Cross-fertilisation in the monoecious *Pellia borealis*, $n = 18$, and spatial distribution of two peroxidase genotypes.*

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A population of *Pellia borealis*, $n = 18$, from Diabli Skok reservation (north west Poland) is polymorphic with respect to two peroxidase alleles PX 1^b and PX 2^b (63 and 37 per cent respectively), the spatial distributions of which are non-random. Thalli growing in a close neighbourhood exhibit only one of the two mutually excluding alleles, as shown by analysis of 54 samples, thus pointing to intense vegetative reproduction of thalli within individual samples. The clonal structure extends beyond the area of the sample and frequently includes the whole carpet of *P. borealis*. Apart from intense vegetative reproduction, differential selective value of thalli may constitute another factor shaping clonal structure of samples and carpets.

Thirty-eight out of 40 analysed progenies were found to be monomorphic with respect to PX 1^b and PX 2^b alleles. Sporangia from which these progenies were obtained have been collected from monomorphic samples of corresponding PX 1^b and PX 2^b phenotypes. The results are interpreted as reflecting self-fertilisation and contrast with earlier data on high frequency of cross-fertilisation noted in *P. epiphylla*, $n = 9$.

INTRODUCTION

Determination of the relation between different reproduction patterns and genetic composition, as well as degree of variability at the population level, represent the principal goals in population genetics of Bryophyta. Intense vegetative reproduction in Bryophyta is influencing the spatial structure, defined by allele and genotype frequency in area of individual colonies, composing a given population.

Our knowledge of population structure is still insufficient, since for a long time bryologists have possessed no effective method for rapid and unambiguous genotype identification. Observations of a mainly morphological and anatomical nature (Longton, 1974; Schuster, 1981) indicate a level of genetic variability in Bryophyta, but a detailed formulation of the variability in genetic terms has presented difficulties, until the introduction of electrophoretic techniques (Taylor *et al.*, 1970; Krzakowa, 1977; Krzakowa and Szweykowski, 1979).

Studies involving electrophoresis have focused, in particular, on determination of variability level in Bryophyta as free-growing organisms, with a haploid gametophyte. Data obtained in this manner have provided little information on clonal structure of the population, as reflected by genotype composition of individual colonies and by genotype distribution within the region of the whole population. However, various authors have presented different opinions as to the meaning of the term "population" as well as to the ways in which it may be studied and in which results may be interpreted. For example, in studies of Krzakowa (1977) and Krzakowa and Szweykowski (1979) a *Plagiochila* population was represented by a single sample and results based on analysis of a large number of thalli of the sample were supposed to reflect genetic structure of the population. Yamazaki (1981) studying *Conocephalum conicum* from Japan accepted continuous distributions along different streams as single populations, and sampled single thalli from them to eliminate the chance of repeated sampling of the same clone. Results obtained in this manner, however, give a poor indication of intensity of vegetative propagation in regions of a colony and a population. The first of the methods mentioned, even if it provides

* Genetic variation and evolution of the liverwort genus *Pellia*, with special reference to central European territory. II.

accurate information on genotype composition of colonies, yields results which pertain to a single colony, and could only doubtfully be extended to the whole population. The second method yields relatively representative data for the whole population but pays no attention to genetic composition of colonies. Even if Yamazaki's approach to estimation of *C. conicum* variability at the population level seems basically adequate, the problem of what is a population in liverworts and, therefore, how such a population should be examined has been raised by Szweykowski *et al.* (1981a). Based on rare variant distribution, the authors concluded that a continuous region of *C. conicum* along a stream corresponds to a Mendelian population in higher plants. In studies of Cummins and Wyatt (1981), Szweykowski *et al.* (1981a, b), Zieliński (1984a), colonies have been shown to exhibit a single or few electrophoretic phenotypes and the same phenotypes can be encountered also in other sites within the region of the population.

To solve the question of whether individual colonies represent clones which have arisen by vegetative reproduction of individual genotypes, and whether other factors shape spatial distribution of genotypes in a population, one should first of all determine distribution of genetic markers in colonies and within a population as a whole. In this study two peroxidase phenotypes have been studied. The distribution of these phenotypes has been examined in a population of *Pellia borealis*, $n = 18$, from Diabli Skok reservation. That population was chosen because it was polymorphic in respect to the peroxidase phenotypes.

