Original Research

Morphological Variability of *Cardaminopsis halleri* (L.) HAYEK Populations from Areas Differing in Anthropopressure Level

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Abstract

Twelve morphometric variables of *Cardaminopsis halleri* individuals from four sites in Upper Silesia province were analyzed to establish the pattern of interpopulational relations. Two hundred plants were collected from two very polluted areas (near zinc-lead and zinc smelters) as well as from two other sites in unpolluted areas within the same bioclimatic region. Stem size, dimensions of leaves and rosettes, as well as flower and seed numbers and individual dry weights were studied using standard biometric methods. Cluster analysis indicated overall differences among populations. Those four populations are different with respect to all variables. Discriminant function analysis confirmed seed number as the most important variable in the evaluation of interpopulational variability.

Keywords: *Cardaminopsis halleri* (L.) HAYEK, morphotypes, PCA, discriminant analysis, zinc smelter

Introduction

Changes in the environment are prompted by human activity connected with occurrence of natural mineral resources. The increase of anthropopressure causes an upsurge in heavy metal contamination [1, 2].

The answer of the plant population to heavy metal pollution is very different [3-5]. Some plants are characterized by natural resistance or tolerance to these extreme conditions, in which they can grow and reproduce [6-9]. The species termed hyperaccumulators [10] is an amazing evolutionary phenomenon in this group. They accumulate over 1% of metal in dry weight [11, 8]. One of the best known Zn and Cd hyperacumulators is *Cardaminopsis halleri* (L.) HAYEK [7, 9, 12-14]. It is also a so-called pseudometallophyte, which means that it grows in both polluted and unpolluted areas [15]. *Cardaminopsis halleri* is an objective of investigations connected with hyperacumulation mechanisms [3, 14, 16, 17], and genetic and molecular systematic problems [11, 18-21], but it is not well known for its morphological variability. Some attempts to explain observed variability have been made by Fiałkiewicz and Rostański [22].

They have claimed that high heavy metal pollution strongly influences the general appearance of this species.

In this paper we try to:

(1) explain morphological variability of *Cardaminopsis halleri* as an adaptation to habitats differing in the level of anthropopressure,
(2) specify traits differentiating investigated populations, and
(3) determine morphological aspects of *Cardaminopsis halleri* life strategy.

Materials and Methods

The Species

Cardaminopsis halleri (*Arabidopsis halleri)* [21] is a self-compatible, insect-pollinated, perennial herb that forms rosettes and stolons which spread to <1m distance

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[8]. Stems are decumbent, simple or branched above. Petiole of basal leaves are 1-5 cm long; leaf blade is orbicular or broadly ovate, 1-9 cm long and 0.5-2.5 cm wide, marginally pinnatifid or lyrate-pinnatifid. Siliques are linear, flattened, torulose, 0.9-2.5 cm long, 0.6-1 cm wide [11, 23]. It flowers and bears fruit from April to August. The chromosome number equals 2n = 16 [11, 18, 24]. It is a mountain plant, occurs in Poland in the Carpathians and the Sudeten, in the adjacent areas of Upper and Lower Silesia as well the Sandomierz Lowlands [24-26]. Its occurrence in the Silesian Uplands is probably connected with the presence of calamine soils [9, 22].

Study Site and Sampling

The individuals of *Cardaminopsis halleri* were sampled in June of 2004 from four sites in the province of Silesia in southern Poland. The first population was located in a zinc-lead dump (DW), the second one was in the vicinity of Zinc Smelter "Miasteczko Śląskie" near Tarnowskie Góry (DM), the third population was in the deciduous forest, in the "Uroczysko Buczyna" Reserve near Chorzów city (UB), and the last one in mixed forests of Bibiela, five kilometers near Miasteczko Śląskie city (LB). Populations from the two last areas were treated as a control, unpolluted group.

The zinc-lead dump can be observed in the northern section of Rów Wełnowiecki, east of the "Silesia" steelworks; the site is situated between two townships: Katowice and Siemianowice (49° 04'N, 18° 55'E). The post--flotation sludge-silt waste tip (33m high) is composed of waste originating from complex zinc and lead ore enrichment processes, comprising dolomites, clays and extremely toxic silts (Zn 1078 mg/kg, Pb 1331mg/kg, Cd 9 mg/kg d.w.soil) [27].

