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Hybrids and polyploidy in the genus *Athyrium* (Pteridophyta) in Europe. 2. Origin and description of two triploid hybrids and synthesis of allotetraploids

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Abstract

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Recently Schneller and Rasbach (1984) showed that *Athyrium filix-femina* (genome formula ff) and *A. distentifolium* (genome formula dd) had given rise not only to a diploid hybrid *A.* × *reichsteinii* (df) but also to triploid hybrids. Based on their cytology and morphology they were interpreted as the genome combinations ddf and dff, respectively. As mentioned in Schneller and Rasbach (1984), the diploid hybrid rarely shows somatic tetraploidization yielding cells with genomic formula ddff. When sporangia develop on such areas, df spores result after meiosis. These spores form regular gametophytes which were used for breeding experiments. It was possible to obtain experimental triploid (dff) and also fertile tetraploid (ddff) progeny. Our results show that formation of triploids without allotetraploid plants is possible in nature.

The two different triploid hybrids are described as new nothosubspecies of *A.* × *reichsteinii,* the triploid with genomic formula ddf as nothosubsp. *microderris,* the triploid with genomic formula dff as nothosubsp. *praetermissum.*

Key words: Hybridization, somatic polyploidization, polyploidy, cytology, *Athyrium,* Pteridophyta.

Introduction

Athyrium filix-femina (L.) Roth and *A. distentifolium* Tausch ex Opiz are two common diploid fern species with a holarctic distribution. Both show 2n = 80 chromosomes in mitosis and $n = 40^{II}$ in meiosis. We express this in their genome formulas, (ff) and (dd), respectively. *Athyrium filix-femina* is the most common fern of Europe. It inhabits woodlands from sea level to the timberline and may even be found up to 2300 m in the alpine region, above the timberline. *Athyrium distentifolium* is restricted to subalpine and alpine areas where it grows in vegetation of tall herbs or on scree or in shady places above

the timberline. In areas where the two species occur sympatrically, they occasionally hybridize and form the diploid sterile hybrid *A.* × *reichsteinii* Schneller et Rasbach (= *A. distentifolium* × *A. filix-femina* (df)). In the meiotic stages of its sporemothercells only, or nearly exclusively, univalent chromosomes are seen. This suggests that the two genomes lack correspondence and that in spite of very similar gross morphology the two species are not closely related. For differentiation it is necessary to examine sori and spores (Schneller and Rasbach 1984). Our intensive search for hybrids revealed that, in addition to the diploid hybrid, triploid hybridogenous plants occur in rare instances (Schneller and Rasbach 1984). Within these triploids two morphologically slightly distinct hybrids could be observed, both with 40^{II} and 40^{I} in meiosis. These two triploid hybrids were interpreted as two different combinations of the genomes of *A. filix-femina* and *A. distentifolium,* that is, dff and ddf, respectively. The occurrence of such triploids puzzled us. Finally, three possibilities were considered (Schneller and Rasbach 1984). In spite of an extensive search no allotetraploid taxon with the putative formula ddff could be found. Our present experiments prove that the key lies in the fact of occasional (rather rare) formation of tetraploidized tissue (ddff) on pinnae or pinnules of *A.* × *reichsteinii* (df). On these parts sori with sporangia containing regular "diplospores" develop, a phenomenon discovered by Marianne Schneller. Such sori become well visible at maturity as conspicuous "dark spots" which differ from the rest of sori found on diploid tissue (Fig. 1).

The diplospores collected from tetraploidized tissue of *A.* × *reichsteinii* were sown for gametophyte production. These gametophytes were crossed with *A. filix-femina* and *A. distentifolium* or used for selfing experiments. We obtained triploid hybrids and many specimens of the allotetraploid plant (ddff); the latter has so far never been observed in nature. Additionally, we give some micromorphological characteristics of the two natural triploid hybrids. The results are also more generally discussed in the light of speciation.

Material and methods

For collecting spores we followed in general the methods described by Reichstein (1981). Because of the size of the fronds we picked only a few pinnae per plant. Spores of *A. filix-femina* and *A. distentifolium* were preferably taken from plants growing in pure stands or from isolated plants. Spores were collected as pure as possible and used for sowing without sterilisation. For collecting the rarely formed diplospores (formula df) we marked several plants of the diploid hybrid *A.* × *reichsteinii* in the field (Black Forest, Germany) with a label stuck in the ground. The location of the plants was also mapped on the corresponding 1:10000 maps. In the years 1983, 1984, and 1985 the plants were visited several times when the spores were mature (July/August). The fronds were very carefully searched for parts of tetraploidized tissue, their sori being characterized by comparatively darker spots. Such spots could be found on some parts of the pinnules or on whole areas of up to about 9 pinnae (see also Schneller and Rasbach 1984, Fig. 7). We cleaned the parts containing the tetraploidized areas from dust and unwanted spores by blowing over them several times. They were then pressed in clean paper (method following Reichstein 1981). In spite of all these precautions the collection proved to contain not only spores of the df type but also few spores of the parent species (either d or f spores or both). A list of the plants used for breeding experiments is given in the appendix.

Measuring the exospore length was done with spores embedded in balsam. The guard cells (stomata) were measured on the (lower) epidermis taken from living plants or from dry (herbarium) plants wettened in hot (nearly boiling) water. Spore dimensions found in the different taxa are given in Tab. 1, those of guard cells in Tab. 2. Cytological investigation was done as mentioned in Schneller and Rasbach (1984) and Manton (1950).

