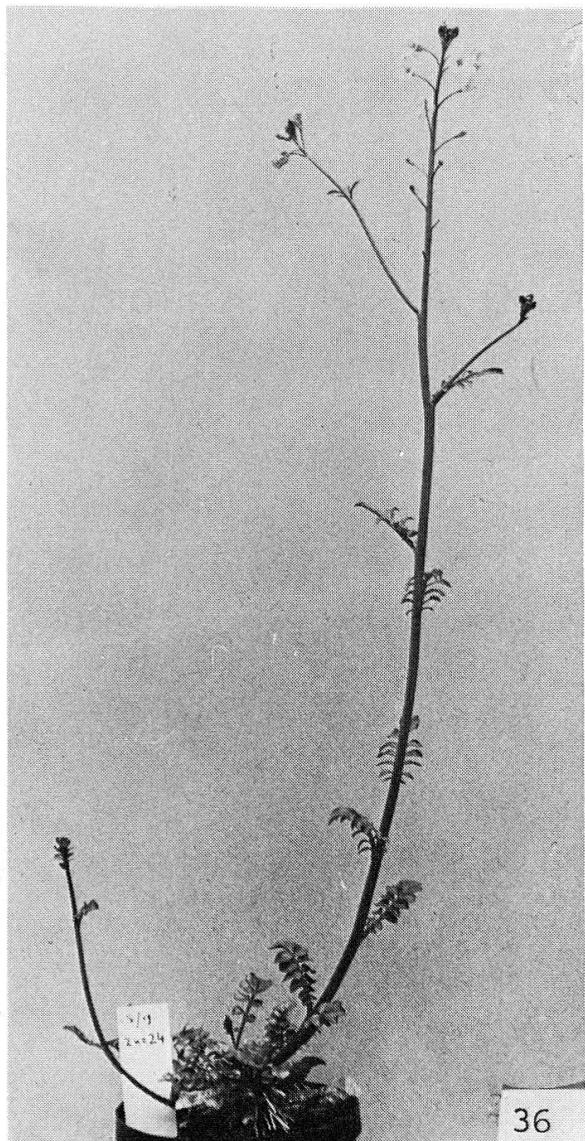
34

Figs 33 - 34. Diploid backcross plants  $2n=16$  (16<sub>R</sub>). 33. Root-tip metaphase (c.) 2000 x. 34. Habitus (about 1/3 life size).

Rhizome thin, rather short. Stems in number 1-4, usually simple, upright, glabrous, up to 40 cm tall. Basal leaves 9-27-foliolate, prostrate, younger ones ascending; their terminal leaflets rather small, about 5-9 mm long, reniform at the base, smooth-edged, glabrous or with sparse hairs usually at the leaflet margin. Surface of the terminal leaflet less than twice as large as that of the nearest lateral leaflet. Lateral leaflets small, orbiculate or obovate, diminishing gradually in size towards the base of the petiole. Cauline leaves pinnate, in number 5-7, their leaflets about 1.7 mm broad. Upper cauline leaves much smaller than the middle ones. The second uppermost cauline leaf usually 9-11-foliolate, its terminal leaflet narrow, 1/2-3/4 times as long as the rest of the leaf. Inflorescence racemose, simple or exceptionally compound. Petals pinkish, 7-10 mm long. Anthers pale-yellow, dehiscent. Style 0.20-0.35 mm thick. Ovary with 12-28 ovules.



35



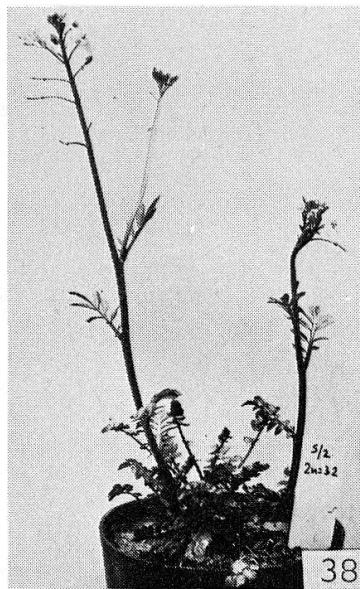
36

Figs 35 - 36. Triploid backcross plant  $2n=24$  (16.8). 35. Root-tip metaphase (c.) 2000 x. 36. Habitus (about  $\frac{1}{3}$  life size).