Cardaminopsis halleri plants that live in (at) the "Uroczysko Buczyna" Reserve (50° 16' N, 18° 57'E) grow at the edge of the forest, which constitutes the fragments of *Tilio-Carpinetum*. These remains of the forest occurred predominantly on brown pozolic soils, which were formed from loamy sand.

The herb layer was well developed with the cover of grasses (*Festuca gigantea, Deschampsia caespitosa*) and *Cardaminopsis halleri* at a level of 80%.

Miasteczko Śląskie and Bibiela city are located in the Tarnogórski Hump (50° 30'N, 18° 56'E). These areas are characterized by the occurrence of the forest complexes with *Calamagrosti villosae-Pinetum*, *Leucobryo-Pinetum* and *Molinio-Pinetum* [28]. In the vicinity of a Zinc smelter, communities are distorted by the influence of industry [28]. The "Miasteczko Śląskie" steelworks produce the largest amount of zinc and lead in Poland.

The four sites mentioned above are situated in the climatic zone of Central Uplands and have maximum precipitation in June (143.7 mm) and minimum in March (28.9 mm) for control areas (UB, FB), and 133.8 mm in June and 21.8 mm in March, respectively, for the polluted areas (DW, DM). July is the warmest month (20.1°C) and December is the coldest (-5.1°C) for the pollution areas. For the forests August is the warmest (19.3°C) and December is the coldest (-5.6°C).

Two hundred individuals were used for investigations (50 plants from each place). Morphological definition of the individual was adopted by Falińska [4]. Each plant was sampled randomly, one meter distance from one another.

Morphological Analysis

For each individual twelve (code of feature in brackets) traits were investigated. The traits were selected on the basis of original papers and our own observations.

- number of stem (1), length of stem (2), number of stem leaves (3);
- diameter of rosette (4), maximal length (5) and width (6) of rosette leaves;
- number of seeds in siliqua (7);
- number of flowers (10) and fruits (8) per individual;
- maximal length of siliqua (9);
- weight of individual dry mass (11) and rosette dry mass (12).

Statistic Analysis

Mean variability of *Cardaminopsis halleri* groups was compared using parametric (after using Shapiro-Wilk test) one-way analysis of variance (ANOVA) for each continuous character with population as a grouping factor. Prior to this, the measurements were subject to log (x+1) transformation for the purpose of stabilization of variance. The cluster analysis was selected as a tool for checking degree of similarity within the habitat. Cluster Analysis and PCA are complementary tools. CA only helps us to build a hypothesis about a problem (in our case: similarity is greater between the sites than individuals; 12 traits per four sites) and the results of such an analysis have no statistical significance. It gives us only a general view. On the other hand, PCA allows confirming this hypothesis in terms of describing the ranges of the variability of groups on the principal components and at the same time by identifying the variables which are responsible for the group discrimination.

A reduction of the number of variables was acquired with the principal component analysis (PCA) in which a smaller number of uncorrelated components were obtained, each of which was equivalent to a certain percentage of total variability [29]. In order to determine which variables discriminated four populations, a discriminant function analysis with the stepwise method was used. Conclusion on the discriminatory power was reached on the basis of Wilks` lambda statistics values, which is the ratio of the intergroup value and the total of squares. Wilks`lamda takes a zero value when the discrimination is complete and is higher (towards unity) when there is no discrimination [30].

Linear regression relationships for all analyzed traits were tested. The analyses were performed in Statistica 6.0 PL software (Statsoft, Poland).

Results

The results of the F- tests, next to the mean values of the measurements, show significant differences in all the features of four morphotypes (Table 1). The following characteristics: length of stem, rosette diameter, dimensions of leaves – were larger in the forest habitat, and number of stems, number of stem leaves, number of flowers and fruits per individual and weight of individual – were larger in the polluted area.

