

A COMPARATIVE STUDY OF THE GAMETOPHYTES OF *ASPLENIUM MAJORICUM* LITARD. (ASPLENIACEAE) AND RELATED TAXA*

by

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Resumen

PRADA, C., E. PANGUA, A. HERRERO & S. PAJARÓN (1996). Estudio comparativo de los gametófitos de *Asplenium majoricum* Litard. (Aspleniaceae) y táxones relacionados. *Anales Jard. Bot. Madrid* 54: 126-136 (en inglés).

Mediante el cultivo de esporas en condiciones controladas de luz y temperatura se ha estudiado el desarrollo y morfología de los protalos y jóvenes esporófitos en el allotetraploide *Asplenium majoricum* Litard. y en sus progenitores diploides *A. fontanum* (L.) Bernh. subsp. *fontanum* y *A. petrarchae* (Guérin) DC. subsp. *bivalens* (D.E. Mey.) Lovis & Reichst., así como en el autotetraploide *A. petrarchae* (Guérin) DC. subsp. *petrarchae* derivado de este último. Los protalos son pelosos. En *A. majoricum* están caracterizados por tener una banda central de células alargadas diferentes de las restantes células del protalo; en *A. fontanum* los tricomas marginales son con frecuencia bicelulares. En todos los casos los primeros gametangios que se forman son los anteridios. Las hojas de los esporófitos jóvenes de *A. majoricum* muestran caracteres intermedios entre los de sus progenitores.

Palabras clave: *Pteridophyta*, *Aspleniaceae*, *Asplenium*, gametófito, morfología.

Abstract

PRADA, C., E. PANGUA, A. HERRERO & S. PAJARÓN (1996). A comparative study of the gametophytes of *Asplenium majoricum* Litard. (Aspleniaceae) and related taxa. *Anales Jard. Bot. Madrid* 54: 126-136.

Morphology and development of prothallia and young sporophytes of the allotetraploid *Asplenium majoricum* Litard., and its diploid progenitors *A. fontanum* (L.) Bernh. subsp. *fontanum* and *A. petrarchae* (Guérin) DC. subsp. *bivalens* (D.E. Mey.) Lovis & Reichst., as well as of the autotetraploid *A. petrarchae* (Guérin) DC. subsp. *petrarchae*, have been studied in spore culture under controlled light and temperature conditions. Prothallia are hairy. In *A. majoricum* they are characterized by a central band of elongate cells different from the other cells of the prothallus. In *A. fontanum* marginal bicellular hairs are often produced. In all taxa antheridia are the first sexual organs formed. Leaves of sporelings of *A. majoricum* have characters intermediate between those of the parents.

Key words: *Pteridophyta*, *Aspleniaceae*, *Asplenium*, gametophyte, morphology.

INTRODUCTION

Asplenium majoricum Litard. is an allotetraploid originating from the crossing of

A. fontanum (L.) Bernh. subsp. *fontanum* and *A. petrarchae* (Guérin) DC. subsp. *bivalens* (D.E. Mey.) Lovis & Reichst., followed by chromosome doubling. It was considered as

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restricted to Mallorca Island, although RIGUAL (1972) and recently PANGUA & *al.* (1989, 1992) and PÉREZ CARRO & FERNÁNDEZ ARECES (1992) mentioned its presence on the mainland.

A. petrarchae subsp. *bivalens* was believed to be an endemic plant from southern Spain (NOGUEIRA & ORMONDE, 1986); PANGUA (1989) mentioned that it exists on the eastern coast of Spain and BENNETT & *al.* (1990) found it in Mallorca also. PANGUA & *al.* (1992) described a hybrid plant between *A. petrarchae* subsp. *bivalens* and *A. fontanum* subsp. *fontanum* from Valencia that was named *A. × protomajoricum*, representing the intermediate hybrid in the formation of *A. majoricum*. *Asplenium petrarchae* subsp. *petrarchae*, the autotetraploid derived from *A. petrarchae* subsp. *bivalens*, has also been included in this study. All the taxa grow on limestone rocks.

While the morphology of sporophytes of this group has been extensively studied, gametophytes have only been examined for their early stages of development and to ascertain some morphological aspects in *A. fontanum* and *A. majoricum* (HENRIET & MOENS, 1976). In this paper we present data on gametophyte development, ontogenetic sequence of gametangia, and morphology of young sporophytes.

MATERIAL AND METHODS

Spores for gametophyte cultures were obtained from the following collections. Voucher specimens are deposited in MABC.

Asplenium petrarchae (Guérin) DC. subsp. *bivalens* (D.E. Mey.) Lovis & Reichst.

