COMPARATIVE STUDY OF *ASPLENIUM BALEARICUM, A. ONOPTERIS* AND THEIR SPONTANEOUS HYBRID *A.* × *TYRRHENICUM*

by

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Resumen

CUBAS, P., J. ANTONI ROSSELLÓ & E. PANGUA (1988). Estudio comparativo de Asplenium balearicum, A. onopteris y su híbrido espontáneo A. × tyrrhenicum. *Anales Jard. Bot. Madrid* 45(1): 75-92 (en inglés).

Asplenium balearicum es una especie endémica de las islas occidentales del Mediterráneo, siendo en Menorca donde es más abundante. A pesar de los datos obtenidos en las plantas de Menorca, que indican una fuerte variabilidad en morfología y tamaño de las frondes, todas las plantas estudiadas son tetraploides con meyosis regular y muestran un patrón fenólico bien definido y una morfología esporal característica. La morfología, citología y patrón fenólico del híbrido espontáneo *A.* × *tyrrhenicum* apoyan fuertemente que este taxon se ha originado por el retrocruzamiento de *A. balearicum* con *A. onopteris*.

Palabras clave: *Asplenium*, morfología, citología, patrón fenólico, Islas Baleares, España.

Abstract

CUBAS, P., J. ANTONI ROSSELLÓ & E. PANGUA (1988). Comparative study of Asplenium balearicum, A. onopteris and their spontaneous hybrid A. × tyrrhenicum. *Anales Jard. Bot. Madrid* 45(1): 75-92.

Asplenium balearicum is an endemic species of the western islands of the Mediterranean Sea, being most abundant in Minorca (Balearic Islands, Spain). Despite new data from Minorca indicating a strong variability in morphology and size of the fronds, all the plants studied are tetraploid with regular meiosis, displaying a well defined phenolic pattern and a characteristic spore morphology. The morphology, cytology and phenolic pattern of the spontaneous hybrid *A.* × *tyrrhenicum* strongly support the origin of this taxon as the result of a backcross between *A. balearicum* and *A. onopteris*.

Key words: *Asplenium*, morphology, cytology, phenolic pattern, Balearic Islands, Spain.

INTRODUCTION

Asplenium balearicum Shivas is an allotetraploid species having the parentage *A. onopteris* L. and *A. obovatum* Viv. (SHIVAS, 1969; LOVIS & *al.*, 1972). Ac-

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cording to SLEEP (1983) it was first described in 1969 with material collected in 1952 by Miss E. O'Nions from an unspecified locality of the Balearic Islands. The plants were initially ascribed to *A. obovatum* and afterwards to *A. billotii* F. W. Schultz. However, conflicting results obtained during the experimental hybridization programmes carried out by SHIVAS (1956) and SLEEP (1966) led to the realization that the plants studied were neither *A. obovatum* nor *A. billotii*, but a further new species. No new localities were reported until NARDI(1983) found scattered populations of *A. balearicum* on some islands of the Tyrrhenian and Sicilian seas. Later on, ROSSELLÓ & *al.* (1986) reported this species in some areas of northeast Minorca (Balearic Islands), growing exclusively on schists and sandstones, and considered its presence on the mostly calcareous island of Majorca improbable.

A recent paper on *A. balearicum* (ROSSELLÓ & SERRA, 1987) noted a strong polymorphism on material from Minorca. A great number of specimens (some of them alive) of *A. balearicum* and *A. onopteris* were therefore collected by the autors in a field trip to Minorca in January 1987.

Cytological, chromatographic and palynological studies justified the proposal of a new triploid hybrid: *A.* × *tyrrhenicum* whose probable parents are *A. balearicum* and *A. onopteris* (CUBAS & *al.*, 1987). The main results of these studies as well as a discussion on the probable origin of this hybrid follow.

MATERIAL AND METHODS

Sampling sites, herbarium specimens employed and collection data are given in the appendix and figure 1. Drawings of macromorphological details were made

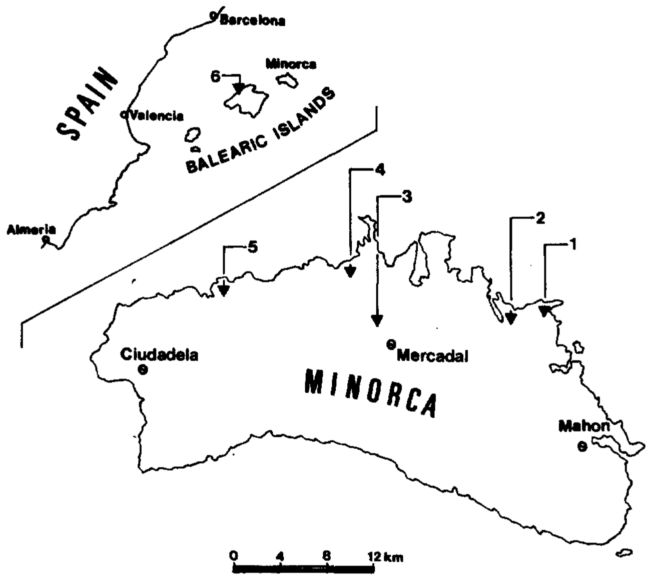

Fig. 1.—Location map of sampling sites. 1-5.—MINORCA: 1, Favaritx; 2, Mongofre Nou; 3, Llinaritx Nou; 4, Binimel.la; 5, Sa Vall; 6, Majorca, Gorg Blau.

with a camera lucida using fronds collected in the field as well as plants kept in cultivation at the Botanical Department of the Faculty of Pharmacy, University of Madrid.

Epidermal details and measurements of stomatal guard cells were obtained from dried herbarium material, rehydrated 24 hours in water, cleared in sodium hypochlorite solution and rinsed in water. The sample size measured for each specimen was 30 cells.

Measurements (perispore excluded) and photomicrographs of untreated spores were made from preparations mounted in glycerojelly, while SEM pictures were taken from gold-coated spores. The sample size was 30 spores and only spores in side view were measured in order to avoid small differences due to slightly oblique positions.

Chromosome preparations were made by fixing developing sporangia in the field and/or in the laboratory and staining according to MANTON (1950).

Two-dimensional chromatograms were run using a procedure based on MABRY & *al.* (1970) and SMITH & LEVIN (1963). Chromatographic patterns were developed on celullose plates (Merck 5552) from methanolic solutions of pressed or fresh plants (usually between 40 and 100 miligrams). The first directional solvent was BAW (butanol, acetic acid, water, 4:1:5, upper phase) and 15 % HOAc (acetic acid) was used as the second solvent. The plates were observed under a U.V. lamp at 360 nm, with and without ammonia fumes, and the spots marked.

