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

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

by

KRYSTYNA URBANSKA-WORYTKIEWICZ

Contents

1. Introduction
2. Material and methods
3. Results
   - 3.1. Vegetative multiplication
   - 3.2. Sexual reproduction
     - 3.2.1. Microsporogenesis and development of pollen
     - 3.2.2. Macrosporogenesis
     - 3.2.3. Remarks on the seed setting after open pollinations
   - 3.3. Experimental crosses
     - 3.3.1. Crosses between *Cardamine rivularis* and *C. amara*
     - 3.3.2. Selfings and cross-pollinations in triploid hybrids
     - 3.3.3. Backcrosses
     - 3.3.4. Further experimental crosses
4. Evaluation of the breeding behaviour of the triploid hybrids
5. Discussion

Summary - Résumé - Zusammenfassung

References

1. Introduction

Natural hybridization between *Cardamine rivularis* and *C. amara* in a subalpine station at Urnerboden (Central Switzerland) was first noted in 1971. A general morphology and chromosome numbers of both parent species as well as the hybrids were then studied. It was found that the hybrids represented a triploid level (2n=24) although both their parent species were diploid with 2n=16. Differences in chromosome size occurring between *C. rivularis* and *C. amara* permitted to distinguish the resp. parent genomes within the triploid set: 16 chromosomes of *C. rivularis* type and 8 of *C. amara* occurred invariably in all 337 studied plants (URBANSKA-WORYTKIEWICZ and LANDOLT 1972, 1974, 1977, URBANSKA-WORYTKIEWICZ 1976, 1977b, URBANSKA-WORYTKIEWICZ unpubl.). Except for a few individuals, the 24chromosomic plant had non-dehiscent anthers or were extremely high sterile (97-98%). None the less, they indiscutably predominated within the population covering about 16 hectares.

The station at Urnerboden was often revisited during 1972-1977; in 1973, fertile hexaploid plants (2n=48) were found within a sector of the population (URBANSKA-WORYTKIEWICZ and LANDOLT 1974, 1977, URBANSKA-WORYTKIEWICZ 1976, 1977b).

The striking numerical supremacy of the triploids as well as the appearance of the hexaploids at Urnerboden suggested that particular mechanisms may favour the production of hybrids and their biological success. A detailed study on ecological genetics was therefore undertaken; the present paper deals with the reproduction of the 24chromosomic plants.

Acknowledgements

Mrs. A. HEGI took an exceptional care of the young experimental plants. Most of the fixations as well as the staining were made by Mrs. M. SIEGL. Photographs were taken by Mr. H. SIGG and Mrs. E. WOHLMANN-BRäM prepared some diagrams. Sincere thanks of the author are presented to all these persons.

2. Materials and methods

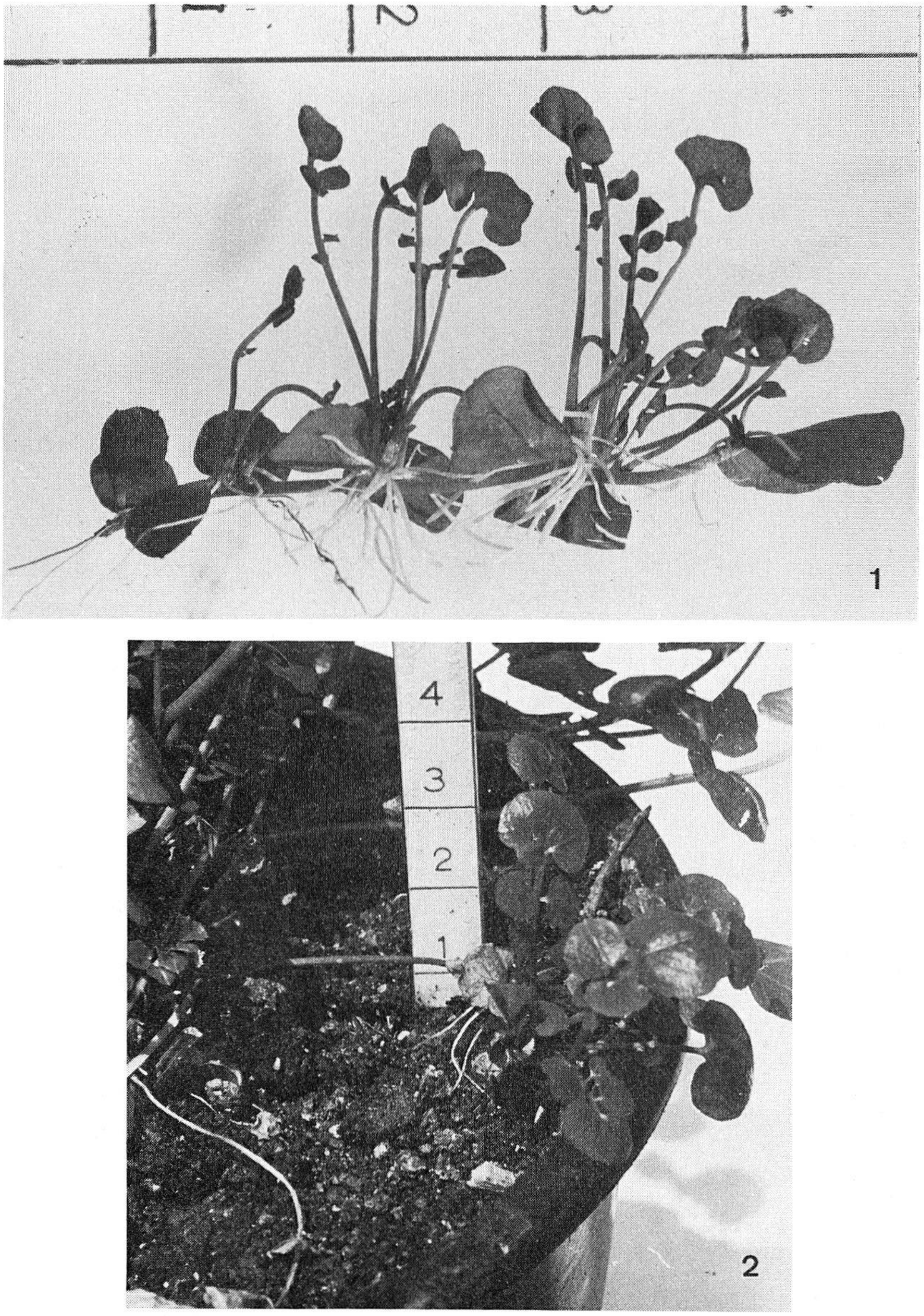
The material for the present study was collected during 1972-1977. Fixations were made both in the field as well as the experimental greenhouse of the Geobotanical Institute. The flower buds were fixed in acetic alcohol (1:3) with a small addition of ferric acetate and acetocarmine. Micro- and macrosporogenesis 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 gemination tests and raising of young plants.