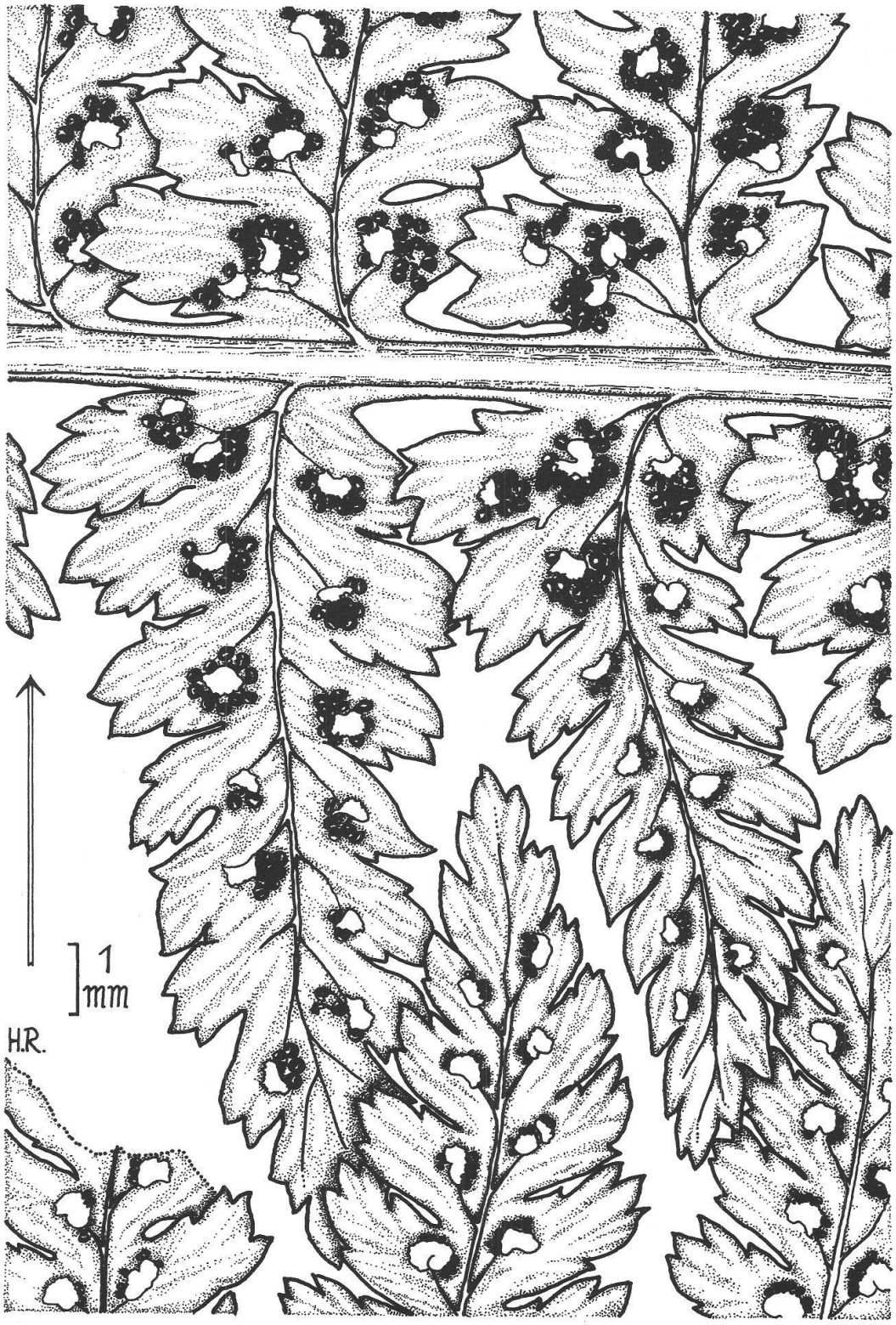


Fig. 1
Athyrium × *reichsteinii* nothosubsp. *reichsteinii* (Ras-240) with tetraploidized tissue and fertile sporangia ("dark spots") above and diploid tissue with sterile sporangia below (drawing H.R.).

The spores were sown in Erlenmeyer flasks containing agar medium (Dyer 1979: 282) slightly modified according to Rasbach and Reichstein (1990). The flasks were kept tightly closed (with "Parafilm M", Amer. Can Comp.) at about 16–22 °C in full daylight (SE-exposed window). When the prothallia reached about 2–3 mm in diameter they were planted (up to 30 prothallia) in small pots (about 5 cm diameter) on limefree soil (Rasbach et al. 1983). These pots were immersed in a bed of sterilized sand in the greenhouse and covered with a transparent plastic cup avoiding direct sunlight, and watered (with drops) every day. Detailed information on the experiments is given in the appendix. When sporophytes appeared and reached a height of 5–10 mm they were pricked out carefully and planted separately into deep narrow pots. They were kept in a cool but frost-free greenhouse during the first two winters; afterwards they were planted in a garden bed outdoors, protected from full sunlight. Unfortunately many plants were lost, especially during the two very dry winters 1985/86 and 1986/87. *Athyrium distentifolium* and the allotetraploid plants are particularly sensitive to drought and cold. The former is protected in nature by abundant snow cover in the places where it grows.

Results

Tetraploidized tissue in A. × reichsteinii

In *A. × reichsteinii* the pinnules or the parts of the pinnae formed by tetraploidized tissue are somewhat larger. This is apparently due to the greater cell size which is also observed when the guard cells are compared (see Schneller and Rasbach 1984, Fig. 9 and Table 2 in this article).

The occurence of this characteristic feature (chimera formation, somatic polyploidization within a plant) is quite rare in nature. It seems to be dependent on environmental conditions and possibly on some regulatory instabilities. We realized that plants which showed tetraploidized tissue in one year did not do so in the next. Somatic tetraploidization, however, was not only seen in different localities of the Black Forest (Feldberg, Germany) but also in Switzerland (Bödmerenwald, Muotatal).

As mentioned earlier, the size of the tetraploid tissue is somewhat variable. Sometimes it covers only parts of a single pinna, sometimes several pinnae. The pattern of this tissue is dependent on the age of differentiation. If cell division of a meristematic cell early in the development is incomplete (and thus leads to polyploid derivatives), the area of tetraploidized tissue will be larger compared to a similar event later in development. The pattern of this tissue may have originated by divisions of a single tetraploidized cell. It may allow some indications on differentiation because of its "marker"-characteristic.

Normal and tetraploidized sporemothercells (the latter in sporangia on tetraploidized tissue) of the same plant of *A. × reichsteinii* (Ras-329) could be analysed cytologically. In the same preparation we were able to find several cells with 80 univalent chromosomes and three cells with 80 pairs. This means that the fixed material contained normal df tissue and tetraploidized ddff tissue, respectively (Figs. 2a, 2b).

