Genetics of apospory in apomictic *Ranunculus auricomus.* V. Conclusion

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Abstract

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Cytogenetics of apospory and its embryological implications have been investigated on the crossing *Ranunculus cassubicifolius* (sexual) × *R. megacarpus* (apomictic) and on the necessary backcrossings. The findings published in this and in previous papers are discussed here in their mutual context.

Aposporous embryo sac formation is caused by a dominant apospory factor A^- the wild allele of which (A^+) does not contribute to apospory. The ♀ meiosis is not affected by A^-. Apomicts are heterozygous for A^+/A^-, sexual plants homozygous for A^+. In polyploids an increasing number of genomes carrying A^+ reduces the penetrance of one A^- more and more, up to pentaploids (genotype $A^+A^+A^+A^+A^-$) with a degree of apospory of only a few percent. The penetrance of apospory is further influenced by the timing of induction of aposporous initials (which is not determined by A^-): only an aposporous induction before or during the ♀ meiosis ensures the suppression of meiotic development (a prerequisite for "total" apomixis); delaying induction towards the end of ♀ meiosis or beyond it increases the chances of development of meiotic embryo sacs.

A^- has no influence on parthenogenesis, but the factors concerned are closely linked: just one single recombinant (**d** in fig. 1) could be obtained.

The dominant apospory factor A^- is a recessive lethal factor. The consequences for apomixis and evolution within the agamic complex, on the diploid and on polyploid levels, are discussed.

1. Introduction

Asexual reproduction by seeds under maintainance of the alternation of generations (life cycle), but without an alternation of nuclear phases, i.e., gametophytic apomixis, would offer inestimable advantages for plant breeding, first of all undoubtedly the possibility to fix heterosis effects genetically. In fact, plant breeders have been trying to introduce apomixis into sexual crop and other cultivated plants (e.g., potato: Hermsen 1980; maize: Petrov 1984). However, the great efforts undertaken in this direction so far have led to a rather moderate success, not least for lack of a thorough understanding of the genetic and physiological background of gametophytic apomixis, this most important anomaly of reproduction in angiosperms. At present, a rather primitive di-
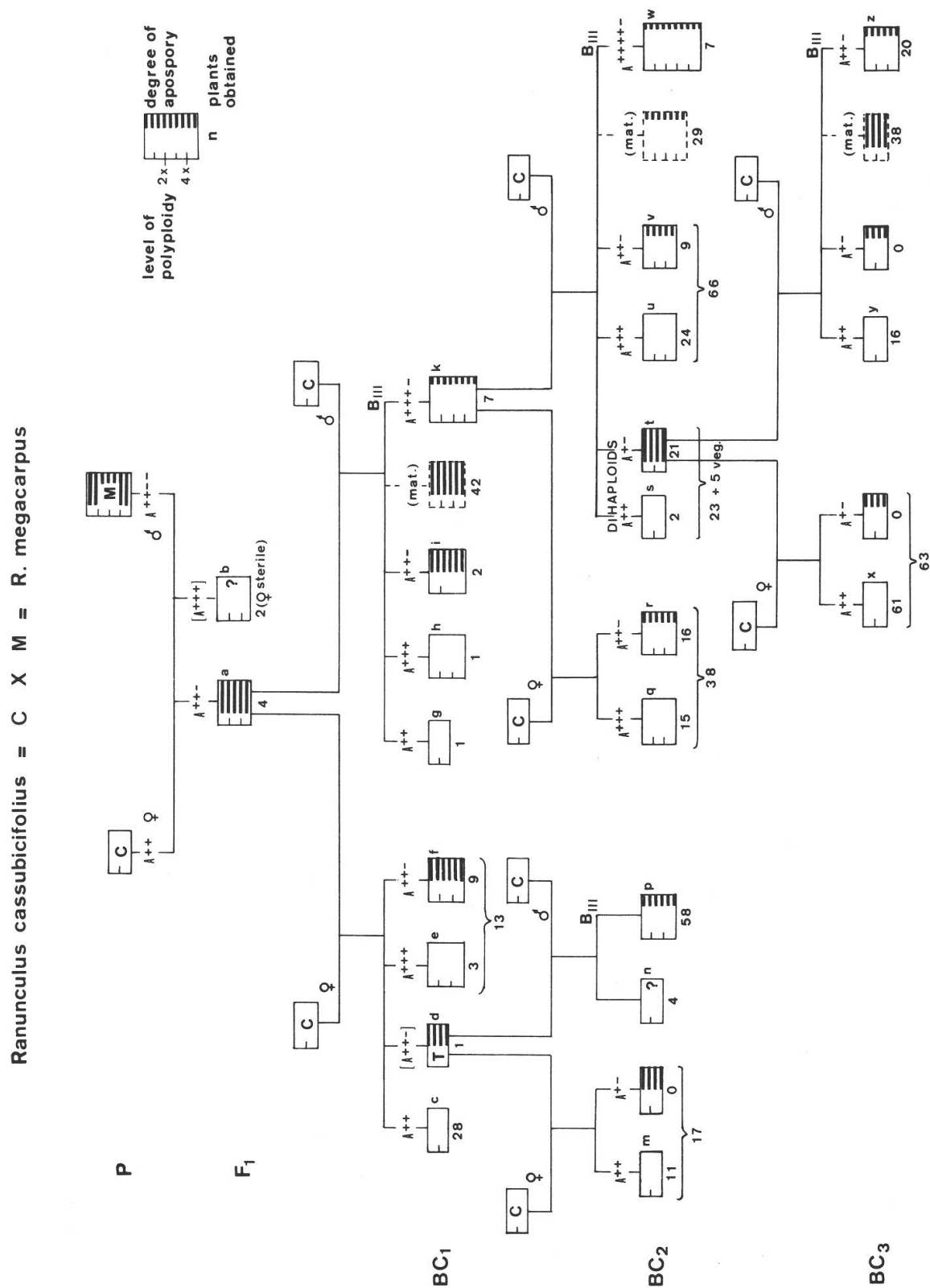
Fig. 1. Genealogical tree of the crossing *Ranunculus cassubicifolius* = C, 2x = 16, meiotic (sexual) × *R. megacarpus* = M, 4x = 32, partially aposporous ("totally" apomictic) and the different backcrossings with the sexual parent C. The number of plants obtained, the level of polyploidy, the approximate degree of apospory, and the genotype are indicated for each offspring.