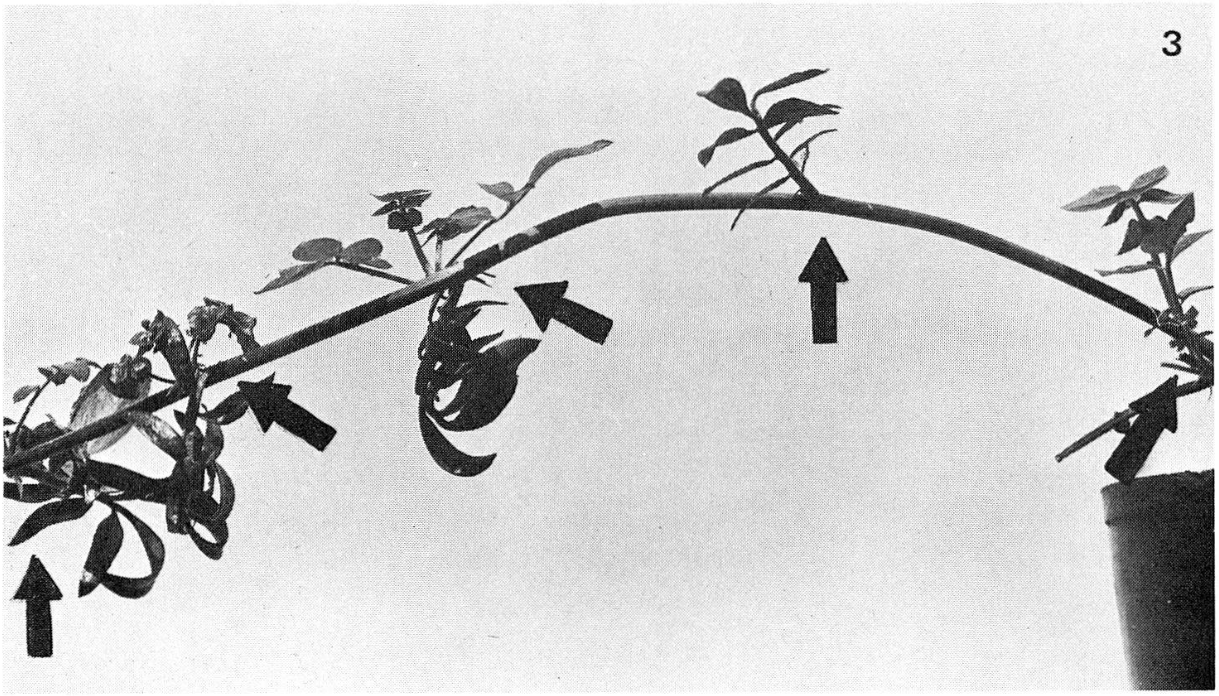
3. Results

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



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



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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 m^2 (URBANSKA-WORYTKIEWICZ and LANDOLT 1977). It can be inferred that vegetative multiplication of the 24chromosomic 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 univalents 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.

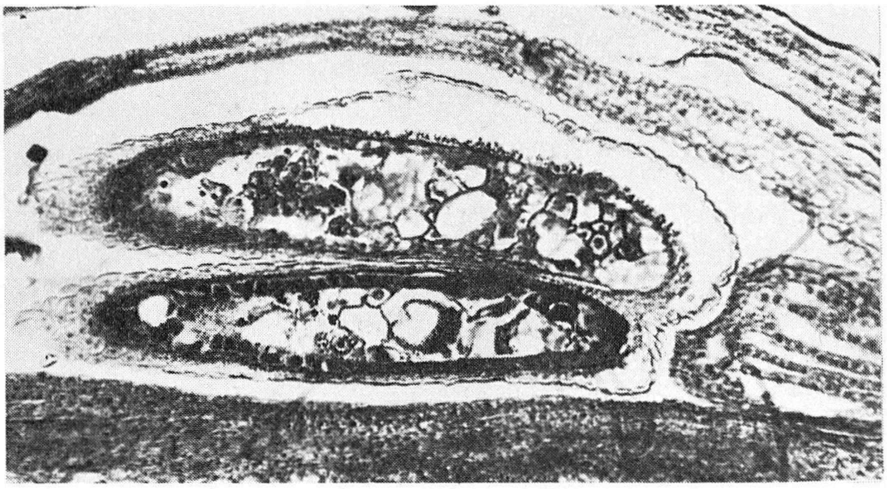
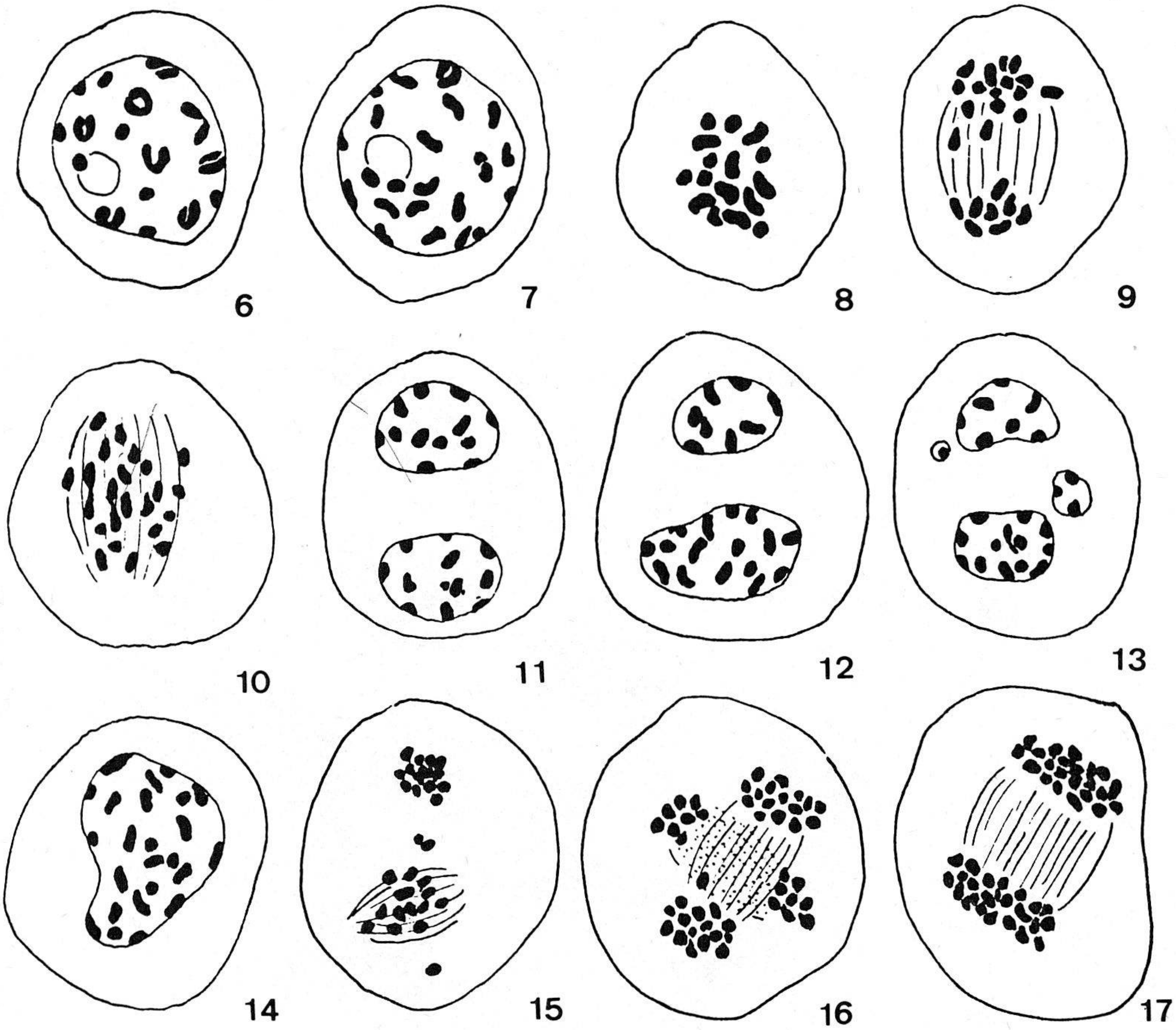