RESULTS AND DISCUSSION

A. balearicum and A. onopteris

a) *Morphology*

Variation is manifested in *A. balearicum* by differences in the lamina length and width, petiole length, frond outline, number of pinna pairs per frond, and the degree of dissection of pinnae. The range of variation closely matches the results of ROSSELLÓ & SERRA (1987); however a brief comment on the morphology of pinnae is worth giving here.

The pinnae outline (fig. 2) varies from short ovate pinnatifid with pinnulae obovate (fig. 2 A) to long triangular pinnatisect with almost elliptic pinnulae (fig. 2 G). The lowermost acroscopic pinnulae of the proximal pinnae are generally longer than the basiscopic ones. Acroscopic pinnulae are usually broader and more lobed than the basiscopic ones. This feature was also observed in plants raised from spores of the original collection used by SHIVAS (1969) to describe *A. balearicum.* The pinnules vary from dentate with numerous acute mucronate teeth to inconspicously dentate with broad obtuse teeth (fig. 2 E).

A. onopteris from the Balearic islands on the other hand presents a characteristic triangular outline with caudate apex, numerous pinnae obliquely inserted, and lanceolate pinnules cuneate at the base, with deep acute teeth (fig. 2 H).

b) *Stomata and epidermal cells*

Drawings of the lower epidermis from cleared fronds are shown in figure 3.

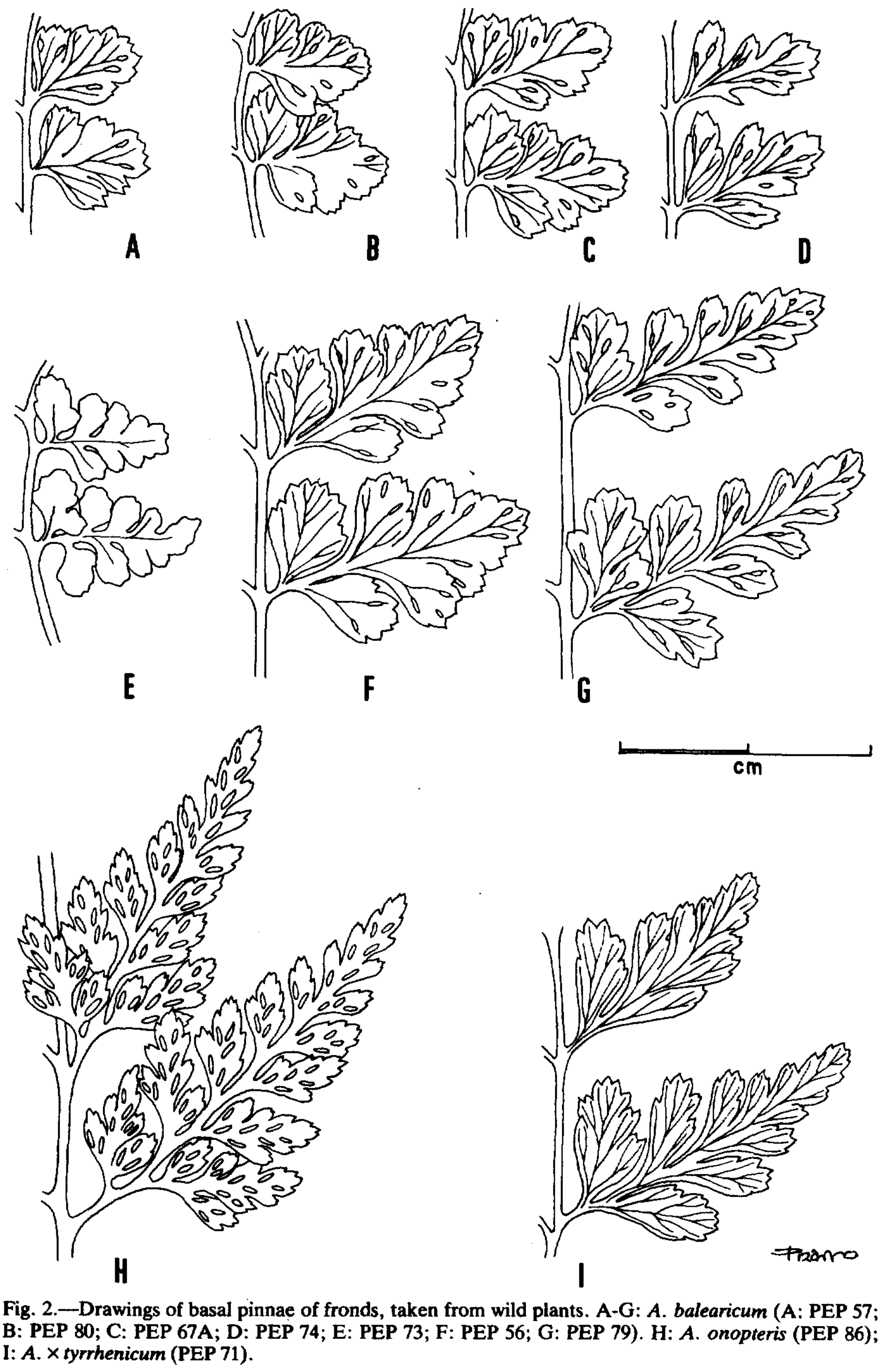

Fig. 2.—Drawings of basal pinnae of fronds, taken from wild plants. A-G: *A. balearicum* (A: PEP 57; B: PEP 80; C: PEP 67A; D: PEP 74; E: PEP 73; F: PEP 56; G: PEP 79). H: *A. onopteris* (PEP 86); I: *A.* × *tyrrhenicum* (PEP 71).