Spores

The spores of *A. filix-femina* and *A. distentifolium* can easily be distinguished under the light microscope (Fig. 4, Table 1). The spores of *A. filix-femina* are somewhat larger and have a thin, smooth perispore, whereas the spores of *A. distentifolium* are smaller and are covered with a thick, rugose perispore. The spores of the allotetraploid (ddff) are larger than those of both diploid species and are covered with a perispore of intermediate

Tab. 1 Exospore dimensions of the different taxa measurements taken from 25 spores each

BF: Black Forest; ZH: Canton Zürich; SZ: Canton Schwyz; art. hybr.: artificial hybrid

Taxon	Exospore length µm	mean µm
A. filix-femina BF Sch-385	37–43–50	42.70
A. filix-femina ZH A-1	35–40–44	39.63
A. distentifolium BF Sch-394	33–38–43	38.92
A. distentifolium SZ Sch-392	31–35–40	35.38
A. × *reichsteinii** BF Sch-369	45–51–55	50.74
A. × *reichsteinii** BF Sch-376	45–52–61	52.48
A. exp. tetrapl. (ddff) TR-5986 (4)	47–54–63	53.58
	Exospore width	mean
A. filix-femina BF Sch-385	24–28–32	27.97
A. filix-femina ZH A-1	24–27–30	26.63
A. distentifolium BF Sch-394	23–27–31	26.79
A. distentifolium SZ Sch-392	24–26–30	25.61
A. × *reichsteinii** BF Sch-369	31–36–41	35.85
A. × *reichsteinii** BF Sch-376	27–35–40	34.59
A. exp. tetrapl. (ddff) TR-5986 (4)	31–36–41	35.62

* Spores from tetraploid tissue

(relatively thin) structure (Schneller 1989). The difference in spore size seems to be correlated with the genome size (Fig. 3).

Guard cells

Measurements of the size (length) of the guard cells of the different taxa and ploidy levels showed a good correlation to exist between ploidy level and cell-size (Table 2).

Results of breeding experiments

As mentioned by Schneller and Rasbach (1984), the diplospores (df) from the "dark spots" (Fig. 1) are viable and produce normal gametophytes which were caryologically

Fig. 2

A. × *reichsteinii* (Ras-329) Meiosis of normal and tetraploidized sporemothercells, both in the same preparation.

a, a′: $n = 1^{II}, 78^{I}$: a = photo, a′ explanatory diagram. b, b′: $n = 80^{II}$; b = photo, b′ explanatory diagram. Bivalents black, univalents outlined.

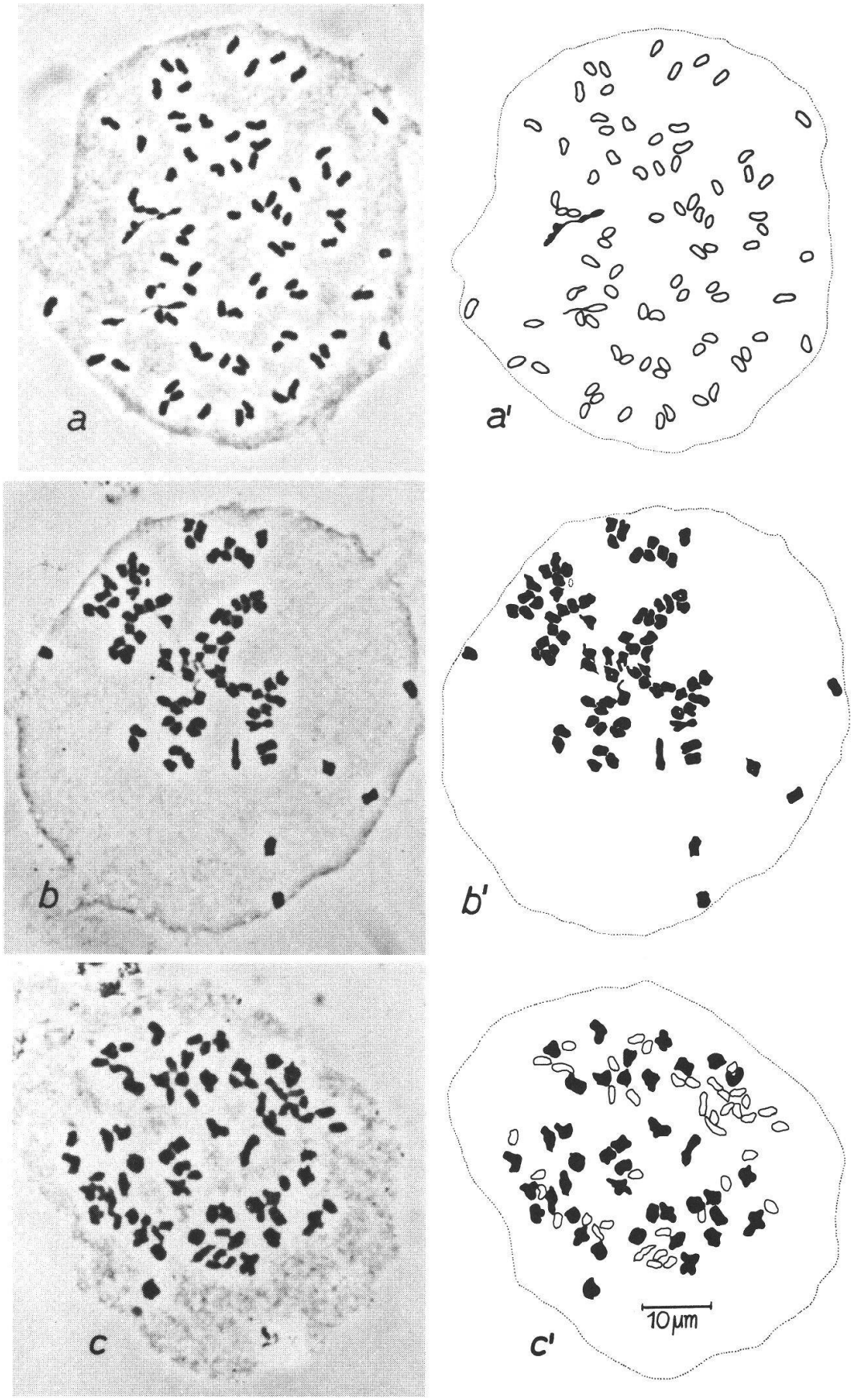
Experimentally produced plants (see also appendix Table 1)

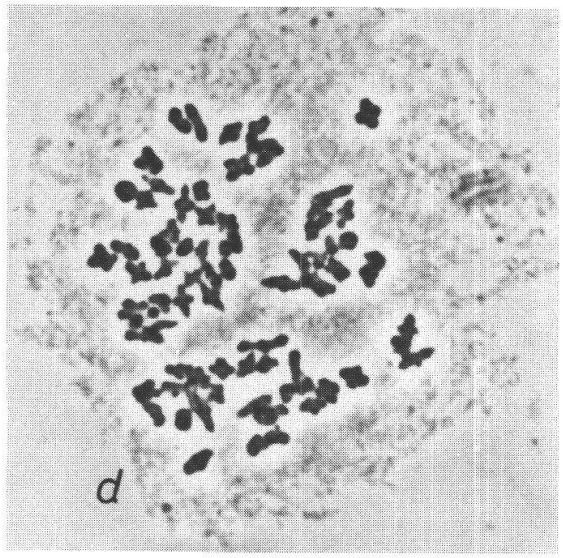
c, c′: TR-5985 (1), experimentally produced triploid hybrid (dff). Sporemothercell at meiosis with $n = 40^{II}$ and 40^{I}, c = photo, c′ = explanatory diagram. Bivalents black, univalents outlined.

