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Gender determination and mating system in the autotetraploid fern *Asplenium septentrionale* (L.) Hoffm.

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Abstract

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Asplenium septentrionale subsp. *septentrionale* is an autotetraploid fern derived from *A. septentrionale* subsp. *caucasicum*, which inhabits cracks in siliceous or decarbonated rocks. By culturing isolated gametophytes and pairs of gametophytes of related and unrelated individuals, from various populations of this taxon, the sequence of gametangia has been established. In all cases, this begins with antheridia, which are followed by archegonia thus forming bisexual gametophytes, with both gametangia functional throughout a prolonged phase, thereby favouring intragametophytic fertilisation. The formation of 62.9% of sporophytes in isolated gametophytes and the lower percentage when the cultures are in pairs or crosses demonstrates the capacity of this taxon to self-fertilise.

Key words: Asplenium, reproductive biology, gametophyte, sex expression.

Introduction

Mating systems play an important role in the evolutionary biology of species. Not only do they act on reproduction itself, but they also significantly influence the genetic structure of populations and species (Soltis and Soltis 1990).

In pteridophytes, the reproductive mechanisms that determine genetic diversity and evolutionary potential are determined by gametophytic generation. The gametophytes of the homosporous pteridophytes can become bisexual and reproduce themselves by self-fertilisation, although they can also develop mechanisms that favour out-

crossing (Klekowski 1969, Lloyd 1974a, Soltis and Soltis 1992). Three different possibilities of gametic exchange exist: intragametophytic selfing, intergametophytic selfing, and outcrossing (Klekowski 1969).

The homosporous pteridophytes are the only vascular plants capable of producing completely homozygous individuals in a single generation, through intragametophytic self-fertilisation. The accumulation of deleterious recessive genes (genetic load) in the genotype of individuals would represent an obstacle to the formation of sporophytes through this type of reproduction. High levels of genetic load suggest intergametophytic mating systems, while low levels, or the absence of genetic load have been associated with intragametophytic self-fertilisation (Klekowski 1973, Lloyd 1974a, Lloyd 1974b).

On the other hand, adaptations such as the sequence of formation of the gametangium or the relative proportions of individuals of each sexual type – male, female or bisexual – in a population of gametophytes may be used to predict mating systems (Klekowski 1969, Raghavan 1989 and references therein).

Asplenium septentrionale (L.) Hoffm. subsp. *septentrionale* is an autotetraploid fern with 2n = 144 chromosomes (Lovis 1964) derived from the diploid *A. septentrionale* (L.) Hoffm. subsp. *caucasicum* Fraser-Jenkins and Lovis. It lives in mountainous areas and colonises cracks in siliceous or decarbonated rocks, tolerating harsh winters and high summer temperatures. It is distributed throughout the temperate regions of Europe, Asia and North America, and inhabits the siliceous mountainous formations of the Iberian Peninsula (Nogueira and Ormonde 1986). Momose (1960), observed that its gametophytes could be bisexual, for which reason intragametophytic selfing is theoretically possible. This paper addresses the study of the mating system of tetraploid *Asplenium septentrionale*, comparing the gender determination of the gametophytes, their mortality and sporophyte production among isolated gametophytes and pairs of gametophytes originating from the same and from different parental sporophytes. Holderegger and Schneller (1994) and Schneller and Holderegger (1996) found little genetic variability and a lack of gene flow among Swiss populations of *A. septentrionale*, indicating a predominantly inbreeding mating system.

The comparative analysis of the studies of the genetic composition of populations, like the ones mentioned above, and characteristics of the mating system in Pteridophytes, carried out on laboratory cultures, can lead to a better understanding of evolutionary processes and population dynamics in this group of plants.

Material and Methods

Material studied

A. septentrionale was sampled from three different populations in the centre of the Iberian Peninsula (Tab. 1). Fertile fronds were collected in each population. The fronds were taken to the laboratory in filter paper envelopes, washed, and then pressed between sheets of satin paper. Subsequently, the spores were extracted from each paper.

Experimental development

Spores of three sporophytes were chosen at random from each population and sown in 5.5-cm plastic Petri dishes (Dyer 1979) on mineral agar medium that had been sterilised in an autoclave at 20 atm, 125 °C, for 20 min. With the aim of preventing con-

Tab. 1. Populations of *A. septentrionale* studied.

Localities	Acronyms	Altitude (m.s.n.m)	Coordinates UTM	Collection date	Collector(s)
Madrid. San Lorenzo de El Escorial	ESC	1250	30TVK0192	july 00	E. Pangua, S. Pajarón and C. F. Aragón
Madrid. Manzanares El Real. La Pedriza	PED	1300	30TVL2310	july 00	C. F. Aragón and R. G. Camacho
Guadalajara. Cerca de Cañamares	GUA	1090	30TWL0261	july 00	E. Pangua and S. Pajarón

tamination, 100 $U \cdot ml^{-1}$ of the antifungal agent Nystatin were added to the medium after it had been autoclaved. Spores were cultured in a chamber at 21 °C with 30 μmol m^{-2} s^{-1} photon flux intensity, with a 16 h light: 8 h dark photoperiod. Six weeks later, a randomly selected sample of developed prothallia, still in the presexual phase, was isolated and transplanted to 10 × 10 cm plastic boxes, divided into 24 cells of 2 × 2 cm. The prothallia were arranged in the boxes in order to pursue the following three experimental lines, adopting the methodology of Korpelainen (1996): 1) Single prothallia from the same sporophyte were transplanted to each of the cells in such a way that only intragametophytic (isolated) fertilisation was possible; 2) pairs of prothallia from the same sporophyte were cultured in the same cell, where there was the possibility of intra- and intergametophytic self-fertilisation (pairs); 3) finally, pairs of prothallia from different sporophytes were cultured in the same cell, thus making intragametophytic self-fertilisation and cross-fertilisation possible (unrelated pairs)