The assumption of a higher degree of similarity within the habitat can be made in view of the results of the cluster analysis (CA) based on the average values of fifty individuals from each side for particular twelve features (Fig. 1). The dendrogram was prepared using Ward's method. It allows fusion of clusters to take place on the basis of minimizing within-cluster variance. The Euclidean distance was taken as a measure of distance between the single objects. In our results this analysis shows a clear distinction between the four clusters. CA only helps us to build a hypothesis about a problem (in our case: similarity is greater between the sites) and the results of such analysis have no statistical significance. We decided to use the PCA method as a complementary tool to CA. On the contrary, PCA allows us to confirm this hypothesis in terms of describing the ranges of group variability with regard to the principal components, which at the same time identify the variables responsible for the groups discrimination. The results of the scree test (Statsoft '98) made it possible to distinguish two components, which explain 95.3 percent of the resulting variability. The features: number of stem (1), length of stem (2), number of stem leaves (3), rosette diameter (4), maximal rosette leaf dimensions (5, 6), number of fruits and flowers per individual (8, 10), weight of individual (11) were found to be the most strongly correlated with the first of the components, and seeds number (7) as well as maximal length of siliqua (9) were correlated with the second one. For these results, ellipses of confidence were drawn encompassing, with a 90% confidence limit, each of the 4 separate groups (Fig. 2, Table 2).

The results of the Discriminant Function analysis have confirmed the existing differences in the morphometrics of four forms (Wilks' lambda 0.01513, F (36.547) =47.597; p<0.01). The partial values of Wilks' lambda in Table 3 have shown that studied *Cardaminopsis halleri* populations are primarily discriminated by seed number. The parameter values of the classification function (not enclosed) were used for *a posteriori* classifications in order to determine whether the empirical data agreed with the model. As a result, 98 percent of the cases were correctly classified as "Uroczysko Buczyna" habitat forms 100 percent as waste dump habitat, 96 percent as the zinc smelter "Miasteczko Śląskie" habitat and 84 percent as the forests of the Bibiela one.

The most important traits in linear regression analysis of investigated microhabitats were the number of fruits and weight of individual dry mass (Fig. 3). Regression relationships between the rest of the analyzed traits were not shown.

Table 1. Summary of the variance analysis for all investigated populations.

Traits	Mean	SD	Coefficient of variability	F	p
[1] number of stem	9.425	2.266	0.240	154.775	p < 0.05*
[2] length of stem	28.513	4.228	0.148	167.854	p < 0.05*
[3] number of stem leaves	27.020	8.300	0.307	64.852	p < 0.05*
[4] rosette diameter	7.026	0.851	0.121	43.471	p < 0.05*
[5] length of maximal rosette leaf	3.695	0.423	0.114	35.098	p < 0.05*
[6] width of maximal rosette leaf	0.748	0.101	0.135	59.581	p < 0.05*
[7] seed number	12.845	1.934	0.150	115.238	p < 0.05*
[8] fruit number / individual	102.320	31.746	0.310	135.460	p < 0.05*
[9] max. length of siliqua	1.788	0.129	0.072	23.957	p < 0.05*
[10] flower number / individual	46.425	14.751	0.317	71.653	p < 0.05*
[11] weight of individual dry mass	2.682	1.078	0.401	168.231	p < 0.05*
[12] weight of rosette	0.079	0.021	0.267	21.240	p < 0.05*