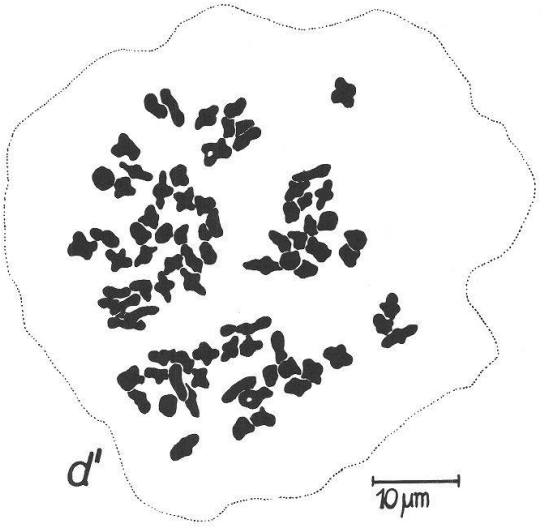
d, d′: TR-5986 (4), experimentally produced tetraploid hybrid plant (ddff). Sporemothercell at meiosis with $n = 80^{II}$, d = photo, d′ = explanatory diagram.

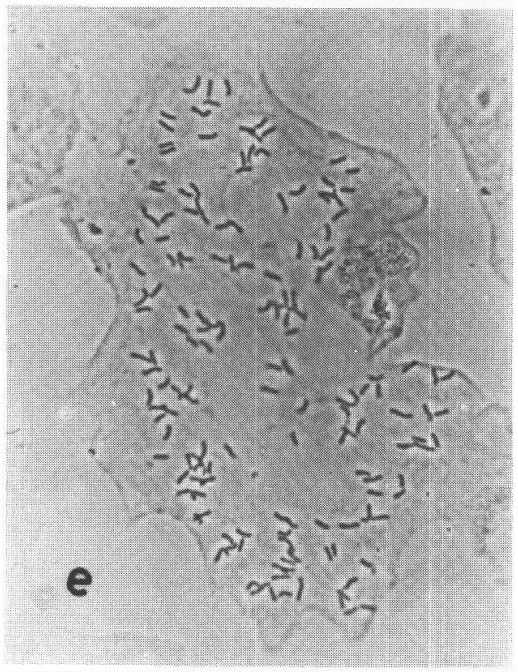
e, e′: Mitosis in roottips of experimentally produced allotetraploid (ddff) (TR-6223 (6)), 2n = 160. e = photo, e′ = explanatory diagram (scale see f′).

f, f′: Mitosis in roottips of experimentally produced allotetraploid (ddff) (TR-6243 (2), 2n = ca. 160 (158 counted), f = photo, f′ = explanatory diagram.

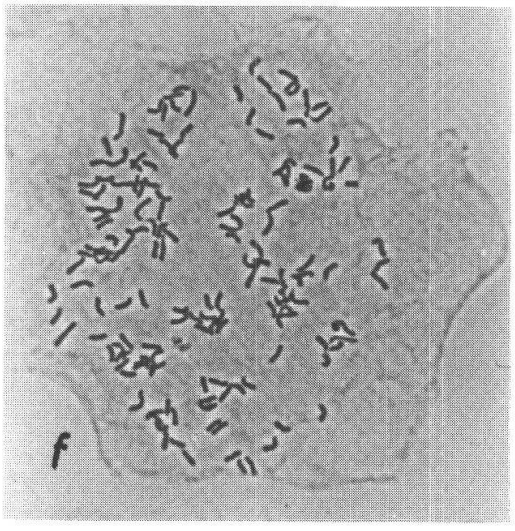

a
a'
b
b'
c
c'
10 µm


d


d'
10 µm


e


e'


f


f'
10 µm

Tab. 2. Length of guard cells of stomata of different plants

Taxon	Length µm	Mean µm
A. filix-femina (ff) A-1	47–52–59	51.69
A. filix-femina (ff) Sch-385	37–46–49	45.54
A. distentifolium (dd) Sch-437	43–51–59	50.67
A. distentifolium (dd) Sch-1569	43–50–55	50.33
A. × *reichsteinii* (df) Sch-434	41–46–53	46.06
A. × *reichsteinii* (df) Sch-1546	43–49–53	49.25
A. × *reichsteinii* (df) Ras-284	41–47–51	46.88
A. × *reichsteinii* (ddff) Ras-284*	55–63–72	63.04
A. × *reichsteinii* (df) Ras-376	43–48–53	47.98
A. × *reichsteinii* (ddff) Ras-376*	55–60–67	59.88
A. × *reichsteinii* (ddf) Sch-424	51–59–67	59.33
A. × *reichsteinii* (ddf) Sch-1557	51–59–67	59.25
A. × *reichsteinii* (dff) Sch-381	53–57–61	57.28
A. × *reichsteinii* (dff) Sch-412	49–58–65	57.91
A. × *reichsteinii* (dff) TR-5985**	49–55–63	55.39
A. × *reichsteinii* (ddff) TR-5986***	57–67–76	67.84

* tetraploidized tissue
** artificial hybrid
*** experimental tetraploid

examined. They contained about 80 chromosomes in their nuclei. They also produced normal archegonia and antheridia but in the first experiment done by one of us (J.S.) in 1983, they failed to produce sporophytes.

We were interested in the synthetic production of tetraploid plants and, therefore, the sowing of diplospores was repeated. The first experiments (sowings TR-5902 and TR-5903) gave similar results. Prothallia were easily obtained on agar medium; after planting in soil they remained green and continued to grow for many months but did not produce sporophytes. As this might be due to self-sterility we attempted "hybridisation" between these prothallia of two different origins (experiments TR-5983 and TR-6243). Besides some inevitable diploids and triploids, both experiments gave some tetraploid plants (see below) but most of the plants of experiment TR-5983 were lost during the winter of 1985/86. One plant of the latter experiment (TR-6243) survived and became fertile and was still alive in June 1990. Only later did we realize that the above-mentioned failure to obtain tetraploid sporophytes from the diplospores (df) must be due to reasons other than self-sterility.

Further planting on soil (27 September 1985) of prothallia raised from diplospores (TR-6223 = Ras-284, sown on 16 May 1985) resulted in seven viable sporophytes potted 16 April 1986; of these root-tips were fixed. Plants no. 2, 4 and 6 were tetraploid, plant no. 1 was triploid, the other three could not be counted (Fig. 2e).