The markers used in the present paper have been described in *Pellia epiphylla*, $n = 9$ and in *P. borealis*, $n = 18$ by Szweykowski *et al.* (1981b) and Zieliński *et al.* (1985). The phenotypes are stable in culture and yield the same patterns as those for material collected directly from the field (Zieliński, 1985a). The markers helped to provide the first information on clonal structure of *P. epiphylla* and to demonstrate cross-fertilisation in the taxon. This provided the basis for interpreting the phenotypes as two alleles of the same locus (Zieliński, 1984a).

In this paper we have aimed to determine the spatial distribution of the peroxidase markers within individual colonies and in the whole population and the frequencies of the markers, defining effects of vegetative and generative reproduction on their distribution. We also make a genetic analysis of the PX 1^b and PX 2^b markers in order to establish whether cross-fertilisation exists in the diploid and bisexual *Pellia* taxon and to relate

clonal structure to frequencies of self- and cross-fertilisation.

MATERIAL AND METHODS

Studies were performed on a population of *Pellia borealis*, $n = 18$, from Diabli Skok reservation, near Wałcz (north west Poland), localised on banks of a stream, with the length of approximately 500 m. In Autumn, 1980, a random sample of the population was taken, represented by 21 samples, each of approximately 10 cm in diameter (Transect I). From each sample, five thalli were taken at random to establish separate clones. The clones were analysed in respect to PX 1^b and PX 2^b phenotypes and in respect to 13 other enzymes (see Zieliński, 1985, in press). As only peroxidase showed two electrophoretic phenotypes in the population (PX 1^b, PX 2^b), the subsequent studies were centred mainly on accurate description of the two peroxidase phenotypes distributions at the level of a sample within a population. The rest of enzymes studied were monomorphic.

In the analysed population, *P. borealis* formed rather extensive carpets at bases of tree trunks. The non-random manifestation of PX 1^b and PX 2^b phenotypes within a sample (demonstrated by analysis of Transect I) may encompass the whole region of the carpets. Therefore during the subsequent collection (24 March, 1983, Transect II) several samples were taken from each carpet. In this way 33 samples were collected from 12 carpets. For each sample, six to ten thalli were analysed. This provided information on distribution of PX 1^b and PX 2^b markers within individual samples as well within each of 12 distinguished carpets, localised within the area of the whole population.

Sporangia were collected from 14 samples originating from 10 carpets. Spores were isolated separately from each sporangium, placed on agar medium and cultured according to the technique described earlier by Zieliński (1984a). After 1.5 years, 40 progenies which had grown up from the spores were analysed in respect to PX 1^b and PX 2^b markers. Segregation in the progeny would indicate that cross-fertilization took place and, taking into account spatial distribution of thalli carrying the markers, would provide information on effectiveness of cross-fertilisation as affected by distance between the thalli.

Electrophoresis and peroxidase staining were performed according the technique described by Szweykowski *et al.* (1981b).

RESULTS

Frequency of thalli of PX 1^b and PX 2^b phenotypes, and spatial position of the phenotypes, were examined in the *Pellia borealis* population by analysing 5 thalli randomly collected from each of 21 samples (Transect I). Among 21 analysed samples, 20 proved monomorphic, carrying either PX 1^b (13 samples) or PX 2^b (7 samples) phenotypes. In a single sample the two phenotypes manifested together (sample No. 19: one PX 1^b thallus and four PX 2^b thalli, see table 1). Out of a total of 105 analysed thalli, PX 1^b phenotype was demonstrated in 66 thalli (63 per cent) and PX 2^b phenotype in 39 thalli (37 per cent).

Both PX 1^b and PX 2^b types formed larger groups with numerous thalli exhibiting the same phenotype. Among 21 analysed samples, the PX 1^b phenotype was demonstrated in five adjoining samples (samples 4 to 8) and PX 2^b phenotype in the following seven adjoining samples (samples 12 to 18). Carpets with thalli PX 1^b and PX 2^b phenotypes occur throughout the area studied (see table 1).