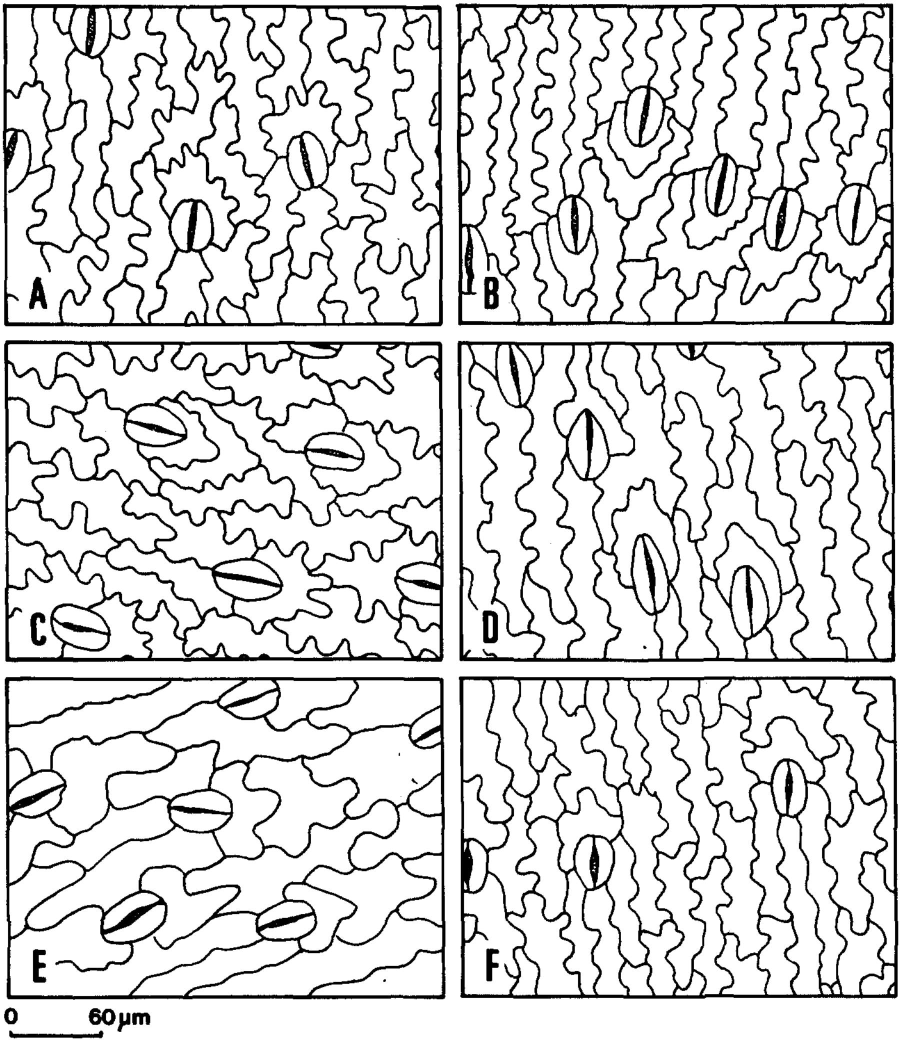

Fig. 3.—Drawings of the lower epidermis from fronds of *A. balearicum* (A-D), *A. onopteris* (E), and *A.* × *tyrrhenicum* (F). A: PEP 75; B: PEP 80; C: PEP 81; D: PEP 92; E: PEP 86; F: PEP 71.

Epidermal cells of both species are elongate, with undulating anticlinal walls. Only two out of the four types of stomata found by VAN COTTHEM (1970) and VIANE & VAN COTTHEM (1977) in *Aspleniaceae* are present in *A. balearicum* (fig. 3 A-D) and *A. onopteris* (fig. 3 E) fronds: anomocytic and polocytic, the former being dominant.

The mean length of the guard cell presents a strong variation within and between samples (table 1), as clearly shown by mean values ranging from 52 to 75 µm in *A. balearicum* (fig. 4) and from 52 to 61 µm in *A. onopteris*. There is no

TABLE 1

SPORE AND STOMATAL PARAMETERS IN *A. BALEARICUM, A. ONOPTERIS* AND *A. × TYRRHENICUM*

	1	2	3	4	5
A. balearicum	PEP 56	38.1±1.8	28.9±1.3	5.8±1.3	60.2±4.0
	PEP 57	37.7±1.6	27.9±1.3	6.2±1.1	60.1±3.3
	PEP 58	37.3±1.6	27.8±1.1	5.5±1.1	62.7±3.6
	PEP 59	35.9±1.7	26.2±1.2	5.8±1.5	53.7±4.2
	PEP 62	36.8±1.8	27.6±1.4	4.7±0.8	55.7±4.0
	PEP 63	36.9±1.7	28.5±1.8	4.8±1.2	57.1±3.9
	PEP 64	37.6±1.6	28.0±1.3	4.5±0.9	59.4±4.0
	PEP 65A	35.3±1.7	26.3±1.2	6.0±0.9	65.1±3.9
	PEP 67A	36.7±2.0	27.7±1.6	5.7±1.3	71.6±4.2
	PEP 67C	36.5±1.7	27.6±1.6	5.7±1.0	61.7±5.3
	PEP 68	36.6±1.6	27.9±1.4	6.2±1.3	69.1±3.7
	PEP 69	35.0±2.2	27.1±2.2	5.2±0.6	60.3±2.6
	PEP 70	–	–	–	62.2±4.6
	PEP 73	35.7±2.0	25.5±1.6	5.2±0.9	56.1±3.7
	PEP 74	36.3±2.3	27.9±1.7	6.2±1.1	60.3±3.6
	PEP 75	34.0±1.7	26.6±2.1	4.6±0.9	59.7±3.5
	PEP 77	37.5±2.3	28.1±1.4	6.3±0.9	52.2±2.9
	PEP 79	35.2±2.1	26.5±1.5	3.5±0.8	60.3±4.0
	PEP 80	34.9±2.1	25.8±1.5	4.0±1.1	63.7±3.0
	PEP 81	33.9±1.6	25.9±1.6	3.1±0.6	75.0±3.2
	PEP 82	36.6±1.5	28.7±1.4	6.3±1.1	57.8±4.3
	PEP 83	36.4±2.3	28.0±1.2	5.8±1.0	57.7±2.6
	PEP 85	37.1±1.6	28.6±1.1	7.0±1.4	61.5±3.6
	PEP 91	36.4±2.0	27.5±2.0	3.6±0.8	63.4±3.1
	PEP 92	36.8±2.0	26.7±1.8	4.2±0.9	65.3±6.2
	PEP 93	35.8±1.5	28.7±1.7	3.4±0.7	55.7±6.6
	TR 1432	37.5±2.0	27.3±1.4	5.1±1.3	65.7±4.1
A. onopteris	PEP 50	30.0±1.2	22.5±0.9	4.1±0.9	53.1±3.1
	PEP 86	32.3±1.6	23.6±0.9	4.9±0.8	60.9±3.6
	PEP 88	29.6±0.9	21.3±0.9	4.4±1.0	52.6±3.0
	PEP 89	30.3±1.1	23.5±1.1	4.5±0.8	55.2±3.8
A. × tyrrhenicum	PEP 71	–	–	–	50.2±2.6

1: Sample. 2: Mean and standard deviation of exospore length. 3: Mean and standard deviation of exospore width. 4: Mean and standard deviation of perispore width. 5: Mean and standard deviation of guard cell length. Sample size = 30.

correlation between stomatal length and spore length in *A. balearicum* (fig. 4). Besides, counter to what has been reported from other polyploid complexes (BARRINGTON & *al.*, 1986), the stomatal length has proved to be of no use to differentiate *A. onopteris* (diploid) from *A. balearicum* (tetraploid).