In all experiments, 24 gametophytes were analysed from each sporophyte. Since 3 individuals were studied from each of the 3 populations, 648 gametophytes were analysed in total.

Initially, the same mineral agar medium was used to cultivate prothallia, the medium was replaced in week 32 by a compost soil (autoclaved under the same conditions). Although the agar facilitates the handling of gametophytes when they are small, it is reasonable to assume that a medium that more closely approximates to natural conditions would facilitate gametophyte development. Distilled water was added to the medium whenever necessary, in order to favour conditions for fertilisation. To avoid desiccation the Petri dishes and the boxes were sealed with Parafilm (American National Can, Chicago).

The developmental state of all gametophytes (presexual, male, female or bisexual), their mortality and the presence of sporophytes was checked after 6 weeks and then every 4 or 6 weeks until 58 weeks of culture, after which no further changes occurred.

Data analysis

An analysis of variance was performed on the data obtained 10, 36 and 58 weeks after sowing (Zar 1999). These dates correspond, respectively, to the moment when

Tab. 2. Levels of significance for the effects of population and experiment on the percentages of bisexual gametophytes, of dead gametophytes, and of bisexual gametophytes that produced sporophytes, 10, 36 and 58 weeks after sowing. MS, mean squared; df, degrees of freedom; ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Variable	Source of variation		week 10		week 36		week 58	
		d.f.	MS	F	MS	F	MS	F
% Bisexuals	Population	2	3744,582	13,426**	2434,543	21,859**	1639,389	6,941*
	Experiment	2	397,415	1,899 ns	41,430	1,203 ns	88,197	0,867 ns
	Population x Experiment	4	216,261	1,082 ns	102,003	2,963 ns	92,221	0,907 ns
	Specimen	6	278,875	1,396 ns	111,376	2,236 ns	236,181	2,323 ns
	Specimen x Experiment	12	199,744	...	34,417	...	101,680	...
%Deads	Population	2	115,010	1,659 ns	1150,309	13,950**	814,364	9,150**
	Experiment	2	55,747	1,395 ns	38,600	1,288 ns	86,378	0,890 ns
	Population x Experiment	4	33,430	0,836 ns	109,913	3,267 ns	87,017	0,896 ns
	Specimen	6	69,333	1,735 ns	82,439	2,751 ns	89,041	0,917 ns
	Specimen x Experiment	12	39,962	...	29,971	...	97,070	...
% Bisexuals	Population	2	0,000	...	101,747	0,671 ns	82,324	1,175 ns
with sporophytes	Experiment	2	0,000	...	215,090	1,675 ns	2868,403	65,789***
	Population x Experiment	4	0,000	...	96,375	0,750 ns	41,070	0,942 ns
	Specimen	6	0,000	...	151,538	1,180 ns	70,071	1,607 ns
	Specimen x Experiment	12	0,000	...	128,440	...	43,600	...

gametangia are observed for the first time, the appearance of the first sporophytes and the end of the experiments. The *population* of origin (ESC, PED, GUA; Tab. 1) and *experiment* (isolated, pairs from the same sporophyte and pairs from different sporophytes) were taken as fixed factors, and the random factor *individual* was nested within the *population* factor. The effect of the factors on three variables has been considered: percentages of developed bisexual gametophytes, of dead gametophytes and of bisexual gametophytes that form sporophytes. In order to normalize data variables, arcsine-transformations were performed according to Zar (1999) and the analysis were carried out separately for each date. The Tukey test was used to identify groups with homogenous means (significance threshold, $p < 0.05$). All analyses were done with the SPSS statistical program (1999).

Results

The proportions of presexual, male, female, bisexual and dead gametophytes in each of populations ESC, PED and GUA in the separate experiments are respectively illustrated in figures 1, 2 and 3. In the first weeks, bisexual and male gametophytes were detected, the latter becoming bisexual within a short time. Exceptionally, in week 10, the presence of a female gametophyte was detected in all populations studied (Figs. 1A, 2C and 3B-C) that also finally became bisexual. It may be said that, in general, the gametophytes of *A. septentrionale* initially develop antheridia, and subsequently rapidly attain the bisexual condition by the development of archegonia.

By week 18 of culture, practically all cases had reached their maximum percentage of bisexuals (Figs. 1–3).

The results of the analyses of variance are shown in Table 2. The interaction between experiment and population factors was not significant in any case. Neither did the factor of individual have any significant effects on any of the variables.