Distribution of thalli with PX 1^b and PX 2^b phenotypes was examined in 12 separate carpets in the area of the whole population (Transect II). The carpets were divided into three size categories. Depending upon their size, from each of them 5-6, 3-4 or 1-2 samples were collected, each 10 cm in diameter (table 2). Again, individual samples were mostly monomorphic: among 33 samples 24 carried exclusively PX 1^b phenotype and 7 exclusively PX 2^b phenotype. Two samples, 6c and 8a (table 2) were polymorphic and contained, respectively, five PX 1^b and three PX 2^b thalli and seven PX 1^b and one PX 2^b thallus. Analysis of samples collected from the separate carpets shows that these aggregations, which were usually large, were mostly monomorphic. As in Transect I, the PX 1^b phenotype was most frequent: it was noted in nine out of 12 analysed carpets while PX 2^b phenotype was encountered in five of the carpets.

Phenotypes of nine randomly chosen thalli were determined for each of 40 analysed progenies (table 3). Thirty-four progenies were found to carry

PX 1^b phenotype, four progenies exhibited PX 2^b phenotype and two were polymorphic and segregated in respect to PX 1^b and PX 2^b markers at a ratio close to 1:1. A complete correlation was evident between phenotypes of samples from which sporangia were collected and phenotypes of the progeny. Each of 34 progenies of PX 1^b phenotype was developed from sporangia collected in samples of the same phenotype and the identical result was obtained for progenies and the parental samples of PX 2^b phenotypes (see table 1 and table 3). Also in polymorphic carpets progeny phenotype corresponded to the phenotype of samples from which sporangia were collected (e.g., 11 progenies of the sample No. 6b).

The two segregating progenies, i.e. 6c and 8a (table 3) were developed from sporangia collected from polymorphic samples and one of the two analysed sporangia collected from the sample 8a was homozygous and exhibited PX 1^b phenotype.

DISCUSSION

The studies conducted have demonstrated clearly that distribution of thalli of PX 1^b and PX 2^b phenotypes in *P. borealis* is non-random. Thalli growing in close together have the same marker. Among 54 samples analysed only 3 samples have been polymorphic. Development of the same phenotype in neighbouring thalli may indicate that the thalli have arisen by vegetative reproduction and constitute, most probably, individual clones.

Analysis of larger *P. borealis* carpets has shown that cloning in the taxon is not restricted to 10 or several tens of neighbouring thalli within the sample region but extends over a larger area, frequently consisting of all thalli on individual tree trunks. Such a situation has been observed in nine out of 12 separately analysed carpets. Even the largest of them contained only one of the two mutually excluding peroxidase phenotypes.

Detection of three polymorphic *P. borealis* carpets has allowed the distribution of thalli within them to be studied. Each of the three samples collected from polymorphic carpet No. 1 was monomorphic, which proves that when both

Table 1 Spatial distribution of *Pellia borealis* thalli of PX 1^b (○) and PX 2^b (×) phenotypes in Diabli Skok population (Transect I). Based on analysis of 5 thalli from each of 21 collected samples

○ × ○ × × × × × ○ ○ × ○ ○ ○ ○ ○ ○ ○ × ○ ○ ○

PX 1^b = 13 samples, PX 2^b = 7 samples, PX 1^b + PX 2^b = 1 sample

Table 2 PX 1^b and PX 2^b phenotype distribution within larger separated groups (carpets) of *Pelia borealis* (based on analysis of 1 to 6 samples, depending upon size of group, Transect II)

