MORPHOMETRIC DIFFERENTIATION OF *CAREX LIGERICA* GAY IN POLAND

LECH URBANIAK

Department of Genetics, Adam Mickiewicz University,
Międzychodzka 5, 60-371 Poznań, Poland

(Received: October 29, 1997. Accepted: June 10, 1998)

ABSTRACT

The experimental material involved 7 *Carex ligerica* Gay populations which were cultured in standardised conditions in a greenhouse before their spikes were collected for morphological studies. Four characters reflecting size of male and female glumes, selected from particular spikes were examined. Mahalanobis distances for each pair of populations were calculated and their significance was estimated using Hotellings T^2 statistics. Dendrite was constructed on the basis of shortest Mahalanobis distances while Euclidean distances provided grounds for hierarchy grouping. The result obtained from a multivariate analysis indicated a definite interpopulation variability within the species. All of the examined populations were found to differ significantly on the grounds of Mahalanobis distances. The dendrograms manifested the distinct character of the populations originating from regions around the lower course of the Vistula river – 5 (Toruń-Wrzesy), 3 (Tychnowy), 2 (Piaski) and 6 (Kadyny), not noted before. Moreover, similarity of two geographically distant populations, the population 1 (Złotaria) from the Central Poland and the population 4 (Szumilowo) from the western part of the country, attracted attention, as well as the individual character of the population 7 (Kopanica) originating from the southernmost location. From the point of view of historical geography of plants, the obtained differentiation pattern may represent sequelae of migration in the postglacial period, which crossed the area of Poland along multiple distinct pathways. The obtained results point to importance of culturing plants in uniform conditions of a greenhouse, which permits to describe genetic variability unbiased by modifying effects of the environment.

KEY WORDS: *Carex ligerica* Gay, morphometric differentiation, female and male glumes, greenhouse culture, multivariate analysis.

INTRODUCTION

In the territory of Poland, *Carex ligerica* Gay, representing one of species from sect., *Ammoglochin*, occurs in small isolated populations, mainly in the northern parts of the country, and along the lower course of the Vistula river, furthermore only rarely in southern parts of the country.

The performed till now studies on interpopulational variability of *C. ligerica* Gay, started with analysis of phenolic compounds, demonstrated the similarity of populations from Western Poland and the individual character of populations originating from North-Eastern part of the country (Urbaniak 1988a).

The result was confirmed in studies on differentiation of populations in respect to anatomical characters of leaves and rhizomes (Urbaniak 1988b). Both studies were performed on plants cultured in similar conditions of a greenhouse.

Variability analysis of morphological characters of spike components which were collected at the site of population origin failed to confirm the above mentioned variability. The reverse proved true: a divergent character of the two populations from the western region was detected (Urbaniak 1990).

This prompted us to investigate again the intraspecific differentiation of *C. ligerica*, on the basis of morphological characters of female and male glumes. However, in this case the studied material was collected from plants, which previously

had been planted in the greenhouse in order to eliminate the differentiating effect of the environment.

Comparing the results of studies on materials originating from different geographical stations of the country with the presented data, an attempt has been made to appraise a modifying effect of the environment on morphological characters. Therefore we may reasonably expect the differences observed in the course of the investigations to have genetic character.

MATERIAL AND METHODS

Rhizomes, representing 7 populations of *Carex ligerica* were dug out in 1976 and in 1977 along the marked transects in such a manner that the distance between the collected samples was 1 m. From each population 30 rhizomes were collected. Sites of population origin are shown in Fig. 1.

The rhizomes were planted in separate pots in a mixture of compost, sand and peat in relation of the parts 1:2:1 and transferred to a greenhouse.

In 1979 mature spikes were collected from 20 plants from the population 1 (Złotaria), in 1980 – 27 spikes from the population 5 (Toruń-Wrzesy), 26 from the population 4 (Szumilowo), 26 spikes from the population – 2 (Piaski) and 27 from population 7 (Kopanica).

Fig. 1. Location of the studied populations

On the other hand, in two populations: 3 (Tychnowy), and 6 (Kadyny) mature spikes in 1979 were present in few individuals only. Therefore, in 1991 rhizomes were again collected from these stands. Following planting to pots and transfer to the greenhouse, after 3 years of culture, i.e. in 1994, from the population 3 (Tychnowy) 24 spikes and from the population 6 (Kadyny) 25 spikes were collected.

