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ORIGINAL RESEARCH PAPER

Genetic diversity of natural psammophilous populations of *Hypogymnia physodes* (L.) Nyl. on Polish seacoast dunes

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Abstract

Hypogymnia physodes is a lichenized fungus of the family Parmeliaceae. The aim of this study was to compare the level of genetic diversity in eight psammophilous and three epiphytic populations of this species from the Baltic coast in Poland, based on randomly amplified polymorphic DNA (RAPD) markers. In the reactions with nine primers, 153 fragments were obtained, of which 133 were polymorphic. In one reaction, from 0 (for lich2 primer) to 55 (for C02 primer) amplicons were obtained. A Dice's genetic similarity index matrix was constructed based on the results of RAPD marker polymorphism examination. The values of similarity indices ranged from 0.00 to 0.73. Results of this study confirm the separateness of all three epiphytic populations from those found on sand dunes (100% support, UPGMA/1000 trees).

Keywords

genetic polymorphism; interpopulation variability; randomly amplified polymorphic DNA; RAPD; *Hypogymnia physodes*; sand dunes

Introduction

Hypogymnia physodes (L.) Nyl. is an epiphytic species of lichenized fungi of the family Parmeliaceae. This taxon also occurs, although less often, on bedrock, mosses, or soil. *Hypogymnia physodes* produces numerous soredia and reproduces mainly vegetatively. Recent molecular studies have shown that genetic diversity in the lichens reproducing vegetatively is high and not lower than in the lichens that reproduce sexually [1–3]. Hypothetically, the preference of lichens for one type of substrate can be reflected in their genetic constitution. Using molecular markers, Mattsson et al. [4] have distinguished among populations of *H. physodes*, growing on different types of trees, the haplotypes that more than others prefer a specific type of substrate.

The primary objective of this study was to evaluate the interpopulation variability of epigeic populations of *H. physodes* growing on the sand dunes of the Polish seacoast and their preference for substrate. The genetic variability of *H. physodes* was studied using randomly amplified polymorphic DNA (RAPD) analyses, although RAPD analyses have been still rarely used in lichenized fungi [5–7], possibly due to their low reproducibility.

Material and methods

Lichen material

Samples of *Hypogymnia physodes* were collected on the sand dunes of the Polish seacoast (Fig. 1) from 11 sites in July 2014. The analysis was performed on dried and cleaned material.

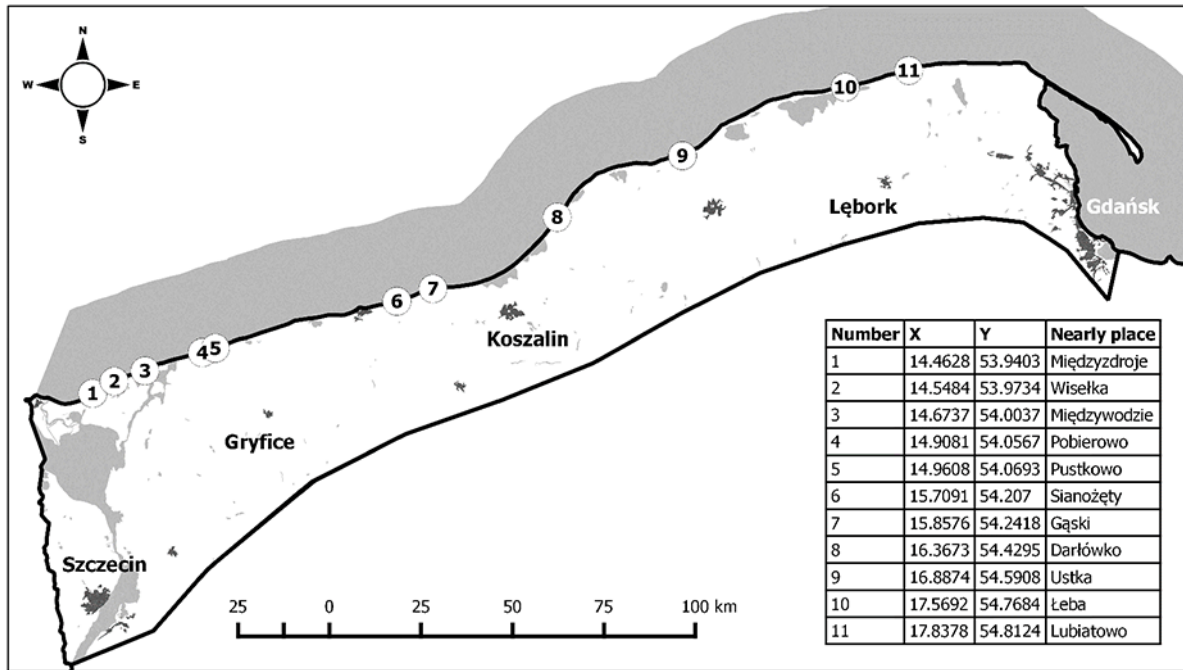


Fig. 1 Study area and location of sampled Populations 1–11 of *Hypogymnia physodes*.