1. VALENCIA: Gandfa, Sierra Falconera, Cova Xurra, 130 m, limestone rock, 17-II-1990, *Pangua & Prada CE87*.
2. VALENCIA: Road between Simat de Valldigna and Barx, limestone rock, 16-II-1993, *Pajarón & al. CE242*.
3. VALENCIA: Road between Simat de Valldigna and El Romeral, limestone rock, 16-II-1993, *Pajarón & al. CE268*.

A. petrarchae (Guérin) DC. subsp. *petrarchae*

4. JAÉN: Sierra de las Villas, valle de Fuentepinilla, crevices on limestone rock, 25-X-1992, *Pajarón & Pangua CE337*.

A. fontanum (L.) Bernh. subsp. *fontanum*

5. ALICANTE: Puig Campana, crevices on limestone rock, 17-II-1993, *Pajarón & al. CE318*.
6. GUADALAJARA: Corduente, valle del Alto Tajo, 30TWL71, 980 m, crevices on limestone rock, 7-IV-1993, *Herrero & al. AH1F*.
7. GERONA: Vallfogona, Sierra de Milany, Castell de Milany, 31TDG46, 1530 m, crevices on calcareous conglomerates, exposure S, 28-XII-1991, *Herrero & al. AH3F*.

A. majoricum Litard.

8. MALLORCA: Biniaraix, 29-I-1987, *Pangua & al. PEP30*.
9. MALLORCA: Balix d'Emming, 28-I-1987, *Pangua & al. PEP21a*.

A. × protomajoricum Pangua & Prada

10. VALENCIA: Benifairó de Valldigna, La Gola, 30SYJ32, 470 m, limestone rock, 17-II-1990, *Herrero, Pangua & Prada CE75*.

Data on percentages of germination, chromosome numbers, spore and guard cells length for each sample are summarized in table 1.

Spores from a single sporophyte of each collection were sown in Petri dishes (6 cm diameter) on mineral agar (DYER, 1979) for study of early stages of gametophyte development; these gametophytes were sampled during the first month after the sowing. From these cultures 25 gametophytes of *A. majoricum* (9) were isolated in the presexual stage in order to determine their capacity for intragametophytic selfing.

Except for *A. × protomajoricum*, which only had a few diplospores, each sample was also sown on sieved soil (a 3:1 mixture of compost and sand) devitalized in a stove at 120 °C for two hours in order to observe complete morphological development and obtain mature prothallia and sporophytes. Sowings of each sample were replicated twice on both agar and soil to produce enough gameto-

TABLE 1

PERCENTAGES OF GERMINATION, CHROMOSOME NUMBERS, EXOSPORE LENGTH AND GUARD CELLS LENGTH FOR EACH SAMPLE
(the exospore and guard cells length in μm)

Sample	Percentage germination	Chromosome numbers	Exospore length	Guard cells length
<i>Apb CE87</i>	82	$2n = 72$	38.7 ± 1.7	39.7 ± 2.1
<i>Apb CE242</i>	56	$2n = 72$	37.3 ± 1.9	40.6 ± 3.5
<i>Apb CE268</i>	36	$2n = 72$	39.4 ± 2.3	39.3 ± 3.8
<i>App CE337</i>	88	$2n = 144$	43.7 ± 2.2	57.5 ± 3.6
<i>Aff CE318</i>	88	$2n = 72$	34.5 ± 2.0	39.5 ± 3.0
<i>Aff AH1F</i>	76	$2n = 72$	33.2 ± 3.7	40.5 ± 2.6
<i>Aff AH3F</i>	80		35.1 ± 2.3	38.8 ± 2.3
<i>Ama PEP30</i>	54		40.6 ± 1.8	48.4 ± 2.8
<i>Ama PEP21a</i>	66		43.3 ± 2.5	48.3 ± 2.7
<i>Apm CE75</i>		$n = 68^f + 2^h$		45.7 ± 2.3

phytes for sampling. The dishes were kept in a growth chamber at 23 °C under continuous illumination with white fluorescent tubes. Soil cultures were watered once a week and were maintained for 20 weeks.

Fifty gametophytes of each sample on soil were sampled weekly after reaching the bidimensional stage, stained with chloral hydrate aceto-carmine, mounted in water and observed under the light microscope in order to obtain the percentages of each sex expression. At the same time, the length and density (number of hairs/perimeter) of marginal hairs were calculated for each sample using an image-analyzing computer, based on thirty measurements taken randomly from 10 mature prothallia about 4.5-5 mm wide. The values were summarized in a box-and-whisker plot.

