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Serological estimation of the level of cross-fertilization in the monoecious liverwort *Pellia epiphylla* n = 9

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Abstract

Using protein antigens as markers, antigenic differentiation of progenies obtained from individual sporangia was examined. The experiments were expected to permit estimation of cross-fertilization frequency in the monoecious liverwort species, *Pellia epiphylla*, n = 9. The results obtained indicated segregation into two serological types, i.e. pointed to cross-fertilization, in approximately, 80% progenies. In correlation with electrophoretic studies, employing two peroxidase alleles and two shikimic acid dehydrogenase alleles as markers, the result made possible the establishment of cross-fertilization frequency at approximately 93%. The data may indicate an absence of self-fertilization in this liverwort species and, thus, self-incompatibility. This may be included among the facors responsible for maintenance of genetic variability in populations of this species, in which haplophase is the prevalent phase of its life-cycle.

Key words: *cross-fertilization, monoecious liverworts, antigenic markers, genetic variability*

INTRODUCTION

Because the haploid (gametophyte) phase takes up most of their life cycle, bryophytes are generally regarded as representing organisms of slow evolutionary processes (Gemmel 1950, Stebbins 1950, Anderson 1963, Khanna 1964, Crum 1972). The prevalence of the haploid generation

is thought to restrict variability since mutations are manifest immediately in the phenotype and, being mostly disadvantageous are likely to be eliminated by natural selection. Studies conducted on variation among bryophytes have, however, shown that particular species exhibit quite substantial variability in their physiological, cytological and chemical traits (for review see Szweykowski 1984). One of the factors which undoubtedly affects the level of genetic variability of *Bryophyta* involves the mating system. However, the reproductive strategies of these organisms remain relatively unknown. This pertains in particular to the mating system of monoecious bryophyte species. Since both antheridia and archegonia develop on the same individual, some authors have considered that in such cases only self-fertilization (autogamous gametophytic selfing) takes place (Gemmel 1950, Lewis 1961, Iwatsuki 1972, Stark 1983). Self-fertilization in *Bryophyta* cannot augment genetic variability, since it results from the mating of identical gametes produced by mitosis. Also cross--fertilization, understood as mating between different individuals of identical genotypes (geitonogamous gametophytic selfing), will yield the same result. The latter situation seems to be particularly frequent in *Bryophyta* due to their intense vegetative propagation which results in the formation of colonies of identical genotypes (clones). Anderson and Lemmon (1974) noted that in the monoecious moss species, *Weissia controversa*, approximately 90% of sporangia are formed by cross-fertilization. The authors failed, however, to demonstrate whether the fertilizations took place between the same or different genotypes. Cross-fertilizations were suggested in monoecious bryophyte species by several authors (Crum 1972, Lazarenko and Lesnyak 1972, Anderson and Lemmon 1974, Smith 1979) but the absence of appropriate genetic markers has made it difficult to prove this phenomenon. The most evident proof for the existence of such a mating system is the detection of interspecific moss hybrids with participation of monoecious species (Anderson and Lemmon 1972). Zieliński (1984) applied isoenzymatic markers to demonstrate cross-fertilizations in the monoecious liverwort species, *Pellia epiphylla*, $n = 9$. The markers involved two peroxidase alleles, *PX* 1^a and *PX* 2^a (see also Szweykowski et al. 1981) and, later on, also two alleles of shikimic acid dehydrogenase, *SHI*^{a}, *SHI*^{b} (Zieliński 1986a, b, c). Genetic analysis of progenies obtained from individual sporophytes collected in the field from polymorphic colonies demonstrated segregation of the markers in approximately 39% of the progenies. Hence the sporophytes from which the progenies were obtained must have been heterozygous and arose due to mating between different genotypes. Progenies exhibiting no segregation of the studied markers might have resulted from self-fertilization of from cross-fertilization, but the segregation could not be proven due to the absence of appropriate isoenzymatic markers.

In this study we have attempted to serologically analyze the progenies obtained from individual *Pellia epiphylla*, n = 9, sporangia, which were previously analyzed electrophoretically. The new markers introduced in the form of antigenic traits of proteins were expected to demonstrate additional differentiation, thus allowing more precise estimation of cross-fertilization frequency.

