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Objekttyp: **Article**

Zeitschrift: **Botanica Helvetica**

Band (Jahr): **94 (1984)**

Heft 1

PDF erstellt am: **21.09.2024**

Persistenter Link: https://doi.org/10.5169/seals-65866

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Botanica Helvetica 94/1, 1984

0253-1453/84/010081-19$1.50 + 0.20/0

Hybrids and Polyploidy in the Genus *Athyrium* (Pteridophyta) in Europe

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Manuscript received April 6, 1983

Abstract

J.J. Schneller and Helga Rasbach. 1984. Hybrids and Polyploidy in the genus Athyrium (Pteridophyta) in Europe. Bot. Helv. 94: 81–99.

The diploid hybrid between *Athyrium filix-femina* (genome formula ff) and *A. distentifolium* (dd) is described here as *A.* × *reichsteinii* hybr. nov. (df). The name *A.* × *cassum* Chiovenda (1929) is on basis of type specimen synonymous to *A. filix-femina.* The meiosis of *A.* × *reichsteinii* shows 76–80 univalents and 0–2 bivalents. Although the parents are known to be diploids, triploid hybrids and also autotriploid *A. filix-femina* (fff) could be found. Morphological differences indicate that two different triploid hybrids occur, one with the probable genome formula ffd and the other with the probable genome formula ddf, both showing 40 bivalents and 40 univalents at meiosis.

A. × *reichsteinii* is confined to areas where the parents are abundant and intermix. The triploid hybrids seem to be rarer. All the hybrids form generally large clones by vegetative propagation.

We found that somatic polyploidization in parts of the diploid hybrids leads to the production of sporangia with «tetraploid» cells (ddff) and as a consequence to df-spores and gametophytes. Hybridization of "df"-gametophytes with "d"- or "f"-gametophytes may result in the triploid hybrids.

Introduction

Three species of *Athyrium* Roth are described in Europe: *A. distentifolium* Tausch ex Opiz, *A. filix-femina* (L.) Roth and *A. flexile* (Newman) Druce. The last one is endemic to Scotland and its taxonomic position is still not clear. All three species are sexual diploids with $2n = 80$ chromosomes (Manton 1950, Löve et al. 1977) corresponding to $x = 40$ as basic number.

A. filix-femina is one of the most common woodland ferns of Europe and has a wide ecological tolerance. *A. distentifolium* is restricted to more mountainous and northern regions (distribution see Jalas & Suominen 1972). There are many areas where *A. filix-femina* and *A. distentifolium* are sympatric, most of them from about 900 m to the timberline in C. Europe or at lower altitudes in northern regions of Europe.

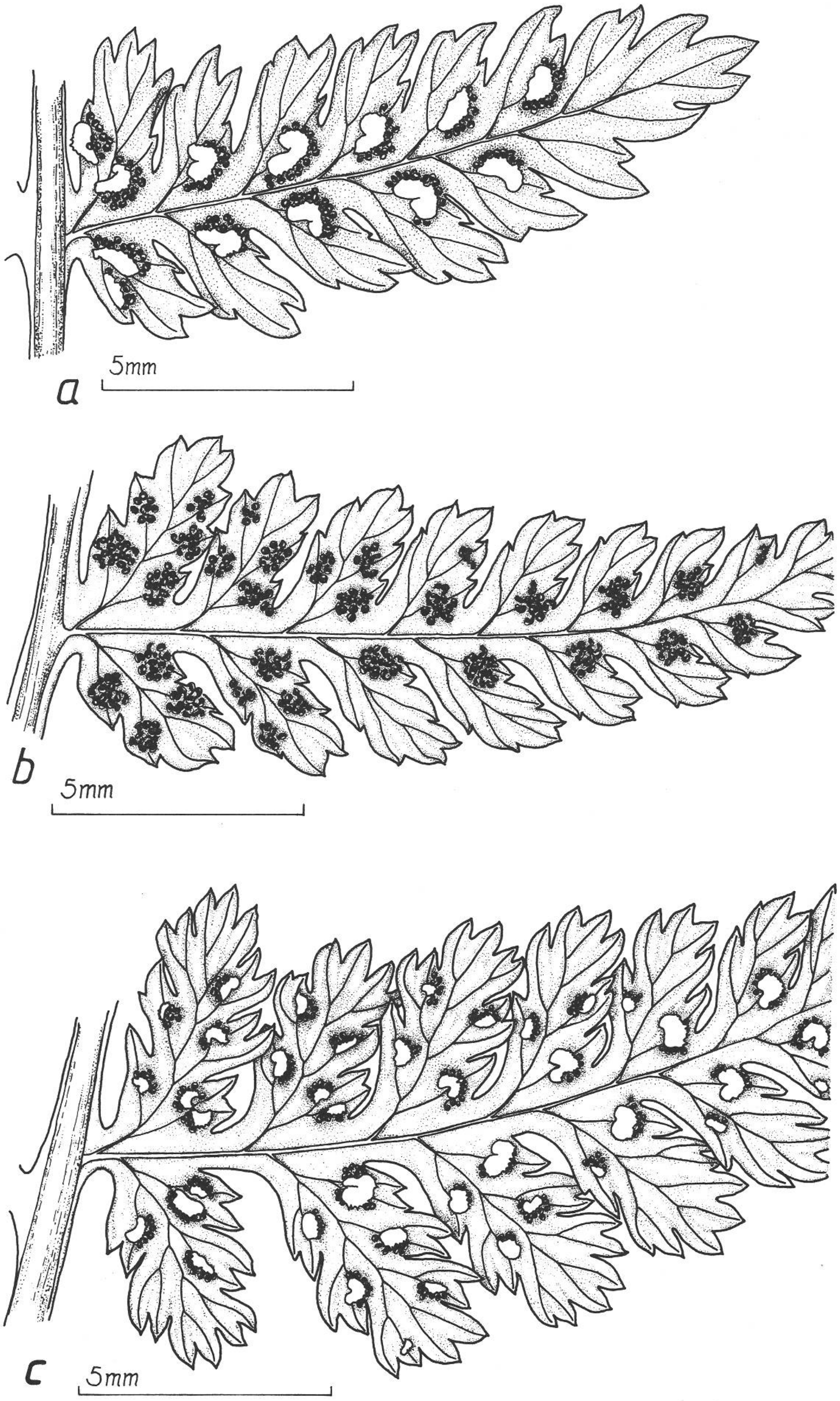
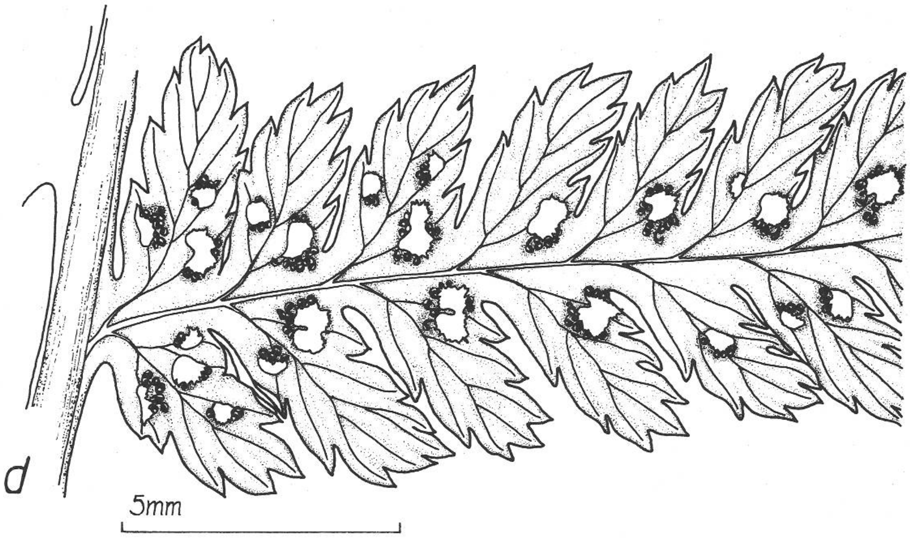


Fig. 1 Pinnules of different genome combinations of *Athyrium*, showing sori and veins.
a) *A. filix-femina* (Ras-286)
b) *A. distentifolium* (Ras-233)
c) *A.* × *reichsteinii*, df (Sch-386)
d) Triploid hybrid, ffd (Sch-342)
e) Triploid hybrid, ddf (Sch-424)
f) Autotriploid *A. filix-femina*, fff (Sch-388)
Drawings: H. Rasbach