RESULTS

Spores started to germinate 9 days after sowing. The *A. × protomajoricum* sporangia had abortive spores along with a few diplospores; the first diplospore germinated one week later than those of the other taxa. The general pattern of development was similar in

all five taxa (fig. 1), following the *Aspidium* type (NAYAR & KAUR, 1969, 1971) in which there is early hair formation in the young gametophyte. The number of cells in the filamentous stage is variable depending on the taxa; in both subspecies of *A. petrarcae* and in *A. fontanum* the filament frequently has 1 to 4 cells, whereas in *A. majoricum* there are 4 to 6 cells.

The first longitudinal cell division which gives rise to the bidimensional stage takes place about three weeks after sowing. Hairs appear when the plate has started to form (fig. 1b-e), except in *A. petrarcae* subsp. *bivalens* and *A. majoricum* which sometimes produce a hair from the apical cell of the filament (fig. 1b', c').

Morphology of mature gametophytes is very much alike in all taxa. They are usually wider than long (fig. 2a), but in *A. fontanum* the gametophytes tend to be longer than wide (fig. 2b). The margins of young prothallia and the lower part of the wings of mature prothallia are frequently irregular (fig. 2c), except in *A. fontanum*.

Asplenium majoricum prothallia are distinguished by a broad central zone of narrow

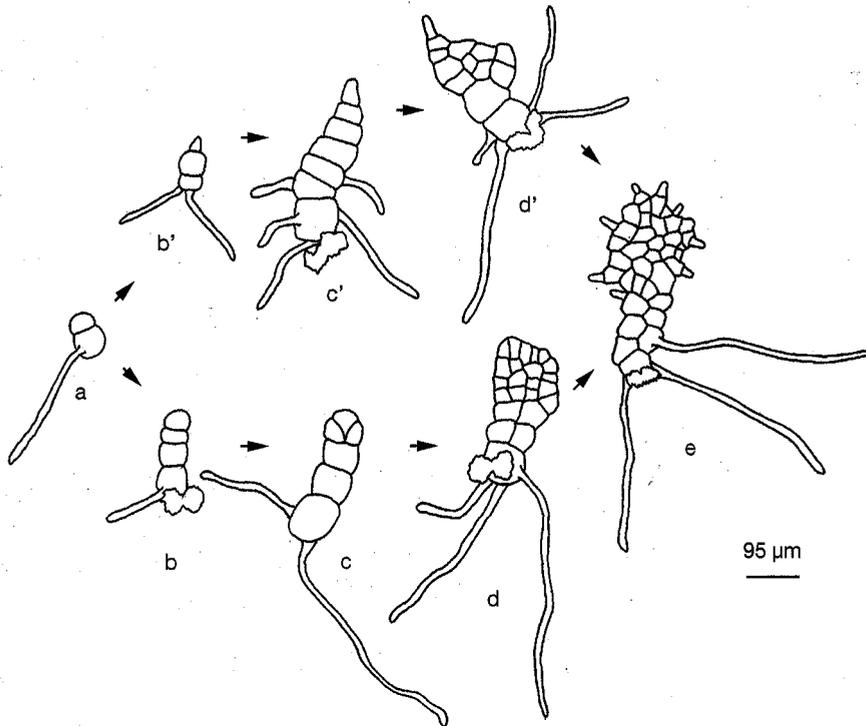


Fig. 1.—Early stages of prothallial development: a, spore cell after the first division (*A. fontanum* CE318); b'-d', early formation of hairs (b', *A. petrarchae* subsp. *bivalens* CE268; c', d', *A. majoricum* PEP21a); b-d, hairs formed after reaching bidimensional stage (b, c, *A. fontanum* CE318; d, *A. petrarchae* subsp. *petrarchae* CE337); e, young gametophyte (*A. fontanum* AH1F).

TABLE 2

MEANS AND STANDARD DEVIATIONS OF THE LENGTHS AND DENSITIES OF MARGINAL HAIRS
(the length of the marginal hairs in μm , and the density in number of hairs/mm)

Sample	Length of marginal hairs		Density of marginal hairs	
	Mean	SD	Mean	SD
<i>Apb</i> CE87	44.33	3.76	8.98	1.06
<i>Apb</i> CE242	40.00	2.79	8.68	1.21
<i>Apb</i> CE268	43.25	3.23	8.61	0.97
<i>App</i> CE337	43.58	2.91	8.05	0.95
<i>Aff</i> CE318	43.83	4.19	6.93	1.62
<i>Aff</i> AH1F	46.33	4.86	11.91	1.93
<i>Aff</i> AH3F	45.17	3.59	9.88	0.33
<i>Ama</i> PEP30	47.50	4.05	6.29	0.63
<i>Ama</i> PEP21a	50.50	4.89	6.18	0.65
<i>Apm</i> CE75	57.08	5.21		