The percentages of bisexual gametophytes and of dead gametophytes varied among populations, although not between experiments (Tab. 2). The results of the Tukey test (Tab. 3) show in both cases that the population ESC made up a homogeneous group distinct from that comprised by PED and GUA. Individuals from the ESC population developed a smaller proportion of bisexual gametophytes, and were the only ones a proportion of whose gametophytes were still male at the end of the experiments (22–37%; Fig. 1). This is also the population whose gametophytes had the highest mortality rate. It should be mentioned that although there was some contamination produced by fungi and algae in all the boxes, those of this population were the most affected.

In all the cultures, the prothallia gave rise to colonies, derived from a continuous vegetative proliferation. Numerous prothallia of the colony subsequently developed antheridia and archegonia, remaining bisexual.

The presence of sporophytes was first detected simultaneously in all boxes of samples corresponding to week 36 (Fig. 4), at which time there were no significant differences between populations and experiments (Tab. 2). The total sporophyte production by the bisexual gametophytes at the end of the experiment (week 58) was similar in all populations, but differed significantly among experiments (Tab. 2). As may be seen in Table 4, the mean percentage of bisexual gametophytes that formed a sporophyte was higher in the case of isolated gametophytes, with a mean of 62.9% for all populations.

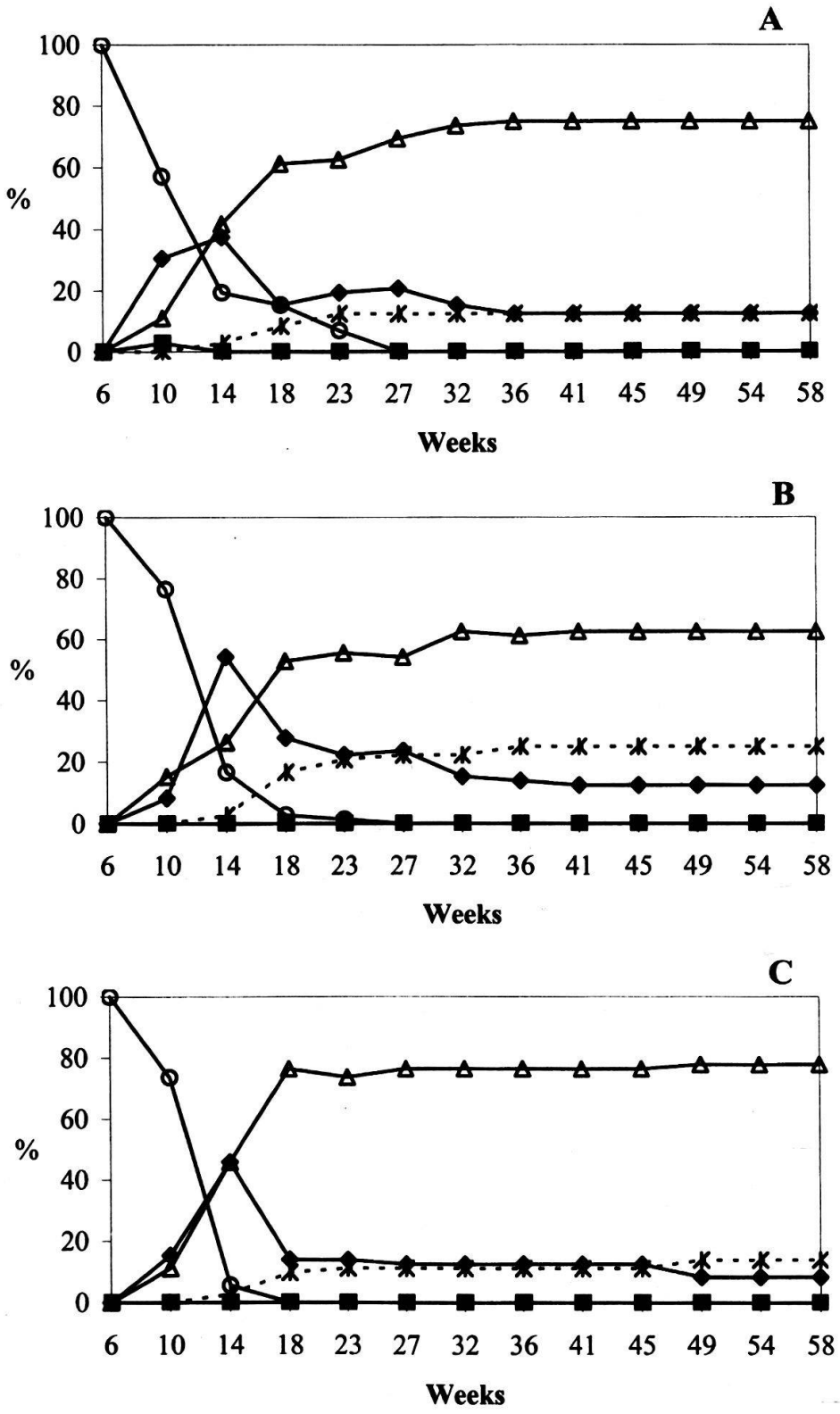

Fig. 1. Percentages obtained over 58 weeks of culture, from presexual gametophytes (○), male (◆), female (■), bisexual (Δ), and dead (×) for population ESC in the experiments with isolated gametophytes (A), pairs of gametophytes from the same sporophyte (B) and pairs of gametophytes from different sporophytes (C).