In all the cases each spike represented a separate plant from a single pot. There female glumes were selected from the central part of each spike and three male glumes from its top part. Each glume was isolated using a preparatory needle and transferred to a microscopical glass and suspended in a drop of water. Covered with a coverslip the preparations were examined at 17.5 x magnification to draw a scheme at which four selected characters were measured (Fig. 2).

The statistical processing of the obtained data included grouping analysis by the nearest neighbourhood method using Euclidean distances (Karoński and Caliński 1973) and Mahalanobis distances with Hotellings T^2 statistic. Moreover, minimum spanning trees (dendrite) on the basis of the shortest Mahalanobis distances were constructed (Caliński et al. 1975).

Fig. 2. Diagram of the glume showing the method of measurements: a – length, b – maximum width, c – distance between the maximum width and the base of the glume, d – width of the glume in one third of its length as measured downwards from the top

RESULTS

Female glumes

The dendrogram of relationships among 7 *C. ligerica* populations constructed on the ground of agglomerative grouping by the method of nearest neighbourhood using Euclidean distances is presented in Fig. 3. Results of grouping analysis was marked on the map of Poland where dendrogram transsections, denoting typical groups of populations, were presented in the form of contour lines (Fig. 3).

The studied material can be subdivided into two groups. The first group is formed of populations from areas adjacent to the lower course of the Vistula river – 2 (Piaski), 6 (Kadyny), 3 (Tychnowy) and 5 (Toruń-Wrzesy). The second group consists of two populations from Western Poland – 4 (Szumitowo) and population 1 (Złotoria), near Toruń. Individual character of population 7 (Kopanica) is marked.

The minimum spanning tree constructed on the basis of the shortest Mahalanobis distances and the Hotellings T^2 statistics are presented in Fig. 4. Comparison of the calculated values of Hotelling's statistics with the appropriate critical value indicated that all the population pairs forming minimum spanning tree are significantly differentiated in respect to the calculated Mahalanobis distances.

The tables of Mahalanobis distances (Tab. 1) indicated that all remaining populations are also significantly different ($T^2_{0.01}=48.96$).

TABLE 1. Mahalanobis distances between 7 populations of *Carex ligerica* calculated on the basis of female glumes characters with T^2 statistics (T^2 critical value at the 0.01 level = 48.96, ** denotes critical Mahalanobis distances)

	1	2	3	4	5	6
2	8.37** 764.63					
3	9.01** 885.74	2.47** 73.76				
4	4.76** 256.79	7.62** 725.93	7.66** 732.75			
5	7.82** 702.75	3.00** 114.82	5.10** 330.48	7.45** 737.01		
6	6.20** 427.32	3.52** 151.76	5.01** 307.39	6.85** 598.86	4.42** 254.03	
7	12.08** 1651.06	4.53** 256.88	5.31** 352.09	1.09** 1558.39	4.77** 301.65	7.83** 782.57

Male glumes

The dendrogram of relationships among 7 *C. ligerica* populations and its projection on the map of Poland are shown in Fig. 5. Similarly to female glumes, populations 5 (Toruń-Wrzesy) and 3 (Tychnowy) as well as 2 (Piaski) and 6 (Kadyny) form a single group. The other group consists of 4 (Szumitowo) and 1 (Złotoria) together with 7 (Kopanica).

On the respective minimum spanning tree (Fig. 6) the pattern is confirmed, together with the specific character of the population 7 (Kopanica).

Similarly to female glumes, the list of Mahalanobis distances and the calculated values of Hotelling's T^2 statistics (Tab. 2) point to a significant differentiation of all the studied populations ($T^2_{0.01}=48.96$).

Fig. 3. Dendrogram of relationships among 7 *Carex ligerica* populations on the basis of female glume characters with its projection on the map of Poland

DISCUSSION

Culture of plants in standardised conditions represents an important step toward description of interpopulation variability within a species. A number of factors of a modifying effects becomes in this way eliminated and the observed variability is of inherited type (Clausen et al. 1940, Clausen and Hiesey 1958, Schlichting and Levin 1984, Via 1987, Black-Samuelsson and Andersson 1996).