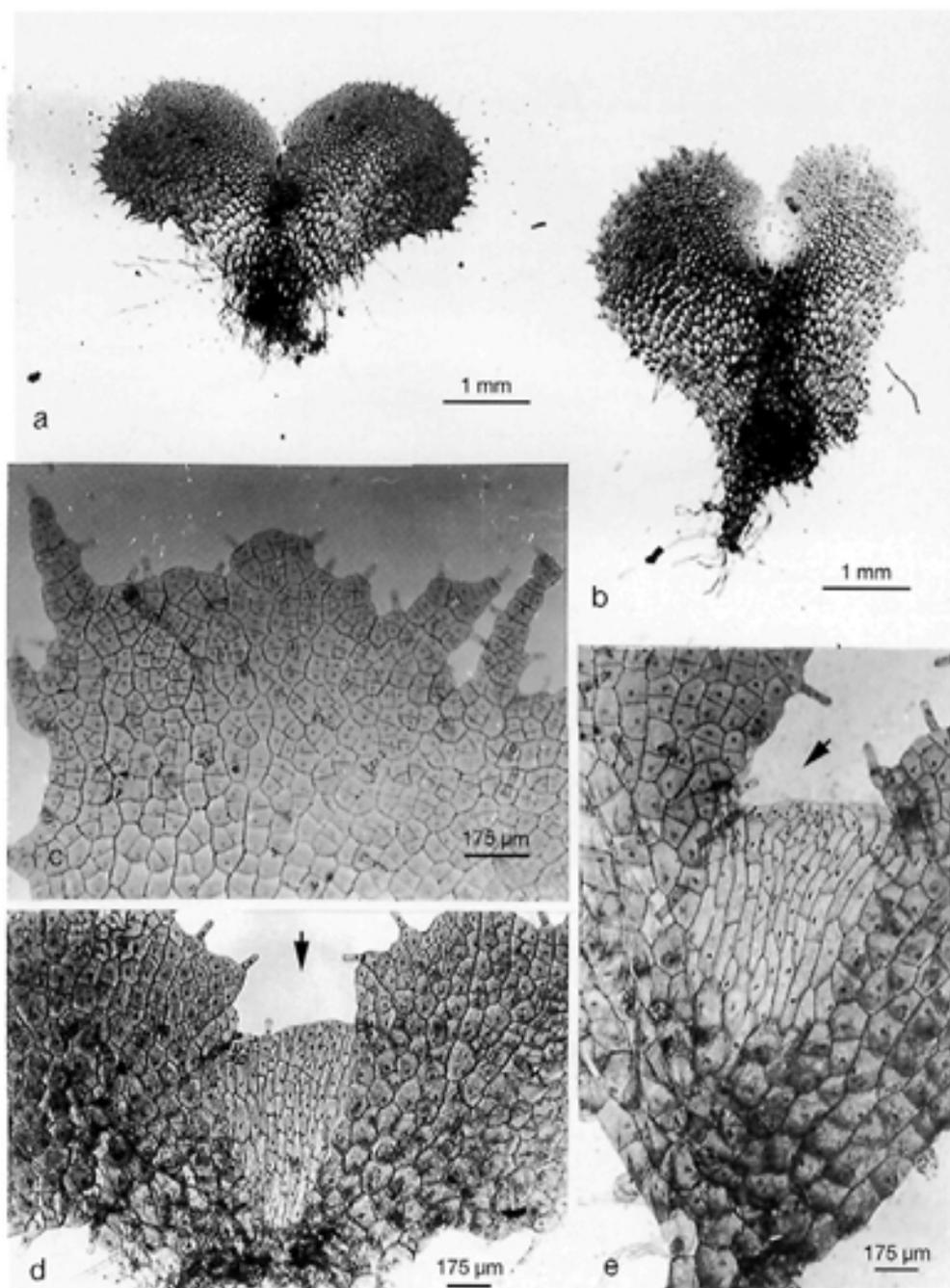


Fig. 2.—Morphology of mature gametophytes: a, *Asplenium petrarchae* subsp. *bivalens* (CE268); b, *A. fontanum* (CE318); c, *A. petrarchae* subsp. *petrarchae* (CE337); d-e, *A. majoricum* (PEP30).

elongated cells, distinct from the other cells of the gametophyte (fig. 2d-e).

As stated above, unicellular hairs are formed at an early stage of development. They are abundant at the margins and on the surface of the mature prothallia. Hairs in all taxa are unicellular and cylindrical; in *A. fontanum* we found bicellular hairs as well as unicellular ones at the margin (fig. 3a-f).

Table 2 shows means and standard devia-

tions of the lengths and densities of marginal hairs. The length of marginal hairs shows a high degree of overlapping among all samples, with *A. × protomajoricum* having the longest hairs. Regarding hair density, samples of both subspecies of *A. petrarchae* were homogeneous, as was *A. majoricum*. However, *A. fontanum* had a higher intrataxon variability. Figure 4 represents length and density values in a box and whisker plot.