d
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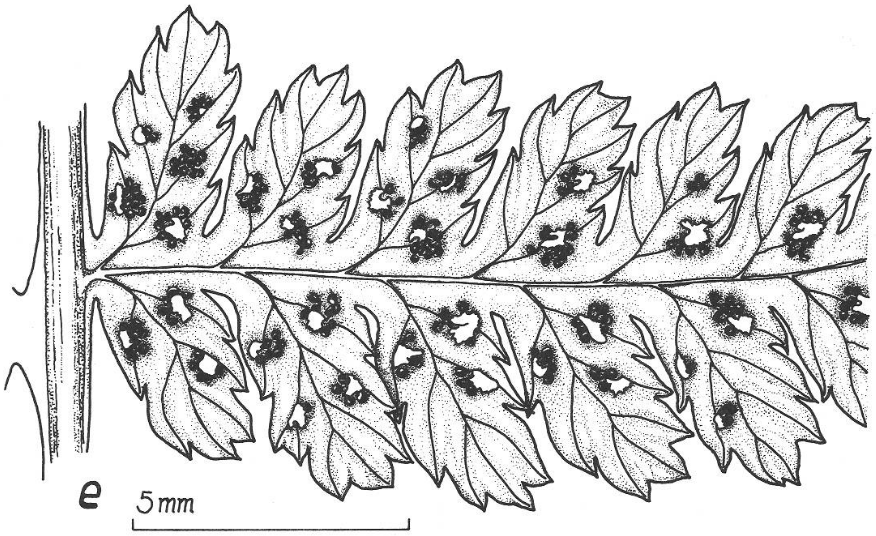
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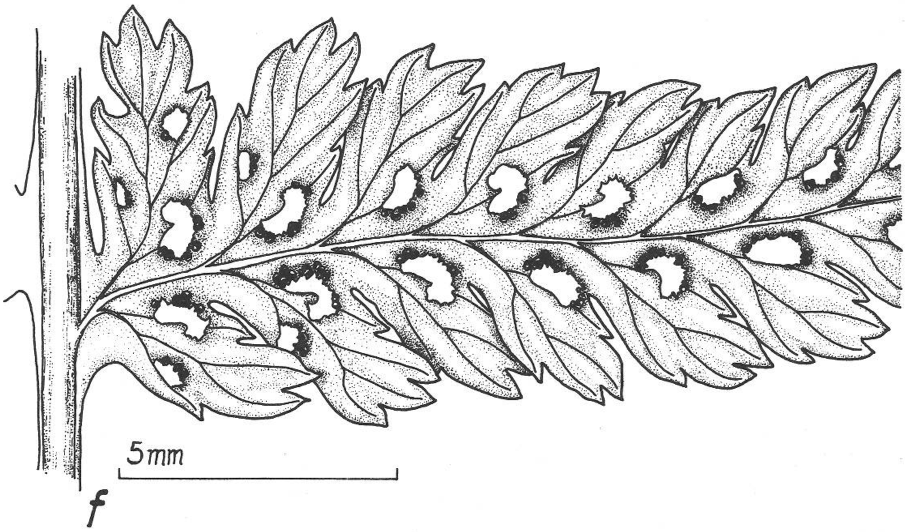
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f

Christ (1900) described the hybrid between *A. filix-femina* and *A. distentifolium* without giving it a formal name. However, until recently the existence of this hybrid was doubted. Since *A. filix-femina* and *A. distentifolium* are very similar – except in their soral and some micromorphological characteristics – the identification of these taxa is difficult. The great intraspecific variability found in *A. filix-femina* (Schneller & Schmid 1982) includes forms which nearly copy *A. distentifolium* in gross morphology. Therefore it is even more difficult to distinguish hybrids from their parents. Such problems of identification have been ascribed to introgression between the two species (Hess et al. 1967). After one of us (J.J.S.) found a diploid hybrid in Switzerland and examined it caryologically, we started a systematic search for hybrids in natural populations. In the centre of our investigation were the questions: What is the ecology of the hybrids? Which conditions are necessary for the formation and what is the vitality of the hybrids? After three years of interesting investigation we present results which strongly influence the interpretation of biology and evolutionary relationships within the genus *Athyrium* in Europe.

Materials and Methods

The plants used in this investigation are quoted in the chapters "Specimens of our collection" and "Specimens in herbaria". Additional notes on mode of investigation were added there. The meiosis was analyzed following the classical acetocarmine method (Manton 1950). Roottips and prothalli were pretreated with 0.1% aqueous solution of cholchicine for 3-5 hours before they were fixed in a mixture of three parts of pure ethanol and one part of glacial acetic acid. They were then stained with a combination of Feulgen reagent and acetocarmine. For the characterization of the different genomes we are using genome formulas. A diploid species like *A. filix-femina* ($2n = 80$) contains two sets of homologous chromosomes in the sporophyte which in this case we abbreviate as "ff" or in the case of *A. distentifolium* as "dd". After meiosis (reductional division) spores with one chromosome set ("f" or "d") result.

Morphological Characteristics and Caryological Examination

As expected, most of the hybrids between the two diploid species were found to be diploid as well. A few plants, however, according to their caryological and morphological state had different ploidy levels.

Diploid hybrids (genome formula df)

Before considering hybrids it is necessary to define the differences between the two species *A. filix-femina* (genome formula ff) and *A. distentifolium* (genome formula dd). Under the same ecological conditions both species are of the same size. The shape of the fronds is very similar and cannot be used for identification. The best discriminating characteristic is the sorus. Sori of *A. filix-femina* are oblong with oblong indusia which are fimbriate at the margin, and which often have the shape of a comma (Fig. 1a). The indusia can always be discerned in the mature state even if the sori are crowded together. In contrast to *A. filix-femina* the sori of *A. distentifolium* are orbicular, and the indusia rudimentary or absent; if they occur they can be observed only at a young stage (Fig. 1b). In *A. filix-femina* the spores are olive-yellow and nearly smooth; sometimes small wartlike structures can be seen. In *A. distentifolium* the spores are brown and wrinkled (Fig. 4a, c).

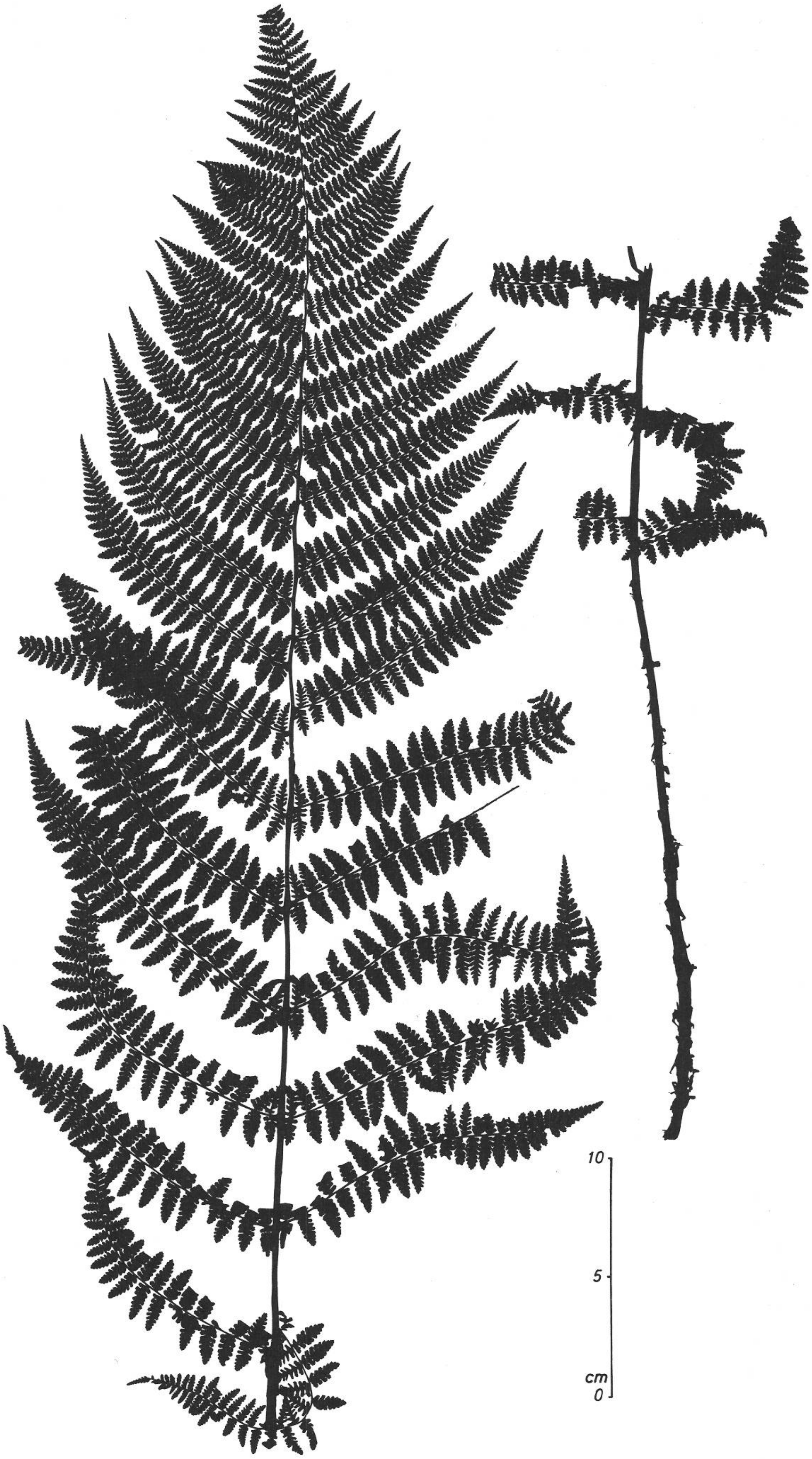

Fig. 2 One leaf of diploid *A.* × *reichsteinii.* Germany, Black Forest, Rinken (Sch-373)

We observed another difference between the two species looking at the vein-endings. A characteristic which was hitherto not described in the literature. The distance between the endings of the veins and the border of the pinnules is strikingly larger in *A. filix-femina* than in *A. distentifolium* (Fig. 10).