In 1990 we had about 12 tetraploid sporophytes (2n = about 160 in root tips), rigorous enough to be likely to survive the following winter outdoors. Two of them (TR-5984 (1) and TR-5986 (4); see appendix) were fertile, showing 80^{II} in meiosis (Fig. 2d) and producing well formed, large spores. Of the triploid hybrids only one plant (TR-5985 (1); see appendix) survived to reach fertility. It showed $n = 40^{II}$ and 40^{I} in meiosis and 2n ca. 120 chromosomes in mitosis (Fig. 2c). This is the same result as found for natural triploids (Schneller and Rasbach 1984). The morphology of the indusia corresponds to

"dff". Root-tips of young triploid hybrids of the combination "ddf" were also examined cytologically. The plants succumbed outdoors during the winter of 1985/86.

It has to be noted that the production of triploids is more successful than that of tetraploids and that the tetraploids (= allopolyploid progeny of the hybrid *A.* × *reichsteinii*) are less vigorous when grown outdoors than the parent species, the diploid hybrid, and the triploids.

When we compared the plants growing side by side, the allotetraploids look slightly crisped with the pinnules somewhat more dissected (Fig. 3). The indusia were very similar to those of the diploid hybrid (df). The spores correspond to those of the somatically tetraploidized pinnules of the diploid hybrid (Fig. 4). Among the other 14 plants which we planted in pots as baby sporophytes and which we grew in the greenhouse during the very dry and cold winters of 1985/86 and 1986/87, there were some tetraploids. They were large enough to survive in the garden bed outdoors but not yet fertile. The tetraploids among them which could be counted (TR-5986 (4) (Fig. 3), TR-6243 (1, 2, 3, 5, 6), TR-6447 (1, 2), TR-6448 (1, 4), and TR-6449 (1, 2)) have exactly the same crisped appearance as the large plant TR-5984 (1).

Because of the fact that triploid hybridogenous taxa occur in nature, and as similar triploids could be produced experimentally, we decided to describe two new nothosubspecies.

Nomenclature and typification

Identification of an *Athyrium* hybrid in the field by examination of the indusia with a hand-lens is nearly always possible for an experienced pteridologist. For some diagnoses examination of the content of sporangia under the light microscope is sufficient. Distinguishing the three hybrids in the field needs more experience but is also possible. Cytological examination is necessary as a final proof.

As we describe here two new nothosubspecies, the diploid hybrid *A.* × *reichsteinii* Schneller et Rasbach 1984 (df) will bear the autonym nothosubsp. *reichsteinii*.

Although the formation of the two triploid hybrids, originating from unreduced spores formed on the diploid hybrid *A.* × *reichsteinii*, is a special case which is not explicitly mentioned in the International Code of Botanical Nomenclature (Greuter et al. 1988) we define the resulting triploids as nothotaxa. The result (triploid hybrids) is the same as when an allotetraploid species is crossed with either one of its diploid parents.

A. × *reichsteinii* nothosubspecies *microderris* Rasbach, Reichstein et Schneller nothosubsp. nov. Filix triploidea chromosomatibus 120, meiosi 40 univalentibus 40 bivalentibus. Habitus inter parentes, Athyrio distentifolio similior; indusia parva et irregularia, diametro maximo 0.5 mm (figura 5a). Sporae fere omnes abortivae. Extremitas venarum a margine medio 140 µm remota. Formula genomatis ddf.

Typus: Germany, Black Forest; Feldberg, Hochkopf, Wanne, ca. 1220 m alt., first found 15/8/1982; coll. 27/7/1990 (Sch-424), 5 fronds (Z).

Intermediate between *A. filix-femina* and *A. distentifolium* but closer to the latter. Fronds up to 1.80 m long. Sori roundish with a small, irregular indusium. Veins in pinnules nearly reaching the edge of the teeth. Triploid, 2n = 120, meiosis with 40^{II} and 40^{I}, genome formula ddf (backcross of ddff with dd). Spores mostly aborted. The name *microderris* was chosen because the plants are characterized by small, more or less roundish indusia, diameter up to 0.5 mm (Fig. 5a). At the locus classicus the hybrid shows clonal organisation, colonizing an area of about 4 m^2 with about 20 plants. Diploid *A.* × *reichsteinii* nothosubsp. *reichsteinii* (Ras-240) is found in close vicinity.

Fig. 3
Frond of experimental allotetraploid (ddff) (TR-5986 (4))

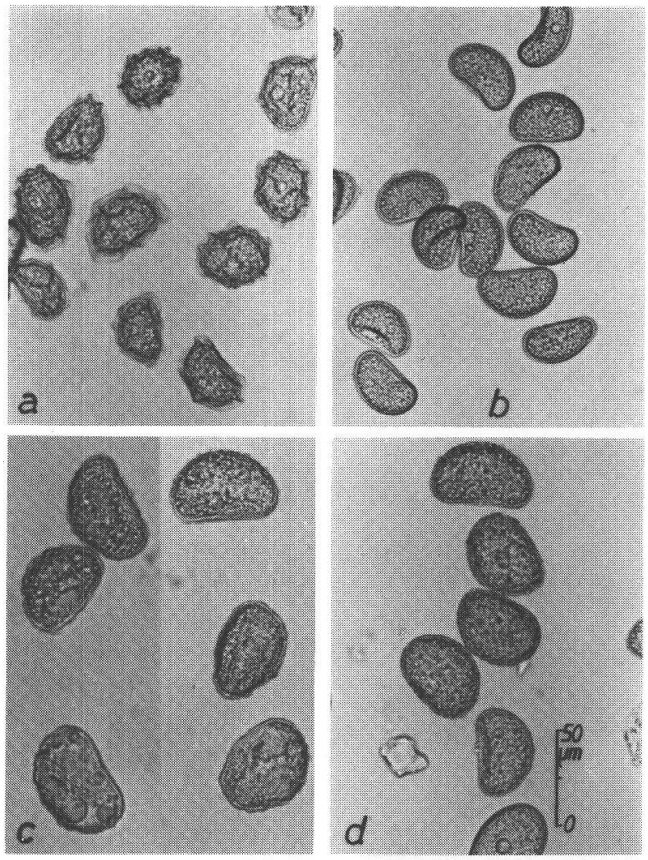


Fig. 4
Spores of a) *A. distentifolium* (d) (Ras-783), b) *A. filix-femina* (f) (Ras-784), c) spores from "dark spots" (tetraploidized tissue) (ddff) (Ras-329), d) spores from experimental allotetraploid (ddff) (TR-5986 (4)).