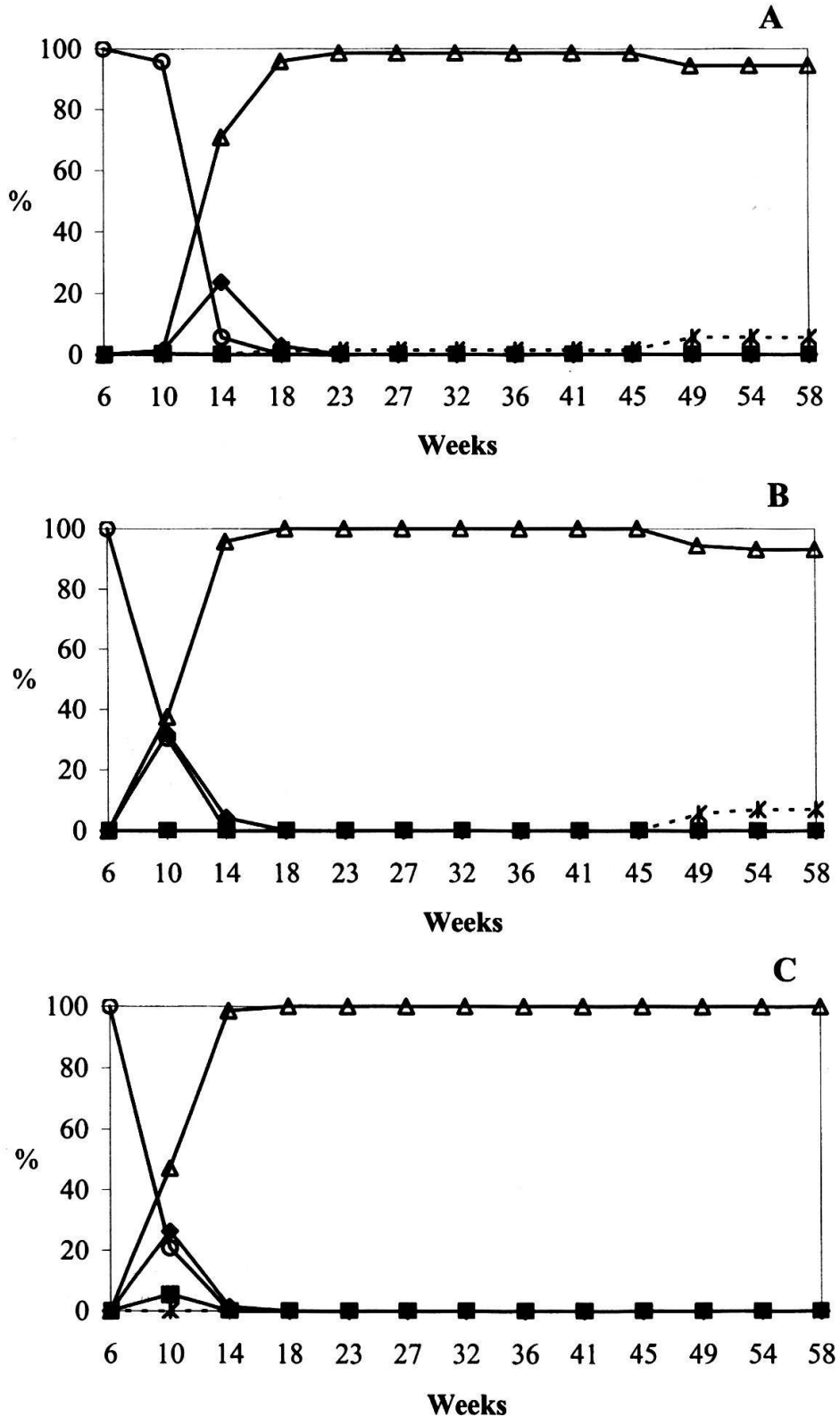

Fig. 2. Percentages obtained over 58 weeks of culture, from presexual gametophytes (○), male (◆), female (■), bisexual (Δ), and dead (×) for population PED in the experiments with isolated gametophytes (A), pairs of gametophytes from the same sporophyte (B) and pairs of gametophytes from different sporophytes (C).