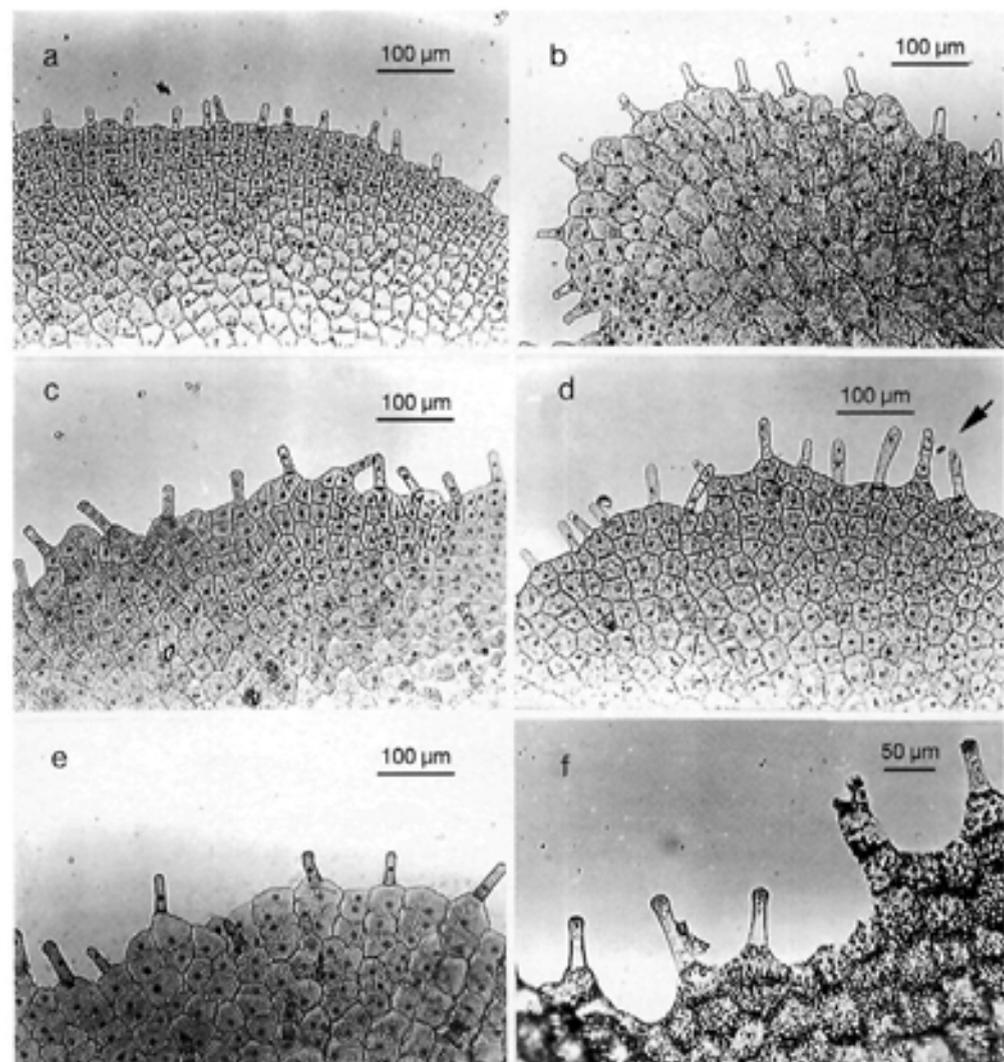


Fig. 3.—Morphology of marginal hairs: a, *Asplenium petrarchae* subsp. *bivalens* (CE87); b, *A. petrarchae* subsp. *petrarchae* (CE337); c-d, *A. fontanum* (CE318 and AH3F); e, *A. majoricum* (PEP21a); f, *A. × protomajoricum* (CE75).