cotyle such as *Ranunculus* may be more suitable for basic research in cytogenetics than a cultivated apomictic plant. In *Auricomi* the basic components of gametophytic apomixis are easy to survey and are not obscured by additional deviations in the generative sphere. These basic components securing the alternation of generations without an alternation of nuclear phases control (1) the unreduced, *aposporous embryo sac* formation and competition, (2) the (unreduced) *parthenogenetic embryo* formation, and (3) the *pseudogamous endosperm* formation and seed compatibility.

Certain individuals obtained in the present material offer a unique opportunity to investigate all three aspects of gametophytic apomixis separately on the same object. With the present paper I am finishing the discussion of the cytogenetics of *apospory* in *Auricomi,* based on results published in 1971–1982 (see the remarks to the genealogical tree fig. 1) and on the present additions and complements thereto. *Parthenogenesis* and *pseudogamy* of *Auricomi* will be treated in future publications.

Terminology. Unreduced egg cells develop after fertilization into B_{III} hybrids or parthenogenetically into *maternal* offspring. – Reduced egg cells develop after fertilization into B_{II} hybrids or parthenogenetically into *polyhaploids* (*dihaploids* from tetraploid mother plants).

2. Material and methods

2.1. The sexual, diploid *R. cassubicifolius* (C, $2x = 16$) originates from Äsch LU, the aposporous, apomictic, tetraploid *R. megacarpus* (M, $4x = 32$) from Rümlang ZH. Crossings and backcrossings with the sexual parent are shown in fig. 1, backcrossings with the aposporous parent are recapitulated in § 3.4. Designation of backcross offspring:

F_1 from the crossing $C \times M \rightarrow CM_{1 \ldots 6}$

BC_1: Backcrossing with $C \rightarrow CM_1C_{1, 2}, \ldots$, with $M \rightarrow CM_1M_{1, 2}, \ldots$, etc.

reciprocal backcrossing: $C(CM_1)_{1, 2}, \ldots$, etc.

BC_2: Backcrossing with $C \rightarrow CM_1C_1C_{1,2}, \ldots$, etc.

Remarks on fig. 1

F_1: **a**: $CM_{1, 2, 3, 6}$, $2n = 24$: Nogler 1971, 1972

    b: $CM_{4, 5}$, $2n = 24$: ibid. and below § 3.1.1

BC_1: **c/d**: B_{II} hybrids, $2n = 16–17$: Nogler 1982

    d: $T = C(CM_3)_{13}$, $2n = 17$: see below § 3.2.1

    e/f: B_{II} hybrids, $2n = 23 \ldots 25$: see below § 3.1.2.

    g: B_{II} hybrid CM_6C_2, $2n = 17$: Nogler 1978 p. 30, 1982

    h: B_{II} hybrid CM_2C_{16}, $2n = 23$: see § 3.1.2

    i: B_{II} hybrids CM_3C_1 and CM_6C_1, $2n = 24$: Nogler 1972

    k: 7 B_{III} hybrids, $2n = 32$: $CM_1C_{1, 11, 20}$, $CM_5C_{2, 5, 8}$, CM_6C_7: Nogler 1973, 1975, 1978

(BC_1 offspring with the aposporous *R. megacarpus* are not included in the genealogical tree: Nogler 1971, 1972 and below § 3.4)