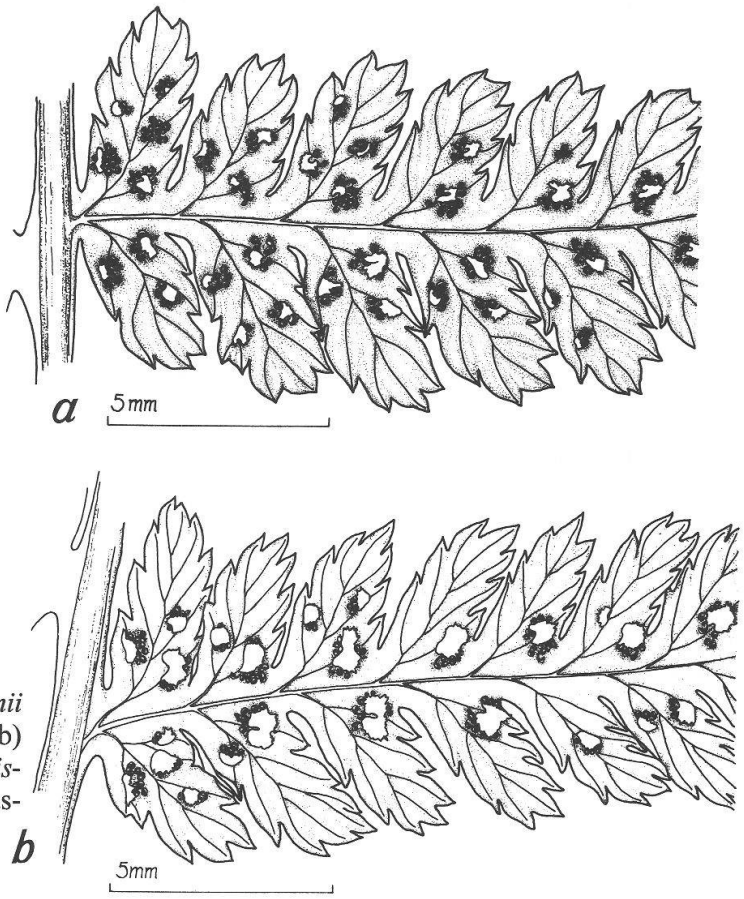


Fig. 5
Pinnules with sori of a) *A.* × *reichsteinii* nothosubsp. *microderris* (Sch-424), b) *A.* × *reichsteinii* nothosubsp. *praetermissum* (Sch-342), from Schneller and Rasbach (1984).

A. × *reichsteinii* nothosubspecies *praetermissum* nothosubsp. nov. Filix triploidea chromosomatibus 120, meiosi 40 univalentibus 40 bivalentibus. Habitus inter parentes, *A. filix-femina* similior. Indusia magis rotundata quam in *Athyrio filix-femina*, extensione maxima 1 mm (figura 5b). Sporae fere omnes abortivae. Extremitas venarum a margine medio 80 µm remota. Formula genomatis dff.

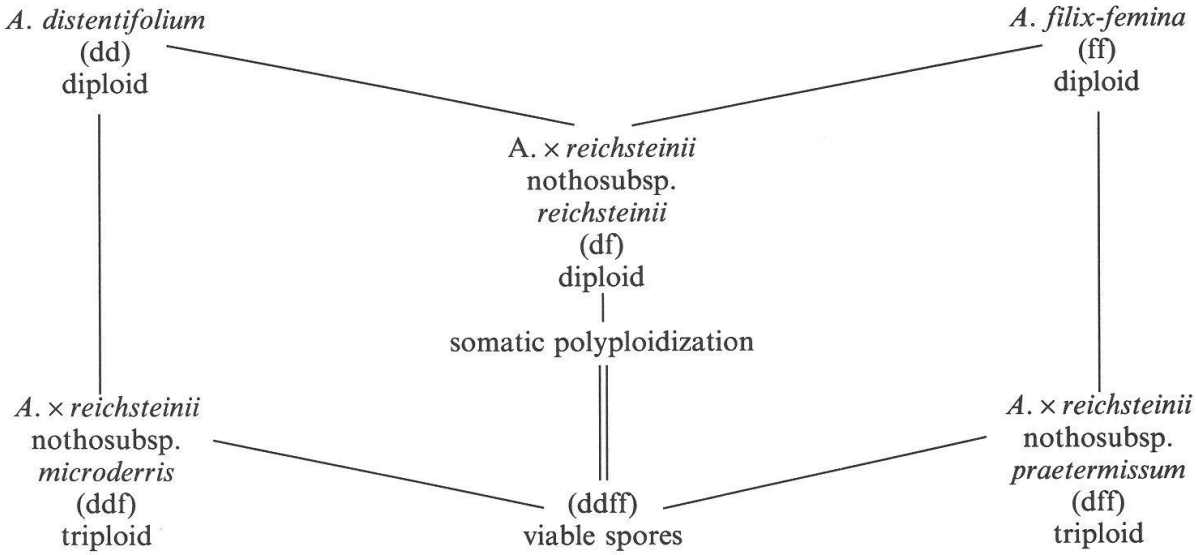
Typus: Germany, Black Forest; Feldberg, Rinken, track to Felsenweg, near Goldersbach, 1290 m alt., first discovered 24/8/81; coll 15/8/1990 (Sch-371), 7 fronds (Z).

Intermediate between *A. filix-femina* and *A. distentifolium* but closer to the former. Fronds up to 1.6 m. Sori similar to those of *A. filix-femina* but somewhat more roundish, and indusium somewhat smaller and more roundish (Fig. 5b), up to 1 mm in length. Veins in pinnules falling short of teeth (as in *A. filix-femina*). Triploid, 2n = 120, meiosis with 40^{II} and 40^{I}, genome formula ffd. Spores mostly aborted.

At the locus classicus the hybrid forms (clonal) a population consisting of many plants covering about 3 m^2. Diploid *A.* × *reichsteinii* nothosubsp. *reichsteinii* grows in close vicinity.

We use the term *praetermissum* because the plant was overlooked until very recently although it forms large clones even close to well-trodden trails.

As a summing-up we add a scheme of the relation between the different taxa dealt with in this investigation



Specimens

We add here a list of diploids and triploids previously referred to by Schneller and Rasbach (1984).