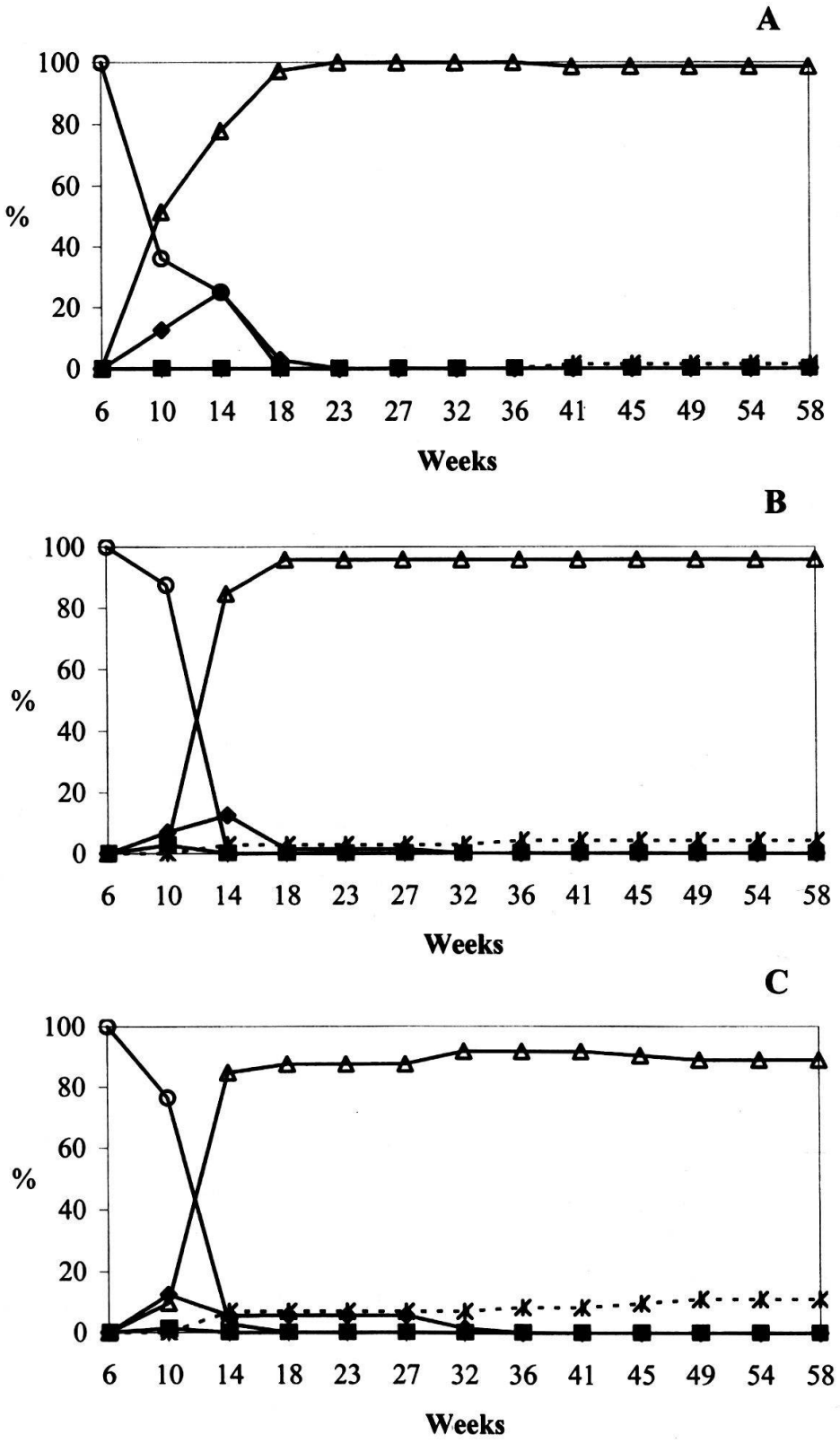

Fig. 3. Percentages obtained over 58 weeks of culture, from presexual gametophytes (○), male (◆), female (■), bisexual (Δ), and dead (×) for population GUA in the experiments with isolated gametophytes (A), pairs of gametophytes from the same sporophyte (B) and pairs of gametophytes from different sporophytes (C).

Tab. 3. Percentages (mean ± standard error) of bisexual gametophytes, of dead gametophytes, and of bisexual gametophytes with sporophytes, 10, 36 and 58 weeks after sowing. Vertical lines group classes with homogeneous means (Tukey test), among which there are no statistically significant differences ($p > 0.05$).

Time (weeks)	Variables					
	% Bisexuals		% Deads		% Bisex. with sporophyte	
10	38,0 ± 7,9	ESC	2,78 ± 1,2	ESC	0	Isolates
	82,4 ± 5,5	GUA	3,2 ± 1,7	GUA	0	Pairs
	88,4 ± 5,8	PED	0	PED	0	Crosses
36	70,8 ± 4,4	ESC	16,21 ± 2,9	ESC	14,7 ± 4,8	Isolates
	95,8 ± 2,2	GUA	4,2 ± 2,2	GUA	5,11 ± 2,2	Pairs
	99,5 ± 0,5	PED	0,5 ± 0,5	PED	6,29 ± 2,5	Crosses
58	71,8 ± 4,4	ESC	17,13 ± 2,5	ESC	62,94 ± 3,2	Isolates
	92,1 ± 2,6	GUA	7,9 ± 4,0	GUA	17,58 ± 4,4	Pairs
	95,8 ± 2,8	PED	4,2 ± 2,8	PED	12,5 ± 2,6	Crosses

In the experiments with pairs of related gametophytes 17.6% formed sporophytes, while in the unrelated group only 12.5% of pairs originated sporophytes.

The Tukey test (Tab. 3) for week 58 showed that the experiments with isolated gametophytes made up a distinct homogeneous group from that made up of related and unrelated pairs.

In each colony of gametophytes, not only the isolated but also those in pairs, more than one sporophyte was produced in different gametophytes of the colony, although always in greater numbers in the isolated prothallia. They were counted as a single sporophyte.