Fig. 5. Microtome section of a non-dehiscent anther. (c.) 100x.

Table 1. Chromosome pairing at diakinesis and I metaphase.

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



Figs 6-17. Microsporogenesis. 6. Diakinesis: $8_{II}8_{I}$. 7. Irregular diakinesis: $3_{II}18_{I}$. 8. I metaphase: $8_{II}8_{I}$. 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. IInd 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.

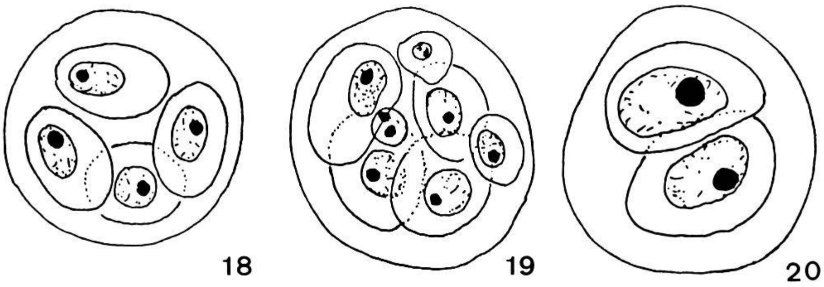
The second meiotic division was frequently very irregular, the chromosomes being scattered along the spindle or eliminated into the cytoplasm (Fig. 15). A regular distribution of chromosomes was observed only in 46 PMCs out of the 114 studied. It is interesting to note that they represented various types: a normal meiosis i.e. four 12chromosomic 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 µm in diameter and 2/ large, apparently unreduced grains (modal value of about 25 µm); 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.

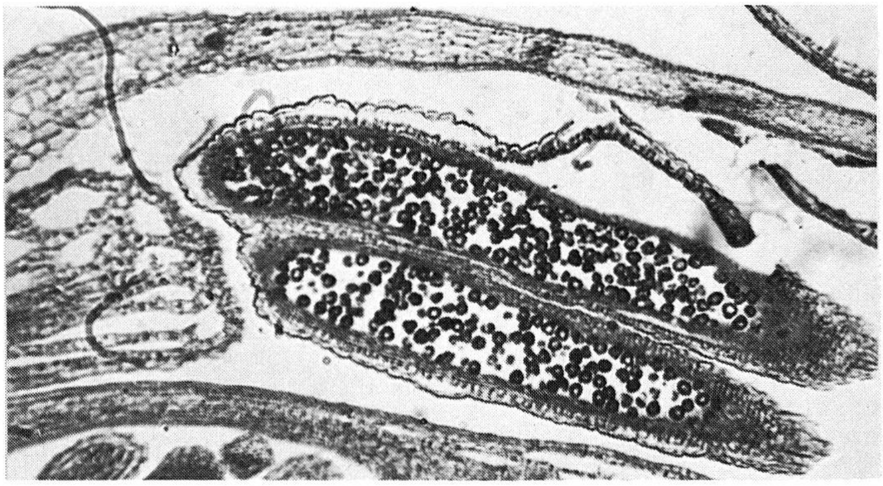
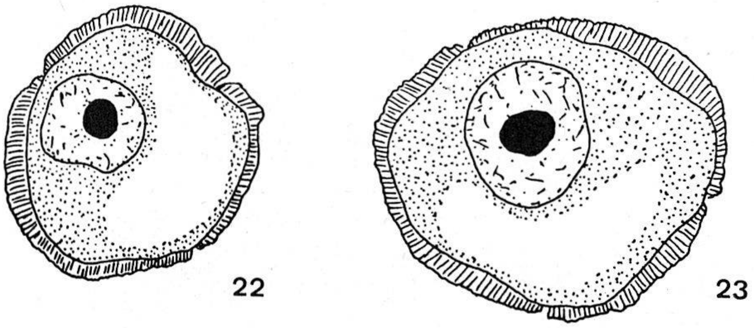


Fig. 21. Microtome section of a dehiscent anther. (c.) 100 x.



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 poolen 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. 12chromosomic grains are in fact aneuploid: they contain one genome of *Cardamine rivularis* but only half a genome of *C. amara* ($8_R 4_R$). 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/ 16chromosomic RA grains containing a single genome of either parent species;

c/ 8chromosomic 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).