A. × *reichsteinii* nothosubsp. *reichsteinii*

Switzerland Klosters (Grisons), east of Klosters, ca. 1600 m, leg. R. Göldi, H. and K. Rasbach and T. Reichstein. 18. 7. 1988;
Switzerland Engelberg, Farenegg, 1250 m, $n = 80^{I}$. leg. H. and K. Rasbach, J. J. and M. Schneller, 27. 7. 1990 (Sch-1547 = Ras-755);
Germany. Feldberg (Black Forest): Rinken, in the vicinity of Sägenbach, 1270 m, $n = 80^{I}$. leg. H. and K. Rasbach, July 1983 (Ras-285);
Zastler Loch below summit of Feldberg, between 1350 and 1370 m. Three small clones. leg. H. and K. Rasbach, July 1984 (Rasbach s.n.);

Baldenweger Weide, 1270 m, leg. H. and K. Rasbach, July 1984 (Rasbach s.n.);
Near Caritasheim, 1200 m, leg. H. and K. Rasbach, 21. 8. 1985 (Ras-483);
Ruckenwald, near Feldberg-Pass, 1300 m, large clone. leg. H. and K. Rasbach, August 1989 (Rasbach s.n.);
Seebachtal, near Bärental, 950 m, leg. H. and K. Rasbach, 14. 8. 1991 (Ras-794).

A. × *reichsteinii* nothosubsp. *microderris*

Switzerland, Engelberg, track to Surental. Farenegg, NE of Restaurant Alpenrösli. 1280 m alt. leg. J. Schneller, H. Rasbach. 27. 7. 1990. Cytologically examined by H. Rasbach, with 40^{I} and 40^{II}, (Ras-746). Further individuals from the same locality (Sch-1555, Sch-1556, Sch-1557, Sch-1558) (guard-cell size like type specimen). Nearby *A.* × *reichsteinii* nothosubsp. *reichsteinii* (df) (cytologically checked by H.R., Ras-755 = Sch-1547) was growing.

A. × *reichsteinii* nothosubsp. *praetermissum*

Germany, Feldberg (Black Forest) between Rinken and Baldenweger Buck. 1210 m, leg. H. and K. Rasbach, 26. 6. 1983. Cytological examination showed $n = 40^{II}$ and 40^{I} (Ras-328).

Discussion

Our experiments show that partial somatic tetraploidization is indeed the mechanism leading to the formation of triploid *Athyrium* hybrids in nature. In the formation of such hybrids somatic polyploidization plays an important role. On tetraploidized tissue tetraploid sporangia and sporemothercells are formed (genome content of cells = ddff), in which meiosis is regular and leads to the formation of spores with the genomic formula df. Using gametophytes originating from such spores, we could obtain triploid hybrids by backcrossing with either parent. Because somatic tetraploidization in *A.* × *reichsteinii* was found several times in nature and because triploid hybrids occur in different areas but always in the same area as the diploid *A.* × *reichsteinii* nothosubsp. *reichsteinii,* we suppose that they were formed like the synthetic hybrids. This type of tetraploidization – although only rarely taking place – may produce numerous spores (depending on the size of the tetraploidized area) and makes the occasional formation of triploids possible.

We also succeded in raising synthetic "allotetraploid" progeny. In view of the fate of the synthetic hybrids in the garden there is some evidence that these tetraploids are somewhat less viable than the triploids. This may be a reason why until now we could not find any allotetraploids in nature. The formation of such allotetraploids may be rare because every one of the few diplospores reaching the ground is surrounded by a large number of spores of one or both parent species so that any diploid gamete will unite with a haploid gamete and form a triploid hybrid. Formation of allotetraploids may be rare because of the gender development. Due to the presence of an antheridiogen system gametophytes are most frequently unisexual (Schneller 1979). Intragametophytic selfing (a possibility for obtaining an allotetraploid ddff from a df gametophyte) seems to be a very rare event. For the formation of an allotetraploid intergametophytic selfing or crossing is therefore necessary. The probability of having two df gametophytes in close proximity is low. The argument that such allotetraploids cannot compete seems to be less convincing, since diploid or triploid hybrids survive and compete quite well with the parent in areas where they occur. It can be shown, however, that in nature there exists a potential for speciation within *Athyrium* through hybridization and polyploidization. As a consequence, it cannot be completely ruled out that an allotetraploid taxon origi-

nating from the cross between *A. filix-femina* and *A. distentifolium* may exist in nature or is going to be formed in future.

The results show a rather uncommon situation for ferns, viz. that triploids exist but tetraploids are apparently lacking. In many well-known cases in ferns the formation of "allotetraploid" species proceeds as follows (see also Lovis 1977): Two different diploid species hybridize to form a diploid sterile hybrid which occasionally forms some diplospores. Gametophytes grown from such spores produce gametes of both sexes which unite (intragemetophytic selfing). The resulting sporophyte is an allotetraploid plant. In flowering plants the formation of allotetraploids is often a two-step process (Lewis 1980). The first step is the union of a reduced (haploid) with an unreduced (diploid) gamete. In a second step the resulting triploid may form some unreduced triploid gametes which fuse with a haploid to form tetraploid offspring.

A similar second step may be possible in *Athyrium* when we assume that "unreduced" spores (ddf or dff) are sometimes formed in the triploid hybrids. This would then be another way resulting in a tetraploid taxon.

The occurrence of somatic polyploidization resulting in chimeras is very rare in higher plants (DeWet 1980). One of the best known cases is *Primula kewensis* (Newton and Pellew 1929). In ferns it was first described by Butters and Tryon (1948) in a natural *Woodsia* hybrid. They were also able to show that spores from polyploidized tissue were viable. Somatic polyploidization was also observed by Vida (1974) in apogamously produced diploid *Cystopteris* and by Schneller (1983) in a haploid sporophyte of *Athyrium*. It is interesting to note that in most cases (flowering plants or ferns) somatic polyploidization is observed in hybrids or in haploidized plants. It may be correlated with some genetic instabilities that arise in hybrids or haploids, as mentioned also by Vida (1974).

The pattern of chimera formation may also allow some interpretation of leaf differentiation.

We thank Dr. K. Rasbach for the photographs and for stimulating discussions, and also Marianne Schneller who as a keen observer discovered the "dark spots". Without this we would have missed an important piece of interesting investigation. We are grateful to Prof. Dr. K. U. Kramer for helping with the Latin diagnoses and for reading and correcting the manuscript.

Zusammenfassung

Wie kürzlich beschrieben (Schneller und Rasbach 1984), bilden die zwei in Europa verbreiteten *Athyrium*-Arten, nämlich *A. distentifolium* Tausch ex Opiz (Genom Formel dd) und *A. filix-femina* (L.) Roth (ff) nicht nur die ziemlich seltene, diploide, sterile Hybride *A.* × *reichsteinii* Schneller und Rasbach (df), sondern überraschenderweise auch zwei triploide Hybriden, die aufgrund der Form der Indusien und der Zytologie als „ddf" und „dff" zu interpretieren sind. Eine allotetraploide „Art" (ddff) konnte aber bis heute nie in der Natur festgestellt werden.