DNA extraction and RAPD analysis

Genomic DNA from each population was isolated using the DNeasy Plant Mini Kit (Qiagen, USA). RAPD analysis was then carried out according to the modified method of Williams et al. [8]. Amplification reactions were carried out in 25 μ L final volume of reaction mixture containing: 50 ng DNA, 1 \times Green GoTaq Reaction Buffer (Promega, USA), 0.2 mM dNTPs (Fermentas, Lithuania), 0.28 mM MgCl₂, 5 mM primers, 0.04 U GoTaq DNA Polymerase (Promega). The thermal profile of the reaction was determined experimentally and included initial DNA denaturation at 95°C for 11 min, 40 cycles of DNA denaturation at 93°C for 30 s, primer annealing at 37°C for 40 s, DNA chain elongation at 72°C for 80 s, and final elongation at 72°C for 5 min. Amplification was carried out twice in an MJ Mini Gradient Thermal Cycler (Bio-Rad, USA) on two samples of DNA from each genotype.

The resulting amplification products were separated on 2.0% agarose gel with ethidium bromide (5 μ g/mL; Sigma-Aldrich, USA).

Data analysis

To visualize, document, and analyze the results obtained, a set of Gel Doc XR and Quantity One 4.6.5 software (Bio-Rad) were used. In the populations, we assumed the existence of Hardy–Weinberg equilibrium. The genetic similarity of the studied populations of *Hypogymnia physodes* was also determined using similarity index, according to Dice's formula (1945), as described by Nei and Li [9]. The genetic similarity matrix was used to construct the UPGMA dendrogram using the FreeTree and TreeView 1.6.6 software [10,11].

Results

This study examined the variation of 11 populations represented by 45 specimens on the basis of 133 loci, i.e., RAPD bands, in which a model of total dominance was assumed. No band was defined as a recessive allele and, on this ground, the probable percentage of recessive alleles in the population was calculated on the basis of Bayesian method that estimates the allele frequency based on the diversity of RAPD fragments, separately in all the analyzed loci in each population. The total number of analyzed PCR products was 153 and included polymorphic products whose size ranged from 155 to 1500 base pairs (Tab. 1, Fig. 2). The number of the polymorphic products amplified by one (individual) primer ranged from 0 in lich2 to 55 in C02. The assay effectiveness index (effective multiplex ratio; EMR) was also determined, as a ratio of the number of polymorphic products to the number of applied primers, which amounted to 14.78.

The highest similarity values were recorded for population from Gąski: 73% similarity to the population from Łeba and 70% similarity to the population from Pobierowo (Tab. 2). The analyses showed the lowest genetic similarity for populations from Wiselka and Międzywodzie versus the populations from Pobierowo and Darłówko (Tab. 2). Overall, the lowest genetic similarity (0–45%) was characteristic for the *Hypogymnia physodes* population from Międzywodzie.

Tab. 1 Primers employed with their sequence, the number of RAPD markers obtained, as well as the number and percentage (P) of polymorphic markers for each primer.

Primer name	Sequence 5'-3'	Total No. of products	No. of polymorphic products	P (%)
C02	GTGAGGCGTC	55	55	100
D03	GTCGCCGTCA	23	23	100
D08	GTGTGCCCCA	24	23	96
E07	AGATGCAGCC	12	9	75
lich2	AACGCGCAAC	6	0	0
lich3	GTAGACCCGT	8	3	37
lich4	AAGAGCCCGT	13	12	92
lich5	CCCGTCAGCA	10	6	60
OPA 18	AGGTGACCCGT	2	2	100
Total		153	133	

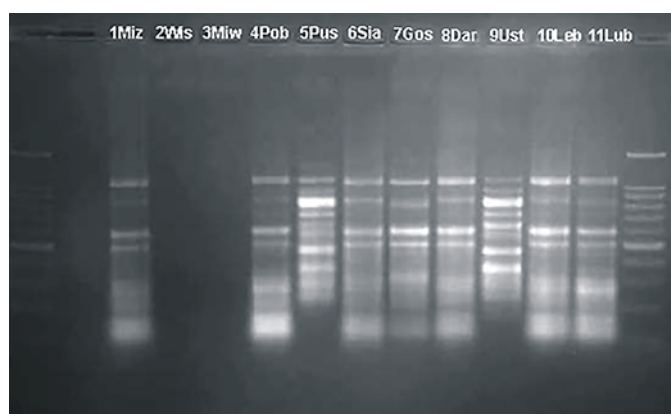


Fig. 2 Agarose gel electrophoresis of PCR product from DNA of *Hypogymnia physodes* obtained with primer C02.