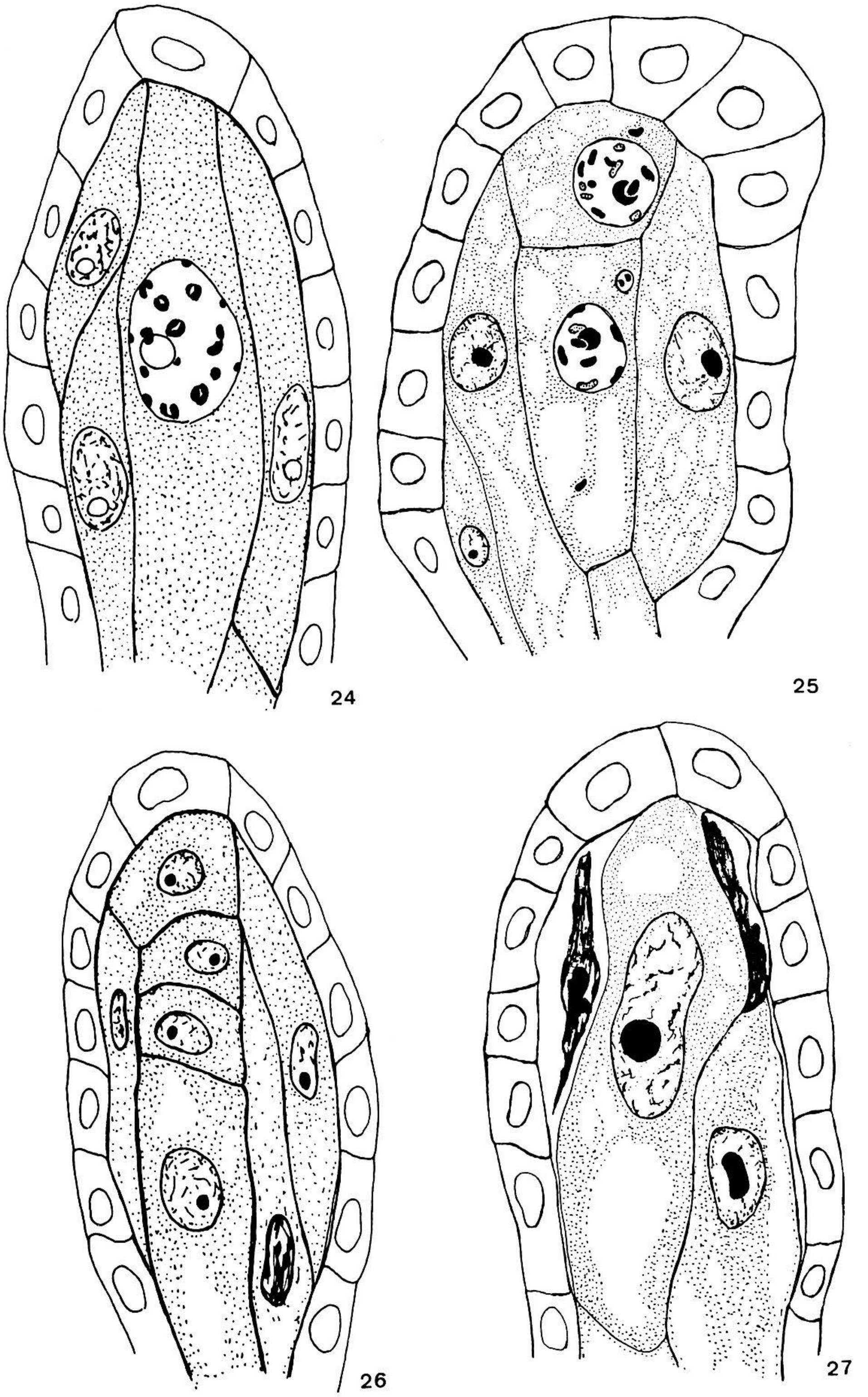
Earlier stages of meiosis were apparently normal; however, at diakinesis, univalents were found in all studied EMCs. Eight bivalents and eight univalents occurred in 9 out of the 11 analysed EMCs (Fig. 24). In the two remaining ones, a pronounced asyndesis was observed.

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.



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 24chromosomic 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 24chromosomic 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 Insitute, 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 mechanismsm 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 24chromosomic 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 24chromosomic 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 slighty 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.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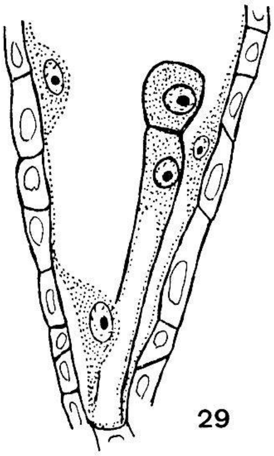
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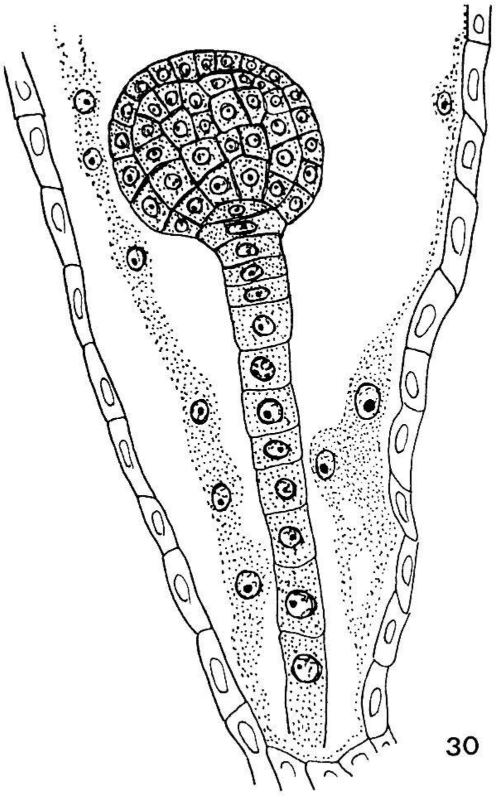
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Figs 28 - 31. Experimental crosses between *Cardamine rivularis* and *C. amara*. 28.A prophase in the suspensor: 2n=16 (8_R8_A).(c.) 2000 x. 29 - 30. Young embryos. (c.) 1000 x. 31. Root-tip metaphase of the experimental hybrid: 2n=16 (8_R8_A). (c.) 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
C. rivularis x *C. amara*	5 days	6	168	19 11.3%
C. rivularis x *C. amara*	12 days	5	110	21 19.1%
C. amara x *C. rivularis*	5 days	5	173	12 6.9%
C. amara x *C. rivularis*	12 days	5	156	16 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
C. rivularis x *C. amara*	65	15(14)	3	16	RA
C. amara x *C. rivularis*	120	243 (6)	1	16	RA

* 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 attemps of LöVKVIST (1956, 1957).

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

Disturbances in micro- and macrosporogenesis observed in the 24chromosomic 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	24	RRA
			1	32	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. 12chromosomic) gametes or result from a combination of two polarized ones, respectively carrying 8(R) and 16(RA) chromosomes. On the other hand, the 32chromosomic plant comprising three genomes of *C. rivularis* and a single genome of *C. amara* does prove the occurrence of 8chromosomic 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 *C. rivularis*	61	32	24
C. rivularis x triploid hybrid	39	11	3
Triploid hybrid x *C. amara*	67	-	-
C. amara x triploid hybrid	65	-	-

Thus,incompatibiliy 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
C. rivularis x triploid hybrid	16	RR	3	12-18%
Triploid hybrid x *C. rivularis*	16	RR	15	6-16% (28%)
	24	RRA	5	nil
	32	RRRA	2	nil
	40	RRRRA	2	52%

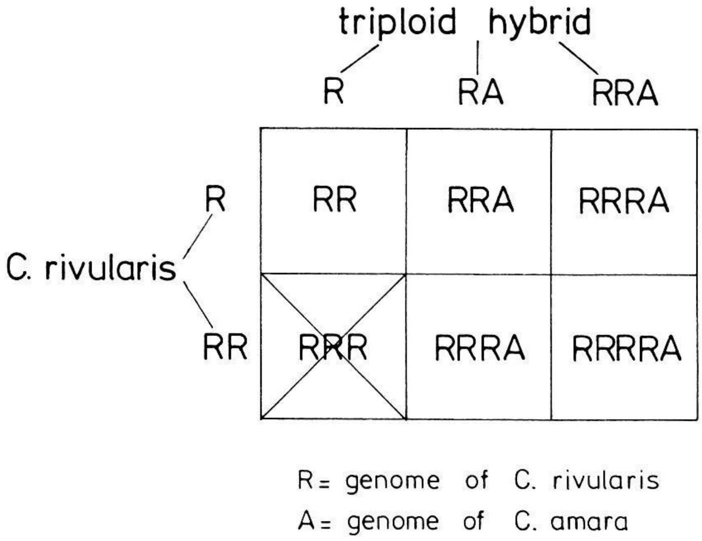

Fig. 32. Behaviour of triploid hybrids in backcrosses to *C. rivularis*.