BC_2: $CT_{1 \ldots 17}$: **m**: B_{II} hybrids, $2n = 16–17$: § 3.2.3

    $TC_{1 \ldots 63}$: **n**: 4 diploid plants, not yet investigated

        p: 59 B_{III} hybrids, 58 with $2n = 25$ and 1 with $2n = 33$: § 3.2.2

    q/r: B_{II} hybrids, $2n = 23 \ldots 25$: Nogler 1975 and below § 3.1.3

    s/t: Dihaploids, $2n = 15 \ldots 18$: Nogler 1982 and below § 4.2

    u/v: B_{II} hybrids, $2n = 23 \ldots 25$: Nogler 1975 and below § 3.1.3

    w: 7 B_{III} hybrids, $2n = 40–41$: § 3.3

BC_3: **x/y**: B_{II} hybrids, $2n = 16–17$: Nogler 1982

    z: B_{III} hybrids, $2n = 23 \ldots 25$: Nogler 1982

2.2. For embryological analyses of the crassinucellate ovules of *Auricomi* the classical paraffin technique (*complete* series, 12–18 μ) and staining with iron haematoxylin Heidenhain is still far superior to modern clearing techniques. For cytological controls root meristems pretreated with 0.002% colchicine fixed in alcohol: acetic acid 3 : 1 and stained with Feulgen give the best results. Crossings were done in the usual manner: carefully emasculated buds were pollinated by hand after anthesis and isolated with nylon bags until maturation to avoid losts of seeds. Most crossing combinations have poor seed setting, and germinability is often very low. The germination may improve after freezing (–5 °C) and defreezing repeatedly, which may help to break seed dormancy.

3. Results

3.1. Triploid B_{II} hybrids

3.1.1. In the F_1 generation 2 of the 6 hybrids described in 1971 deserve to be reconsidered: CM_4, highly ♀ sterile, and CM_5, completely ♀ sterile (**b** in fig. 1). Many nucelli of both these triploid hybrids, when stained with iron haematoxylin, showed groups of darker chalazal cells (which were neither enlarged nor vacuolated). At that time they were interpreted as "potential aposporous initials". Since then such darker cells have repeatedly been observed in many ovules, even in purely meiotic hybrids, during a short transitory stage at the end of the ♀ meiosis. This preferential staining of certain chalazal cells, however, disappears when the functional megaspores become vacuolated. Thus in young stades an enlargement and beginning vacuolation of nucellar cells is the only reliable embryological criterion for apospory. A reexamination of the old slides did not confirm apospory, neither for CM_4 nor for CM_5. Moreover, no tendencies towards apospory have shown up in an embryological investigation of older stades (fixed in 1961) nor was this the case by pollination of the sexual parent with pollen of the ♀ sterile CM_5 (effectuated in 1971): the 2 diploid and the 6 triploid B_{II} hybrids arisen from this backcrossing (not in fig. 1) were all purely meiotic. The only endosperm ever obtained from CM_5 (Nogler 1972) had arisen from an unreduced embryo sac, but this is not necessarily a proof of apospory, as sexual plants may form, exceptionally, meiotic restitution nuclei developing into unreduced embryo sacs. Unfortunately, a reinvestigation of CM_4 and CM_5 is no longer possible, as both plants have died long ago.

3.1.2. In the *first backcross generation (BC$_1$)* triploid B_{II} hybrids occurred in both reciprocal backcrosses (fig. 1: **e/f** and **h/i,** respectively), the majority of which, but not all of them, being aposporous. In 4 cases out of 15 the diploid gametes of the respective aposporous F_1 plant did not transmit apospory. Especially noteworthy is the meiotic hybrid CM_2C_{16} (**h** in fig. 1, arisen in 1977, 2n = 23) because its mother plant CM_2 was still found to be "totally" aposporous in 1972.

3.1.3. In the *second backcross generation (BC$_2$)* triploid B_{II} hybrids were formed by the tetraploid, partially aposporous B_{III} hybrids **k** (fig. 1) in both reciprocal backcrosses with the sexual parent. Unfortunately, not all triploids produced enough buds for an embryological analysis, so that segregation ratios are based on too small numbers.

    Using the tetraploids as pollen parents, meiotic and partially aposporous triploid hybrids arose in similar proportions (**q/r** in fig. 1, and tab. 1 – certain crossing combinations, however, show this less clearly). In contrast to this, in the reciprocal backcrosses (**u/v,** and tab. 2) the majority of the triploid hybrids were meiotic, and relatively

few were partially aposporous. But not all reduced egg cells form B_{II} hybrids: often they develop parthenogenetically into dihaploids (**t** in fig. 1) – which are, unlike the B_{II} hybrids, nearly all aposporous (Nogler 1982). Summing up the B_{II} hybrids *and* the dihaploids (and considering that aneuploid dihaploids are even less viable than aneuploid B_{II} hybrids) reveals equally indeed, that gametes transmitting apospory or not occur in similar proportions (tab. 2).