The diploid hybrid has the same size as the parents and is morphologically intermediate. There is no vegetative characteristic which allows a reliable identification (Fig. 2, 3). The vein characteristic is not very different from *A. filix-femina* showing some tendency for smaller values (Fig. 10). The sizes of the guard cells in the hybrid correspond with those of the parents. The sori, however, are very characteristic in the hybrid. They are almost as round as in *A. distentifolium* but with an indusium which is smaller than in *A. filix-femina* (Fig. 1c). Caryological examination showed, that in meiosis nearly all of the chromosomes are unpaired. Sometimes one or two bivalents can be distinguished (Fig. 5a, 6a). The formation of the spores is irregular. Often the sporangia shrink before they are ripe. Most of the spores abort (Fig. 4b). In experiments no germinating spores could be observed.

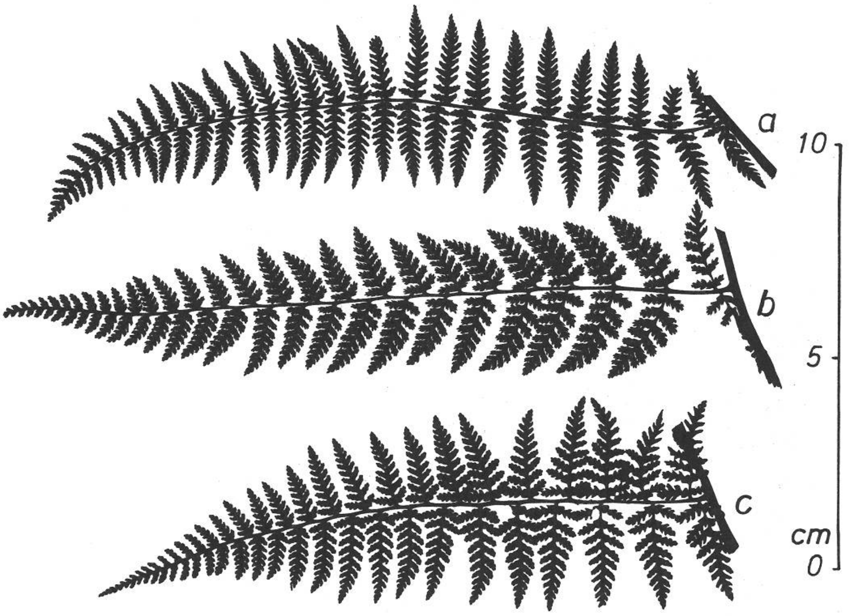

Fig. 3 Silhouettes of pinnules. a) *A. filix-femina* (Ras-234) b) *A.* × *reichsteinii* (Sch-386) c) *A. distentifolium* (Ras-233)

Evidence for somatic polyploidization in the diploid hybrid

Some of the hybrid plants collected in Switzerland (Muotatal, Kt. Schwyz, Sch-434) and in Germany (Black Forest, Sch-369, Sch-376, Ras-284, Ras-329) showed a very peculiar characteristic.* Parts of leaves developed wider pinnae, wider section of pinnae or wider pinnules which showed a darker green than the rest of the leaf (Fig. 7). The sori on these pinnae and pinnules had larger sporangia which contained well-developed spores (Fig. 4h). Such spores look more like those of *A. filix-femina* than those of *A. distentifolium*. On the remaining of the leaf normal hybrid sori with aborted spores

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* Thanks to the very critical and precise observation of Marianne Schneller this characteristic was recognized.

are formed (Fig. 4g). Comparison of the well developed spores of the diploid parents and comparison of guard cells (Fig. 9) lead to the conclusion that the wider leaf parts are polyploid. Partial somatic polyploidization was described recently for haploid sporophytes of *A. filix-femina* (Schneller 1983, incl. further references). The regular spores of polyploid pinnules in the hybrids are viable and develop regular prothalli. Such prothalli could be caryologically examined. They have about n = 80 = 2x chromosomes in their cells. However, up to April 1983 no sporophytes have developed on such prothalli.

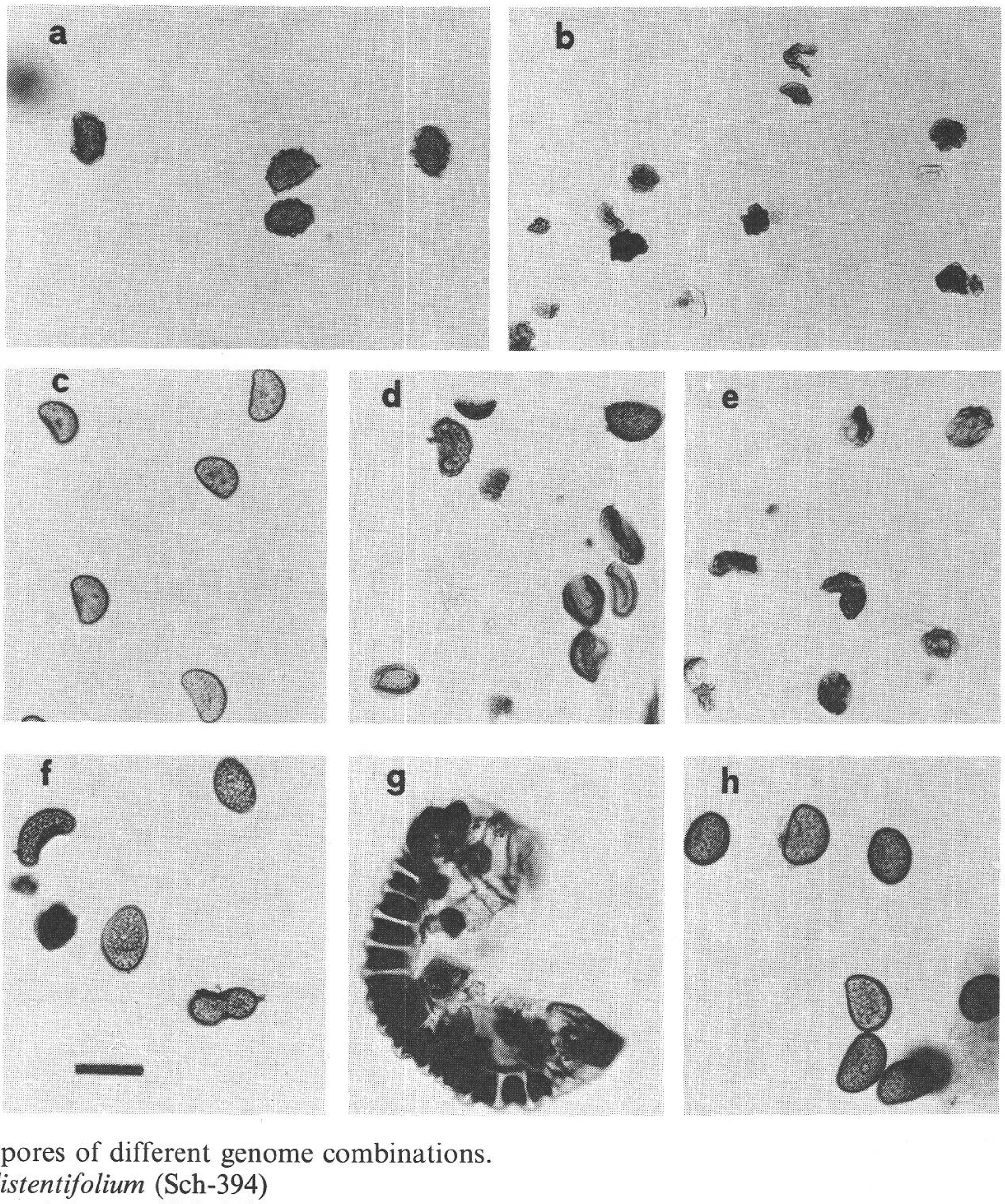

Fig. 4 Spores of different genome combinations.
a) *A. distentifolium* (Sch-394)
b) *A.* × *reichsteinii* (Sch-350)
c) *A. filix-femina* (Sch-A30)
d) Triploid hybrid, ffd (Sch-366)
e) Triploid hybrid, ddf (Sch-424)
f) Autotriploid, fff (Sch-388)
g) *A.* × *reichsteinii* (Sch-434), sporangia with aborted spores on diploid tissue
h) Same plant as g), regular spores, originating from polyploidized tissue

Triploid hybrids

Exploring many populations with both species of *Athyrium* present, it was a great surprise to find not only diploid but also triploid hybrids. So far triploids have been found only in the Black Forest (Germany). The triploid hybrids again are morphologically intermediate between the parents, but we have evidence to distinguish two different types of triploids, both of which show hybrid vigour. For one type we propose the hypothetical genome formula ffd. It looks more like *A. filix-femina* because of the soral