It seems probable that the functioning of gametes in some backcross combinations is influenced either by certation or some other selective mechanisms. The 8chromosomic 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

Figs 33 - 34. Diploid backcross plants 2n=16(16_R). 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, deshiscent. Style 0.20-0.35 mm thick. Ovary with 12-28 ovules.

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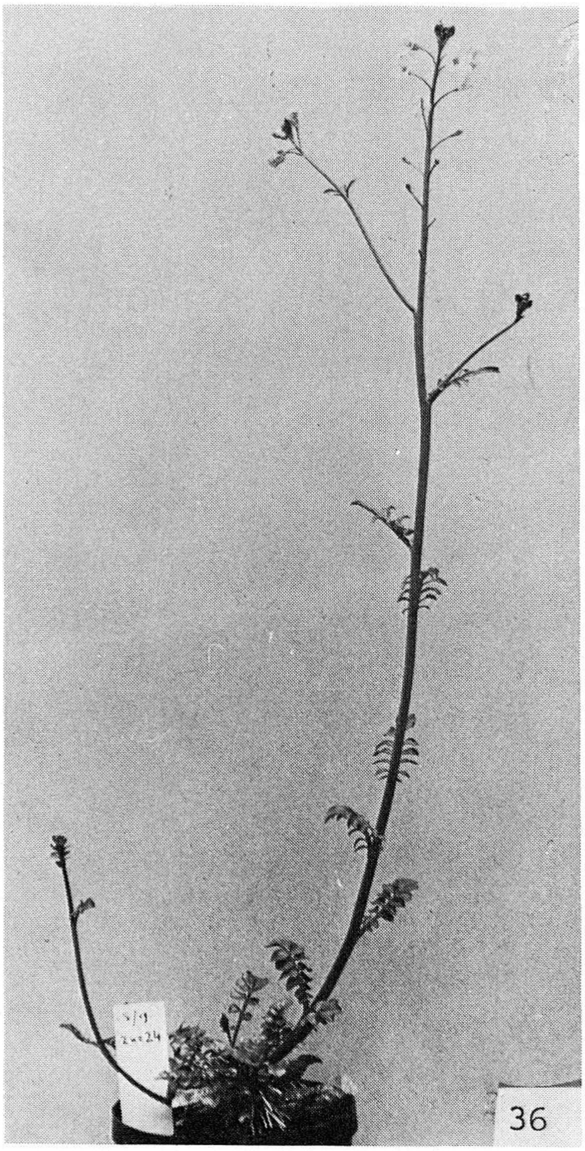

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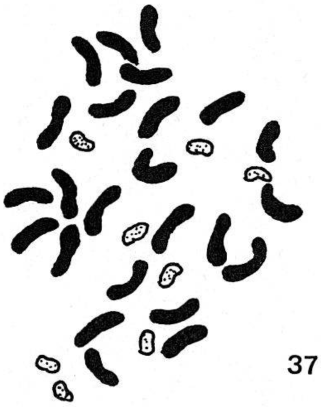
Figs 35 - 36. Triploid backcross plant $2n=24(16_R8_A)$. 35. Root-tip metaphase (c.) 2000 x. 36. Habitus (about 1/3 life size).

Plants with short stolons. Stems most frequently branched, in number 1-4, upright, glabrous, up to 43 cm tall. Basal leaves prostrate, 7-15-foliolate; 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.



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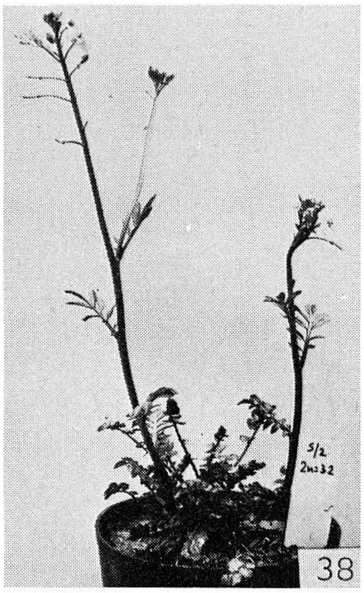

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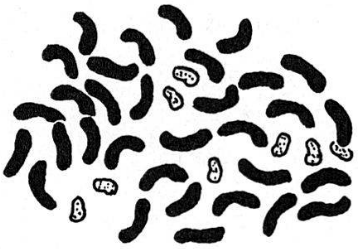
Figs 37 - 38. Tetraploid backcross plant $2n=32(24_R 8_A)$. (c.) 2000 x.
37. Root-tip metaphase. 38. Habitus (about 1/3 life size).

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.



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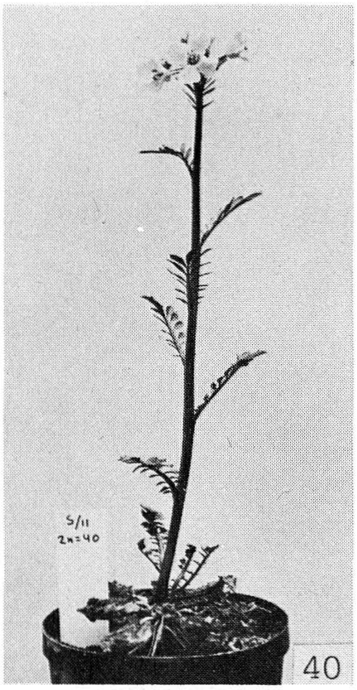

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Figs 39 - 40. Pentaploid backcross plant $2n=40(32_R8_A)$. 39. Root-tip metaphase. (c.) 2000 x). 40. Habitus (about 1/3 life size).

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 16chromosomic 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 diploid	47	12	9	16	RR
Diploid x pentaploid	29	-	-	-	-
Triploid x diploid	18	4	1	16	RR
Triploid x pentaploid	15	-	-	-	-
Tetraploid x diploid	31	-	-	-	-
Tetraploid x pentaploid	19	-	-	-	-
Pentaploid x diploid	17	-	-	-	-
Pentaploid x pentaploid	14	2	-	-	-

Table 12. Further crosses with backcross plants involved

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

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 24chromosomic 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 24chromosomic 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, 12chromosomic gametes in the triploid hybrids; the 24chromosomic 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.

It should be kept in mind that the sexual reproduction in the triploid hybrids is apparently limited; just these limitations, however, stress the importance of an anomalous breeding behaviour as a factor of stabilizing the hybridity, structural and numerical one alike.

Formation of gametes containing whole genomes and subsequently functioning in a complementary way might fortuitously appear in any sexual hybrid; by contrast, only a few well-documented cases are known where this mechanism represents a principal factor influencing patterns of variation and evolution. For the purpose of comparison, three classic examples are characterized below.