Dendrogram (Fig. 3) revealed two separate clusters: the first group is formed by the lichen populations from Międzyzdroje, Międzywodzie, and Wiselka, found on the bark of pine trees, whereas the second one includes the other analyzed populations of *H. physodes*, occurring on sand. This is confirmed by clade stability tests, with the use of bootstrap resampling methods.

Discussion

The study of Molnár and Farkas [12], in which the five loci sequences were analyzed, found no significant genetic diversity of the *H. physodes* population. This is also confirmed by studies by Mattsson et al. [4].

Tab. 2 Dice's similarity matrix of *Hypogymnia physodes* populations.

Population	1Miz	2Wis	3Miw	4Pob	5Pus	6Sia	7Gas	8Dar	9Ust	10Leb
2Wis	0.48									
3Miw	0.64	0.43								
4Pob	0.28	0.00	0.00							
5Pus	0.58	0.32	0.39	0.28						
6Sia	0.50	0.20	0.36	0.62	0.35					
7Gas	0.63	0.46	0.45	0.48	0.70	0.55				
8Dar	0.41	0.06	0.08	0.66	0.53	0.54	0.51			
9Ust	0.44	0.23	0.30	0.40	0.60	0.58	0.54	0.46		
10Leb	0.58	0.35	0.32	0.48	0.62	0.62	0.73	0.64	0.66	
11Lub	0.56	0.33	0.36	0.62	0.41	0.40	0.68	0.45	0.25	0.48

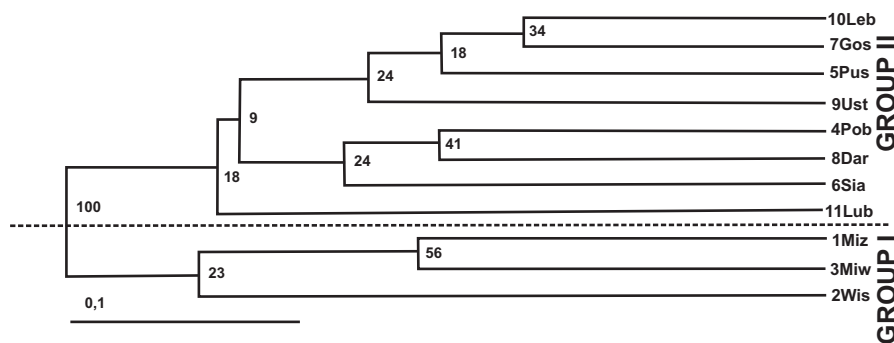


Fig. 3 UPGMA dendrogram of *Hypogymnia physodes* populations based on RAPD markers.

However, our analyzes showed a high population differentiation depending on the presence on the bark or on the dunes. As a result of the reactions being performed, 87% of the products were polymorphic, which indicates a high genetic diversity of the analyzed populations of *Hypogymnia physodes*. Among the lichen populations growing on tree bark, forming the first group, the highest genetic similarity was observed between individuals from Międzywodzie and Międzyzdroje (64%); a lower genetic similarity was found between other populations, not exceeding 50% (Tab. 2). Such a high genetic diversity between local populations of lichens has been already described [2,13]. Moreover, another studies have shown even higher intraspecific genetic diversity than noted in our research, e.g., in the lichen species *Lobothallia alphoplaca* and *L. radiosa* [14] or in the fungus *Pisolithus tinctorius* [15]. The reason of such high genetic may be the habitat whose isolation reduces the flow of genes between populations [16–18]. The high variation is an important trait because it plays a role under environmental changes, determining the survival of organisms [14,19,20].

The evaluation of two analyzed groups of *H. physodes*, the first growing on the bark of pine trees and the second on the sand, confirms the hypothesis that lichens are sensitive to substrate features [21]. It is believed that some genotypes are closely related to a preferential substrate, which can be associated with the process of colony formation [22]. The fact that the type of substrate can affect lichen variation is confirmed by other studies of *H. physodes*, in which an intraspecific diversity resulting from different amounts of nutrients in the substrate has been shown [4]. A high diversity, as in *H. physodes*, between populations preferring different substrate was also observed in *Xanthoria parietina* [17]. Sequence analysis of the intergenic spacer (IGS) and internal transcribed spacer (ITS) sequences revealed significant differentiation between seven populations

Xanthoria parietina in different habitat (on the bark and rock) but, in contrast to our results, it was not high between the studied populations within the same habitat type [17]. Results reported by Gaya et al. [23] show that genetic diversity linked with habitat preferences of a given species is not always related to a single factor. It may result from a random combination of many biotic and abiotic factors.

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