Discussion

The sequence of appearance of gametangia with the initial formation of antheridia, followed by the archegonia, reaching a prolonged bisexual phase, is considered to be an adaptation that favours self-fertilisation (Klelowski 1969, Klekowski and Lloyd 1968). This sequence, in our case, is independent of the gametophytes that are isolated or in pairs, and is common in other pteridophytes, such as *Adiantum capillus-veneris* (Masuyama 1972) and some species of *Athyrium* and *Asplenium* (Masuyama 1975, Pangua et al. 1994).

The patterns of sexual expression may vary between populations (Cousens 1988), for which reason it is unsurprising that the ESC population is slightly different in this respect. The bigametophytic system formed by the presence of male and bisexual gametophytes favours intragametophytic selfing (Klekowski 1969), at least in the relative proportions in which they have appeared. As has already been suggested in the results section, the higher mortality in the ESC population could be due to the contamination that, in large part, affected gametophytes of this population. However, it might also be an effect of interpopulation genetic variability.

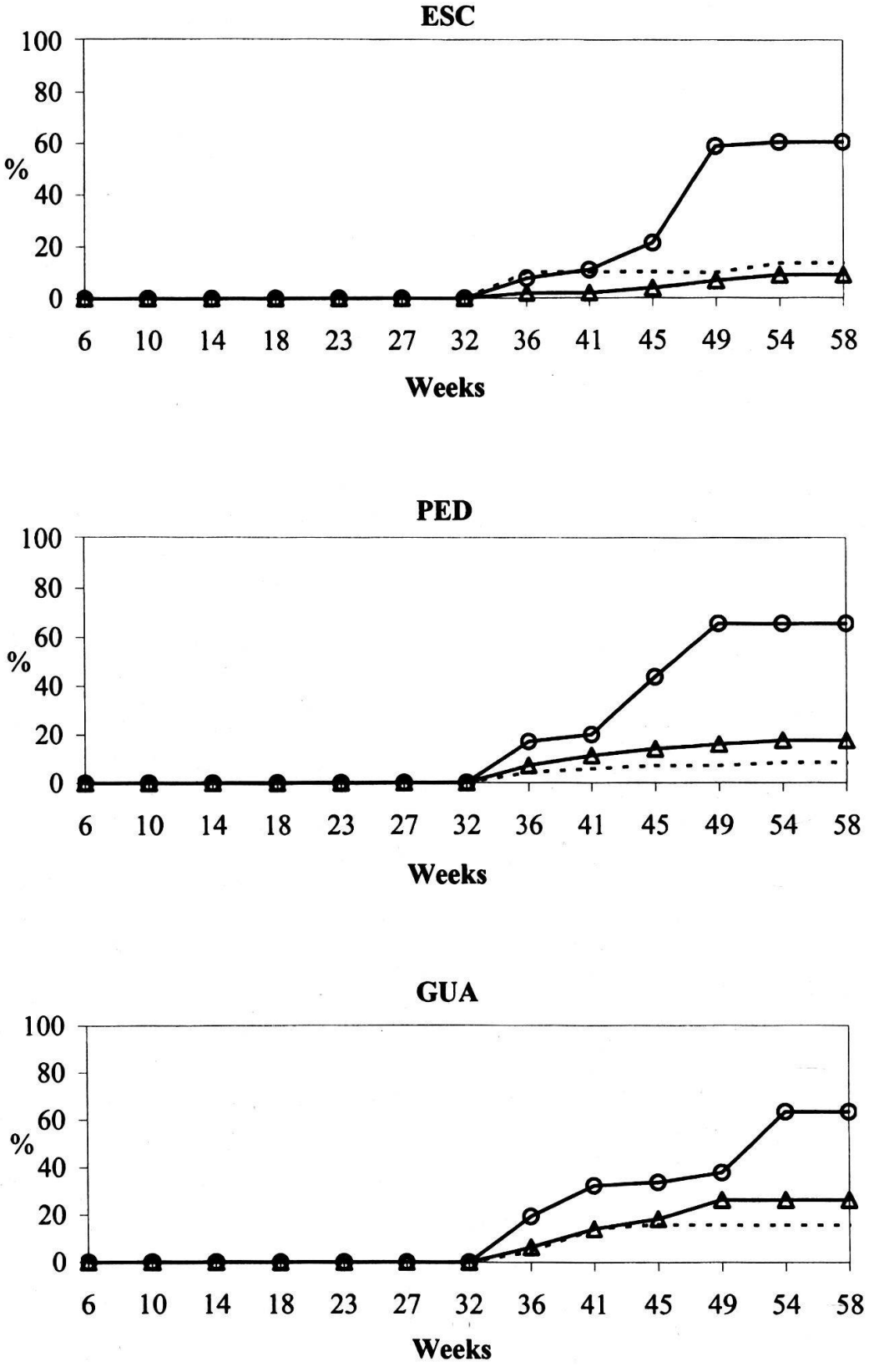

Fig. 4. Percentages of sporophytes from each population throughout the experiments with isolated gametophytes (○), with pairs of gametophytes from the same sporophyte (Δ) and with pairs of gametophytes from different sporophytes (---).

Tab. 4 Mean percentages of bisexual gametophytes that formed sporophytes by the end of the experiments (week 58) in the different populations and experiments. (mean ± standard error).