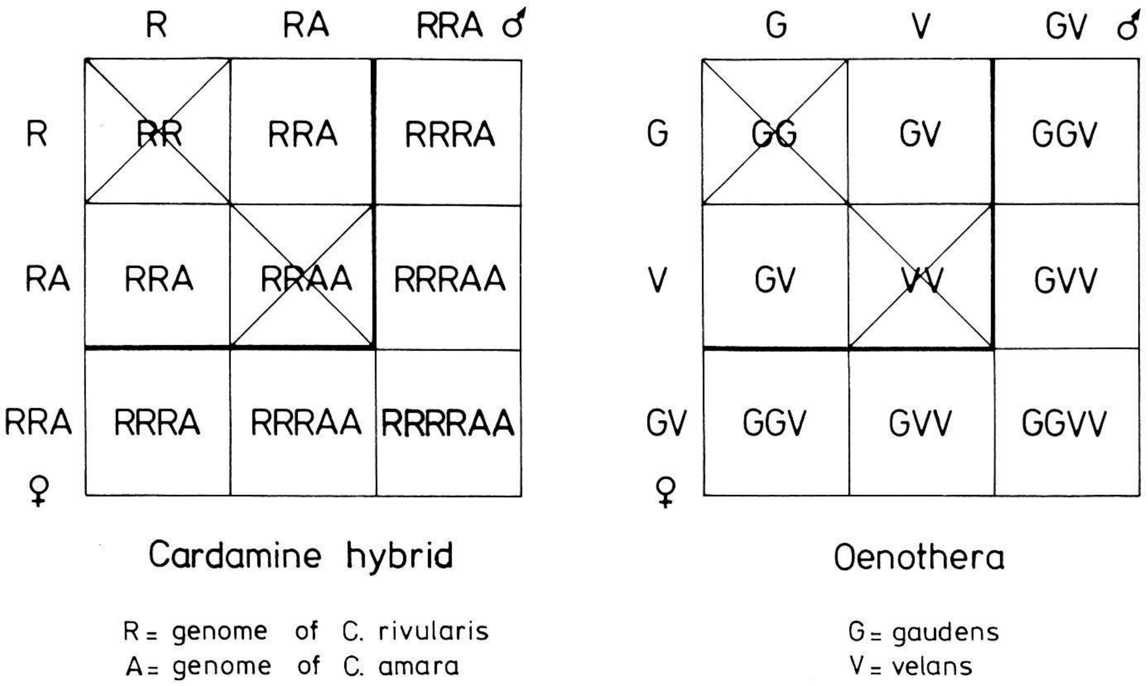
The anomalous breeding behaviour of the *Oenothera biennis* group (2n=14) and, in particular, that of *O. Lamarckiana* was extensively investigated (DE VRIES 1917, RENNER 1917, 1925 etc.; for detailed references see CLELAND 1972). These diploid taxa are permanent heterozygotes for successive translocations on all or nearly all chromosomes of the complement. The formation of chromosome rings followed by a polarized segregation results in two differentiated genomes ("the RENNER complexes") being reassembled intact at the end of meiosis; all spores produced by a given *Oenothera* taxon have accordingly the same reduced chromosome number n=7, but in fact represent two genetically distinct classes, both on the male and the female side. However, the plants breed true for their specific heterozygous structure, all other combinations being eliminated in selfings or sib crosses by a system of balanced lethals that operates either in gametes (e.g. *O. muricata*, DARLINGTON 1937) or zygotes (e.g. *O. Lamarckiana*). On the other hand, crosses between various true breeding lines often produce segregating progeny in the F_1; one, two or four classes of progeny may appear depending whether one or both parents produce one or two kinds of functional sperms and eggs (CLEVELAND 1962). A comparable behaviour was recorded in some *Oenothera* species of temperate South America (HAGEN 1950, HECHT 1950), in *Gayophytum heterozygum* (LEWIS et al. 1958, LEWIS and SZWEYKOWSKI 1964), *Gaura biennis* and *G. triangulata* (RAVEN and GREGORY 1972), *Rhoeo discolor* (SAX 1931), *Paeonia californica* and *P. Brownii* (STEBBINS and ELLERTON 1939, WALTERS 1942) and partly in *Isotoma petraea* (JAMES 1956, 1970); however, in most of these cases it is not known whether the translocation heterozygosity is associated with balanced lethals.

The *Rosa canina* group represents a different aspect. Most taxa are pentaploid (2n=35). At meiosis seven bivalents and twenty-one univalents occur both in PMCs and EMCs; however, the distribution of chromosomes at I anaphase is quite different in anthers and ovules. A random segregation of univalents occurring in PMCs results mostly in aneuploid, abortive microspores; the only functioning pollen grains are those with a basic set of 7 chromosomes derived from a normal distribution of bivalents. On the other hand, a polarized segregation occurring in the ovules results in the formation of two uneven groups comprising 7 and 28 chromosomes, respectively; the only functioning macrospores are 28chromosomic. Pentaploid chromosome number is subsequently restored through the fertilization of a 28chromosomic female gamete with 7chromosomic sperm. Thus, the heterogamic representatives of the *Rosa canina* group breed true for their odd polyploid condition (TäCKHOLM 1922, HURST 1931, DARLINGTON 1937, FAGERLIND 1940).

Leucopogon juniperinus is, to our knowledge, the only case comparable to the *Rosa canina* group although a random segregation does not occur; furthermore, the pattern of microspore selection being very special, there is no reduction in the total output of a good pollen. In this heterogamic allotriploid (2n=12, the genomic constitution AAB), both PMCs and EMCs form four bivalents and four univalents. As a result of a polarized segregation at I anaphase, 8 and 4 chromosomes occur in the respective groups. A subsequent elimination of some micro- and macrospores taking place, the embryo sacs in *Leucopogon* always carry eight chromosomes corresponding to a genomic constitution AB, whereas the functioning microspores contain only four chromosomes representing the genome A. Fertilization restores the original chromosome number and the proportion of the parent genomes (SMITH-WHITE 1948, 1955, 1959b).

As far as the *Cardamine* hybrids are concerned, eight bivalents and eight univalents occurred rather frequently during meiosis; it may be supposed that the bivalents corresponded to the two genomes RR of *C. rivularis*, whereas the univalents represented the only genome A of *C. amara*. A polarized segregation at I anaphase resulted in two uneven groups comprising respectively eight and sixteen chromosomes; the meiotic behaviour observed in some part of the studied material seems thus comparable both to *Leucopogon* as well as the *Rosa canina* group. However, gametes of either sex formed in the above mentioned taxa are of a single kind, whereas both polarized types of sperms and eggs i.e. R and RA apparently occur in the *Cardamine* hybrids; in this respect, the studied plants correspond to the *Oenothera Lamarckiana* group.

Recurrent patterns appearing in experimental crosses strongly suggest three categories of gametes operating in the 24chromosomic hybrids from Urnerboden: apart from the polarized gametes, unreduced RRA ones are functional. However, not all theoretically possible combinations were realized.