Carpet No.	Samples	Number of examined thalli	Thalli phenotypes			
			PX 1 ^b	PX 2 ^b	PX 1 ^b +	PX 2 ^b
1*	a	7	7	-	-	-
	b	9	-	9	-	-
	c	10	10	-	-	-
2	a	8	8	-	-	-
	b	9	9	-	-	-
	c	7	7	-	-	-
	d	6	6	-	-	-
	e	6	6	-	-	-
	f	6	6	-	-	-
3	a	10	-	10	-	-
4	a	5	-	5	-	-
	b	7	-	7	-	-
5	a	7	-	7	-	-
	b	8	-	8	-	-
6*	a	8	8	-	-	-
	b	8	8	-	-	-
	c†	8	-	-	5	3
7	a	6	6	-	-	-
	b	7	7	-	-	-
	c	8	8	-	-	-
	d	7	7	-	-	-
8*	a†	8	-	-	7	1
	b	7	7	-	-	-
	c	7	7	-	-	-
	d	8	-	8	-	-
9	a	8	8	-	-	-
10	a	7	7	-	-	-
11	a	7	7	-	-	-
	b	7	7	-	-	-
	c	7	7	-	-	-
	d	8	8	-	-	-
	e	8	8	-	-	-
12	a	8	8	-	-	-
12	33		24	7	2	-

* Polymorphic carpet. † Polymorphic sample.

phenotypes were present, thalli carrying each of the phenotypes form large homogenous units and show no tendency to mixing. A similar situation occurred in carpet No. 8 in samples b, c and d while sample 8a was taken from the region of direct contact of the phenotypes.

The absence of a tendency for mixed growth may result from selective differences between phenotypes. It is known that peroxidase isoenzymes play a significant role in growth and development (Scandalios, 1974) so that phenotypes may have different adaptation value, differing, for example, in growth rate. This is partially corroborated by the fact that PX 1^b progenies were analysed first since they first grew faster in culture.

The monomorphic structure of individual samples indicates also that sexual reproduction and, linked to it, a vast production of spores of PX 1^b and PX 2^b phenotypes, do not contribute to increasing heterogeneity of the samples and of large *P. borealis* aggregations. Spores produced within a single colony probably have only a restricted chance of germinating within the area of a colony due to competition from the thalli already growing in compact aggregations.

Segregation analysis performed on 40 progenies has demonstrated that two progenies have segregated in respect to PX 1^b and PX 2^b phenotypes thus indicating that their sporangia were heterozygous and arose by cross-fertilisation.

Table 3 Analysis of progeny thalli obtained from spores isolated from individual sporangia with respect to PX 1^b and PX 2^b markers (based on analysis of 9 thalli in each progeny)

Site of sporangia collection		Number of separate progenies	Number of analysed progenies (n thalli)		
Carpet No.	Sample		PX 1 ^b	PX 2 ^b	PX 1 ^b :PX 2 [*]
2	a	2	1 (9)	—	—
	b	2	2 (2×9)	—	—
	c	4	3 (3×9)	—	—
4	b	2	—	1 (9)	—
5	a	1	—	1 (9)	—
6*	b	12	11 (11×9)	—	—
	c	4	1 (9)	—	1 (10:10)
7	c	2	2 (2×9)	—	—
8*	a	3	2 (2×9)	—	1 (5:4)
	d	2	—	2 (2×9)	—
9	a	3	3 (3×9)	—	—
10	a	2	2 (2×9)	—	—
11	a	3	3 (3×9)	—	—
12	a	6	4 (4×9)	—	—
10	14	48	34	4	2

* Segregation of PX 1^b and PX 2^b markers in progeny thalli obtained from individual sporangium

Cross-fertilisation therefore does take place in *P. borealis*, as has been noted earlier in *P. epiphylla* (Zieliński, 1984a). It is worth noting that frequency of cross-fertilisation is manyfold lower in *P. borealis* (5 per cent) than that observed in *P. epiphylla* (Zieliński, 1984a; Zieliński, 1985b, c, d, in press).

Methodological differences between the two studies, however, make it impossible to relate this difference to the haploid status of *P. epiphylla* as compared with the autodiploid *P. borealis* (Zieliński, 1984b).

Samples, which in *P. borealis* populations from Diabli Skok are generally monomorphic, produce vast sporangia containing mature and well germinating spores. If the samples represent in fact individual clones, as indicating by the analysis, this suggests that generative reproduction in the population usually involves self-fertilisation. In *P. borealis* as a whole generative reproduction results equally well from self- or cross-fertilisation and the actual fertilisation mechanism reflects the genotype of the nearest thallus. In other *P. borealis* populations, in which distribution of thalli with divergent genotypes is of a more random type, more frequent cross-fertilisations may occur.

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