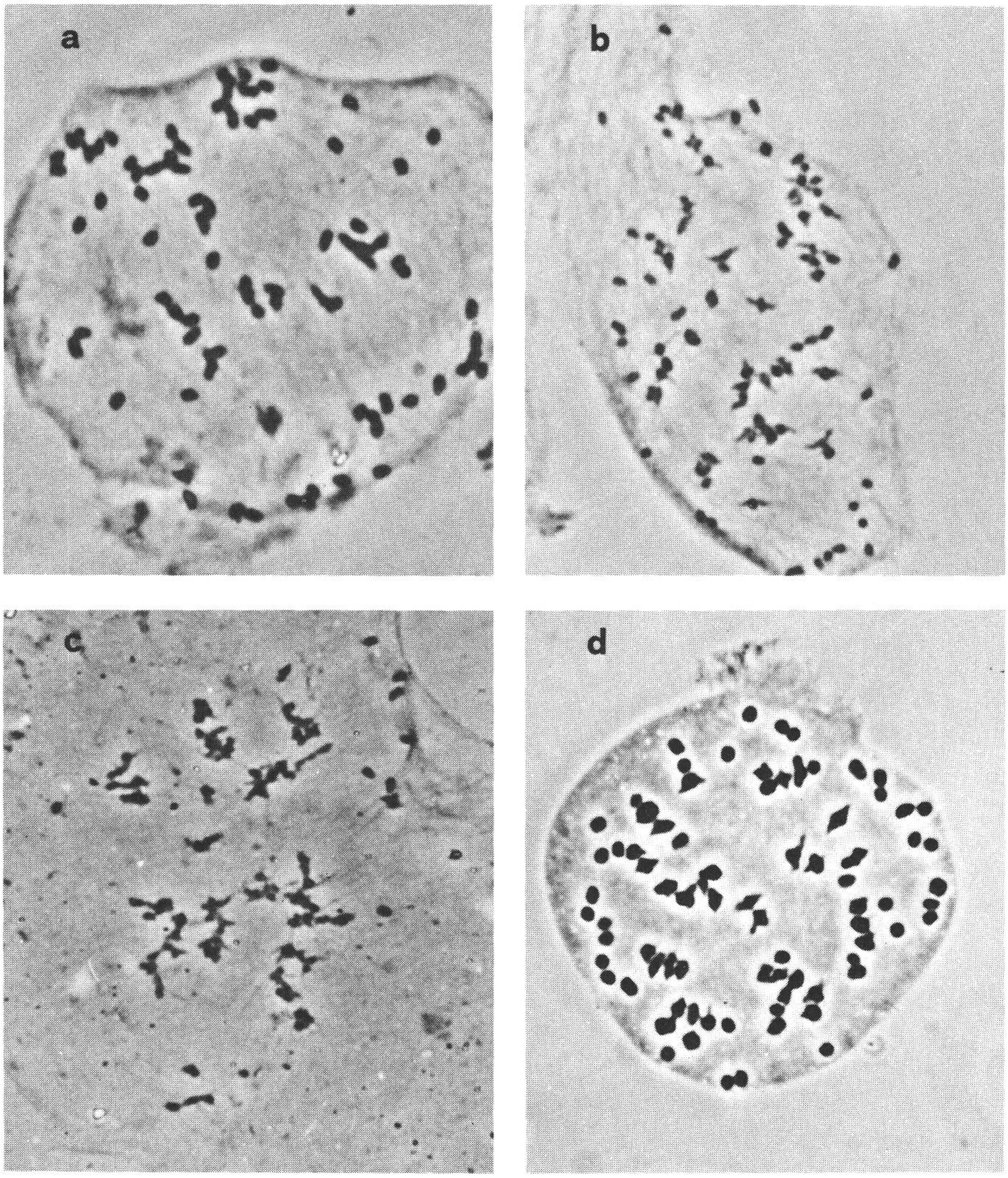

Fig. 5 Cytological results.

a) *A.* × *reichsteinii,* meioisis, metaphase I (Sch-438)
b) Triploid hybrid, ffd, meiosis, metaphase I (Sch-366)
c) Autotriploid *A. filix-femina,* fff, meioisis, metaphase I (Sch-388)
d) Triploid hybrid, ddf, meiosis, metaphase I (Sch-424)
(For explanatory diagram see Fig. 6)

characteristics (shape of the sorus somewhat elongated and indusium well developed but somewhat smaller) (Fig. 1d), and the ending of the veins in the pinnules (Fig. 10). This triploid is intermediate between *A. filix-femina* and the diploid hybrid.

The second type of triploids is closer to *A. distentifolium* (Fig. 1e) in both the soral characteristics (showing a more round sorus and a very small indusium) and the type of nerve-ending (which is very close to the border) (Fig. 10). We give this type the hypothetical genome formula ddf.

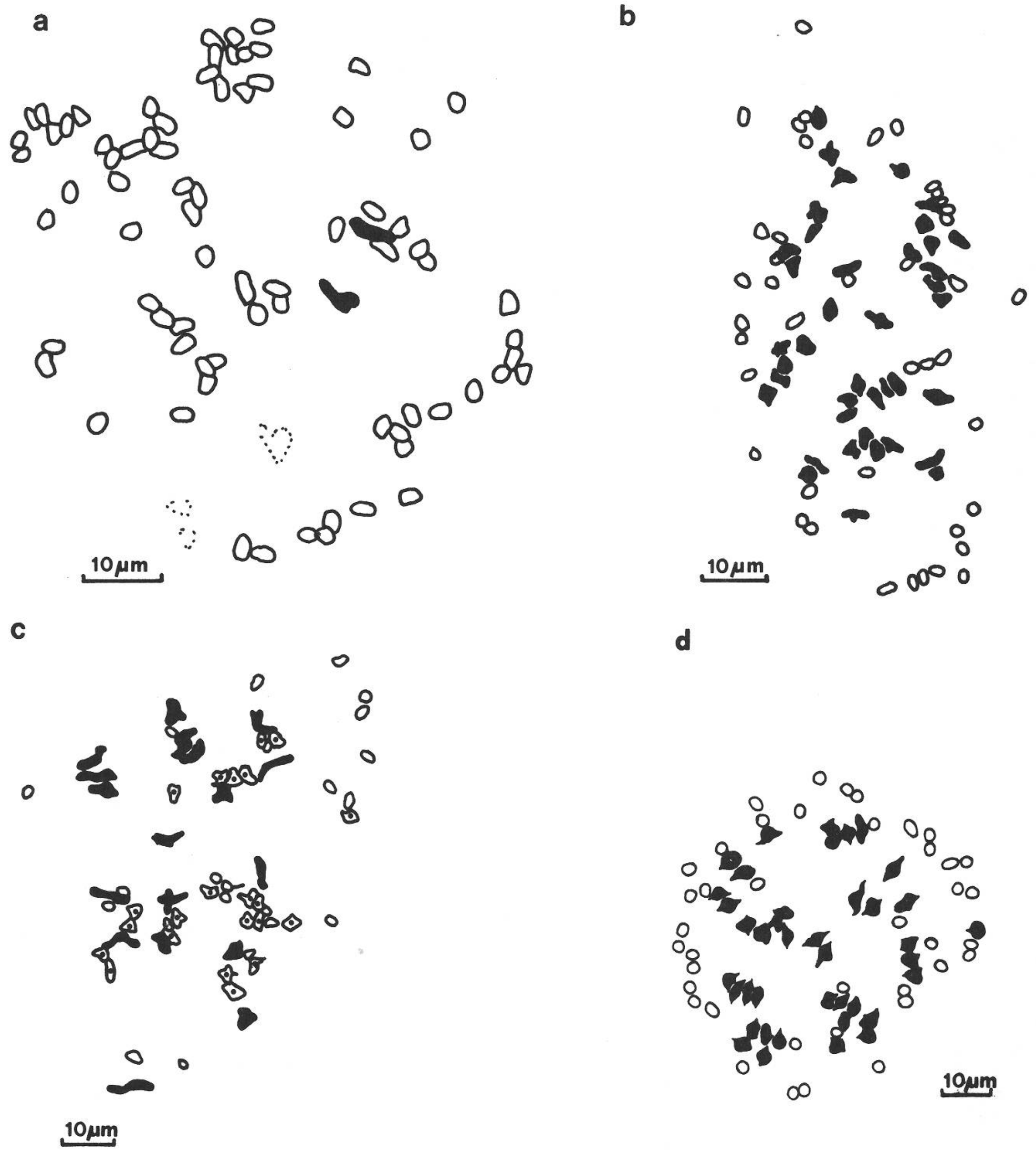

Fig. 6 Explanatory diagram to Fig. 5.

a) Metaphase I in *A.* × *reichsteinii* showing 76 univalents and 2 bivalents
b) Metaphase I in triploid hybrid, ffd, showing 40 univalents and 40 bivalents
c) Metaphase I in autotriploid *A. filix-femina,* fff, showing about 22 univalents, 22 bivalents, and 18 trivalents
d) Metaphase I in triploid hybrid, ddf (Sch-424) showing 40 univalents and 40 bivalents (univalents in outline, bivalents in black or in outline with point, trivalents in black)

For the identification of the triploids micromorphological characteristics such as size of guard cells and shape of the spores are helpful (Figs. 4b, d, e; 9). However, caryological investigations are usually necessary to confirm the state of a hybrid.

Both types of triploid hybrids show at metaphase I of meiosis about 40 bivalents and 40 univalents (Figs. 5b, d; 6b, d). In the root tips of both types about 120 chromosomes could be counted. The spores are aborted but the degree of abortion is less than in diploid hybrids and some spores have a more or less normal shape (Figs. 4d, e). There seems to be a small difference in the sculpturing of the spore wall in the two different types. In the "ffd"-type a small percentage of the spores is viable and germination occurred to some degree. (Spore viability was not examined in the "ddf"-type). The resulting gametophytes were in most cases irregularly formed. This seems to be due to aneuploidy. Similar observations have been made in *Dryopteris* × *tavelii* (Schneller 1975) and *Dryopteris* × *ambroseae* (unpubl.).