Plants with short stolons. Stems most frequently branched, in number 1-4, upright, glabrous, up to 43 cm tall. Basal leaves prostrate, 7-15-foliate; the terminal leaflet medium large, 9-12 mm long, dentate (usually 5 teeth), glabrous or with sparse hairs. Surface of the terminal leaflet usually more than twice as large as that of the nearest lateral leaflet. Lateral leaflets dentate, diminishing gradually in size towards the base of the petiole. Cauline leaves pinnate, in number 7-8, their lateral leaflets about 2.60 mm broad. Upper cauline leaves only slightly smaller, or of the same size as the middle ones. The terminal leaflet of the second uppermost cauline leaf usually dentate with 3 teeth, about 1/3-1/2 times as long as the rest of the leaf. Inflorescence racemose simple or compound. Petals pink, 6-8 mm long. Anthers uniformly dark-purple, non-dehiscent. Style 0.25-0.40 mm thick. Ovary with 9-36 ovules.



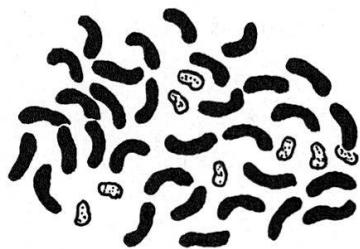
37



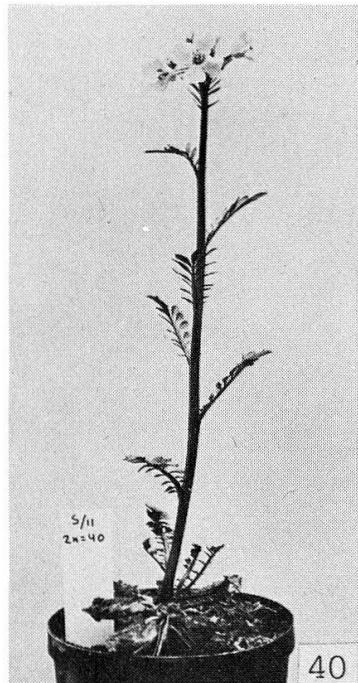
38

Figs 37 - 38. Tetraploid backcross plant  $2n=32$  (24<sub>R</sub> 8<sub>A</sub>). (c.) 2000 x.  
37. Root-tip metaphase. 38. Habitus (about 1/3 life size).

Plants with short stolons. Stems in number 1-4, simple or branched, upright, glabrous, about 21 cm tall. Basal leaves prostrate, 5-13-foliolate. The terminal leaflet 8-14 mm long, reniform at the base, smooth-edged or dentate (5 teeth), usually glabrous. Surface of the terminal leaflet often nearly as large as that of the nearest lateral leaflet. Lateral leaflets diminishing gradually in size towards the base of the petiole. Cauline leaves in number 4-6, pinnate, their petioles broader than those of the diploid and the triploid sibs. Upper cauline leaves of about the same size as the middle ones. The second uppermost cauline leaf 9-foliolate, its terminal leaflets slightly shorter than the rest of the leaf. Lateral leaflets of the cauline leaves 2.5-3 mm broad, diminishing rather abruptly in size towards the base of the petiole, the uppermost ones nearly as long as the terminal leaflet. Inflorescence racemose simple, or compound. Petals pale-pink, 6-9 mm long. Anthers pale-yellow, non-dehiscent. Style 0.60-0.80 mm stick. Ovary with 12-34 ovules.



39



40

Figs 39 - 40. Pentaploid backcross plant  $2n=40$  (32<sub>R<sub>A</sub></sub> 8). 39. Root-tip meta-  
phase. (c.) 2000 x. 40. Habitus (about 1/3 life size).

Plants with short stolons. Stems simple, in number 1-2, glabrous, up to 20 cm tall. Basal leaves prostrate, 9-15-foliolate. The terminal leaflet 9-12 mm long, reniform at the base, dentate with 5 teeth, usually glabrous. Surface of the terminal leaflet a little larger than that of the nearest lateral leaflet. Lateral leaflets diminishing slightly in size towards the base of the petiole, the uppermost ones nearly as long as the terminal leaflet. Cauline leaves pinnate, petiole rather broad, number of cauline leaves 8-9. The uppermost cauline leaves of about the same size as the middle ones. The second cauline leaf 9-foliolate, its terminal leaflet about 1/2 as long as the rest of the leaf. Inflorescence racemose simple. Petals pink, 8-12 mm long. Anthers yellow with faint reddish patches, dehiscent. Style 0.45-0.55 mm thick. Ovary with 18-36 ovules.