♀ \ ♂	R	RA	RRA ♂
R	~~RR~~	RRA	RRRA
RA	RRA	~~RRAA~~	RRRAA
RRA ♀	RRRA	RRRAA	RRRRAA

Cardamine hybrid

R = genome of C. rivularis
A = genome of C. amara

♀ \ ♂	G	V	GV ♂
G	~~GG~~	GV	GGV
V	GV	~~VV~~	GVV
GV ♀	GGV	GVV	GGVV

Oenothera

G = gaudens
V = velans

Fig. 41. Breeding behaviour of the triploid *Cardamine* hybrids compared to the *Oenothera Lamarckiana* type. RRRA - tetraploid found in the wild as well as obtained experimentally; RRRAA = pentaploid obtained from crosses between triploid hybrids and autoallohexaploids (URBANSKA-WORYTKIEWICZ, in press); RRRRAA = natural autoallohexaploid (URBANSKA-WORYTKIEWICZ and LANDOLT 1974, 1977, URBANSKA-WORYTKIEWICZ 1976, op. cit.). GGV and GVV = *O. semigigas* (STOMPS 1912, DE VRIES and BOEDIJN 1924, RENNER fide EMERSON 1935); GGVV = *O. gigas* (LUTZ 1907, 1908, EMERSON 1935).

Crosses between various triploid individuals resulted mostly in likewise triploid RRA plant; in addition, a single tetraploid of RRRA type was obtained. We are inclined to think that the triploid offspring derived from polarized gametes R and RA functioning in a complementary way; on the other hand, the tetraploid plant might have been issued from the union of an unreduced RRA gamete and a polarized R one. The triploid hybrids from Urnerboden appear thus to breed true for their particular genomic constitution, bar the cases where unreduced gametes occasionally contribute to some new combinations. This unusual breeding behaviour might be double-controlled by a gametophytic and a zygotic selection, the latter mechanism comparable to the balanced lethals system operating in *Oenothera Lamarckiana* (Fig. 41). It seems therefore that several ordinarily deleterious mechanisms add up in the *Cardamine* hybrids to a system with high survival value in which plants are able not only to bred true but also to profit from maximum hybrid vigour and to multiply prolifically.

5. Discussion

The rarity of hybrids between *Cardamine rivularis* and *C. amara* in the wild apparently is a measure of an isolation occurring between the two species (URBANSKA-WORYTKIEWICZ and LANDOLT 1972). The present investigations revealed that internal barriers of the reproductive isolation play the main rôle, strong incompatibility blocks operating both at pre- and postmating stages.

Natural hybrids born from crosses at the homoploid level between sexual species usually have the same chromosome number as their parents, particularly in diploid taxa. The large triploid population at Urnerboden issued from the diploid parents represents thus an exceptional case. To some extent comparable are the peculiar "lolioid" and "festucoid" hybrids (2n=21, the genomic constitution LLF and FFL, respectively) between *Lolium perenne* (2n=14) and *Festuca pratensis* (2n=14), studied by a succession of authors (e.g. PETO 1934, JENKIN 1933, 1955, WIT 1959, 1963, 1964, WHITTINGTON and HILL 1961, ESSAD 1962, 1964, 1966, 1968, GYMER and WHITTINGTON 1973a, 1973b, 1975a, 1975b). A single triploid hybrid (2n=33) between *Primula* *veris* (2n=22) and *P. elatior* (2n=22), apparently formed with a part of an unreduced gamete of the latter species, belongs also to this rare category (VALENTINE 1966). However, in the above mentioned cases both diploid and triploid hybrids occurred within the populations, whereas diploid hybrids of *Cardamine* have so far been sought unsuccessfully among the hundreds of triploids at Urnerboden.

Unreduced pollen grains were previously observed in *Cardamine rivularis* from various parts of its distribution range, including Urnerboden (URBANSKA-WORYTKIEWICZ and LANDOLT 1972, 1974, 1977). They proved to be functioning in some crosses performed in the course of the present study: two pentaploid individuals (2n=40, RRRRA) obtained in backcrosses to *C. rivularis* could only have been formed through the union of unreduced gametes, RRA and RR respectively. Therefore a possibility cannot be ruled out that triploid plants with a genomic constitution RRA might occasionally appear in natural populations as a direct result of crosses between *C. rivularis* and *C. amara*.

It is generally believed that the sterility of hybrids depends most frequently either upon their genic balance or chromosomic structure; interesting remarks in this subject were presented among others by REUSCH (1960), AHLOOWALIA (1965) as well as GYMER and WHITTINGTON (1975a) dealing with the triploid hybrids between *Lolium* and *Festuca*. A similar interpretation might apply to the *Cardamine* hybrids from Urnerboden: some rare triploids developed from crosses between *C. rivularis* and *C. amara* were more likely to contain a full series of complementary genes than their diploid siblings; it is also possible that small translocations and deficiences that mainly account for chromosomic sterility might occur less frequently in triploids than in diploids. The particular genomic constitution of the 24chromosomic RRA hybrids might accordingly have conferred them some chance of reproduction and survival within the population whereas the diploid RA plants remained genetically passive and eventually disappeared.

The above presented hypothesis might explain an initial phase of development of the triploid hybrid population at Urnerboden; however, it is not very helpful when the characteristic abundance of the 24chromosomic plants with non-dehiscent anthers is being considered. To begin with, it is rather difficult to accept a recurrent hybridization between the diploid *Cardamine rivularis* and *C. amara* always resulting solely in triploids. Secondly, sexually reproducing triploids of the F_1 generation should normally produce various intermediate, aneuploid segregates; however, all the 337 studied triploids have invariably represented an exactly euploid level and the same genomic constitution RRA, in spite of the fact that some slight morphological variation occurred and the rare pollen-producing individuals showed various degrees of the pollen fertility (26%-62%).

Observations on meiosis in the triploid hybrids as well as the results obtained in experimental crosses contribute to a better understanding of the unusual stabilization of the triploid hybrids from Urnerboden. Three different types of chromosome segregation were observed at I anaphase: 1/ a normal segregation 12 : 12 was rather infrequent; 2/ a random segregation, characteristic for the studied material and 3/ the most peculiar, polarized or preferential segregation i.e. $8_R 8_A$ vs 8_R rather frequently occurring both in anthers and ovules. The diversified meiosis followed by elimination of numerous micro- and macrospores and probably by a zygotic selection as well, proved to have a variety of effects upon the breeding behaviour.

An apparently normal sporogenesis should first be commented. It cannot be excluded that the triploid hybrids may sometimes segregate truly reduced viz. 12chromosomic micro- and macrospores. However, the functioning of such gametes seems rather doubtful given their unbalanced genomic constitution: they should carry one genome of *Cardamine rivularis* plus only a half the genome of *C. amara*. It should be emphasized that the results of our experimental crosses positively indicate three euploid gametic types i.e. R, RA and RRA functioning in the hybrids, no definite proof, however, has been obtained for the reduced, aneuploid gametes.