Autotriploidy in *Athyrium filix-femina*

During intense search for hybrids, one, possibly two autotriploids were found. Their morphology is not different from *A. filix-femina*. At metaphase I of meiosis trivalents, bivalents and unpaired chromosomes could be distinguished (Figs. 5c, 6c). From late prophase stages to metaphase the degree of pairing seems to change and just before separation of chromosomes the amount of trivalents was reduced. In Fig. 6c about 18 trivalents, 22 bivalents, and 22 univalents are visible. In late metaphase only about 5 trivalents, 35 bivalents and 35 univalents were present in one case. The spores were mostly irregular in shape (Fig. 4f), but some of them germinated. The resulting gametophytes are in most cases irregular. As in the triploid hybrids this seems to be due to aneuploidy. Unreduced triploid prothalli may occur.

The guard cells are of about the same size as those of the triploid hybrids (Fig. 9).

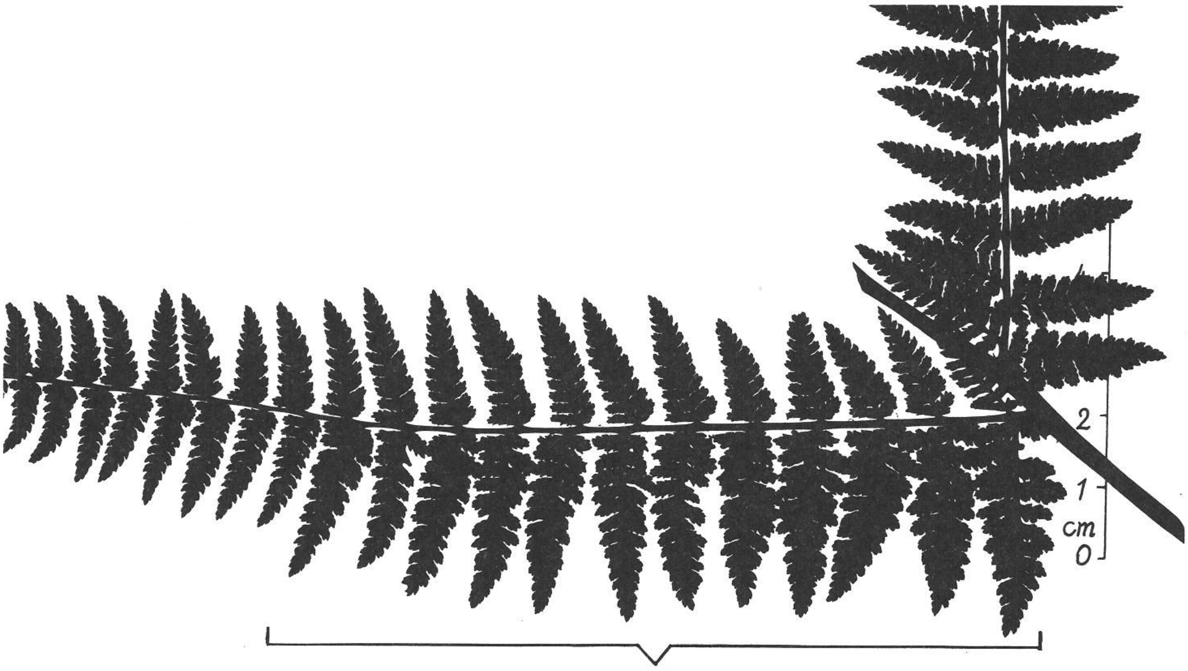

Fig. 7 Part of a leaf of diploid hybrid (Sch-376) showing polyploidized tissue on one pinna, marked by the bracket.

Comparison of Micromorphology

Comparison of pinnules shows that the hybrids with different genome formulae are morphologically very similar (Fig. 8). If a caryological examination is not possible some micromorphological characteristics are helpful for an identification. It could be seen that the size of the guard cells is correlated with the ploidy level (Fig. 9). (Haploids of *A. filix-femina* which were investigated by Schneller (1983) were also used for comparison).

Additionally the ending of the nerves in the pinnules was compared. This characteristic is very useful to separate the parent species and may be of value for recognizing one of the two triploid hybrid-types (Fig. 10).

There are some differences in the sporewall differentiation. The parent species show very clear and specific characteristics. The hybrids differ mainly in the amount of irregularity but also in the sculpturing of the sporewall (Fig. 4b, d, e).

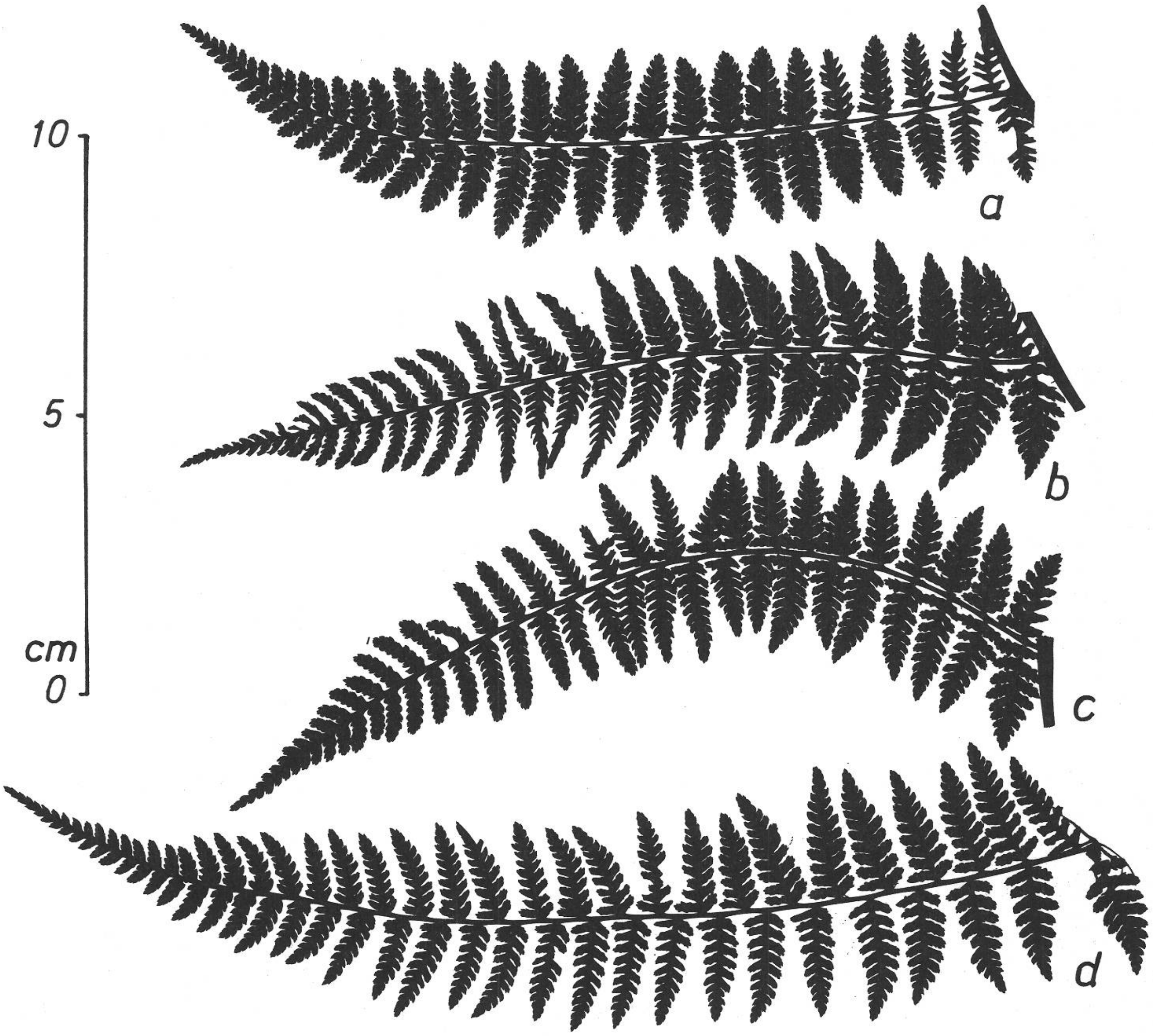

Fig. 8 Comparison of pinnules of different hybrids.
a) *A.* × *reichsteinii* (Sch-373)
b) Triploid hybrid, ffd (Sch-341)
c) Autotriploid *A. filix-femina,* fff (Sch-388)
d) Triploid hybrid, ddf (Sch-424)

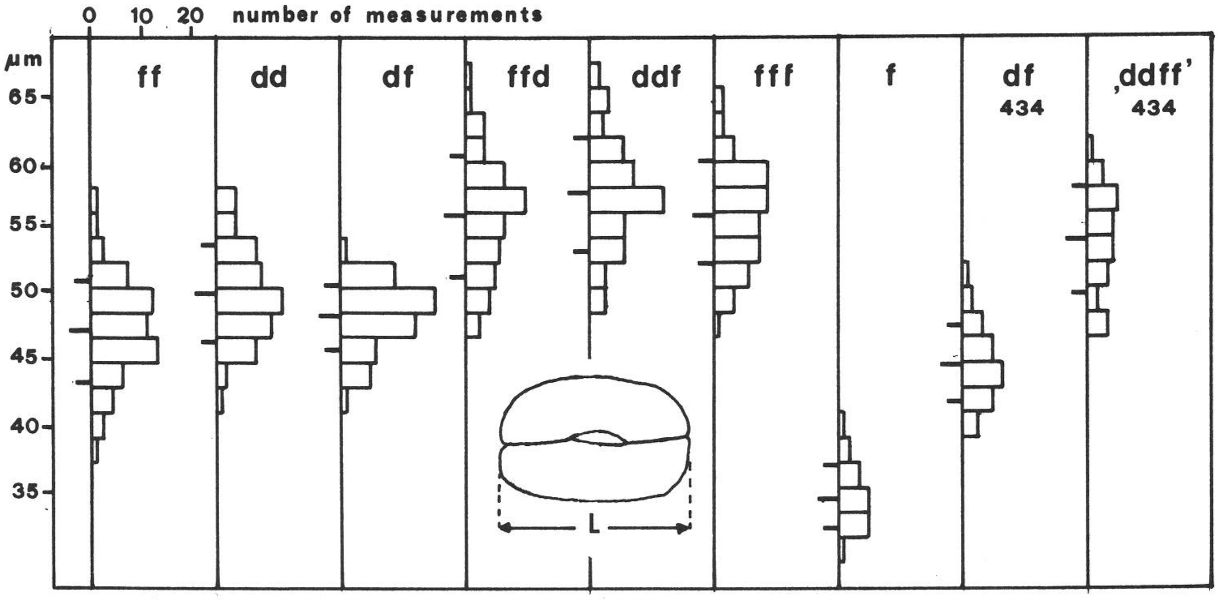

Fig. 9 Sizes of guard cells in the different genome combinations; with mean and standard deviation; df-434 and "ddff"-434 are diploid and polyploidized cells in plant Sch-434.