Dihaploids are highly aposporous and induced very early (even earlier than *R. megacarpus*), long before completion of the ♀ meiosis (Nogler 1982). Contrary to this, most of the aposporous triploid hybrids in BC_2 have a rather low degree of apospory (about 10–30%) and a retarded and highly asynchronous aposporous induction. In meiotic triploids, on the other hand, the embryo sac development is largely synchronous and perfectly regular, and aposporous initials can never be observed, not even in older buds.

Tab. 1. Backcrosses of tetraploid, partially aposporous B_{III} hybrids (**k** in fig. 1) as pollen parents with the diploid, sexual parent *R. cassubicifolius* (C)

♀ ♂	meiotic B_{II} hybrids (triploid)	aposporous B_{II} hybrids (triploid)
C × CM_1C_{11}	5	4
C × CM_5C_8	6	10
C × CM_6C_7	4	2
total	15	16

Tab. 2. Backcrosses of tetraploid, partially aposporous B_{III} hybrids (**k** in fig. 1) as mother plants with the diploid, sexual parent *R. cassubicifolius* (C)

♀ ♂	B_{II} hybrids (triploid)		Dihaploids		total	
	meiotic	aposporous	meiotic	aposporous	meiotic	aposporous
CM_1C_1 × C	2	–	–	–	2	–
CM_1C_{11} × C	6	2	1	5	7	7
CM_1C_{20} × C	2	1	–	3	2	4
CM_5C_5 × C	2	–	–	1	2	1
CM_5C_8 × C	5	1	–	2	5	3
CM_6C_7 × C	7	5	1	3	8	8
total	24	9	2	14*	26	23

(enlarged from Nogler 1978)

* **t** in fig. 1: 7 other plants arisen from backcrosses not considered in this table

3.2. The trisomic hybrid T (**d** in fig. 1)

3.2.1. The hyperdiploid B_{II} hybrid T (2n = 17) arose from the crossing C × CM_5 and was formerly designated as $C(CM_5)_{13}$. It is the only *aposporous* diploid hybrid which has

ever been obtained – all the other 110 diploid hybrids being meiotic (Nogler 1982): **c+g+x+y** (fig. 1)+4 B_{II} hybrids from selfing or crossing aposporous dihaploids inter se.

An embryological analysis shows that about ¾ of the ovules of the hybrid T have contained aposporous embryo sacs. The aposporous induction took place much earlier than in the triploid B_{II} hybrids mentioned (**f, r, v** in fig. 1), but not quite so early as in dihaploids.

3.2.2. The 63 backcross offspring of T as mother plant, designated as $TC_1 \ldots TC_{63}$ (**n/p** in fig. 1) have not yet been analyzed embryologically. Nearly all of them are B_{III} hybrids: 58 triploids with 2n = 25 and 1 tetraploid with 2n = 33 chromosomes (obviously a 2n + 2n hybrid). Only 4 plants are diploid (2 of these with 2n = 16 and 2 with 2n = 17 chromosomes), probably 3 of them being B_{II} hybrids and 1 maternal (his can no longer be established, as the mother plant T has died). The degree of apospory of the plant T was, when calculated from offspring, even higher than when established embryologically, viz. at least 94% (reduced embryo sacs having little chances of developing), whereas its degree of apomixis was 0 or at most 2%. Details concerning the hybrids from the backcrossing T×C will appear in a later paper.

3.2.3. The reciprocal backcross C×T resulted in 17 plants, $CT_1 \ldots CT_{17}$ (**m** in fig. 1), all, as expected, diploid B_{II} hybrids (14 having 2n = 16 and 3 having 2n = 17 chromosomes). Upto now only 11 have been investigated embryologically – all being meiotic like the 110 cases mentioned in § 3.2.1. Another of these B_{II} hybrids, CT_{13} (2n = 16), is most probably also meiotic, but shall be investigated more in detail: About 60 ovules had only meiotic development, but a single ovule from a very young bud contained, besides an embryo sac mother cell in early prophase, an 1-nucleate embryo sac with a large vacuole which was probably aposporous, but induced long before meiosis. This is very atypical in *Auricomi* in which aposporous induction takes place only during meiotic prophase or even later on. Moreover, in CT_{13} all the other ovules show a synchronous and perfectly regular embryo sac development in the centre of the nucelli, which is very typical for meiotic but not at all for partially aposporous hybrids. It is conceivable that meiotic restitution nuclei are not the only possibility of sexual plants to produce, exceptionally, unreduced embryo sacs.

3.3. Pentaploid B_{III} hybrids (**w** in fig. 1)

originated in the BC_2 generation from partially aposporous tetraploid hybrids **k** (fig. 1, viz. CM_1C_1, CM_1C_{11} and CM_3C_5) by pollinating with the sexual parent. Six out of the 7 pentaploids in question were investigated embryologically: all were predominantly meiotic with traces (5–10%) of apospory. Fewer ovules were aposporous than in the respective tetraploid mother plants (**k**), and induced noticeably later, so that even ovules with 1-2-nucleate meiotic embryo sacs have only small vacuolate aposporous initials, if any.