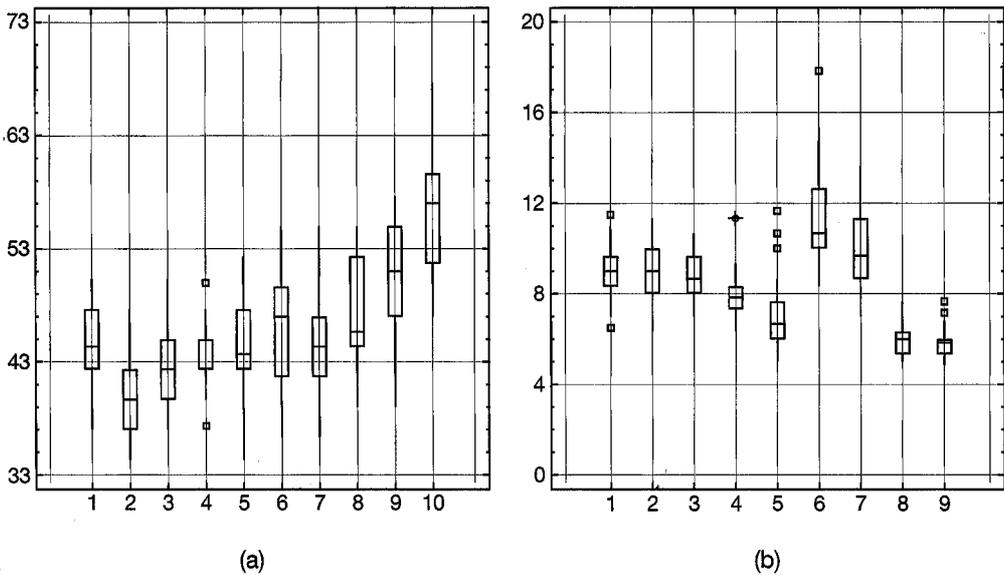


Fig. 4.—Multiple box and whisker plot of (a) length of the marginal hairs and (b) density of the marginal hairs: 1, *Asplenium petrachae* subsp. *bivalens* (CE87); 2, *A. petrachae* subsp. *bivalens* (CE242); 3, *A. petrachae* subsp. *bivalens* (CE268); 4, *A. petrachae* subsp. *petrachae* (CE337); 5, *A. fontanum* (CE318); 6, *A. fontanum* (AH1F); 7, *A. fontanum* (AH3F); 8, *A. majoricum* (PEP30); 9, *A. majoricum* (PEP21a); 10, *A. × protomajoricum* (CE75). The length of the marginal hairs in µm, and the density in number of hairs/mm.

Six weeks after sowing, gametangia appeared in all taxa except *A. majoricum*, where they had developed one week previously. Antheridia formed first in all cultures. Female gametophytes were detected later, along with bisexual ones. Figure 5 shows percentages of each sex expression, calculated at 42, 56, 77 and 119 days after sowing.

Young sporophytes started to appear 15 weeks after sowing. Isolated cultures of *A. majoricum* reached 100% of sporophyte production.

The first leaves had marginal hairs very similar to the ones found on gametophytes (fig. 6). *Asplenium fontanum* had young leaves with triangular lobes separated by deep incisions; their margins had unicellular and bicellular hairs as in the gametophytes, but in this case they are curved instead of straight. Both subspecies of *A. petrachae* showed less pronounced lobes with unicellular marginal hairs frequently arising on a protruding epidermal cell. Young leaves in *Asplenium majoricum* have intermediate characteristics. The

cross section of the petiole is plano-convex in *A. fontanum* and *A. majoricum*, whereas in both subspecies of *A. petrachae* it is more or less circular. Gametophytes obtained from the few diplospores of *A. × protomajoricum* did not undergo fertilization, in spite of the active formation of both kinds of gametangia. They produced many new plates growing from marginal and surface cells of old prothallia.

DISCUSSION

Gametophyte development is similar in all taxa of the group. NAYAR & KAUR (1971) indicated that the *Aspidium*-type development of gametophytes is common in *Aspleniaceae*, mainly in species of *Asplenium* and *Camptosorus*. Morphological differences were found in *A. majoricum*, which has a filamentous stage with a higher number of cells than in the other taxa, and a striking central band of elongate cells; we do not know any reference in the literature to this cellular dimorphism in