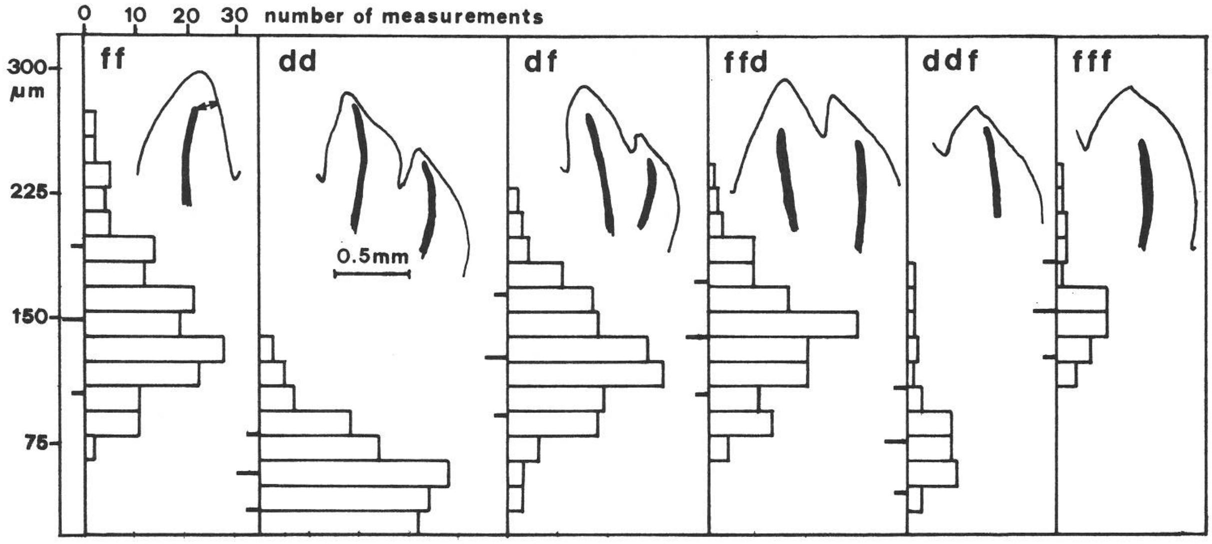

Fig. 10 Distances between vein ending and pinnule border of different genome combinations; with mean and standard deviation.

Nomenclature and typification

Chiovenda (1929) has proposed the name *Athyrium* × *cassum* for the hybrid between *A. filix-femina* and *A. distentifolium*. When we had the opportunity to study the type specimen in Bologna we arrived at the clear conclusion that it is *A. filix-femina*. Chiovenda's description is basing on a not fully ripened plant, showing young sporangia. Therefore *A.* × *cassum* is a synonym of *A. filix-femina*. In 1924 Chiovenda collected a plant (Val Onsernone, in BOLO) which he named *A. filix-femina* var. *multidentatum* and which is a true hybrid (see chapter "Specimens in herbaria").

Christ's description (1900) characterizes the hybrid unambiguously but he did not propose a binomen. It was not possible (in the Paris herbaria where Christ's collection is deposited) to find any hybrid plant mentioned by him in 1900. But we could find true hybrids identified by Christ in the herbaria of Geneva and Karlsruhe (see page 95).

Because of these facts and the finding not only of diploid hybrids but also of triploids, we are publishing a new name for the diploid F-1 hybrid between *A. filix-femina* and *A. distentifolium*. This allows also the choice of caryologically established material for a type specimen, which is in this case particularly important since the identification is very difficult and since morphologically very similar triploid hybrids occur.

The origin and formation of the triploid hybrids has not yet been clarified. Therefore we feel that it is best not to propose new names for these triploid forms until further investigations have been made. Following the convention in fern taxonomy, binomina may be given to triploid backcrossings (which are very common, see Lovis 1978, Reichstein 1981).

Athyrium × *reichsteinii* Schneller et Rasbach hybr. nov. Planta hybrida inter *A. filix-femina* diploideum et *A. distentifolium* diploideum. Habitus inter parentes intermedius. Planta saepe clones formans; differt ab *A. filix-femina* soris rotundis, indusiis minoribus; ab *A. distentifolio* indusiis maturitate praesentibus; sporis abortivis; chromosomatibus 80, meiosi 76–80 univalentibus, 0–2 bivalentibus.

Athyrium × *reichsteinii** (*A. filix-femina* (L.) Roth (2n = 80) × *A. distentifolium* Tausch ex Opiz (2n = 80).) The morphology of this hybrid is intermediate between the parents. In many cases dense clones can be observed. The hybrid differs from *A. filix-femina* in the more roundish sori and the smaller indusia. It differs from *A. distentifolium* in the presence of an indusium at the mature stage. The spores are abortive. The chromosome number is 2n = 80. At meiosis 76–80 univalents and 0–2 bivalents can be distinguished.

Type specimen: Germany, Baden-Württemberg; Black Forest, Feldberg, Felsenweg, SE of the Baldenweger Köpfle, on the left side of a rivulet, in sycamore-beach-spruce forest. – 1320 m, NE exposed. Meiosis showing 76 univalents and 2 bivalents. Coll. 20.8.1981, leg. J. J. Schneller, H. Rasbach and T. Reichstein, Nr. Sch-367 (= TR-5515), (Z).

Specimens of our collection

A list of the material investigated here and examined in herbaria is presented. We have examined the spores of each specimen, so no further indications on spores will be given. Only the numbers and the origin of our collection are given; in brackets the type of investigation is added. The signs used are:

Sch-Nr. = collection nr. Schneller
Ras-Nr. = collection nr. Rasbach
TR-Nr. = collection nr. Reichstein
(cytol. ...) = explanations on cytology, meiosis or mitosis, I = univalents, II = pairs, III = trivalents.
(guard cells) = size of guard cells measured.
(cult. Z, TR) = cultivated at botanical garden in Zürich or in private garden (Reichstein) in Basel.
genome formulas: f = genome of *A. filix-femina*, d = genome of *A. distentifolium*.

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*Dedicated to Prof. Dr. T. Reichstein, who has added very much to the knowledge of the ferns.

Athyrium × reichsteinii (genome formula df)

Kt. St. Gallen: Ricken, Egg, ca. 1220 m: coll. 13.8.80: Sch-286 (cytol. ca. 76 I, 2 II); Sch-290 (cult. Z). – coll. 12.8.81: Sch-334 (cult. TR-5502): Sch-336. All the numbers are of the same clone.

Kt. Schwyz: Muotatal, Bödmerenwald: coll. 2.9.82: Sch-432 (guard cells), ca. 1530 m; Sch-433 (guard cells), ca. 1530 m; Sch-434 (guard cells) (cult. Z and TR-5704), ca. 1450 m; Sch-435 (guard cells), ca. 1420 m; Sch-436 (guard cells), ca. 1390 m.

Kt. Tessin: Val di Campo, Cimalmotto. coll. 29.9.82: Sch-450 (guard cells) (cult. Z), ca. 1440 m; Sch-451 (guard cells), (cult. TR-5728), ca. 1510 m; Sch-452 (guard cells), ca. 1490 m; Sch-453 (guard cells), ca. 1470 m. – Valle Blenio, Campo Blenio. coll. 30.9.82: Sch-454 (guard cells) (cult. Z and TR-5729), ca. 1530 m.

Kt. Graubünden: Valle di Calanca, Valbella. coll. 1.10.82: Sch-458 (guard cells) (cult. Z), ca. 1520 m.