One of these pentaploids (viz. $CM_1C_{11}C_{24}$, 2n = 40) produced, when pollinated again with the sexual parent, 2 B_{II} hybrids with 2n = 25 and 2n = 26 chromosomes, and, when selfed, 29 plants, of which 5 were lethal, 16 had 2n = 36 … 39, 5 had 2n = 41 … 45, and only 3 had 2n = 40 chromosomes like the mother plant. One of them was morphologically aberrant and highly aposporous, thus also a B_{II} hybrid, one was probably maternal, and the third could not be examined. Thus the progenies of

$CM_1C_{11}C_{24}$ confirm the embryological findings, as at least 29 or 30 out of 31 offspring were B_{II} hybrids, at most 2 maternal, and none a B_{III} hybrid.

3.4. Backcrosses with the aposporous, apomictic parent R. megacarpus (M)

Few backcrossings have ever been effectuated with *R. megacarpus* (which has, of course, only been used as male parent); these backcrossings do not figure in the genealogical tree (fig. 1) and are, therefore, recapitulated here.

BC₁ generation. The F_1 hybrids CM_1 and CM_2, both triploid and highly aposporous, produced, besides maternal offspring, 3 hybrids. The degree of apospory of 2 of them (viz. the B_{II} hybrid CM_1M_8, $2n = 30$, and the B_{III} hybrid CM_1M_4, $2n = 41$) was remarkably low compared with CM_1, in spite of the increase of the number of genomes of the aposporous parent M (Nogler 1973). The degree of apospory of the pentaploid B_{III} hybrid CM_2M_2 ($2n = 41$: $CM_2 + 2$ genomes of M) equalled that of its triploid mother plant (Nogler 1971).

BC₂ generation. Tetraploid, partially aposporous hybrids (**k** in fig. 1, viz. CM_1C_1, CM_1C_{11}, and CM_1C_{20}, as well as one of its offspring from free pollination, the tetraploid B_{II} hybrid CM_1C_1F – not in fig. 1) were pollinated with *R. megacarpus* and have produced (besides maternal plants and dihaploids) a total of 19 tetraploid B_{II} hybrids, 14 of which were, as expected, aposporous in various degrees, but 5 of them were, surprisingly enough, purely meiotic (Nogler 1975). Two of these meiotic tetraploids still survive; these have recently been backcrossed again with the aposporous parent and produced, as expected, exclusively B_{II} hybrids ($2n = 31 \ldots 33$). Random tests from these large progenies have shown that most of them are aposporous in various degrees, some of them, however, are again purely meiotic, thus proving once more that *R. megacarpus* is heterozygous for the genetic factor causing apospory.

4. Discussion

The genetic factor causing apospory in the apomictic *R. megacarpus* is present only in 2 of its 4 genomes. This heterozygosity follows from the different backcrosses with *R. megacarpus* recapitulated in § 3.4. All apomicts hitherto investigated genetically are known to be heterozygous also in respect to morphological and other characters – this in contrast to their sexual relatives (for *Auricomi*: Rutishauser 1960, 1965). Heterozygosity of *R. megacarpus* for the apospory factor has already been assumed in 1971, as megaspores, or even 1-2-nucleate meiotic embryo sacs, develop in many ovules side by side with aposporous embryo sacs, or sometimes even without an aposporous competition (Izmaiłow 1965, Nogler 1971). (Nevertheless, neither B_{II} hybrids nor dihaploids have ever been observed in *R. megacarpus* which is, therefore, considered to be "totally" apomictic.)

4.1. Inheritance of apospory

This heterozygosity suggests a simple scheme for the inheritance of apospory in *Auricomi,* on the basis of one pair of alleles A^+/A^-. The apospory factor A^- allows the regular induction of embryo sacs from unreduced, somatic cells of the nucellus. Its wild allele A^+ does not contribute to apospory but may function in the normal, sexual life

cycle. One could imagine that the allele A^+ leads to the conditions for the activation of genes involved in the differentiation of the embryo sac, which normally (i.e., in sexual plants, homozygous for the wild allele A^+) prevail only in meiospores. In the presence of the allele A^- these conditions also prevail in certain somatic cells of the megasporangium (nucellus).