Wir geben diesen zwei triploiden Hybriden jetzt eigene Namen und zeigen, wie sie unter experimentellen Bedingungen entstehen können. Wie von Schneller und Rasbach (1984) erwähnt, bilden sich auf der diploiden Hybride (df), verursacht durch somatische Polyploidisierung, gelegentlich kleine Bereiche von tetraploidem Gewebe (ddff) mit Sori. Im Sommer (Juli/August) können diese an kleinen Gruppen „dunkler Pünktchen" am leichtesten erkannt werden. Es handelt sich um Sori mit Sporangien, die reife, keimfähige

Diplosporen enthalten. Kreuzungen der daraus erhaltenen Prothallien (df) mit solchen von *A. distentifolium* bzw. *A. filix-femina* ergaben die zwei triploiden Hybriden. Die eine davon (dff) konnte bis zur Reife aufgezogen werden und war von natürlichen triploiden Pflanzen nicht zu unterscheiden. Die zweite triploide Form ist leider vorzeitig eingegangen. Aus den Diplosporen konnten außerdem fertile, allotetraploide Pflanzen (ddff) erhalten werden, die aber in der Natur bisher nicht beobachtet wurden.

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Appendix

Origin of spores for sowings and hybridization experiments. The TR number given first is the sowing number. We use the term "dark spots" for the sporangia with mature diplospores borne on the small, somatically tetraploidized area of the diploid hybrid *A.* × *reichsteinii* (df).

A. distentifolium (dd). TR-5900 ex Ras-338, leg. H. and K. Rasbach 14 August 1983, Germany, Black Forest, Feldberg, near Caritasheim, c. 1200 m alt.

A. filix-femina (ff). TR-5901 ex Ras-335, leg. H. Rasbach, 23 June 1983, Germany, Black Forest Fürsatz near Hinterzarten, c. 1100 m alt.

A. × *reichsteinii* (df). TR-5902 = diplospores (df) ex "dark spots" from 12 pinnules of Ras-240 = Sch-376, leg. H. Rasbach, 31 Juli 1983, Germany, Black Forest, Feldberg, between Felsenweg and Emil Thoma Weg, c. 1300 m alt. (see also TR-6341).

A. × *reichsteinii* (df). TR-5903 = diplospores (df) ex "dark spots" from c. 34 pinnules of Ras-238 = Sch-369, leg. H. and K. Rasbach, 30 July 1983, Germany, Black Forest, Rinken, c. 1200 m alt.

A. × *reichsteinii* (df). TR-6223 = diplospores (df) ex "dark spots" from c. 9 pinnae of Ras-284, leg. H. and K. Rasbach, 7 August 1983, Germany, Feldberg, Hochkopf, c. 1200 m alt.

A. × *reichsteinii* (df). TR-6339 = diplospores (df) ex "dark spots" from 4 pinnules of Sch-386, leg. H. Rasbach. 27 August 1985, Germany, Black Forest, between Rinken and Zastlerloch, c. 1200 m alt.

A. × *reichsteinii* (df). TR-6340 = diplospores (df) ex "dark spots" from two pinnules of Sch-367 (type), leg. H. Rasbach 27 August 1985, Germany, Black Forest, Feldberg, near Felsenweg, c. 1320 m alt.

A. × *reichsteinii* (df). TR-6341 = diplospores (df) ex "dark spots" from two pinnules of Sch-376 = Ras-240; leg. H. Rasbach, 27 August 1985.

A. × *reichsteinii* (df). TR-6342 = diplospores (df) ex "dark spots" from 7 pinnules of Ras-483, leg. H. Rasbach, 21 August 1985, Germany, Black Forest, Feldberg, below Caritasheim, c. 1200 m alt.

List and dates of experiments

A	B f		C df	D	E	F	G	H	I
5985 15. 2. 84	5901 (3) 11. 12. 83	×	5902 (6) 11. 12. 83	9 3. 5. 84	9 27. 9. 84	4	3	–	2
5987 8. 5. 84	5901 (6) 11. 12. 83	×	5903 (10) 11. 12. 83	2	2	2	–	–	–
	d		df						
5984 15. 2. 84	5900 (5) 11. 12. 83	×	5902 (5) 11. 12. 83	6 3. 5. 84	6 18. 9. 84	2	1	1	2
5986 24. 2. 84	5900 (10) 11. 12. 83	×	5903 (8) 11. 12. 83	10 2. 5. 84	8 27. 9. 84	3	4	1	–
	df		df						
5983 15. 2. 84	5902 (8) 11. 12. 83	×	5903 (8) 11. 12. 83	8 2. 5. 84	7 16. 9. 84	5	–	1	1
6242 21. 7. 85	5902 (15) 16. 2. 85	×	6223 (30) 16. 2. 85	lost					
6243 21. 7. 85	5903 (15) 16. 2. 85	×	6223 (30) 16. 2. 85	22 Nov. 1985	6	–	–	5	1
6447 29. 3. 86	6339 (35) 7. 12. 85	×	6340 (30) 7. 12. 85	16[1] 21. 1. 86	2			2	–
6448 29. 3. 86	6339 (15) 7. 12. 85	×	6341 (15) 7. 12. 85	25[2] Jul. 86	7			2	5
6449 29. 3. 86	6339 (16) 7. 12. 85	×	6342 (16) 7. 12. 85	19[3] 7. 8. 86	2			2	–
6450 29.3.86	6340 (35) 7.12.85	×	6341 (40) 7.12.85	28[4] 5.10.86					
6451 29. 3. 86	6340 (13) 7. 12. 85	×	6342 (35) 7. 12. 85	16[4] 5. 10. 86					
6452 29. 3. 86	6341 (35) 7. 12. 85	×	6342 (35) 7. 12. 85	14[4] 5. 10. 86					

A = Registration no of experiment. Date of planting of gametophytes on soil
B, C = Registration no of gametophytes of the partners with number of gametophytes used (in brackets). Date of sowing
D = Number of sporophytes which were potted, with date
E = Number of plants of which root fixings were taken, with date.
F = Number of diploids found
G = Number of triploids found
H = Number of tetraploids found
I = Number of fixings which gave no result

[1] two plants still living outdoors 1990, not yet caryologically examined
[2] seven plants still living outdoors in good condition, June 1990
[3] two plants still living outdoors in good condition, June 1990
[4] all died in winter 86/87