Such a procedure has been applied for examination of the interpopulational variability of *C. ligerica* based on phenolic

TABLE 2. Mahalanobis distances between 7 populations of *Carex ligerica* calculated on the basis of male glumes characters with T^2 statistics (T^2 critical value at the 0.01 level = 48.96, ** denotes critical Mahalanobis distances)

	1	2	3	4	5	6
2	7.26** 575.97					
3	11.22** 1375.52	4.81** 277.66				
4	3.33** 125.35	4.29** 230.0	7.91** 782.46			
5	10.11** 1175.18	3.75** 179.00	2.94** 110.36	6.91** 632.65		
6	8.78** 858.38	3.20** 125.46	5.37** 354.06	6.24** 496.36	5.86** 446.86	
7	15.48** 2837.64	8.75** 956.66	7.36* 676.29	12.99** 2196.01	7.52** 749.87	8.28** 873.92

Fig. 4. Dendrite (minimum spanning tree) for the female glume characters showing the ordination of investigated populations according to shortest Mahalanobis distances. Each distance of two populations is described additionally by Hotelling's T^2 statistics. $T^2_{0.01}$ denotes critical value of Hotelling's T^2 statistics

compounds and anatomical characters of leaves and rhizomes (Urbaniak 1988a, 1988b). Results of both studies have proven consistent and the conclusion stressed similarity of the populations from the area of Western Poland (Szumilowo and Kopanica) and from central part of the country (Złotonia near Toruń). Individual characters have been demonstrated by the population Tychnowy.

A distinct procedure has been applied upon analysis of *C. ligerica* populations based on morphological characters. In this case mature spikes were collected in the original sites (Urbaniak 1990). Results obtained on the material have not

Fig. 5. Dendrogram of relationships among 7 *Carex ligerica* populations on the basis of male glume characters with its projection on the map of Poland

point to grouping of populations according to their geographic origin. However, it has been shown that populations from Western Poland-Szumitowo and Kopanica which resembled each other in earlier comparisons this time have demonstrated a significantly distinct character.

Fig. 6. Dendrite (minimum spanning tree) for the male glume characters showing the ordination of investigated populations according to shortest Mahalanobis distances. Each distances of two populations is described additionally by Hotelling's T^2 statistics. $T^2_{0.01}$ denotes critical value of Hotelling's T^2 statistics.

The divergent results have prompted to re-investigate the morphological characters. This time, however, the experimental material was collected from the plants following a greenhouse culture. Comparison of the two studies on morphological characters offers a potential of appraising the modifying effect of the environment.

The individual character of the two populations of *C. ligerica*, growing in Western Poland (4 – Szumitowo and 7 – Kopanica), described on the basis of the material collected in the place of origin, seems evident (Urbaniak 1990). In the presented study, the minimum spanning trees and the dendrograms point also to a divergent character of these two populations and, moreover, is shown also by the remaining populations (Figs 3, 4 and 5, 6).

The connection of population 1 (Złotoria) from central part of the country with population 4 (Szumitowo) from the area of Western Poland also deserves attention. The pattern of observed similarity seems reproducible and pertains to both dendrograms (Figs 3 and 5). Such ordering of the populations also has been obtained in studies on phenolic compounds and anatomical characters of leaves and rhizomes, in which populations – Szumitowo, Kopanica and Złotoria are linked by Mahalanobis distances of low values.

Both populations originate from stands positioned along the pathway of plant migration, associated with the Atlantic climate and heading eastwards along Toruń – Eberswald proglacial stream valley. As a consequence, the common past may be at present manifested by similarity of morphological characters which, being analysed in plants following a greenhouse culture, reflect the genetic structure of the species.

It should be stressed that a group of populations from the lower course of the Vistula river can be distinguished –

5 (Toruń-Wrzosy), 3 (Tychnowy), 2 (Piaski) and 6 (Kadyny), (Figs 3 and 5). In the case of male glumes, the populations geographically closer resemble each other (Fig. 5). The pattern indicates geographical grouping of neighbouring populations of *C. ligerica* from this region, not noted till now.

The latter pattern of population similarity may also reflect the common past in the earlier history. The pathway of plant migration, associated with the Atlantic climate, lead also from South Sweden through Öland and Gotland islands to Finland and, then, eastwards, along the southern coast of the Baltic Sea to the area of Pomerania (Czubiński 1950). Exhaustive explanation of the problem would require, however, that additional research material is investigated, originating from the neighbouring countries.