The aposporous parent *R. megacarpus* (M, $2n = 32$) is heterozygous and has the genotype $A^+A^+A^-A^-$; the sexual parent *R. cassubicifolius* (C, $2n = 16$) is homozygous and has the genotype A^+A^+. In the F_1 generation ($2n = 24$) the 4 predominantly aposporous plants (**a** in fig. 1, viz., $CM_{1,2,3,6}$) have the genotype $A^+A^+A^-$, which has been confirmed by backcrossings (**e/f** and **h/i** in fig. 1). Most probably the ♀ sterile plant CM_5 is meiotic and thus has the genotype $A^+A^+A^+$, and possibly the same applies to the highly ♀ sterile CM_4. Apparently not the entire F_1 is aposporous, contrary to what was assumed in 1971 (see § 3.1.1.). In aposporous hybrids containing one allele A^- every addition of a genome C containing the wild allele A^+ further diminishes the degree of apospory (established embryologically from ovules shortly after completion of the ♀ meiosis): in triploid F_1 hybrids (**a** in fig. 1: $A^+A^+A^-$) well over half the ovules show an aposporous development besides the megaspore; in tetraploid BC_1 hybrids (**k:** $A^+A^+ A^+A^-$) ¼ of the ovules or even less are already aposporous; in the pentaploid BC_2 hybrids (**w:** $A^+A^+A^+A^+A^-$), finally, only a few percent of the ovules contain aposporous initials at this early stage. Thus even four A^+ cannot suppress the effect of one A^- completely. The clearest refutation of the original working hypothesis, which assumed recessivity of apomixis (Nogler 1971), is given by the aposporous dihaploids (**t** in fig. 1) in which one allele A^- imposes apospory against one wild allele A^+.

Plants homozygous for the allele A^- are not known.

The penetrance of apospory is influenced largely by the average *timing* of aposporous induction in relation to the meiotic development in the bud in question. The sooner an aposporous initial is induced, the fewer are the chances of development of functional megaspores, as was already shown in 1943 in the classical investigation by Christoff and Papasova on *Potentilla: P. adscharica* with very early induction is totally aposporous, whereas in *P. recta* with delayed induction meiotic embryo sacs function in about 3% of the ovules. Suppression of meiotic embryo sac development by aposporous competition, which, strictly speaking, amounts to a suppression of sexuality, is a prerequisite to "total" apomixis in wild apomicts like *Auricomi* and many others. The average timing of aposporous induction is a purely quantitative factor and does not depend directly on the number of genomes carrying the allele A^-. Probably environmental factors (day length?) do influence it also to a certain extent – a thorough investigation hereof is being carried on.

Crossing *R. megacarpus* with sexual *R. cassubicifolius* (as ♀ parent!) delays aposporous induction; and each backcrossing delays it further, and this, subsequently, diminishes the degree of apospory more and more. This is best demonstrated by the different triploid B_{II} hybrids with the same genotype $A^+A^+A^-$, which occur in the F_1 (**a** in fig. 1, aposporous induction during the ♀ meiosis), in the BC_1 (**f, i,** induction at the end of the ♀ meiosis) and in the BC_2 (**r, v,** induction after the ♀ meiosis).

If this working hypothesis – that apospory is being caused by a factor A^- without the contribution of its recessive wild allele A^+ – holds good, then a $1:1$ segregation of partially aposporous ($A^+A^+A^-$) versus meiotic individuals ($A^+A^+A^+$) can be expected for the triploid B_{II} offspring in BC_2 (**q/r** and **u/v** in fig. 1). The numerical data presented in tab. 1 and 2 point to this assumption, but, unfortunately, do not allow to prove it, because too many plants were not viable enough for an embryological analysis.

4.2. Dihaploids and diploid apomicts

Conspicuously most (but not all) dihaploid plants are aposporous (A^+A^-), in contrast to the B_{II} hybrids which in their majority are meiotic ($A^+A^+A^+$). Obviously the reduced egg cells of tetraploids carrying the apospory factor A^- (genotype A^+A^-) have a far greater tendency to develop parthenogenetically than egg cells which are homozygous for the wild allele (A^+A^+). In other terms, apospory and parthenogenesis in *Auricomi* are rather closely linked.

Dihaploids are known of many other apomictic angiosperms, but most authors describe them to be meiotic (sexual) and often weak or even sublethal. Female sterile dihaploids are supposed to be aposporous (deWet 1965). Surprisingly, they are often disregarded in embryological investigations, and this can falsify the results.

In a population of tetraploid, apomictic *R. argoviensis,* two aposporous dihaploids were found in 1971, but they were weak and so poorly fertile that no crossing experiments could be effectuated. Crossings have been possible only thanks to the highly aposporous dihaploids which arose in the BC_2 under discussion (**t** in fig. 1). They are surprisingly viable and ♀ and ♂ fertile and thus have enabled the cultivation of both reciprocal backcrosses and other offspring. This has led to two substantial findings concerning diploid apomicts (cf. Nogler 1982):

1) Aposporous dihaploids give rise to maternal (and B_{III}) offspring; consequently, they are di(ha)ploid *apomicts.*

2) The apospory factor A^- is neither transmitted by monoploid ♂ nor by monoploid ♀ gametes. Thus the dominant allele A^- is a recessive *lethal factor.* (One can imagine a partial breakdown in the existing gene system, most likely in connection with embryo sac initiation.)