### 3.3.4. Further experimental crosses

The following series were involved: a/ crosses between various backcross types; b/ crosses between natural triploid hybrids and some backcross progeny and c/ crosses between *Cardamine rivularis* and some backcross plants.

Crosses between various backcross plants yielded seedlings only in two combinations (Table 11). It is interesting to note that diploid RR offspring was obtained not only from crosses between 16chromosomal plants but also from a combination: triploid x diploid with the triploid used as a female parent.

Table 11. Crosses between various backcross plants

Cross	Number of pollinated flowers	Number of seeds	Seedlings		
			Number of plants	2n	Genomic constitution
Diploid					
x	47	12	9	16	RR
diploid					
Diploid					
x	29	-	-	-	-
pentaploid					
Triploid					
x	18	4	1	16	RR
diploid					
Triploid					
x	15	-	-	-	-
pentaploid					
Tetraploid					
x	31	-	-	-	-
diploid					
Tetraploid					
x	19	-	-	-	-
pentaploid					
Pentaploid					
x	17	-	-	-	-
diploid					
Pentaploid					
x	14	2	-	-	-
pentaploid					

Table 12. Further crosses with backcross plants involved

Cross	Number of pollinated flowers	Number of seeds	Seedlings		
			Number of plants	2n	Genomic consti- tution
Diploid backcross plant					
x	23	3	-	-	-
natural triploid hybrid					
Natural triploid hybrid					
x	18	8	3	16 24	RR(2) RRA(1)
diploid backcross plant					
Diploid backcross plant					
x	38	14	6	16	RR
<i>Cardamine rivularis</i>					
<i>Cardamine rivularis</i>					
x	32	21	4	16	RR
diploid backcross plant					
Triploid backcross plant					
x	14	3	1	24	RRA
natural triploid hybrid					
Triploid backcross plant					
x	11	2	1	24	RRA
<i>Cardamine rivularis</i>					
Tetraploid backcross plant					
x	12	-	-	-	-
natural triploid hybrid					
Tetraploid backcross plant					
x	16	1	-	-	-
<i>Cardamine rivularis</i>					
Pentaploid backcross plant					
x	22	-	-	-	-
natural triploid hybrid					
Natural triploid hybrid					
x	12	-	-	-	-
pentaploid backcross plant					
Pentaploid backcross plant					
x	29	-	-	-	-
<i>Cardamine rivularis</i>					
<i>Cardamine rivularis</i>					
x	24	-	-	-	-
pentaploid backcross plant					

Further crosses involving backcross plants as well as natural triploid hybrids and *Cardamine rivularis* were performed in 12 series (Table 12). The obtained results bring about a further experimental evidence as to

the functioning of polarized R and RA gametes in the 24chromosomal hybrids, individuals from the wild as well as those experimentally reconstructed behaving alike. Furthermore, the experiments indicate that diploid backcross plants may replace a genuine *C. rivularis* in crosses with the triploids.

#### 4. Evaluation of the breeding behaviour of the triploid hybrids

The results obtained in the course of the present study strongly suggest a non-Mendelian segregation occurring in the 24chromosomal plants from Urnerboden. It is conceivable that two complementary phases i.e. a selective sporogenesis and a zygotic selection are involved, the former mechanism eliminating all aneuploid spores or most of them, whereas the latter one may favourize only some of euploid zygotes.

No positive proof was obtained as to the functioning of truly reduced, 12chromosomal gametes in the triploid hybrids; the 24chromosomal plants obtained in our experimental crosses might as well have arisen from an union of polarized gametes with the respective chromosome numbers 8 and 16. On the other hand, as much as 21 plants out of the total 25, might only have been formed with a part of one or both polarized gametes (Table 13).

Table 13. Plants obtained in various experimental crosses with triploid(s) used as one or both parents; the latter cases marked by a double asterisk (\*\*).

Chromosome number	Genomic structure	Possible way of origin*	Number of plants
2n=16	RR	R + R	15
2n=24	RRA	RA + R	5
2n=24**	RRA	RA + R or R1/2A + R1/2A	5
2n=32**	RRRA	RRA + R	1
2n=32	RRRA	RRA + R	2
2n=40	RRRRA	RRA + RR	2

\*putative gametes of triploids listed first.