	Isolated	Pairs	Crossed pairs
ESC	60,1 ± 5,7	8,9 ± 1,5	13,4 ± 7,6
PED	65,3 ± 7,7	17,4 ± 4,2	8,3 ± 2,4
GUA	63,5 ± 4,7	26,4 ± 11,9	15,8 ± 1,9
Mean	62,9 ± 3,2	17,6 ± 4,4	12,5 ± 2,6

The results from the experiments with isolated gametophytes, in which a mean of 62.9% of the bisexual gametophytes formed sporophytes, reveals this taxon's capacity for intragametophytic self-fertilisation. This percentage is lower in the case of related and unrelated pairs.

Since gametophytes of this species have shown themselves to be capable of self-fertilising, the fact that sporophyte production was significantly less in cultures of pairs than in those of isolated gametophytes, the percentages of bisexuals developed being similar from one culture to the next, cannot be explained in terms of the mating system. This same observation has been made in similar studies of other species (Pangua and Vega 1995, Korpelainen 1996, Korpelainen 1997) and could be due to the competition between gametophytes cultured in pairs for space and resources, since both prothallia develop colonies that end up overlapping and whose proliferations are densely arranged. However, this should be proved with other experiments. Korpelainen (1994) mentioned that to estimate genetic load, the comparison between isolated gametophytes and those in pairs can only be used if there are no effects of antheridiogens in the medium. We can discount the possibility that our results from the pair cultures were due to the interaction between gametophytes, since no differences in sexual expression have been noted between the distinct experiments.

It is intriguing that the first fertilisations were observed simultaneously in all boxes. This synchrony of the commencement of sporophyte production could be associated with the change of culture medium effected at the same time as the examination of the gametophytes of the immediately prior date. We cannot be certain about this, but the new medium, with its characteristics closer to those of natural conditions, had an obvious effect on the gametophytes, which took on a more vigorous appearance. The production of more than one sporophyte in each proliferated gametophyte coincides with the results of Cousens (1979) from isolated gametophytes of *Blechnum spicant*. Nevertheless, these did not proliferate in moderately or very dense cultures and, when fertilisation occurred, a single sporophyte was produced in each case. Miller (1968, and references therein) cited cases of polyembrony of up to 15 sporophytes in isolated prothallia bearing adventitious outgrowths. The plates initially set up by us at a moderate density, for subsequent isolation of the gametophytes, maintained their characteristic morphology, which indicates that a low density makes them proliferate and form sex organs to reproduce themselves and to ensure fertilisation when conditions are opportune (Raghavan 1989).

The results concerning the mating system of *A. septentrionale* imply low genetic loads for populations of this taxon, where the production of sporophytes by intragametophytic selfing indicates that lethal recessive genes could not have been accumulated. These characteristics would allow this species to establish new populations rap-

idly from individual spores, preserving a favourable genotype. This is an advantage for colonising places where conditions suitable for reproduction persist only for a short time. It has been shown that dominant species in pioneer environments usually have intragametophytic selfing systems, while species that develop in more stable environments have the tendency to develop intergametophytic systems (Lloyd 1974b).

The low intrapopulation genetic variation of *A. septentrionale* and the existence of a certain variability among isolated populations has been demonstrated by Schneller and Holderegger (1996) and Holderegger and Schneller (1994) in Swiss populations, supposing a intragametophytic selfing system and low genetic loads for this taxon. Other taxa studied, from similar habitats to those of *A. septentrionale*, such as the tetraploids *A. csikii* and *A. trichomanes* subsp. *quadrivalens* (Vogel et al. 1999, Suter et al. 2000), also exhibited the capacity to reproduce by intragametophytic selfing. These results are consistent with the idea that, in general, there is a marked tendency towards selfing in polyploid pteridophytes (Masuyama and Watano 1990), although this form of reproduction is not exclusive to this type of species (Korpelainen 1997).

As Haufler and Soltis (1984) have already demonstrated, the analysis of gametophytes in the laboratory may allow what happens in nature to be predicted. Our laboratory findings concerning the mating system point towards the fact that the genetic structure in the Spanish populations studied probably coincide with that found in the other populations mentioned above.

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Résumé

Asplenium septentrionale subsp. *septentrionale* est une plante autotétraploide dérivée d'*A. septentrionale* subsp. *caucasicum*, qui pousse dans les fissures de roches siliceuses ou décalcifiées. En utilisant des cultures de gamétophytes isolés ou de pairs de gamétophytes d'individus issus du même sporophyte ou pas et dérivés de populations variés de ce taxon, la séquence d'apparition des gamétanges a été établit. Dans tous les cas les anthéridies, formées les premiers, sont suivies d'archégones donnant ainsi des gamétophytes bisexuées sur lesquelles les deux types de gamétanges sont fonctionels pendant une phase prolongée ce qui favorise la fécondation intragamétophytique. La formation de sporophytes sur 62,9% des gamétophytes isolés, comparée à un pourcentage plus bas en pairs ou dans des croissements, démontre la capacité d'autofécondation de ce taxon..

References

Cousens M. I. 1979. Gametophyte ontogeny, sex expression, and genetic load as measures of population divergence in *Blechnum spicant*. Amer. J. Bot. 66: 116–132.