A random segregation during meiosis is a well-known feature of hybrids, often accounting for their reduced fertility and giving rise to aneuploid offspring in the F_2. A noteworthy example represents the 35chromosomic hybrid between *Agrostis gigantea* (2n=42) and *A. tenuis* (2n=28): this low-fertile pentaploid forms 14 bivalents and 7 univalents, but an apparently random segregation results in aneuploid backcross- and F_2-individuals, both in the wild as well as in experiments (STUCKEY and BANFIELD 1946, JONES

1956, WIDEN 1971). The random segregation mostly resulting in various abortive aneuploid spores in the *Cardamine* hybrids speaks well, on the one hand, for their status of interspecific hybrids; on the other hand, it represents an important evolutionary mechanism contributing to the gamete pool of hybrids, for it may lead to the formation of unreduced gametes. The autoallohexaploids (2n=48, RRRRAA) spontaneously formed at Urnerboden represent the best argument in favour of unreduced gametes functioning in triploid hybrids (URBANSKA-WORYTKIEWICZ and LANDOLT 1974, 1977, URBANSKA-WORYTKIEWICZ, in press).

Incidentally, balanced gametes of R and/or RA type might also be formed as a result of a random segregation. On this occasion, the 9chromosomic hybrid between *Hypocoeris radicata* (2n=8) and *H. glabra* (2n=10) should be mentioned. Viable gametes of this plant contain respectively four, five or rarely nine chromosomes; all these gametic types are functioning in ovules, as indicated by results of backcrosses to *H. radicata* (HOLMBERG 1930, SANDWITCH 1954, BENOIT 1959, PARKER 1971). In view of the fact that two classes of gametes viz. n=4 and n=5 correspond numerically to constituent genomes of the *Hypochoeris* hybrid, it would be very interesting to get still more data concerning mechanism of its segregation.

The polarized segregation occurring in triploid hybrids resulted in the formation of euploid gametes, respectively carrying a single genome of *C. rivularis* (R) and two genomes, one from either parent species (RA). The functioning of such gametes was confirmed in various experimental crosses.

Diploid and triploid backcross plants deserve a special attention, for they show striking affinities to the plants found in the wild. The 16-chromosomic RR plants resembled *Cardamine rivularis* in a spectacular way. The only distinct difference was their pronounced pollen sterility ranging with a single exception from 84 to 94 per cent, whereas a genuine *C. rivularis* produced on average only 13 per cent of abortive pollen. In view of these results, some highly sterile diploid plants of a *C. rivularis* type, recently found at Urnerboden are of a special interest (URBANSKA-WORYTKIEWICZ and LANDOLT 1977). Triploid backcross individuals, apparently resulting from polarized RA gametes of the hybrids fertilized by normal sperms of

C. rivularis corresponded exactly to the 24chromosomic plants predominating at Urnerboden, both in their morphology as well as the complete male-sterility.

Three tetraploids (2n=32, RRRA) obtained in the course of the present study apparently were formed with a part of unreduced gametes of the hybrids, fertilized either by normal sperms of *Cardamine rivularis* or polarized R gamete operating in the 24chromosomic plant. It is interesting to note that a single plant of the same kind was recently found at Urnerboden.

In conclusion, an attempt may be undertaken to explain the origin of plants forming the population at Urnerboden. It can be assumed that the rare triploids producing pollen in a frequency higher than 2-3%, represent the first hybrid generation i.e. a direct product of crosses between *Cardamine rivularis* and *C. amara*. On the other hand, hundreds of the male-sterile plants occurring at Urnerboden might correspond to further generations issued from backcrosses to *Cardamine rivularis* as well as the sexual reproduction of triploid hybrids themselves. The same could be said for the tetraploid RRRA plants, very seldom appearing within the population. Diploid plants of *C. rivularis* morphology might in turn represent not only the genuine parent taxon but also backcross individuals. The seed production in triploid plants is, admittedly, very limited, but might be partly compensated by the great total number of flowering shoots; recurrent backcrosses and allogamy of the hybrids might accordingly contribute to the development of the population, effects of the sexual reproduction with little or no segregation being reinforced by an extremely strong vegetative multiplication.

The two aspects of the reproduction of triploid hybrids apparently have a bearing upon the population structure. Demographical studies carried out at Urnerboden revealed a diversified density of population: some sectors situated within cut manured meadows were particularly dense, whereas hybrid stands within open pastures and drainage canals were much less compact (URBANSKA-WORYTKIEWICZ and LANDOLT, in press). Vegetative multiplication,promoted by the cutting of the meadows,apparently represents an optimal adaption of triploid hybrids to this biotope. Reproduction by seeds seems there rather difficult for two reasons: 1/ some meadows may be cut before the seed development is completed; 2/ establishment of seedlings might anyway be problematic in some densely inhabited stands. On the contrary, open pastures offer more favourable conditions for seed setting and their dispersion, for 1/ grazing is not very intensive and 2/ light conditions are better for young plants. One might accordingly suppose that triploid hybrids use a different reproductive strategy in different ecological niches, vegetative multiplication being the only mechanism acting within some cut, manured meadows, whereas both sexual and vegetative reproduction are occurring within the pastures and drainage canals.

The question remains of what changes in the variation pattern might be expected within the population at Urnerboden. The reproductive behaviour of the triploid hybrids is characterized by an exceedingly reduced recombination potential. In this way, adaptively valuable properties of F_1 are not lost in later generations. On the other hand, it might be expected that a production of essentially new variants will be critically hampered in the long run. However, the 24chromosomic hybrids have already generated an apparently successful variant through doubling of the chromosome number: the autoallohexaploids (2n=48, RRRRAA), recently found at Urnerboden, are fertile, vigorous and actually expanding within the population. Further investigations in this most interesting subject are continued.

Summary

Reproduction of the triploid *Cardamine* hybrids from Urnerboden comprises 1/ strong vegetative multiplication and 2/ sexual reproduction characterized by an extremely reduced recombination potential. The two mechanisms add up to an unusual system in which adaptive properties of the first hybrid generation become stabilized, the plants being thus able to profit from maximum hybrid vigour. Structure of the population from Urnerboden as well as its possible evolutionary trends are discussed.

Résumé

Reproduction des hybrides triploïdes de *Cardamine* d'Urnerboden comprend 1/ multiplication végétative très importante et 2/ reproduction sexuée caractérisée par un potentiel de recombinaison très réduit. Il en résulte un système étonnant stabilisateur des propriétés adaptives de la première génération des hybrides, les plantes pouvant ainsi profiter d'une vigueur hybride maximale. Sont discutés la structure de la population d'Urnerboden et ses changements évolutifs possibles.

Zusammenfassung

Die Fortpflanzung der triploiden *Cardamine*-Bastarde vom Urnerboden umfasst: 1. eine sehr erfolgreiche vegetative Vermehrung und 2. eine sexuelle Fortpflanzung,die durch stark reduzierte Rekombinationsmöglichkeiten charakterisiert ist. Daraus ergibt sich ein erstaunlich stabiles System von adaptiven Merkmalen der ersten Bastardgeneration, die den Pflanzen ein ausserordentlich üppiges Wachstum erlaubt. Die Populationsstruktur der Pflanzen vom Urnerboden und deren Entwicklungsmöglichkeiten werden diskutiert.

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