c) *Spore size and exospore pattern*

The perispore in the specimens of *A. balearicum* studied shows a flaviform

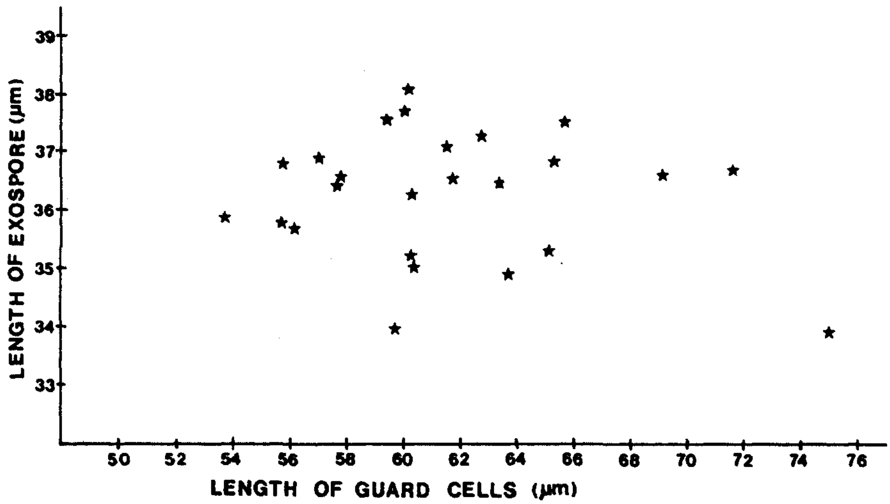

Fig. 4.—Mean length of guard cells vs. mean length of exospore in different samples of *A. balearicum*.

pattern (as defined by PUTTOCK & QUINN, 1980), with narrow ridges that rise to acute crests covered in minute teeth, smooth fold sides with numerous perforations at the base; ill-defined areolae bearing irregular projections which anastomose at the apices; and a straight supralaesural fold reaching the ends (plate 1 A-G). These results agree with the type material (TR 1432) and with those of PANGUA & PRADA (1988) for the two specimens from Minorca.

The spores of *A. onopteris* (plate 1 H-J) can be distinguished from those of *A. balearicum* by their angular rather than dentate crests, bigger and clearly defined areolae, and undulate supralaesural fold shorter than that of *A. balearicum*.

The results of the length and width of the measured exospores are shown in table 1 and figure 5. Some differences are evident when comparing these values with those of SHIVAS (1969), NARDI (1983) and ROSSELLÓ & SERRA (1987). However these may be due to the different mounting media used or to the inclusion of the perispore length, or to analytical error (BARRINGTON *& al.*, 1986).

The graphical gap between *A. balearicum* and *A. onopteris* (fig. 5) can be a morphological expression of their different level of ploidy, i.e. *A. balearicum* (tetraploid) has bigger spores than *A. onopteris* (diploid), as observed in other *Asplenium* complexes (WAGNER, 1954; LOVIS, 1964; ROBERTS, 1979; BENNERT *& al.*, 1982).

d) *Cytology*

The cytological study of both *A. balearicum* and *A. onopteris* gave the following results. Nine plants of *A. balearicum* from different localities of Minorca (PEP 56, 57, 67A, 67B, 70, 73, 79, 80 and 91) proved to be tetraploid and showed regular meiosis with 72 bivalents at metaphase I (plate 2 A, B; fig. 6 A, B). These

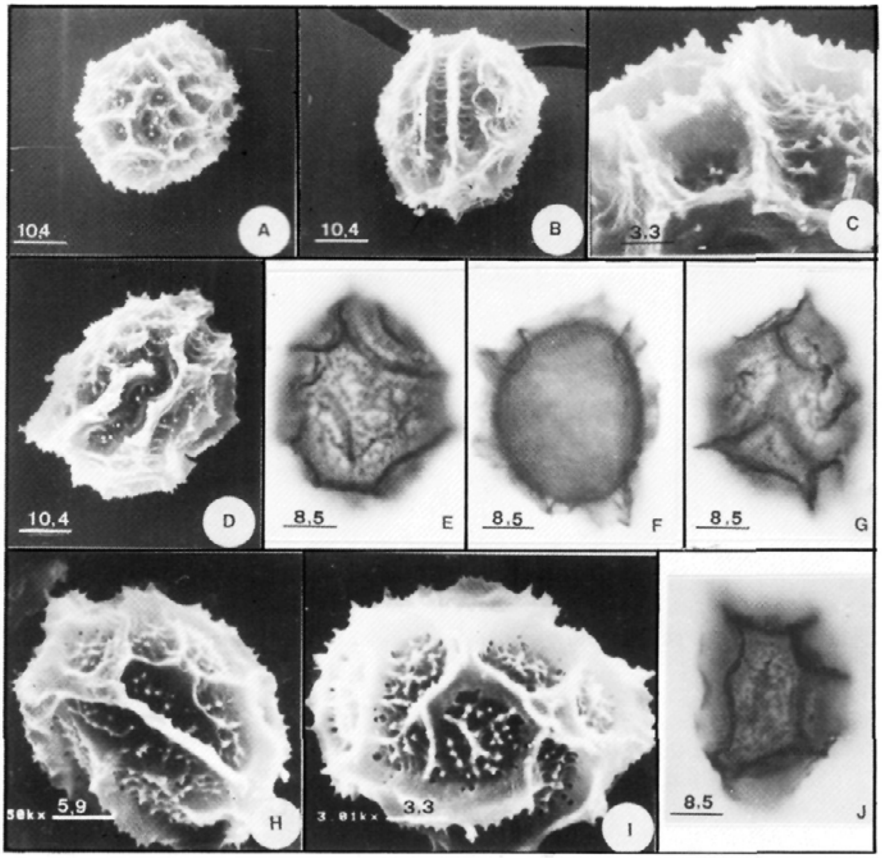

Plate 1.—Spores of *A. balearicum:* A-D. *A. onopteris:* H-J. A-C: PEP 68; D: PEP 83; E: PEP 58; F and G: PEP 67 A; H-J: PEP 86. Scale: in µm.

results agree with those obtained by SHIVAS (1969) and LOVIS & *al.* (1972) using plants from the original collection of *A. balearicum,* and with those of NARDI (1983), who studied plants from Pantelleria (Italy).

The cytological results obtained from plants PEP 50 and 89 confirmed their morphological identification, i.e. *A. onopteris.* Both of them are diploids and show regular meiosis with 36 bivalents at metaphase I (plate 2 C; fig. 7 A) which matches the results obtained by SHIVAS (1969) for plants from Majorca.

e) *Chromatographic pattern*

In the chromatographied alcoholic extracts of ten plants of *A. balearicum* eighteen spots were detected (fig. 8 A), and their chromatographic characteristics are detailed in table 2.