Monoploid gametes gave rise to a total of 121 diploid B_{II} hybrids (see § 3.2.1. and 3.2.3.) which were all purely meiotic. Besides these only a single B_{II} hybrid was aposporous, the hyperdiploid (2n = 17) plant T (**d** in fig. 1, formerly designated as $C(CM_3)_{13}$). Herefrom can be concluded that the hybrid T inherited the apospory factor A^- from a disomic pollen grain (genotype A^+A^-) and was, therefore, trisomic, having the genotype $A^+A^+A^-$. As expected, its monoploid ♂ gametes as well do not transmit apospory (**m** in fig. 1), this being a valuable support of the finding just alluded to. The hybrid T is one of the extremely rare examples of aposporous or diplosporous plants mentioned in the literature, which nearly or completely lack the faculty of parthenogenesis, i.e., which have a high degree of apospory (or diplospory) and, at the same time, a very low degree of apomixis. Müntzing (1958) has described one such plant in *Potentilla argentea.* Backcross offspring from the plant T will allow to analyse separately and on the same object both the main components of gametophytic apomixis, i.e., apospory *and* parthenogenesis. Work in progress concerns the environmental influences on apospory and parthenogenesis, and the cytogenetics of parthenogenesis.

The two findings mentioned, concerning diploid apomicts, explain why diploid apomicts have hardly ever been found in nature. They cannot be formed as hybrids, but only as dihaploids. Once formed, apomictic dihaploids may produce *meiotic* B_{II} hybrids, if any, but they cannot maintain apospory at the diploid level through hybridization. This would only be possible by maternal reproduction, i.e., apomictically – for which, however, apomictic dihaploids are mostly too weak, so that apospory gets lost. On the *diploid* level apomicts are deprived of the possibility of sexual recombination. In 1939 Darlington had assumed that apomixis is a blind alley of evolution. For polyploids this has since then been disproved repeatedly; but, as it becomes clear from the

foregoing, on the diploid level apomixis *is* a blind alley of evolution – at least in *Auricomi*. Nevertheless, even weak and poorly fertile aposporous dihaploids may play an important role for the microevolution within agamic species complexes. Starting from unreduced egg cells of diploids (dihaploids) the tetraploid level is directly achievable in one step: tetraploid B_{III} hybrids can originate either as $2n + 2n$ hybrids by unreduced pollen from di(ha)ploids or – which is more probable – as $2n + n$ hybrids by reduced pollen from tetraploid apomicts, most likely from the same population. All apomicts hitherto examined genetically being extremely heterozygous, one can imagine the effectiveness of such an interlink on the diploid level for the creation of new tetraploid, apomictic biotypes (which may revert again to more or less ephemeral dihaploids, etc.). Such a cycle: apomictic tetraploid – aposporous dihaploid – apomictic tetraploid – aposporous dihaploid – etc., however, does not seem to occur in most of the other apomictic genera, because the aposporous dihaploids that they may form are reported to be female sterile, in so far as they are not lethal. Wherever only *meiotic* dihaploids are available, "reversible tetraploidization" = "(tetraploid) – diploid – tetraploid – di(ha)ploid cycles" (deWet 1971) can be considered as a comparable microevolutionary mechanism. Here also, the decisive steps are reversion to dihaploidy on the one hand, and B_{III} hybridization on the other, but, as in other meiotic (sexual) plants, the unreduced egg cells needed to reestablish tetraploidy occur only exceptionally. Moreover, in tetraploidization starting from meiotic dihaploids, apospory is transmitted only by male gametes. In contrast to this, in the variant of "reversible tetraploidization" with *aposporous* dihaploids discovered here in *Auricomi*, (1) unreduced egg cells as well can transmit apospory, and (2) they are more frequent even if an aposporous dihaploid is only poorly fertile. However, aposporous dihaploids need not necessarily be weak, as has been demonstrated with the help of *Auricomi*. It is even conceivable that the rare diploid apomictic biotypes found in nature (e.g., in *Potentilla argentea:* Müntzing 1958, or in *Hierochloë australis:* Weimarck 1967) are in fact dihaploids which are unusually successfull.

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It is, of course, an open question in how far the circumstances found here in *Auricomi* may apply also to other apomictic genera. Unfortunately, the most significant finding – that the apospory factor is not transmitted by monoploid gametes and is, therefore, a recessive lethal factor – cannot be verified in other apomicts due to the female sterility of aposporous dihaploids. In *Parthenium argentatum* (Gerstel et al. 1953), *Dichanthium aristatum* (d'Cruz and Reddy 1971) and *Taraxacum* (Richards 1970, Müller 1972) certain cytogenetical results point to probable analogies (cf. Nogler 1982).

The cytogenetics of apospory clarified here for *Auricomi* correspond basically to those clarified by Savidan (1980, 1982) for *Panicum maximum* (Gramineae). Comparable cases are, as far as can be judged from the summary results, *Hypericum perforatum* (Noack 1939), *Hieracium aurantiacum* (Christoff 1942), *Parthenium argentatum* (Gerstel et al. 1953), *Bothriochloa/Dichanthium* (Harlan et al. 1964), *Beta lomatogona* (Cleij et al. 1976) and others (cf. Nogler 1984). Unfortunately, certain investigations had to be abandoned in a premature stage – a thorough comparison thus hardly being possible.