The specific position, occupied by the southernmost population – 7 (Kopanica), which is distinct from the remaining populations, deserves comments. Its geographical situation may indicate that it originates from a separate, distinct migration pathway, which reached the area of Poland from the South, to the western parts of Lower Silesia.

The pattern of observed similarity stresses the role which can be played in the case of morphological analyses by cultures of the compared populations at standardized conditions.

One of the conclusions, which stems from the performed till now studies on interpopulational variability of *C. ligerica* in the region of Poland, pertains the observed definite intra-specific differentiation within the species (Urbaniak 1992a, 1992b). This has proven particularly evident in comparison with the taxonomically related species of *Carex arenaria* L. These earlier reached conclusions have been fully confirmed by the present results (Tables 1 and 2). They point to unequivocal interpopulational differentiation since all the examined populations differed from each other in a significant manner, as shown by the Mahalanobis distances ($T^2_{\text{calc}} > T^2_{0.01}$).

Isolation of the small, geographically distant populations of the species has been suggested as the cause of the differences. The populations are separated from each other, as a rule, by distances of tens or even hundreds kilometers and such distances effectively prevent gene flow (Bradshaw 1959, 1972).

The intense vegetative propagation represents another factor which may promote an extensive intraspecific variability. The process may significantly restrict the number of individuals which in given conditions most intensely propagate in the vegetative way. Immunological analysis aimed to evaluate the intensity of sexual reproduction in *Carex arenaria* has shown that among 30 examined plants in two populations, 10 to 12 serological types can be distinguished (Urbaniak 1984). The result draws our attention to the importance which should be ascribed to proportions between intensities of vegetative and sexual reproduction in this group of plants.

The observed significant variations in size of the occupied area typical for individual populations of *C. ligerica* is equally important and related to reduction or increase in the number of plants in consecutive vegetation seasons (Urbaniak 1988b). This process may have an important influence on genetic structure and effective population size (Rannala 1996).

Thus, it seems that the observed variability in *C. ligerica* should be ascribed to parallel action of all the above mentioned factors.

As mentioned previously, the material for the present study has been collected at three different times – in 1979, 1980, 1994. This poses a question of how this time interval in the

spikes' collection might have affected the obtained results. The unequivocal grouping of the populations from the lower course of the Vistula river despite spikes' collection taking place in different years, as well as partly the consistency of results with those obtained earlier seems to remove the doubts.

ACKNOWLEDGEMENT

The author is grateful to Mrs Aneta Starosta for her skillful technical assistance.