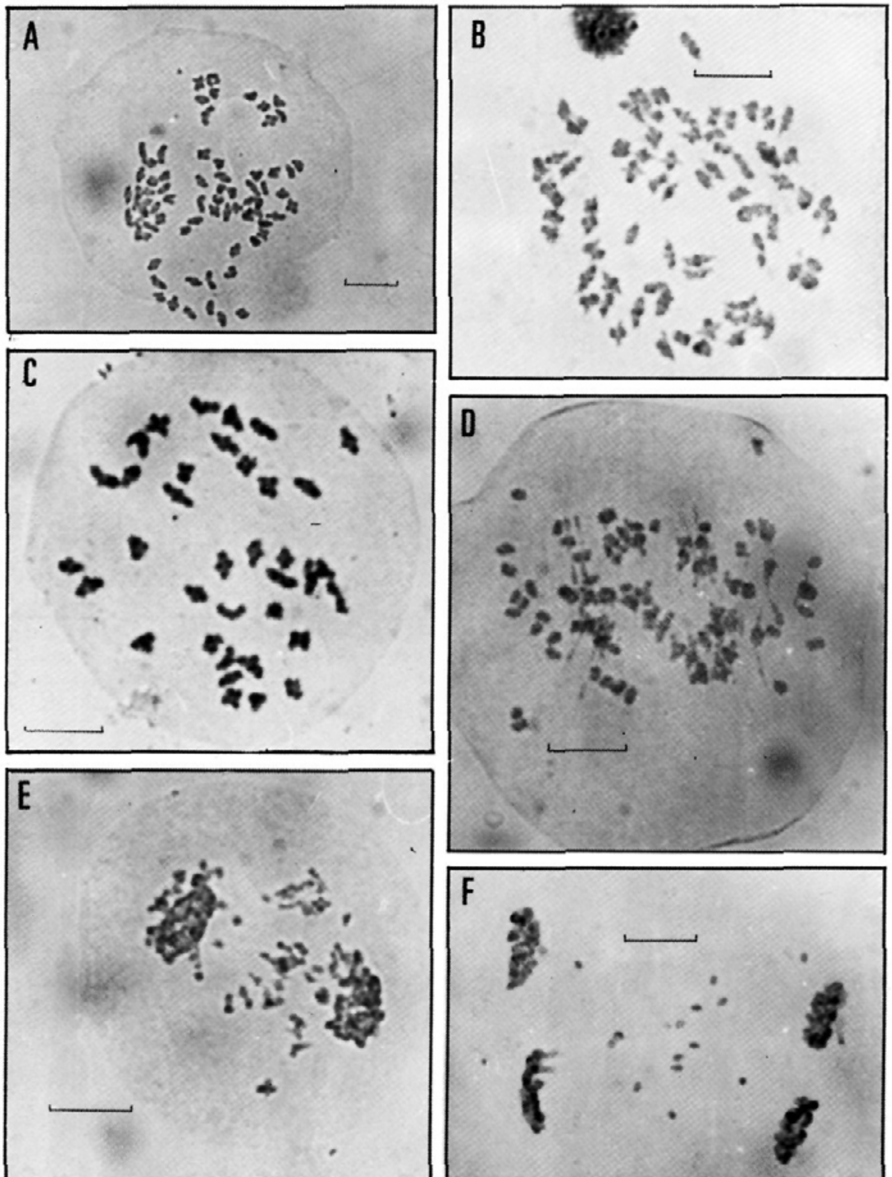

Plate 2.—Photomicrographs of spore mother cells at meiosis. A: *A. balearicum,* PEP 91 ii, 72 bivalents; B: *A. balearicum,* PEP 67B, 72 bivalents; C: *A. onopteris,* PEP 89, 36 bivalents; D-F: *A.* × *tyrrhenicum,* PEP 71, D: 35 bivalents and 38 univalents, E and F: anaphase-telophase I and II with numerous lagging chromosomes. Scale: 10 µm.

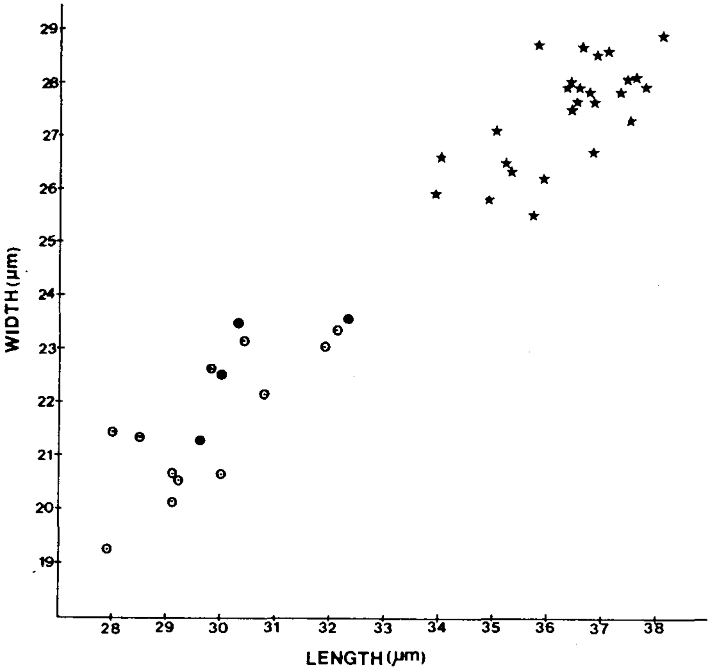

Fig. 5.—Scatter diagram of variation in spore size per plant (mean values). ★: *A. balearicum*; ●: *A. onopteris* (Balearic Islands); ⊙: *A. onopteris* (Iberian Peninsula; data from PANGUA & PRADA, in press).

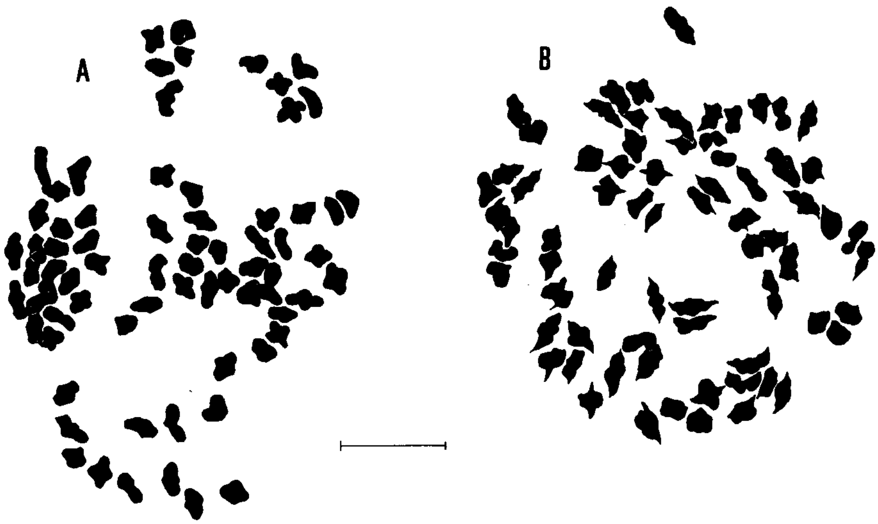

Fig. 6.—Explanatory diagrams for photomicrographs of plate 1. A: *A. balearicum*, PEP 91 ii, 72 bivalents at diakinesis. B: *A. balearicum*, PEP 67B, 72 bivalents at first metaphase. Scale: 10 µm.