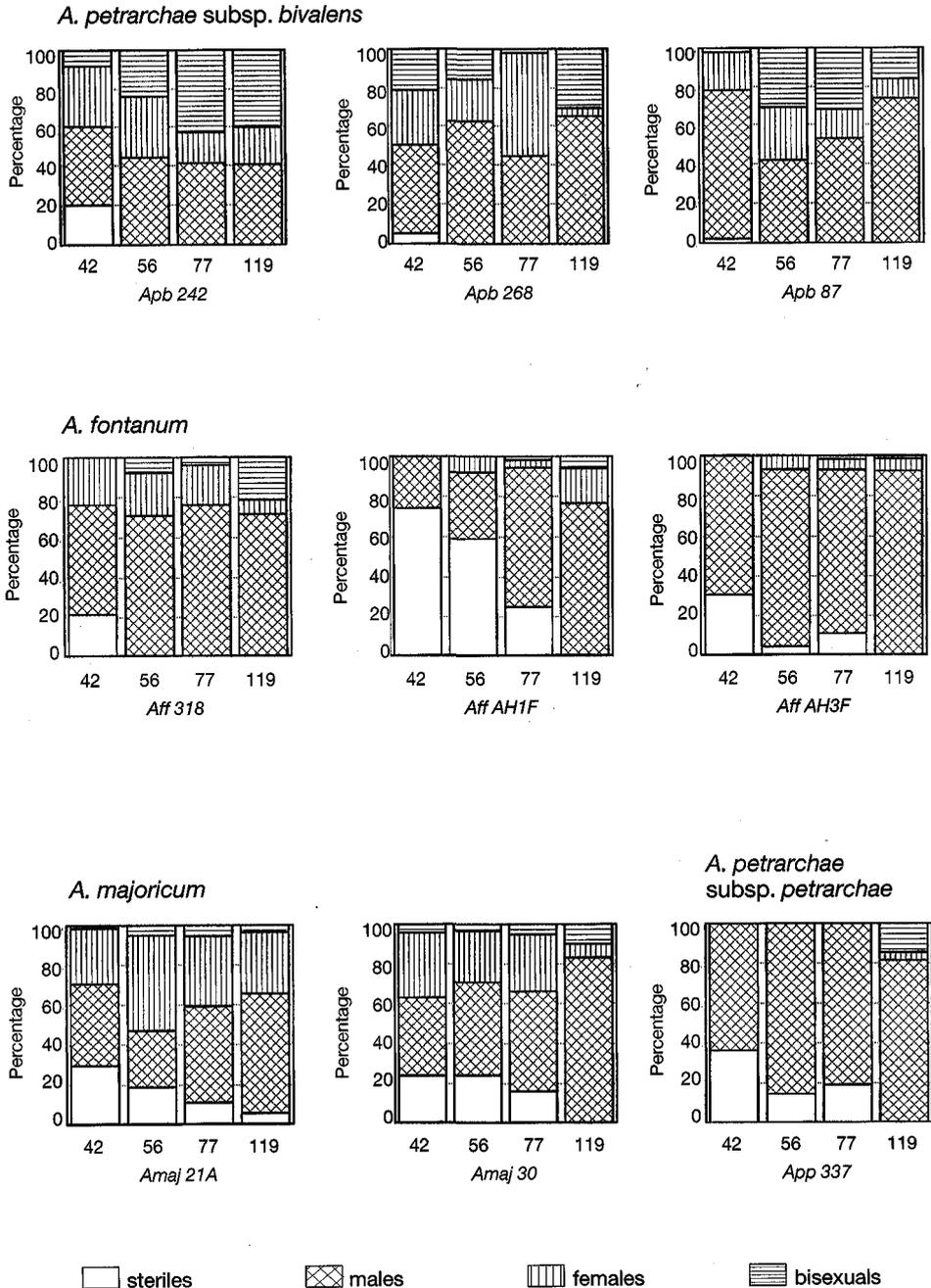


Fig. 5.—Bar diagrams showing sexual expression percentages scored in 42, 56, 77 and 119 days old cultures.