N = 200; untransformed data. * significant differences P<0.05

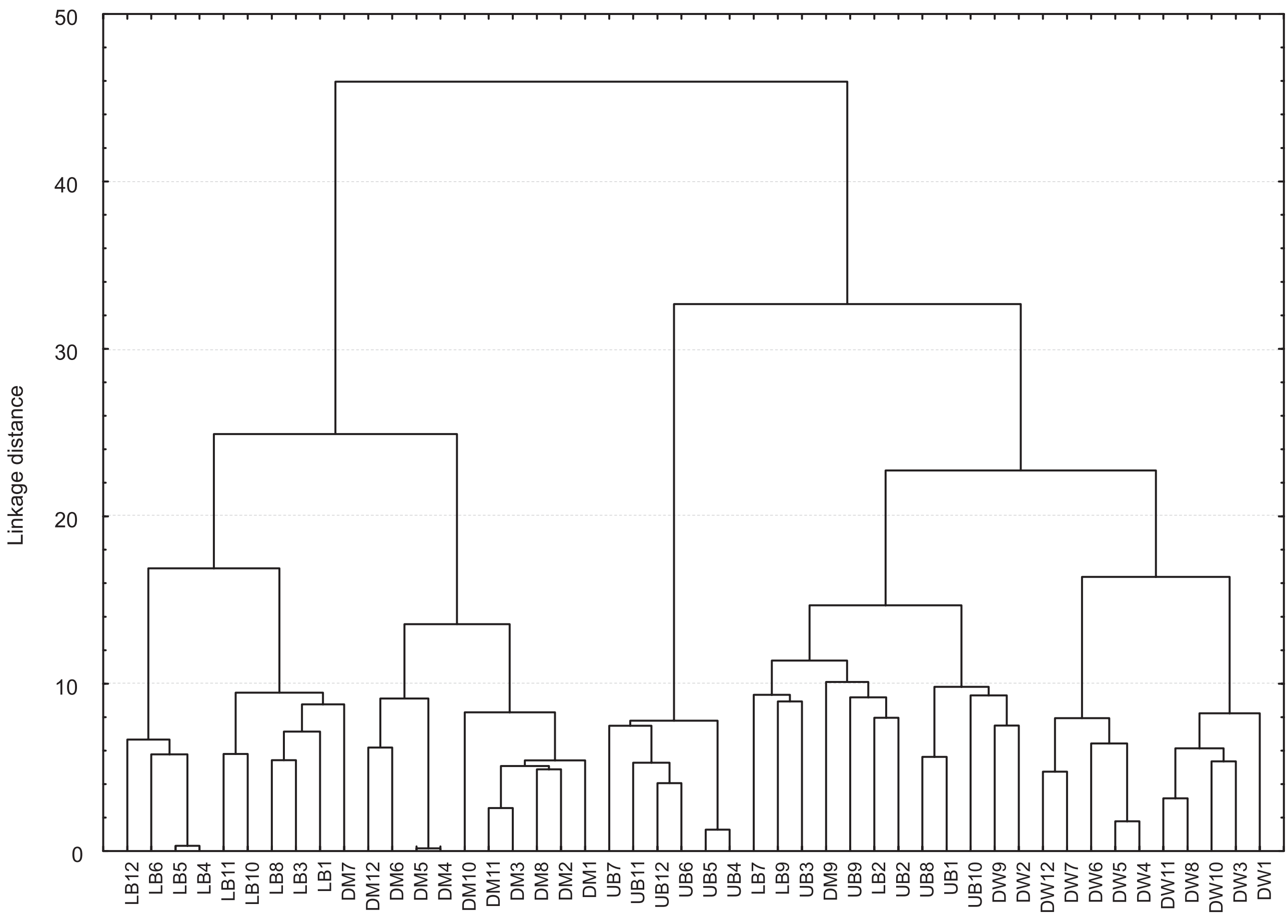


Fig. 1. Cluster analysis for all measured traits of *C. halleri* populations from four studied microhabitats. Dendrogram based on Euclidean distance using Ward's method.

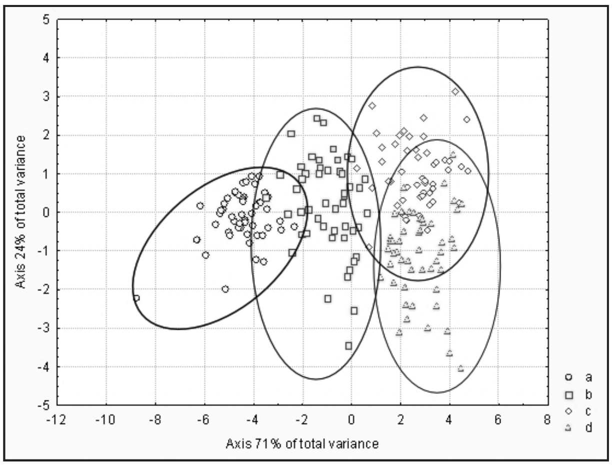


Fig. 2. Relationship between scores on PC1 and PC2 of the biometric traits of investigated *Cardaminopsis halleri* populations (a-DW, b-UB, c-DM, d-LB). Ellipses encompass 90% confidence limits for each population.