Baden-Württemberg: Black Forest, Feldberg. coll. 16.8.81: Sch-340 (guard cells), Wanne, ca. 1200 m; – coll. 20.8.81: Sch-367 (cytol. ca. 76 I, 2 II), Felsenweg, ca. 1320 m; – coll. 24.8.81: Sch-369 (cytol. ca. 78 I, 1 II) (cult. Z), Rinken, ca. 1260 m; Sch-370 (cytol. ca. 78 I, 1 II) (cult. TR-5519), belongs to the same clone as Sch-369; Sch-373 (Ras-244) (cytol. ca. 78 I, 1 II) (cult. Z), Rinken, 1295 m; Sch-375 (guard cells), Felsenweg, ca. 1320 m; Sch-376 (cytol. ca. 76 I, 2 II), Felsenweg, ca. 1330 m; – coll. 26.8.81: Sch-377 (guard cells) (cult. TR-5518), Rinken, ca. 1250 m; Sch-379 (guard cells), Rinken, ca. 1300 m; Sch-380 (guard cells), Felsenweg, ca. 1340 m; Sch-382 (cytol. 80 I; 76 I, 2 II) (cult. Z and TR-5524), Felsenweg, ca. 1290 m; – coll 27.8.81: Sch-386 (guard cells), Rinken, ca. 1190 m; – coll. 29.8.81: Sch-387 (cytol. ca. 78 I, 1 II) (cult. Z), Rinken, ca. 1200 m; – coll. 19.7.82: Sch-406 (Ras-275) (cytol. 76–80 I, 2–0 II), Rinken, ca. 1200 m; – coll. 13.8.82: Sch-410 (guard cells), Rinken, ca. 1250 m; Sch-415 (guard cells), Felsenweg, ca. 1310 m; – coll. 14.8.82: Sch-417 (guard cells), Rinken, ca. 1200 m; Sch-418 (guard cells), Rinken, ca. 1200 m; Sch-419 (guard cells), Rinken, ca. 1200 m; – coll. 15.8.82: Sch-421 (guard cells), Hochkopf, ca. 1220 m; Ras-284, Hochkopf, ca. 1240 m; – coll. 24.7.83: Ras-329 (cytol. 76–80 I, 0–2 II), near Hinterzarten.

Black Forest, Kandel. coll. 17.8.81: Sch-344 (cytol. ca. 76 I, 2 II), ca. 1200 m; Sch-345, same clone as Sch-344; Sch-347 (cytol. ca. 76 I, 2 II) (cult. TR-5503), Sch-348, Sch-349, Sch-350, Sch-351 (cytol. ca. 78 I, 1 II), Sch-352 (cytol. ca. 76 I, 2 II), belong to the same clone as Sch-347; Sch-353 (cytol. ca. 76 I, 2 II), ca. 1200 m; Sch-354 (cytol. 78 I 1 II) ca. 1200 m.

Black Forest, Belchen. coll. 9.9.82: Sch-438 (cytol. ca. 80 I; 76 I, 2 II) (cult. Z and TR-5718), Rübgartenwald, ca. 1220 m; Sch-439 (guard cells) (cult. TR-5719), Rübgartenwald, ca. 1210 m; Sch-440 (guard cells), Rübgartenwald, ca. 1210 m; Sch-442 (guard cells) (cult. TR-5720), Rübgartenwald, ca. 1230 m; Sch-443 (guard cells), Rübgartenwald, ca. 1240 m.

Black Forest, Notschrei. coll. 11.9.82: Ras-287 (guard cells), ca. 1120 m; Ras-288 (guard cells), ca. 1100 m.

Bavaria: Bayerischer Wald. coll. 23.9.82: Ras-306 (guard cells) (cult. TR-5741), Grosser Arber, ca. 1040 m.

Triploid hybrids (probably with genome formula ffd)

Baden-Württemberg: Black Forest, Feldberg. coll. 5.9.80: Sch-292 (cytol. ca. 40 I, 40 II) (cult. Z), Felsenweg, ca. 1330 m; Sch-293 (cytol. ca. 40 I, 40 II), Sch-294, Sch-295 (cytol. ca. 40 I, 40 II), Sch-296, all belong to the same clone as Sch-292; – coll. 16.8.81: Sch-338 (cytol. ca. 40 I, 40 II) (cult. TR-5506), Wanne, ca. 1160 m; Sch-341 (guard cells), Wanne, ca. 1210 m; Sch-342 (cytol. ca. 40 I, 40 II) (cult. TR-5508), Wanne, ca. 1160 m; Sch-343 (cytol. ca. 40 I, 40 II), belongs to the same clone as Sch-342; – coll. 20.8.81: Sch-366 (cytol. ca. 40 I, 40 II) (cult. TR-5514), Felsenweg, ca. 1330 m; Sch-368 (guard cells), Felsenweg, ca. 1320 m; – coll. 24.8.81: Sch-371 (cytol. ca. 40 I, 40 II) (cult. Z and TR-5520), Rinken, ca. 1290 m; – coll. 26.8.81: Sch-381 (cytol. ca. 40 I, 40 II) (cult. Z), Felsenweg, ca. 1340 m; – coll. 13.8.82: Sch-411 (guard cells), Rinken, ca. 1280 m; Sch-412 (guard cells) (cult. Z), Rinken, ca. 1280 m; Sch-413 (guard cells) (cult. Z), Rinken, ca. 1280 m.

Triploid hybrid (probably with genome formula ddf)

Baden-Württemberg: Black Forest, Feldberg. – coll. 15.8.82: Sch-424 (cytol. 40 I, 40 II, 2n ca. 120, guard cells) (cult. Z and TR-5700), Hochkopf, ca. 1240 m.

Autotriploids (probably with genome formula fff)
Baden-Württemberg: Black Forest, Feldberg. coll. 26./29.8.81: Sch-378 (guard cells), Rinken, ca. 1290 m; Sch-388 (cytol. ca. 5–8 III, 40 II, 16–35 I; ca. 18 III, 22 II, 22 I) (cult. Z), Rinken, ca. 1290 m, probably identical with Sch-378; – coll. 14.8.82: Sch-420 (guard cells) (cult. Z), Rinken, ca. 1210 m.

Specimens in herbaria
Athyrium × reichsteinii

Germany: Bavaria, Allgäu, near Oberstaufen, ca. 840 m. 19.7.82, leg. A. Eschelmüller, Nr. AE-40 (Herbarium Eschelmüller). Det. H. Rasbach and J. J. Schneller.

France: Haut-Rhin, Vosges, Hohneck, 1100 m. 2.8.1908, leg. Emile Walter, det. A. Lösch (! H. Christ), sub *A. alpestre × filix-femina* (KR). – Environs de Genève, la Faucille. 17 bre 1879. Herbier Delessert. Donné par M. Auguste Guinet (Dec. 1896). Det. H. Christ, Bale, sub *A. alpestre × filix-femina* (G).

Italy: Prov. Novara, Val Onsernone, Fra Bagni di Craveggio, la Boschetta di S. Antonio, ca. 1350 m. 15.9.1924, leg. E. Chiovenda, sub *A. filix-femina* var. *multidentatum*. Det. J. J. Schneller and H. Rasbach (BOLO).

Poland: Sokozowsko (= Gröbersdorf) near Watbrzych (= Waldenburg). 8.1874, leg. M. Firle, sub *A. alpestre*. Det. J. J. Schneller (P).

Sweden: Jaemtland: Are s:n, Enafors, Hoegasen. S of point 641 m in moist spruce forest ca. 600 m. 4.8.1960, leg. H. Smith, No. 3824, sub *A. alpestre × filix-femina* (BM, G). – Jaemtland: Are s:n, Enafors, Hoegasen, spruce forest, 640 m. 3.8.1959, leg. H. Smith, sub *A. alpestre × filix-femina* (BM).

Switzerland: Kt. St. Gallen, Flumserberg. Chatzeberg, Fichtenwald. 10.8.1982, leg. H. Nägeli. Det. J. J. Schneller (Herbarium Nägeli).

Triploid hybrid (ffd?)

Germany: Baden-Württemberg, Black Forest, Feldberg, über dem Feldsee. 23.9.1959, leg. D. E. Meyer, sub *A. alpestre × filix-femina*. Det. J. J. Schneller (guard cells) (B).

Notes on Vegetative Growth of the Hybrids

Since the distribution area of *A. distentifolium* in Central Europe is limited to about 900–1200 m up to about 2200 m there is a natural limitation of the occurrence of diploid (and triploid) hybrids. The hybrids examined in this investigation were found between 840 m (Allgäu) and about 1650 m (Flumserberg), in shady places of deciduous and coniferous mountain forests, which are rich of perennials. The clay soil is nearly free of limestone, loose and stony. Hybrids normally occur as clones of very different size. That is, single genotypes consist of many shoots which are more or less tightly packed together.

It is probable that different genotypes may be found close together. Sometimes the spatial structure of a clone can be determined. One triploid hybrid (Sch-371) could be recognized by a rare morphological characteristic, it has a red rachis and unicellular hairs on both sides of the rachis and the costae. The maximum diameter of this clone was more than 15 m. A diploid hybrid (Sch-373) with another morphological marker (darker rachis and characteristic pinnula shape) occupied an area of 8 × 18 m. In many cases the clones are so dense that they form pure stands.

Discussion

The diploid hybrid between *A. filix-femina* and *A. distentifolium* seems to be more abundant than previously reported in the literature. It occurs here and there in sites where both parents grow in abundance. However, there seems to exist some hindrance for forming such hybrids. Within populations with many parent plants they are infrequently found (it was not possible also to produce artificial hybrids).

The hybrids seem to compete successfully with the parents and other species. Although hybridization is a rare event, the hybrids, once formed, can persist for a long time by means of vegetative propagation.

The pairing behaviour of chromosomes at meiosis in the diploid hybrids indicates that the two species *A. filix-femina* and *A. distentifolium* are genetically well separated and therefore not closely related. On the other hand one has to bear in mind that the observed meiotic pattern may be due only to rearrangement of basically the same genetic material or to the action of some genes regulating the pairing. Our caryological results do not support the view that introgression between the two species occurs (Hess et al. 1967).