TABLE 2

CHROMATOGRAPHIC CHARACTERISTICS OF THE SPOTS FOUND IN THE *A. BALEARICUM*, *A. ONOPTERIS* AND *A.* × *TYRRHENICUM* PLATES

Spot	Rf (×100)		Colour	
	BAW	HOAc 15 %	UV	UV/NH$_3$
1	13.5±2.8	11.8±2.5	Orange	Yellow
2	25.9±3.0	10.9±2.2	Orange	Yellow
3	45.6±2.7	32.6±2.9	Orange	Yellow
4	40.8±0.8	47.5±0.0	White	White
5	46.3±2.3	54.9±1.7	Deep Purple	Yellow
6	55.0±1.1	59.6±0.4	Deep Purple	Deep Purple
7	22.4±5.3	59.2±1.4	Orange	Yellow
8	19.0±4.7	63.6±0.1	Orange	Yellow
9	57.2±5.2	65.5±1.8	Deep Purple	Deep Purple
10	60.5±3.6	66.5±3.1	Blue	Yellowish
11	44.7±1.1	70.6±7.3	Deep Purple	Deep Purple
12	38.7±1.5	71.6±1.5	Deep Purple	Deep Purple
13	47.3±3.7	75.0±6.7	Deep Purple	Deep Purple
14	68.7±2.0	85.7±1.4	Invisible	Light Blue
15	60.7±3.0	83.8±1.7	Deep Purple	Deep Purple
16	37.0±4.2	80.6±1.7	Deep Purple	Deep Purple
17	42.6±1.7	90.8±0.7	Deep Purple	Deep Purple
18	5.1	81.9	Yellow	Yellow

Of these eighteen spots only one (number 5) is present in all the plants studied, while nine spots were found in over 50% of the samples (table 3). Although the concentration of spots varies widely between plates and depends of the weight of the extracted frond and on the quantity of the extract applied to the plates, the most conspicuous spots are number 5, 3, 15 and 17.

Difficulties were encountered in delimiting bright blue spots accurately (usually with a Rf in the BAW solvent higher than 0.5) near the acetic front and between spots 5 and 15. They never result in a well defined spot and their shape is irregular, and they are usually not consistent among replicates of the same extract, therefore, they were ignored when drawing the phenolic pattern.

We have not, at present, made any effort to identify the partial or total chemical structure of any spot, but the colour appearance under the UV lamp suggests that the deep purple ones are flavonoid glycosides, the orange-yellow are C-glycosil xanthones and most of the blue ones are probably phenolic acids (MABRY & *al.*, 1970).

The chromatographic variation of the *A. balearicum* spots (table 3) is not correlated with the morphology or the geographical distribution of the plants studied.

Unfortunately, we have been able to study only one plant of *A. onopteris* (PEP 89, cytology checked) from Minorca; however, the phenolic pattern of this plant matches well the results obtained from Iberian and Majorcan samples (ROSSELLÓ & *al., in prep.)*. Five spots (fig. 8 B) were clearly seen in the Minorcan

TABLE 3

CHROMATOGRAPHIC VARIATION OF THE *A. BALEARICUM* SAMPLES

Sample	Spot Number																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
PEP 68	+	+	+	–	+	–	–	–	–	+	–	+	+	–	+	–	+	–
JAR 87-424	+	–	+	–	+	+	+	+	+	+	+	+	+	+	–	+	+	–
JAR 87-425	–	+	+	–	+	+	+	+	–	+	–	+	–	–	+	+	+	–
PEP 73	+	+	+	–	+	–	+	–	+	–	–	+	+	+	+	+	–	–
PEP 82	–	+	+	+	+	+	+	+	+	+	–	+	+	–	–	–	+	+
PEP 84	–	+	+	–	+	–	+	+	+	+	–	+	+	+	–	–	+	–
PEP 91	+	+	+	–	+	–	+	–	+	–	–	–	–	–	+	+	–	–
PEP 93	+	+	+	–	+	–	+	–	+	–	–	–	+	–	+	+	–	–
JAR 87-426	–	+	+	+	+	+	+	–	+	–	–	+	+	–	+	+	–	–
JAR 87-427	–	+	–	–	+	+	+	+	+	–	+	+	–	–	–	–	+	–

plant, and all of them are also present in the *A. balearicum* samples, which supports: *a)* the cytological evidence indicating that both plants shared a common genome (SHIVAS, 1969; LOVIS & *al.*, 1972), and *b)* the additive inheritance of the phenolic pattern in these plants as previously found in other *Asplenium* complexes (SMITH & LEVIN, 1963).

A. × **tyrrhenicum**

a) *Morphology*

One plant (PEP 71) did not match the typical appearance of *A. balearicum* and has been recently described as *A. × tyrrhenicum* (CUBAS & *al.*, 1987), probably derived from a cross between *A. balearicum* and *A. onopteris.*

This plant has a narrowly triangular lamina, with a caudate apex and 7-9 pairs of pinnae per frond, the lowermost triangular and obliquely inserted (fig. 2 I). The morphology of *A. × tyrrhenicum* is intermediate between the two species presumably involved in its origin, showing the serrations of *A. onopteris* (fig. 2 H) as well as the cuneate base of its pinnules, while the *A. balearicum* influence is depicted by the rounded apices of pinnules (fig. 2 A-G). The silhouette of this wild hybrid is clearly different from that of the synthetic *A. balearicum* × *A. onopteris* (SHIVAS, 1969), i.e. while the latter is closer to *A. onopteris* the former approaches the morphology of *A. balearicum.*

b) *Stomata and epidermal cells*

Only anomocytic stomata were observed in the *A. × tyrrhenicum* fronds (fig. 3 F) and the mean length of the guard cells (50.2 μm) is smaller than that of *A. balearicum* and *A. onopteris.*