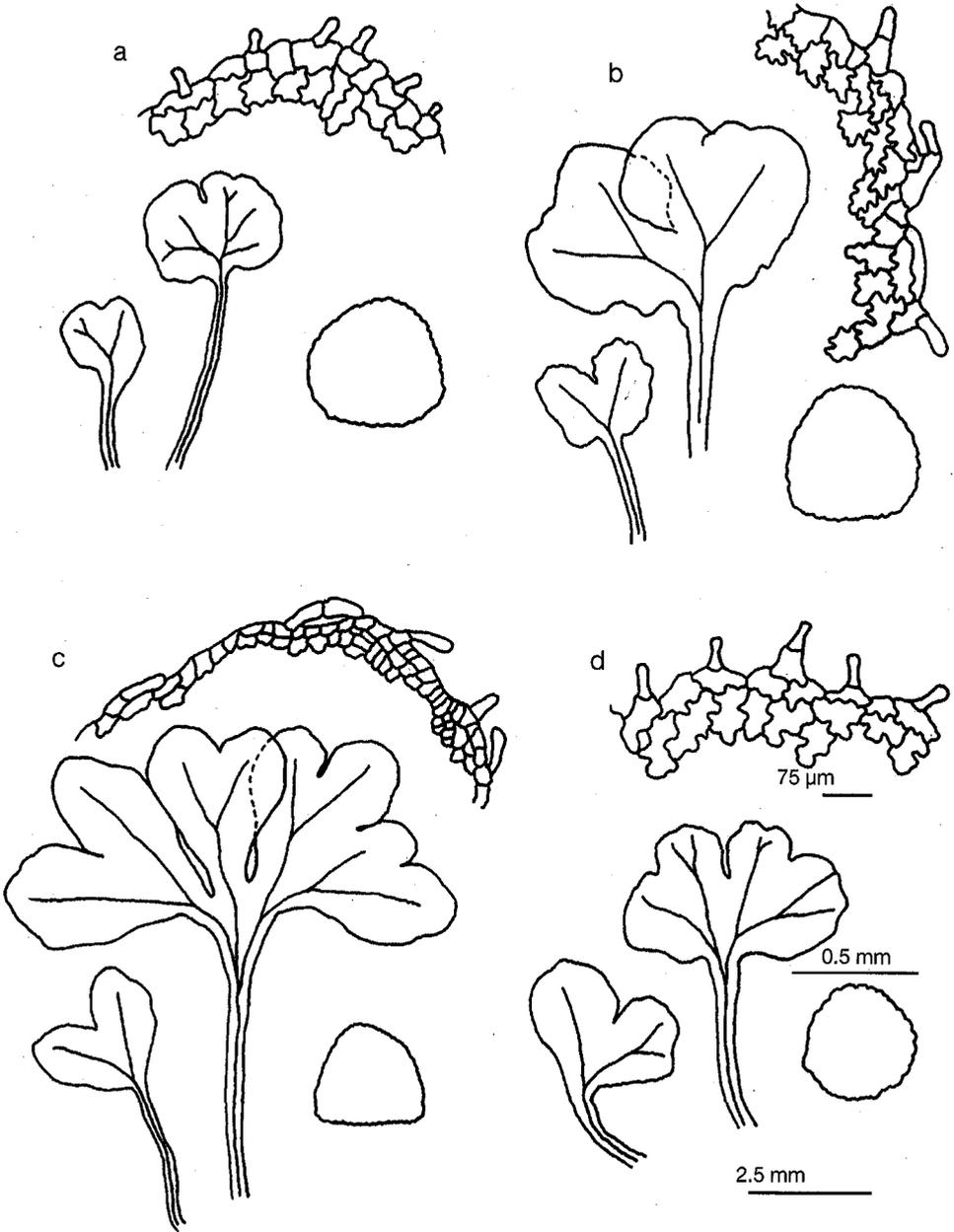


Fig. 6.—First leaves, marginal hairs and section of petiole of the young sporophytes: a, *Asplenium petrarchae* subsp. *bivalens* (CE268); b, *A. majoricum* (PEP21a); c, *A. fontanum* (AH1F); d, *A. petrarchae* subsp. *petrarchae* (CE337). Scale bars are the same in figures a-d.

gametophytes. HENRIET & MOENS (1976) did not mention this character for *A. majoricum*, probably because they studied very young prothallia. This may also explain their reporting of multicellular hairs in this taxon, which we interpret in young gametophytes as the irregular segments of the margin ending in a single hair.

A peculiar characteristic of the gametophytes of *Asplenium fontanum* is the presence of bicellular hairs; this kind of hairs was described in other *Aspleniaceae*, namely in the American tetraploid cytotype of *Phyllitis scolopendrium* (ATKINSON & STOKEY, 1964) as well as in *A. onopteris* (PRADA & al., 1995). Both diploid and tetraploid *A. petrarachae* have unicellular hairs of similar length and density. *Asplenium majoricum* has slightly longer hairs than its diploid parents, but they are more scattered. The sequence of formation of gametangia is constant throughout the group, as in other *Asplenium* species (HERREIRO & al., 1993; PRADA & al., 1995), with antheridia appearing before archegonia in all cultures. This is the most common sequence in ferns (KLEKOWSKY, 1969; MASUYAMA, 1972; COUSENS, 1979; SCHNELLER, 1979).

Since male, female and bisexual prothallia coexist in well-established gametophyte populations, a trigametophytic system exists in which inter- and intragametophytic unions are possible.

Percentages of intragametophytic selfing reaching 100% in isolated cultures of *A. majoricum* indicate that this taxon is able to establish itself from a single spore. On the other hand, the presence of the hybrid *A. × protomajoricum* in different localities of eastern Spain (PANGUA & al., 1992; PÉREZ CARRO & FERNÁNDEZ ARECES, 1992) shows that gametophytes of *A. fontanum* and *A. petrarachae* subsp. *bivalens* may interchange gametes easily when they grow in close proximity. The great capability for regeneration observed in gametophytes of *A. × protomajoricum* obtained from diplospores suggests another potential source of formation of new plants. These possibilities can account for the scattered presence of *A. majoricum* on the mainland.

The young sporophytes have hairs with the

same morphology as those of the gametophytes, which have been mentioned by NAYAR (1965) in Drynarioid ferns. Degree of leaf dissection and cross-sectional shape of the young sporophyte leaf petiole in *A. majoricum* show intermediate characteristics between those of the diploid parents. *Asplenium petrarachae* subsp. *bivalens* and its autotetraploid *A. petrarachae* subsp. *petrarachae* do not show differences in these characters.

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