The occurrence of triploid hybrids cannot be explained at present. Triploid hybrids (of unknown origin) have also been reported for North-America by Wagner & Wagner (1966). There are three hypotheses in discussion.

The first postulates that an allotetraploid species exists (with the genome formula ffdd) which is very difficult to recognize. We think that its morphology should be very close to *A.* × *reichsteinii* except that viable spores (resulting from regular meiosis) could be formed. If allotetraploids occur, the triploid hybrids (with the probable genome formula ffd and ddf) may represent backcrossings with either diploid parent. The occurrence of regular spores (genome formula df) observed on polyploidized pinnules in some diploid hybrids (Sch-369, Sch-376, Sch-434, Ras-284, Ras-329) would allow the formation of such an allotetraploid.

However, some facts do not support this hypothesis. In the polyploidized regions of diploid hybrids, sporangia and sporemothercells with the genome formula ddff are formed. After meiosis spores result having the same diploid chromosome set (df) as the diploid tissue of the "motherplant". Such spores from one plant are all of the same genetic composition. Although in one experiment they formed gametophytes of both sexes, no sporophytes resulted. Self-incompatibility factors may put barriers to intragametophytic selfing.

Investigations on *A. filix-femina* (Schneller 1979) have shown that in nature outbreeding is the rule in this species (because of pheromonal and genetic conditions). For the formation of an allotetraploid "species" two different "df"-prothalli (of the same or in the case of self-incompatibility of two different diploid hybrids) would be necessary. In nature it seems to be very unlikely that such two prothalli are growing close to each other (because of the rarity of diploid hybrids and especially of "df"-spores).

Although we searched very carefully for tetraploids in the vicinity of triploids we could not find any. If allotetraploids really occur they must be very rare.

The second hypothesis postulates that unreduced prothalli occur within the parent species *A. filix-femina* and *A. distentifolium*. One condition which could lead to the formation of unreduced prothalli is the occurrence of "diplospores" (unreduced spores). Diplospores may originate through incomplete meiotic divisions or when somatic polyploidization occurs, in parts of the plants. Somatic polyploidization was – as mentioned before – observed in some diploid hybrids and in a haploid *A. filix-femina* (Schneller 1983); it may be found also within the two diploid species.

In other cases unreduced prothalli resulting from apospory (outgrowth of prothalli from a sporophytic cell) were found in culture (Farmer & Digby 1907). One of us

(J. J. S.) observed aposporous prothalli on young leaves from progeny of Canadian *A. filix-femina* (Sch-400).

The occurrence of autotriploid *A. filix-femina* is evidence for the existence of unreduced prothalli (and gametes). The autotriploid then is formed by the reunion of an unreduced with a reduced gamete of *A. filix-femina*. If unreduced prothalli occur in either species (*A. filix-femina*, *A. distentifolium*) autotriploids or triploid hybrids like those we have observed, can be formed. Although we can conclude that unreduced prothalli within *A. filix-femina* occur and may be found within *A. distentifolium* as well, crossings of diploid gametes of one species with haploid gametes of the other seem to be unlikely. Even the combination of the two diploid species is uncommon.

The third (in our opinion most realistic) hypothesis postulates that the "unreduced" viable "df"-gametes (from spores originating from polyploidized pinnules of diploid hybrids) fuse with gametes of either parent, and the triploid genotypes "ffd" and "ddf" result. If a "df" prothallus and "df" gametes are formed, it is most likely that they will be combined with gametes of one of the diploid species (*A. filix-femina*, *A. distentifolium*), which are the most common neighbours. Such a «backcrossing» must be easily formed, backcrossings are very common in many fern genera such as e.g. *Dryopteris, Asplenium*.

Triploid hybrids were always found in populations where the diploid hybrids occur. The observation that triploid are much rarer than diploid hybrids supports this third hypothesis.

Zusammenfassung

Lange Zeit bezweifelten die Botaniker das Vorhandensein einer Kreuzung zwischen den beiden europäischen *Athyrium*-Arten *A. filix-femina* und *A. distentifolium*, obwohl Christ (1900) erstmals eine solche Kreuzung erkannte und beschrieb, ohne dieser allerdings einen besonderen Namen zu geben. Chiovenda (1929) publizierte dann den Namen *Athyrium* × *cassum* für diese Kreuzung. Nach genauer Untersuchung des von Chiovenda gewählten Typus kommen wir zu dem Schluss, daß es sich dabei um *A. filix-femina* handelt. *A.* × *cassum* ist also als Synonym von *A. filix-femina* anzusehen.

Nachdem einer von uns (J. J. S.) in der Schweiz (Kt. St. Gallen, Ricken) eine diploide Hybride gefunden und diese zytologisch untersucht hatte, wurde der Frage nachgegangen, ob diese Kreuzung häufig sei oder ob sie zu den seltenen Funden gerechnet werden müsse. Nach mehr als drei Jahre laufenden Untersuchungen, die in Gebieten mit reichen Vorkommen der beiden Frauenfarn-Arten durchgeführt wurden, kommen wir zu dem Ergebnis, daß die Kreuzung zwischen diploider *A. filix-femina* und diploider *A. distentifolium* relativ verbreitet, aber im Vergleich zu dem oft massenhaften Vorkommen der Eltern doch recht selten ist. Die Hybride scheint sich schwer zu bilden, und es ist bis heute nicht gelungen, sie künstlich herzustellen. Die diploide Hybride mit der Genomformel df ist am besten an den abortierten Sporen zu erkennen. Sie wird hier als *Athyrium* × *reichsteinii* Schneller et Rasbach beschrieben, zu Ehren von Prof. Dr. T. Reichstein, der so viel für die Farnforschung geleistet hat.

Überraschenderweise zeigte es sich, daß unter den Hybriden nicht nur diploide, sondern auch triploide Pflanzen vorkommen. Die Ploidiestufe wurde durch zytologische Untersuchungen festgestellt; morphologische und anatomische Unterscheidungsmerkmale wurden erarbeitet.

Triploide Hybriden könnten aus einer hypothetischen allotetraploiden «Art» mit der Genomformel ddff als Rückkreuzungen mit einer der Elternarten entstanden sein. Nach der Gestalt der triploiden Hybriden wäre eine solche Entwicklung vorstellbar, da zwei verschiedene Formen triploider Hybriden gefunden wurden. Trotz intensiver Suche gelang es bis jetzt jedoch nicht, allotetraploide Pflanzen zu finden.

Ein zweiter Erklärungsversuch für das Entstehen triploider Hybriden liegt in der Möglichkeit, daß unreduzierte Prothallien bei den Elternarten vorkommen. In diesem Fall könnte eine Befruchtung zwischen einem unreduzierten und einem reduzierten Gameten erfolgen. Das Auffinden einer, möglicherweise zweier, autotriploider Pflanzen mit der Genomformel fff könnte die Bestätigung für das Auftreten solcher unreduzierter Prothallien sein. Derartige unreduzierte Prothallien können die Folge von Diplosporie oder Aposporie sein.

Nach den bisher erfolgten Untersuchungen scheint uns jedoch eine dritte Möglichkeit für die Entstehung triploider Hybriden am wahrscheinlichsten: Bei den diploiden Hybriden (*A.* × *reichsteinii*) werden gelegentlich «unreduzierte» Sporen gebildet, die keimfähig sind. An einigen Pflanzen (Sch-369, Sch-376, Sch-434, Ras-284, Ras-329) konnten die folgenden Beobachtungen gemacht werden: kleine Zonen zeigten eine somatische Polyploidisierung mit keimfähigen Sporen. Bei Sch-434 resultierten daraus regelmässige Prothallien mit n = ca. 80 = 2 Chromosomensätzen. Derart entstandene Prothallien mit funktionsfähigen Gameten könnten mit den Elternarten hybridisieren, d.h. es könnten sich triploide Formen bilden: df + f = ffd (3x) oder df + d = ddf (3x). Auf Grund morphologischer Kriterien glauben wir, diese beiden triploiden Zytotypen gefunden zu haben.

Beobachtungen an den natürlichen Standorten zeigten, daß viele der *Athyrium*-Hybriden als Klone auftreten, die ein beachtliches Ausmaß annehmen können (z.B. ca. 15 × 15 m bei Sch-381) und deshalb zum Teil sehr alt sein dürften. Die Fähigkeit zu vegetativer Vermehrung erlaubt es einer einmal (wohl eher selten) gebildeten Hybride, sich gegenüber den Elternarten zu behaupten.

We thank the following persons for sending, or presenting us with material: F. Badré (P), Dr. P. Geissler (G), A. Eschelmüller (Sulzberg/Allgäu), A.C. Jermy (BM), the late Dr. D.E. Meyer (B), Dr. H. Nägeli (Zürich), Dr. G. Philippi (KR). We thank Prof. Dr. C. Ferrari and Dr. D. Ubaldi (BOLO) for giving us the opportunity to study *Athyrium* × *cassum*, Prof. Dr. K.U. Kramer for valuable discussion of taxonomic problems and for the latin diagnosis, the Regierungspräsidium Südbaden, Freiburg i. Br., for the permission to work and collect material in the nature preservation area "Feldberg", and Dr. Mary Fallen for reading and corrections. Our special thanks go to Prof. Dr. T. Reichstein, Dr. K. Rasbach and Marianne Schneller for help, accompany and encouragement.

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