c) *Sporangial content*

The sporangial content of *A. × tyrrhenicum* consists of nearly opaque misshapen

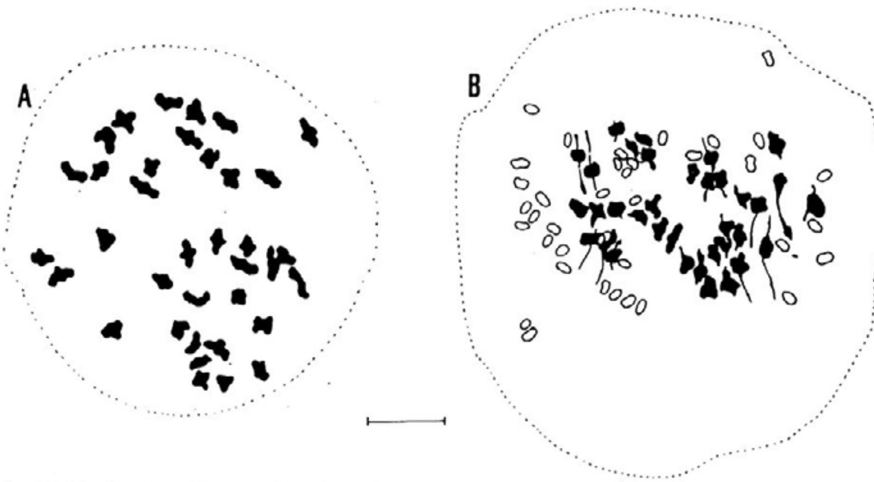

Fig. 7.—Explanatory diagrams for photomicrographs of plate 1. A: *A. onopteris,* PEP 89, diploid showing 36 bivalents at diakinesis. B: *A.* × *tyrrhenicum,* PEP 71, triploid hybrid showing 35 bivalents and 38 univalents at metaphase I. Scale: 10 µm. Bivalents, in black; univalents, in outline.

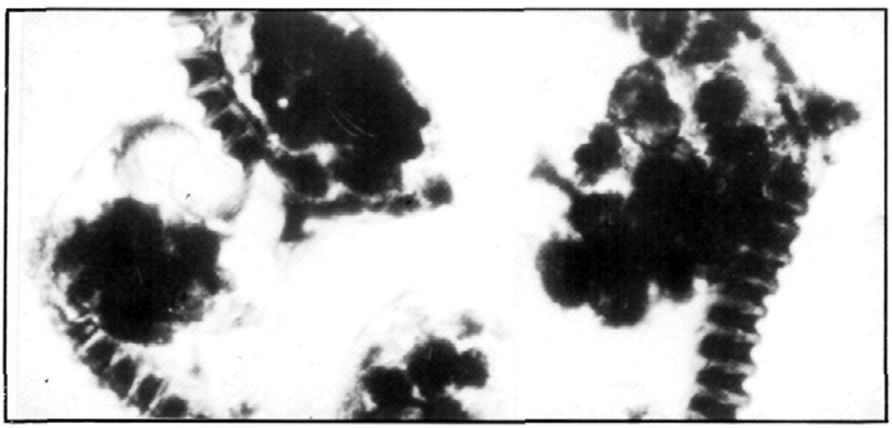

Plate 3.—Sporangia of *A.* × *tyrrhenicum* containing aborted spores.

spores and a dark undifferentiated material (plate 3), which is a good indication of hybridity, as suggested by REICHSTEIN (1981) and WAGNER & WAGNER (1986).

d) *Cytology*

The plant PEP 71 proved to be a triploid hybrid, showing irregular meiosis, with up to 36 bivalents and 36 unpaired chromosomes at metaphase I (plate 2 D; fig. 7 B; table 4). Some further irregularities were detected, and the most conspicuous was lagging chromosomes at anaphase-telophase I and II (plate 2 E, F).

TABLE 4

CHROMOSOME PAIRING IN *A.* × *TYRRHENICUM*

Number of cells	Bivalents	Univalents
2	36	36
1	c. 36	c. 35
1	c. 35	c. 36
1	35	38
1	34	40

This plant behaves in meiosis like the synthetic hybrid *A. balearicum* × *A. onopteris* (SHIVAS, 1969). This cytological behaviour indicates that this plant has two similar genomes (which account for the 36 pairs) and a different genome whose chromosomes remain unpaired.

e) *Phenolic pattern*

The only specimen of this hybrid studied so far has a chromatographic pattern of twelve spots (many of them at very low concentrations, especially spot 5). All the spots found in *A.* × *tyrrhenicum* (fig. 8 C) are present in *A. balearicum* plates, and five of them are also present in the *A. onopteris* plate.

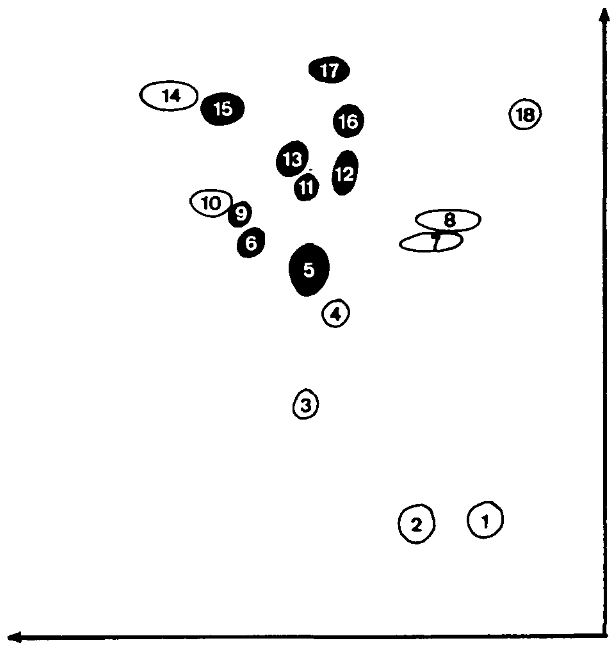

Fig. 8 A.—Chromatographic pattern of *A. balearicum*. Deep purple spot in black.

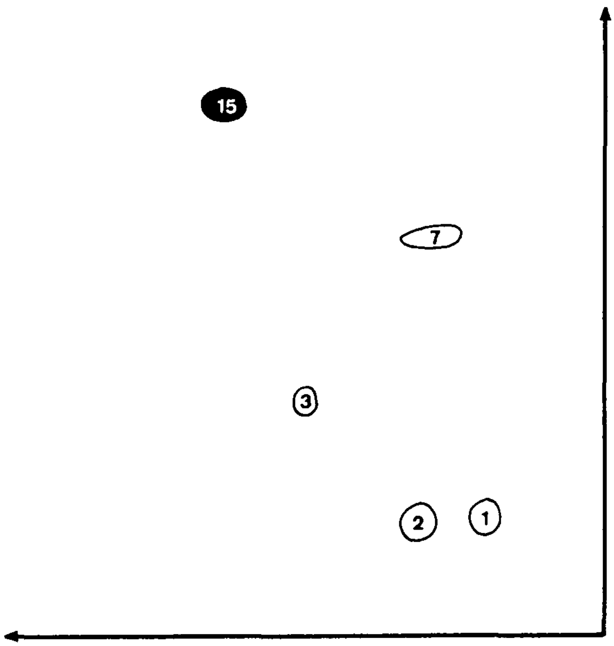

Fig. 8B.—Chromatographic pattern of *A. onopteris*. Deep purple spot in black.