MATERIAL AND METHODS

The samples of *P. epiphylla* were collected from a stand in the Karkonosze Mountains (Zieliński 1984). Sporangia from colonies showing polymorphism with respect to peroxidase and shikimic acid dehydrogenase were collected and spores were sown separately from each sporangium in glass jars with Benecke's agar supplemented with microelements. The culture on agar was grown for 3 months. Subsequently, small thalli, originating from individual spores, were transferred from glass jars to pots filled with peat and the culture was continued in a greenhouse (Zieliński 1984). After a year, 12 thalli were taken at random from each of the 14 cultures and cultured in cuvettes on peat, each thallus in a separate plot, for approximately one year to obtain a sufficient amount of plant material for conducting the serological analysis.

Extraction of antigens was performed in porcelain mortars by grinding the material with quartz sand into a homogeneous mixture according to the following ratio: 1 part of thalli +4 parts (w/w) extraction buffer, pH 7.4, composed of TRIS, H_3BO_4, EDTA and PVP (M. W. 40.000 45 mg 1 cm^{-3} of buffer). Before use, the extraction buffer was supplemented with 2-mercaptoethanol (7 cm^3 per 1 dm^3 of buffer). The homogenate was left for approximately 20 min in a refrigerator and centrifuged. Antigens were analyzed by double immunodiffusion as described by Szweykowski et al. (1981), Prus-Głowacki and Nowak (1982).

Agarose gel (1.5%) (type C, Pharmacia) in veronal sodium-sodium acetate-HCl buffer, pH 6.75, 0.135 M was used as a medium for precipitation. Each well was loaded with 100 mm^3 extract, while grooves were each filled with 900 mm^3 rabbit antiserum against a protein mixture from *Pellia borealis*, n = 18, the natural diploid of *Pellia epiphylla*, n = 9. Antigens for immunizations were extracted from thalli of *P. borealis* n = 18 pooled from several stands to obtain a wide spectrum of antibodies. The extraction procedure corresponded to that used to obtain the samples for analysis. The immunodiffusion patterns obtained were interpreted on the basis of the number of precipitation arcs, their pattern and intensity.

RESULTS

In the majority of progenies from individual sporangia, two types of serological patterns were observed. An immunodiffusion plate with serological types distinguished within a progeny obtained from an individual sporangium is presented in Fig. 1. The observed differences between the distinquished serological types involved the presence of an additional precipitation arc, its absence or differences in staining intensity and in the shape of the precipitation arcs (see Fig. 1).

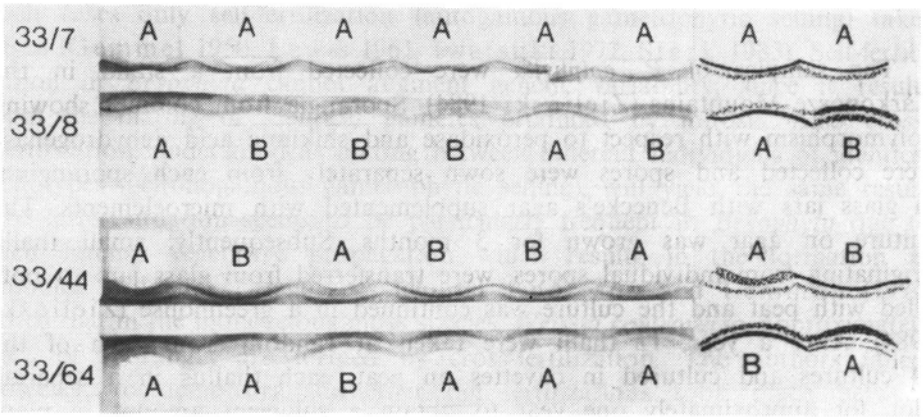

Fig. 1. Example of immunodiffusion plates with serological types (A and B) distinguished within progeny obtained from individual sporangia (33/7 — one type, 33/8, 33/44 and 33/64 — segregation into two serological types)

Only two out of the 14 progenies analyzed, i.e. nos. 33/5 and 33/7, showed no antigenic differentiation. The remaining 12 progenies demonstrated evident differentiation of thalli into the two antigenic patterns. The numerical data on serological differentiation demonstrated within individual progenies are presented in Table 1. The results of serological differentiation within *Pellia epiphylla* progenies inicate that at least 85% of the examined progenies originated from cross-fertilizations.