LITERATURE CITED

- BLACK-SAMUELSSON S., ANDERSSON S., 1996. Reaction norm variation between and within populations of two rare plant species, *Vicia pisiformis* and *V. dumetorum* (Fabaceae). *Heredity* 79: 268-276.
- BRADSHAW A.D., 1959. Population differentiation in *Agrostis tennis* Sibth. Morphological differentiation. *New Phytol.* 58: 208-227.
- BRADSHAW A.D., 1972. Some evolutionary consequence of being a plant. *Evol. Biol.* 5: 25-47.
- CALIŃSKI T., CZAJKA S., KACZMAREK Z., 1975. Principle component analysis and its application. (In Polish). *Roczniki Akademii Rolniczej w Poznaniu. Algor. Biometr. Statyst.* 36: 159-185.
- CLAUSEN J., KECK D.D., HIESEY M.W., 1940. Experimental studies on the nature of species. I. Effect of varied environments on Western North American plants. *Carnegie Inst. of Washington Publ.* No. 520, Washington, D.C. Gibson Brothers, Inc. Standard Engraving Co. Washington, D.C.
- CLAUSEN J., HIESEY M.W., 1958. Experimental studies on the nature of species. Genetic structure of ecological races. *Carnegie Inst. of Washington Publ.* 615, Washington, D.C. The Lord Baltimore, Maryland.
- CZUBIŃSKI Z., 1950. Badania fizjograficzne nad Polską zachodnią. Nr 2, zeszyt 4. PTPN. Poznań.
- KAROŃSKI M., CALIŃSKI T., 1973. Grouping in multivariate populations on the basis of Euclidean distances. (in Polish). *Algor. Biometr. Statyst.* 17: 117-129.
- RANNALA B., 1996. Gene genealogy in a population of variable size. *Heredity* 78: 417-423.
- SCHLICHTING C.D., LEVIN D.A., 1984. Phenotypic plasticity of annual *Phlox*: testes of some hypotheses. *Am. J. Bot.*, 71: 252-260.
- URBANIAK L., 1984. Genetic variation of *Carex arenaria* L. In Poland. *Wyd. Naukowe UAM*
- URBANIAK L., 1988a. The interpopulational variability of *Carex ligerica* Gay in Poland. Part I. Variation of phenolic compounds as revealed by paper chromatography. *Bull. Soc. Amis Sci. Lett. Poznań, Ser. D. Biol.* 27: 43-52.
- URBANIAK L., 1988b. The interpopulational variability of *Carex ligerica* Gay in Poland. Part II. Variation of anatomical characters-rhizomes and leaves. *Bull. Soc. Amis Sci. Lett. Poznań, Ser. D. Sci. Biol.* 27: 53-59.
- URBANIAK L., 1990. The interpopulational variability of *Carex ligerica* Gay in Poland. Part III. Variation of morphological characters. *Bull. Soc. Amis Sci. Lett. Poznań, Ser., Sci. Biol.* 28: 27-39.
- URBANIAK L., 1992a. A comparison between *Carex arenaria* L. and *Carex ligerica* Gay-sedges from the *Arenariae* group based on leaf characteristics. *Acta Soc. Bot. Pol.* 61: 265-
- URBANIAK L., 1992b. The comparison between *Carex arenaria* L. and *Carex ligerica* Gay sedges from *Arenariae* group by phenolic compounds analysis. *Bull. Soc. Amis Sci. Lett. Poznań, Ser. D. Sci. Biol.* 29: 41-49.
- VIA S., 1987. Genetic constraints on the evolution of phenotypic plasticity. In: Loeschcke, V. (ed.) *Genetic Constraints on Adaptive Evolution*, pp. 47-71. Springer-Verlag, Berlin.

ZRÓŻNICOWANIE MORFOMETRYCZNE *CAREX LIGERICA* GAY W POLSCE

STRESZCZENIE

Materiał badawczy stanowi 7 populacji *Carex ligerica* Gay, z których po hodowli w wyrównanych warunkach w szklarni zebrano kłosa do badań morfologicznych. Analizowano 4 cechy przysadek wypreparowanych z poszczególnych kłosów żeńskich i męskich, obrazując ich rozmiary. Dla każdej pary populacji obliczono odległości Mahalanobisa, ich istotność oszacowano za pomocą statystyki T^2 Hotellinga. Dendryt skonstruowano na podstawie najkrótszych odległości Mahalanobisa, podczas gdy dendrogram będący graficznym obrazem analizy grupowania powstał w oparciu o odległości Euklidesa. Wynik uzyskany na podstawie wielocechowych analiz wskazuje na zdecydowane zróżnicowanie międzypopulacyjne w obrębie tego gatunku. Wszystkie badane populacje różnią się w sposób istotny statystycznie na podstawie odległości Mahalanobisa (tab. 1 i 2). Na dendrogramach analizy grupowania zaznacza się odrębność populacji pochodzących z okolic dolnego biegu Wisły – 5 (Toruń-Wrzosy), 3 (Tychnowy), 2 (Piaski) i 6 (Kadyny), na co dotychczas nie wskazywano. Ponadto zwraca uwagę podobieństwo dwóch populacji odległych geograficznie – 1 (Złotoria) z terenów centralnych i 4 (Szumilowo) z zachodniej części kraju, a także indywidualny charakter populacji pochodzącej ze stanowiska wysuniętego najdalej na południe – 7 (Kopanica) (ryc. 3 i 5). Z punktu widzenia historycznej geografii roślin uzyskany obraz zróżnicowania może stanowić następstwo migracji w okresie postglacjalnym, która wiodła na teren Polski wieloma odrębnymi szlakami. Ponadto uzyskane wyniki stanowią o wadze jaką należy przykładać do hodowli roślin w wyrównanych warunkach, w szklarni, co pozwala na opisanie zmienności dziedzicznej bowiem wyeliminowano modyfikacyjny wpływ środowiska.

SŁOWA KLUCZOWE: *Carex ligerica* Gay, zróżnicowanie morfologiczne, przysadki żeńskie i męskie, kultury szklarniowe, analizy wielocechowe.