Discussion

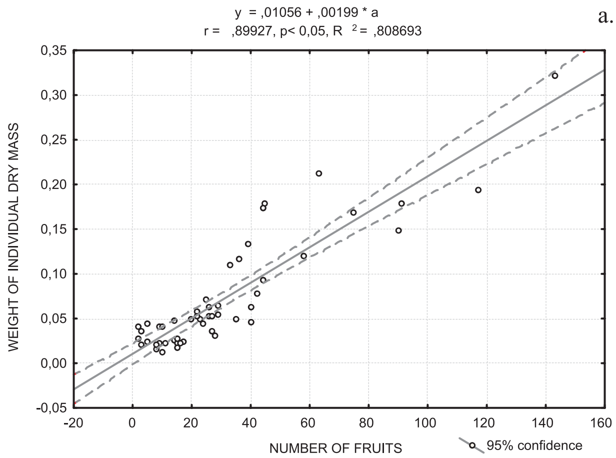
Cardaminopsis halleri is a widely spread species occurring in strongly variable habitats. Adaptation to mountain and forest conditions as well as to high heavy metal pollution conditions is a phenomenon which has triggered amazing morphological variability.

Growth and survival of plants in nature depends on their ability to respond to prevailing environmental conditions. Physiological processes and biochemical pathways leading to the maintenance or recovery of cellular homeostasis in cells under stress form the basis of stress-tolerant genotypes [31]. We cannot talk about new genotypes in *Cardaminopsis halleri* populations, because it demands more long-term investigations, but we have noticed some microevolutionary processes. In each investigated area *Cardaminopsis halleri* adapts to various conditions of the environment and proves great phenotypic plasticity. As a product of plasticity, this species creates four morphotypes. On this level of knowledge plasticity explains observed variability, because it is often the response of plant to environmental variation [5]. It is a functionally appropriate adjustment of the phenotype that acts to enhance fitness under current environmental conditions. This adaptive plasticity is the product of selection for different genetically based trait values in different environments [5].

Links between differential morphology and various environmental conditions has been established. Cluster analyses and PCA confirms our suggestions (Fig.1, Table 2).

Table 2. Factor loadings, eigenvalues and cumulative percentage of variance of PCA (N=200, log-transformed data).

Number and name of features	Factor loadings		
	1	2	3
[1] number of stem	-0.858	0.243	-0.252
[2] length of stem	-0.835	-0.246	-0.222
[3] number of stem leaves	-0.775	0.241	-0.240
[4] rosette diameter	-0.800	-0.362	0.416
[5] length of maximal rosette leaf	-0.744	-0.425	0.461
[6] width of maximal rosette leaf	-0.750	-0.435	0.307
[7] number of seeds	0.039	0.746	0.530
[8] fruit number / individual	-0.807	0.332	-0.349
[9] max length of siliqua	-0.404	0.518	-0.088
[10] flowers number / individual	-0.775	0.189	-0.066
[11] weight of individual (dry mass)	-0.934	-0.047	-0.174
[12] weight of rosette (dry mass)	-0.376	0.509	0.620
eigenvalues	9.113	3.085	0.599
Cumulative% variance	71.2	95.3	100



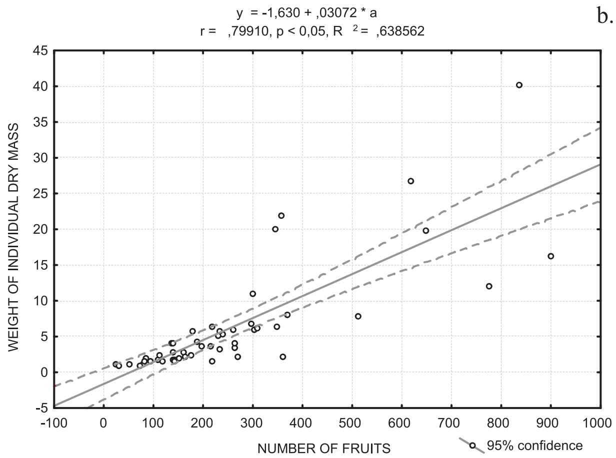


Fig.3. Regression relationships between number of fruits and weight of individual (dry mass) in (a) DW and (b) DM populations of *C. halleri* from contaminated areas (n=50, p < 0.05).