Although the mechanisms involved in gametophytic apomixis may differ to some extent from genus to genus, there is little doubt that the results obtained in *Auricomi* will not differ essentially from those which are to be expected of investigations on other apomictic genera.

Zusammenfassung

Apomiktische Fortpflanzung i.e.S. ist asexuelle Fortpflanzung durch Samen, wobei der Generationswechsel beibehalten ist, nicht aber der Kernphasenwechsel. Dabei entstehen aus unreduzierten Embryosäcken bzw. Eizellen auf parthenogenetischem Wege maternelle (muttergleiche) Nachkommen. Die unreduzierten Embryosäcke werden bei apomiktischen *Auricomi* aus somatischen Zellen des Nuzellus gebildet, d. h. aus aposporen Embryosackinitialen. Die Zytogenetik der Aposporie – untersucht an der Kreuzung *Ranunculus cassubicifolius* (meiotisch und sexuell) × *R. megacarpus* (apospor und apomiktisch) mit den nötigen Rückkreuzungen – war Gegenstand einer Reihe von Publikationen, die mit der vorliegenden abgeschlossen wird. Die Ergebnisse werden hier im Zusammenhang diskutiert.

Aposporie beruht auf einem dominanten Faktor A^-, dessen Wildallel A^+ nicht zur Aposporie beiträgt. Apospore *Auricomi* sind heterozygot für A^+/A^-, meiotische (sexuelle) homozygot für A^+. In Polyploiden wird die Penetranz eines Allels A^- gegenüber einer steigenden Anzahl von A^+ sukzessive schwächer, seine Wirkung ist aber sogar gegen vier A^+ noch gut nachweisbar. A^- beeinflußt die ♀ Meiose nicht; weil aber bei Apomikten apospore Initialen meist bereits vor oder zu Beginn der ♀ Meiose induziert werden, erhalten apospore Embryosäcke einen entscheidenden Entwicklungsvorsprung, so daß Makrosporen oder frühe Stadien reduzierter Embryosäcke konkurrenziert und verdrängt werden – bei „total" apomiktischen Wildformen in bis zu 100% aller Samenanlagen. Bei gewissen Rückkreuzungsbastarden kann diese apospore Induktion mehr oder weniger verzögert sein, so daß auch meiotische Embryosäcke Entwicklungschancen erhalten, was die Penetranz der Aposporie zusätzlich beeinträchtigen kann.

A^- hat keinen Einfluß auf die Parthenogenese, doch sind die betreffenden Erbfaktoren eng gekoppelt. Rekombinanten scheinen allgemein bei apomiktischen Angiospermen äußerst selten zu sein; im vorliegenden Material trat eine einzige auf (**d** in Fig. 1), dank welcher es nun erstmals möglich wird, an ein und demselben Objekt die Zytogenetik sowohl der Aposporie als auch der Parthenogenese zu verfolgen.

Der dominante Aposporiefaktor A^- ist ein rezessiver Letalfaktor; er kann nicht durch monoploide ♂ oder ♀ Gameten (1 Genom mit A^-) vererbt werden, nur durch heterozygote diploide (A^+A^-) oder polyploide. Auf dem diploiden Niveau hat dies Konsequenzen, die sehr wohl der Grund sein könnten, warum diploide Apomikten in der Natur praktisch nie gefunden werden. Diploide apospore *Auricomi* können nämlich gar nicht als Bastarde entstehen, höchstens durch parthenogenetische Entwicklung von Eizellen mit dem Genotyp A^+A^-, gebildet als reduzierte Eizellen Tetraploider (z. B. **k/t** in Fig. 1), d.h. also als apospore *Dihaploide*. So weit diese nicht ganz steril sind, übertragen natürlich auch hier die reduzierten, monoploiden Gameten (hauptsächlich ♂) nur A^+, während A^- nur durch reduzierte Eizellen mit dem Genotyp A^+A^- weitergegeben werden kann. Dies bedeutet, daß sich Aposporie auf dem diploiden Niveau nur bei materneller (apomiktischer) Fortpflanzung halten kann, wozu aber apospore Dihaploide kaum je vital genug sein werden. So wird der rezessive Letalfaktor A^- auf dem diploiden Niveau früher oder später eliminiert.

Eine sexuelle Rekombination ist also bei diploiden (im Gegensatz zu polyploiden) Apomikten ausgeschlossen; hingegen kann Befruchtung von unreduzierten Eizellen des Genotyps A^+A^- unter günstigen Umständen zu neuen polyploiden aposporen Biotypen führen. Abschließend werden die Bedeutung dieser Möglichkeit für die Mikroevolution innerhalb agamer Artkomplexe erörtert und Vergleiche mit genetischen Befunden an anderen Apomikten gezogen.

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