DISCUSSION

Serological analysis of antigens obtained from thalli representing progenies of individual sporangia revealed that segregation into two serological patterns occurred in the majority of the progenies. The progeny from one sporangium no. 33/5 provided a homogeneous pattern while the result

Table 1

Segregation of serological types observed in progenies of *Pellia epiphylla*

Sporangium	Number of plot (clones)												Ratio of serological types A : B
	1	2	3	4	5	6	7	8	9	10	11	12	
23/40	A^1	B^1	B^1	B^1	A^1	A^1	A^1	A^1	A^1	A^1	–	A^1	8 : 3
26/2	A^2	B^2	B^2	B^2	A^2	A^2	A^2	–	A^2	A^2	A^2	A^2	8 : 3
26/4	A^3	B^3	B^3	A^3	A^3	A^3	A^3	A^3	A^3	A^3	A^3	A^3	10 : 2
26/11	A^4	A^4	B^4	A^4	A^4	B^4	B^4	A^4	A^4	B^4	–	B^4	6 : 5
33/1	–	A^5	B^5	B^5	B^5	B^5	B^5	B^5	A^5	A^5	B^5	B^5	4 : 8
33/2	A^6	B^6	B^6	A^6	A^6	A^6	A^6	B^6	A^6	B^6	B^6	B^6	6 : 6
33/3	A^7	A^7	A^7	A^7	A^7	B^7	A^7	B^7	A^7	A^7	B^7	A^7	9 : 3
33/5	–	A^8	A^8	A^8	A^8	A^8	A^8	A^8	A^8	A^8	A^8	A^8	11 : 0
33/7	A^9	A^9	A^9	A^9	A^9	A^9	A^9	A^9	A^9	A^9	A^9	A^9	12 : 0
33/8	A^{10}		A^{10}	B^{10}	–	–	B^{10}	B^{10}	B^{10}	A^{10}	B^{10}	B^{10}	3 : 6
33/44	A^{11}	A^{11}	B^{11}	A^{11}	B^{11}	B^{11}	A^{11}	A^{11}	–	B^{11}	B^{11}	B^{11}	5 : 6
33/64	A^{12}	A^{12}	A^{12}	B^{12}	A^{12}	B^{12}	B^{12}	B^{12}	A^{12}	B^{12}	B^{12}	B^{12}	5 : 7
48/12	A^{13}	B^{13}	A^{13}	B^{13}	B^{13}	A^{13}	B^{13}	B^{13}	A^{13}	A^{13}	A^{13}	B^{13}	6 : 6
48/13	–	A^{14}	–	A^{14}	B^{14}	A^{14}	A^{14}	–	B^{14}	A^{14}	A^{14}	A^{14}	7 : 2

Table 2

Electrophoretical and serological data concerning ratio of segregation for progenies obtained fron single sporangia of *Pellia epiphylla*, n = 9

Sporangium	Electrophoretic data				Serological data	
	Enzymatic phenotype of progenies (peroxidases)	Segregation ratio	Enzymatic phenotype of progenies (SHI) *	Segregation ratio	Serological types	Ratio of serological types
23/40	$PX1^a$: $PX2^a$	23: 27	$SHI1^a$: $SHI1^b$	59: 15	A^1: B^1	8: 3
26/2	$PX1^a$: $PX2^a$	6: 5	$SHI1^a$	–	A^2: B^2	8: 3
26/4	$PX2^a$	–	$SHI1^a$	–	A^3: B^3	10: 2
26/11	$PX2^a$	–	$SHI1^a$	–	A^4: B^4	6: 5
33/1	$PX1^a$: $PX2^a$	6: 5	$SHI1^a$: $SHI1^b$	13: 38	A^5: B^5	3: 8
33/2	$PX2^a$	–	$SHI1^a$: $SHI1^b$	23: 27	A^6: B^6	6: 6
33/3	$PX2^a$	–	$SHI1^a$: $SHI1^b$	4: 5	A^7: B^7	9: 3
33/5	$PX2^a$	–	$SHI1^b$	–	A^8	–
33/7	$PX2^a$	–	$SHI1^a$: $SHI1^b$	25: 25	A^9	–
33/8	$PX2^a$	–	$SHI1^b$	–	A^{10}: B^{10}	3: 6
33/44	$PX2^a$	–	$SHI1^a$	–	A^{11}: B^{11}	5: 6
33/64	$PX2^a$	–	$SHI1^a$: $SHI1^b$	25: 25	A^{12}: B^{12}	5: 7
48/12	$PX1^a$	–	$SHI1^b$	–	A^{13}: B^{13}	6: 6
48/13	$PX1^a$	–	$SHI1^b$	–	A^{14}: B^{14}	7: 2