Cousens M. I. 1988. Reproductive strategies of Pteridophytes. In: Lovet and Lovet (eds.) Plant reproductive ecology. Patterns and strategies. Oxford University Press. New York: 307–328.

Dyer A. F. 1979. The culture of fern gametophytes for experimental investigation. In Dyer A. F. (ed.) The experimental biology of ferns. Academic Press, London: 253–305.

Haufler C. H. and Soltis D. E. 1984. Obligate outcrossing in a homosporous fern: Field confirmation of a laboratory prediction. Amer. J. Bot. 71: 878–881.

Holderegger R. and Schneller J. J. 1994. Are small isolated populations of Asplenium septentrionale variable? Biol. J. Linn. Soc. 51: 377–385.
Klekowski E. J. 1969. Reproductive biology of the Pteridophyta. II. Theoretical considerations. Bot. J. Linn. Soc. 62: 347–359.
Klekowski E. J. 1973. Genetic load in Osmunda regalis populations. Amer. J. Bot. 60: 146–154.
Klekowski E. J. and Lloyd R. M. 1968. Reproductive biology of the Pteridophyta. I. General consideration and a study of Onoclea sensibilis L. Bot. J. Linn. Soc. 60: 315–324.
Korpelainen H. 1994. Growth, sex determination and reproduction of Dryopteris filix-mas (L.) Schott gametophytes under varying nutritional conditions. Bot. J. Linn. Soc. 114: 357–366.
Korpelainen H. 1996. Intragametophytic selfing does not reduce reproduction in *Dryopteris filix-max*. Sex Plant Reproduction 9: 117–122.
Korpelainen H. 1997. Comparison of gametophyte growth, sex determination and reproduction in three fern species from the tropics. Nordic J. Bot 17(2): 133–143.
Lloyd R. M. 1974a. Reproductive biology and evolution in the Pteridophyta. Ann. Mo. Bot. Gard. 61: 318–331.
Lloyd R. M. 1974b. Mating systems and genetic load in pioneer and non-pioneer Hawaiian Pteridophyta. Bot. J. Linn. Soc. 69: 23–35.
Lovis J. D. 1964. Autopolyploidy in *Asplenium*. Nature 203: 324–329.
Masuyama S. 1972. The sequence of sex expression in the prothallia of *Adiantum pedatum* L. and *A. capillus-veneris* L. J. Jap. Bot. 47: 97–106.
Masuyama S. 1975. The sequence of the gametangium formation in homosporous fern gametophytes. II. Types and their taxonomic distribution. Sci. Rep. Tokyo Kyoiku Daigaku Sec. B 16: 71–87.
Masuyama S. and Watano Y. 1990. Trends of inbreeding in polyploid Pteridophytes. Plant Species Biology 5: 13–17.
Miller J. H. 1968. Fern gametophytes as experimental material. Bot. Rev. 34: 361–440.
Momose S. 1960. The prothallia of Aspleniaceae (4). J. Jap. Bot. 35 (8): 229–235.
Nogueira I. and Ormonde J. 1986. Asplenium. In: Castroviejo S., Laínz M., López González G., Montserrat P., Muñoz Garmendia F., Paiva J. and Villar L. (eds.) Flora Ibérica. Vol. 1. Real Jardín Botánico, C.S.I.C. Madrid: 90–104.
Pangua E. and Vega B. 1995. Comparative study of the gametophyte development in *Cosentinia* and *Anogramma* (Hemionitidaceae) and *Cheilantes* (Sinopteridaceae). In: Camus J. M., Gibby M. and Johns R. J. (eds.) Pteridology in perspective. Kew, London: 497–508.
Pangua E., Lindsay S. and Dyer A. 1994. Spore germination and gametophyte development in three species of *Asplenium*. Ann. Bot. 73: 587–593.
Raghavan V. 1989. Developmental biology of fern gametophytes. Cambridge University Press, Cambridge.
Schneller J. J. and Holderegger R. 1996. Genetic variation in small, isolated fern populations. J. Veg. Sci. 7 (1): 113–120.
Soltis D. E. and Soltis P. S. 1990. Polyploidy, breeding system, and genetic differentiation in homosporous pteridophytes. In: Soltis D. E. and Soltis P. S. (eds.) Isozymes in plant biology. Dioscorides Press, Portland: 241–258.
Soltis D. E. and Soltis P. S. 1992. The distribution of selfing rates in homosporous ferns. Amer. J. Bot. 79: 97–100.
SPSS. 1999. SPSS para Windows, versión 10.0.6. Chicago: SPSS Inc.
Suter M., Schneller J. J. and Vogel C. 2000. Investigation into the genetic variation, population structure, and breeding system of the fern *Asplenium trichomanes* subsp. *quadrivalens*. Int. J. Plant Sci. 161 (2): 233–244.
Vogel J. C., Rumsey F. J., Rusell S. J., Holmes J. S., Cox C., Bujnoch W., Stark C., Barret J. A. and Gibby M. 1999. Genetic structure, reproductive biology and ecology in isolated populations of *Asplenium csikii* (Aspleniaceae, Pteridophyta). Heredity 83: 604–612.
Zar J. H. 1999. Biostatistical analysis. 4th edn. Prentice Hall, New Jersey.