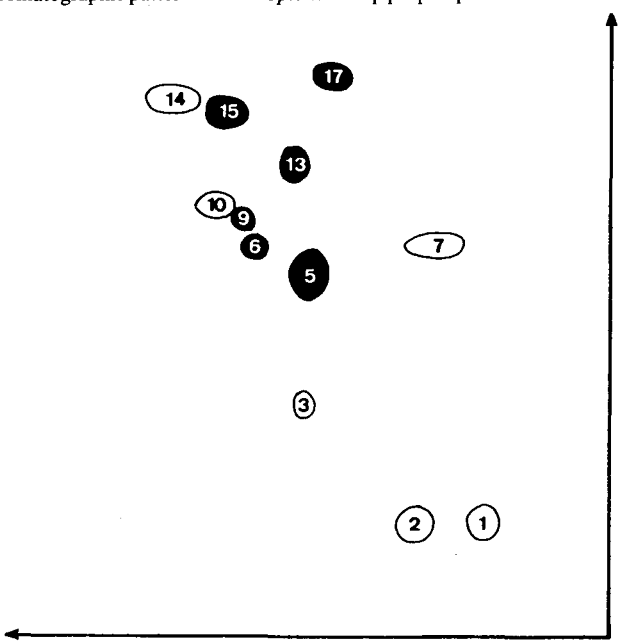

Fig. 8C.—Chromatographic pattern of *A.* × *tyrrenicum*. Deep purple spot in black.

The absence in this hybrid of some spots found in *A. balearicum* can be due to: *a)* very low concentration of the compounds, not detected with the TLC method used; *b)* the blockage of some metabolic pathway in the hybrid (MEARS, 1980), or *c)* one of the part-parental plants of *A.* × *tyrrhenicum* i.e. *A. balearicum* could have been a chemotype having a small number of phenolic compounds.

When more plants of *A.* × *tyrrhenicum* become available perhaps its phenolic pattern will not be distinguishable from that of *A. balearicum*, as suggested in the classic paper of SMITH & LEVIN (1963).

CONCLUSIONS

Despite the variability found in *A. balearicum* specially with respect to the frond size, number of pinnae per frond and degree of dissection of the pinnae, this taxon can be characterized by the triangular lamina, the rounded apices usually with obtuse teeth, and the perispore morphology. Also the phenolic pattern is highly characteristic and can be used to differentiate this taxon from closed forms of *A. onopteris*. This pattern supports that *A. onopteris* is part-parental of *A. balearicum*.

Some factors have to be considered prior to formulating the mode of genetic origin for *A.* × *tyrrhenicum:* I) the cytological behaviour of *A.* × *tyrrhenicum* indicates that this plant has either resulted from a cross between an autotetraploid and a non related diploid, or from a backcross between an allotetraploid and one of its ancestors; II) the phenolic pattern of *A.* × *tyrrhenicum* indicates that no different genomes are present in the hybrid when compared to *A. balearicum* (Ob and On) and *A. onopteris* (On); III) considering I) and II), only three possibilities can be taken into account to explain the origin of *A.* × *tyrrhenicum:* a) *A. billotii* × *A. onopteris* (ObObOn); b) *A. balearicum* × *A. onopteris* (ObOnOn), and c) *A. balearicum* × *A. obovatum* (ObOnOb); IV) neither *A. billotii* nor *A. obovatum* have been found in the Balearic Islands, while both, *A. balearicum* and *A. onopteris* are relatively abundant in Minorca. In fact, *A. balearicum* grew together with *A.* × *tyrrhenicum*, and *A. onopteris* was collected nearby; and V) the morphological characteristics of *A.* × *tyrrhenicum* are intermediate between *A. balearicum* and *A. onopteris*.

We conclude that the evidences presented above strongly support an origin for the triploid wild hybrid *A.* × *tyrrhenicum* from the cross between *A. balearicum* and *A. onopteris*.

Concerning the epiontology of *A. balearicum*, as earlier suggested by ROSSELLÓ & SERRA (1987) and PICHI SERMOLLI (1987), there is not a conclusive evidence to decide whether *A. balearicum* is an old species or a recent one. On the other hand, a possible polytopic origin should be investigated by enzyme electrophoresis on plants sampled from its full range of distribution, together with a comprehensive survey on the *A. balearicum* cytological and morphological characteristics throughout its distribution area.

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APPENDIX

List of localities

1. MENORCA: Favaritx
   - Cape Favaritx, 31SFE0628, 20 m, schist fissures near the road, 31-I-1987, *P. Cubas, E. Pangua & J. A. Rosselló. A. balearicum:* PEP 56, 57, 58, 59, 60, 61, 62).
   - Between Cape Favaritx and Cala Presili, 31SFE0627, 20-30 m, in the crevices of schists, 31-I-1987, *P. Cubas, E. Pangua & J. A. Rosselló (A. balearicum:* PEP 63, 64, 65 A, 65 B, 66, 67 A, 67 B, 67 C, 68).
   - Ibídem, 15-XI-1984, *J. A. Rosselló (A. balearicum:* JAR 87-424).
2. MENORCA: Mongrofe Nou
   - Near Mongofre Nou, 31SFE0326, 25 m, in fissures and ledges of banks of Triassic sandstones, 31-I-1987, *P. Cubas, E. Pangua & J. A. Rosselló (A. balearicum:* PEP 69, 70, 72; *A.* × *tyrrhenicum:* PEP 71).
   - Ibídem, 15-XI-1984, *J. A. Rosselló (A. balearicum:* JAR 87-425).
3. MENORCA: Llinaritx Nou
   - Near Llinaritx Nou, 31SEE9128, 60 m, crevices and ledges of banks in an evergreen oak wood, 1-II-1987, *P. Cubas, E. Pangua, J. A. Rosselló & J. L. Villalonga (A. balearicum:* PEP 73, 74, 75, 76, 77, 78, 79, 87; *A. onopteris:* 86, 88, 89).
4. MENORCA: Binimel·la
   - 31TEE9034, 50 m, banks and crevices of schists, 1-II-1987, *P. Cubas, E. Pangua & J. A. Rosselló (A. balearicum:* PEP 80, 81, 82, 83, 84, 85).
5. MENORCA: Sa Vall
   - Sa Bassa Verda d'Algaierens, 31TEE7934, 100 m, in crevices of Triassic sandstones, 1-I-1987, *P. Cubas, E. Pangua, J. A. Rosselló & J. L. Villalonga (A. balearicum:* PEP 91, 92, 93, 94).
   - Ibídem, XI-1985, *M. Mus & J. L. Villalonga (A. balearicum:* JAR 87-426).
   - Near Algaierens, 31TEE7933, 20 m, evergreen oak wood, XI-1985, *M. Mus & J. L. Villalonga (A. balearicum:* JAR 87-427).
6. MALLORCA: Gorg Blau
   - 650 m, 30-I-1987, *P. Cubas, E. Pangua, J. A. Rosselló & J. Vicens (A. onopteris:* PEP 50).

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