* SHI – Shikimic acid dehydrogenase.

of the analysis for the progeny of 33/7 sporangium was doubtful. The segregation ratio of serological patterns noted within a progeny ranged from 6: 6 to 2: 10. The deviation from the expected 1: 1 ratio may reflect non-random sampling and a too small number of analyzed progenies from an individual sporangium. The serological differentiation demonstrated in thalli of the progenies developed from individual sporangia provides evidence of cross-fertilization. On the other hand, the homogeneous pattern obtained for some of the progenies may be interpreted in the following ways:

1. Self-fertilization took place and, therefore, all spores and thalli originating from them were uniform.
2. Non-random sampling resulted in selecting thalli for culture of the same type.
3. Cross-fertilization took place but antigenic differentiation of the progenies could not be demonstrated due to insufficient specificity of the serological technique.

Comparing results obtained in an enzymatic analysis with those yielded by the serological analysis (Table 2), it should be mentioned that among the 14 progenies examined in this study, 7 had already been demonstrated to be polymorphic, by segregation in respect to peroxidase isoenzymes (23/2), isoenzymes of shikimic acid dehydrogenase (33/2, 33/3, 33/7, 33/64)

or the two types of isoenzymes together (33/1, 23/40) (Zieliński 1984, Zieliński 1986a, b, c). In all the cases, except that of the sporangium 33/7, segregation has also been confirmed serologically. Among the remaining 7 progenies failing to segregate in respect to enzymatic markers, 6 demonstrated evident antigenic segregation. Only in the case of sporangium 33/5, did none of the methods applied detect cross-fertilization. Thus, the cross--fertilization frequency in *Pellia epiphylla*, $n = 9$, calculated exclusively on the grounds of the serological analysis, amounts to approximately 85%. Joining serological and electrophoretic data the frequency may be augmented to 93% (Table 2). This seems to point to an absence of self-fertilization in the majority of the studied *Pellia epiphylla* samples and thus, to the possibility of self-incompatibility in this species. This result contradicts those authors who have regarded *Pellia epiphylla* as a representative of an obligatorily self-fertilizing species (Lewis 1961, Iwatsuki 1972). The high proportion of cross-fertilizations observed among *Pellia epiphylla* progenies may explain the relatively high level of genetic variability observed in the species, due to the possibility of recombination.

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Badanie poziomu zapłodnień krzyżowych u obupłciowego gatunku wątrobowca Pellia epiphylla n = 9 za pomocą metod serologicznych

Streszczenie

Za pomocą metod serologicznych badano zróżnicowanie antygenowe potomstwa uzyskanego z pojedynczych zarodni *Pellia epiphylla*. Uzyskane wyniki wskazują na segregację większości potomstwa na dwa typy serologiczne, co pozwala sądzić, że mieliśmy tu do czynienia z zapłodnieniem krzyżowym. Korelując uzyskane wyniki analiz serologicznych z danymi elektroforetycznymi dla alleli peroksydazy i dehydrogenazy kwasu szikimowego, krzyżowe zapłodnienie obserwowane jest w 93% przypadków. Dane te wskazywać mogą na brak samozapłodnień u tego gatunku wątrobowca, a więc na zjawisko samoniezgodności. Mechanizm ten postulowany jest jako jeden z czynników utrzymujących zmienność genetyczną u tego gatunku wątrobowca.