Table 3. Parametres of the discriminant function (12th step).

Number and name of traits	Lambda Wilksa	Partial Lambda Wilksa	F remove	p	Toler.	1-Toler.
[1] number of stem	0.016	0.930	4.617	P<0.01*	0.388	0.611
[2] length of stem	0.019	0.790	16.328	P<0.01*	0.562	0.437
[3] number of stem leaves	0.017	0.863	9.718	P<0.01*	0.558	0.441
[4] rosette diameter	0.018	0.835	12.180	P<0.01*	0.023	0.976
[5] length of maximal rosette leaf	0.018	0.812	14.187	P<0.01*	0.022	0.977
[6] width of maximal rosette leaf	0.016	0.909	6.111	P<0.01*	0.416	0.583
[7] number of seeds	0.025	0.600	41.049	P<0.01*	0.700	0.299
[8] fruit number / individual	0.017	0.872	9.041	P<0.01*	0.398	0.601
[9] max length of siliqua	0.017	0.869	9.250	P<0.01*	0.718	0.281
[10] flowers number / individual	0.017	0.858	10.160	P<0.01*	0.730	0.269
[11] weight of individual (dry mass)	0.016	0.931	4.503	P<0.01*	0.345	0.654
[12] weight of rosette	0.017	0.865	9.615	P<0.01*	0.546	0.453

* significant differences P<0.01

It has already been written about areas which are polluted by heavy metals and about their impact on morphological variability of *Cardaminopsis halleri*. The results of discriminant analysis indicate that number of seeds (trait 7) discriminate investigated populations in the most remarkable way. Number of seeds is different in each investigated habitat, so it implies that this trait is connected with other biotic and abiotic factors, not only with anthropopressure level. PCA outlined four microhabitats, which determined morphology of studied species.

Investigated individuals are characterized by high morphological variability between populations from polluted and unpolluted areas. Biometrical comparisons have shown significant differences with regard to number of stems, number of stem leaves, number of fruits and flowers per individual, weight of individual for the populations from Miasteczko Śląskie and the zinc-lead dump. Similar results of investigations connected with plants that occur on contaminated soils are shown by other specialists [9, 22, 32]. However, weight of individuals from heavy metal-contaminated territories was seriously greater contrary to results of other authors.

The observation causes difficulty in the interpretation of the phenotypic responses to the environment. As a result of physical and biochemical effects on developmental processes, plants in extreme environments show reduced growth. Vegetative reproduction is expensive, which is evidenced by high allocation of mass to production of vegetative progeny, but this kind of reproduction increases the chances for adaptation to the environment and ensures almost 100% reproduction success. The situation illustrates the rule that greater investment in the progeny increases their chance to adapt to the environment and successful development in particular in much stressful conditions [33].

It is also noticed that "polluted populations" are characterized by high value of regression coefficient between number of fruits and individual weight (Fig. 3). This interesting fact shows *Cardaminopsis halleri* untypical "r" life strategy. The most probable mechanism of selection existing in anthropogenic habitats which allows Cardaminopsis halleri to survive is various reproductive strategies. The individuals start reproduction in the second year of life and the occurrence of them on anthropogenic habitats is smaller.

The dynamic changes in the selection pressure can be seen mostly in the environment where abiotic and biotic factors don't change gradually but jump wisely. It can even be noted within the same vegetation season. Such populations cannot be unambiguously placed in the continuum of life strategies. The set of these features suggests that the selection pressures do not change gradually but jump wisely at the moment of transformation of pioneer habitats. These changes can be characterized by strong dynamics detectable even within the same vegetation season [34].

Conclusion

Cardaminopsis halleri shows high morphological variability in each of investigated microhabitats, but the anthropopressure level is not the only factor, which has caused the described changes. In our opinion all groups of biotic and abiotic factors influence the general appearance and behaviour of *Cardaminopsis halleri*.

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