10.2478/v10067-012-0010-3

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. LXVII, 1 SECTIO C 2012

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Xerothermic species of the genus *Campanula* in Poland – a model for the phylogeographical assessment of reconstruction of post-glacial migration routes

Kserotermiczne gatunki z rodzaju *Campanula* w Polsce – model dla filogeograficznej próby rekonstrukcji postglacjalnych szlaków migracyjnych

SUMMARY

Migration routes of the representatives of particular geographical and ecological groups ('elements') forming the contemporary Polish flora have been an object of botanical studies for more than a century. The present paper introduces a research project (being now carried out by the author) attempting to address this problem using phylogeographical approach (i.e. correlating present geographical distribution pattern with genetic relationships of populations) to confirm and/or correct reconstructions of the 'steppic' element migration routes, established by means of classical methods to date. As a model, four species of the genus *Campanula* (namely: *C. sibirica*, *C. bononiensis*, *C. cervicaria* and *C. glomerata*) were proposed, representing an ecological gradient (varied degree of the connection to dry and thermophilous habitats).

STRESZCZENIE

Zagadnienie dróg migracji poszczególnych elementów geograficznych i ekologicznych flory Polski stanowi od przeszło stulecia stały przedmiot zainteresowania botaników. W pracy przedstawiono realizowaną obecnie przez autora propozycję badań zmierzających przy użyciu metod filogeograficznych (korelacji wzorców współczesnego rozmieszczenia z pokrewieństwem genetycznym, w omawianym przypadku szacowanym przy użyciu analiz DNA: AFLP i sekwencjonowania) do potwierdzenia i/lub korekty przyjmowanych na podstawie rozważań klasycznych, rekonstrukcji szlaków migracyjnych na teren Polski elementu „stepowego". Zaproponowano użycie do tego celu 4 gatunków z rodzaju dzwonek *Campanula* (*C. sibirica*, *C. bononiensis*, *C. cervicaria* i *C. glomerata*), tworzących gradient ekologiczny (pod względem stopnia przywiązania do zbiorowisk kserotermicznych).

Keywords: *Campanula*, xerothermic flora, migrations, phylogeography, Poland

Abbreviations:
AFLP = Amplified Fragment Length Polymorphism
cpDNA = chloroplast DNA
PCR = Polymerase Chain Reaction

INTRODUCTION

The issue of migration routes of thermophilous, light-demanding and drought-resistant species constituting the "steppic" element in the present flora of Poland (as defined e.g. by Kozłowska in (6), previously named "pontic element" by Raciborski in (17)) has been often discussed in the literature at least since the beginning of the 20th century (e.g. (17), (6), (7), (8), (15), (21), (3), (5), (2), (16), (1), (10), (14) and the literature cited there). Locations of putative refugia were suggested, where these species could survive the adverse climatic conditions during consecutive Pleistocene glaciations and several routes were proposed by which they could have advanced afterwards to their extant (or recorded historical) locations. Among the most recognized were (Fig. 1): [1] the route from Podolia along the northern edge of the Carpathians, [2] the route from the Pannonian Basin via the passes of the Western Carpathians (Beskid Niski and/or Moravian Gate), [3] the route from Thüringen and German Lowland via the Toruń-Eberswalde Spillway. Few authors proposed also: [4] the route from the northern Alpic foothills and south of today's Germany via the northern edge of the Sudetes and [5] the north-eastern route, from today's Belorussian and Lithuanian area via the Masurian Lakeland and moraine hills. It is also possible that the present distribution of the xerothermic species may be an effect of several migration waves from various directions, taking place over the present or even earlier interglacials.

The wide availability of sensitive molecular techniques, being presently an off-the-shelf tools, often allows drawing more secure conclusions concerning the issues of historical formation of the contemporary ranges, than speculative analyses of the present distribution or studies of scarce palaeobotanical material, that were the only ways to address these problems until recently. Four species from the genus *Campanula*, belonging to the same section of the genus and representing an ecological gradient with a variously strong connection to thermophilous plant communities (see below for details), were selected for this study. Based on this biological model, the main aims of the project being presently carried out by the author are: (i) addressing the question of the nature of geographical discontinuities of the present distribution of the studied species (purely ecological or also historically conditioned), (ii) demonstrating analogies (or their lack) in genetical variability patterns of the ecologically and taxonomically close species at the country's scale, (iii) an attempt at reconstruction of particular post-glacial migration routes with regard to their potential divergence driven by autecological differences among the study species. Phylogeographical studies will be based

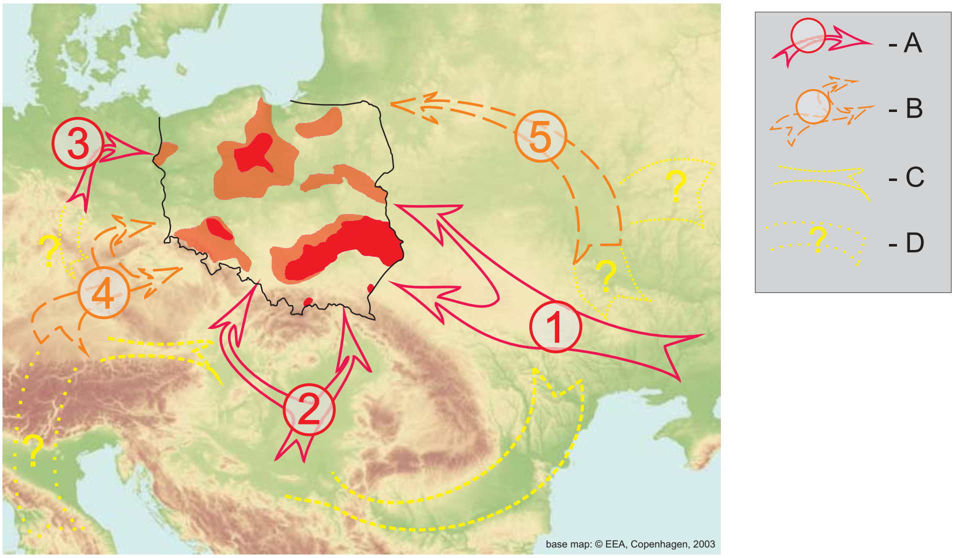


Fig. 1. Review of the putative post-glacial migration routes of 'steppic' element into Poland, based on classical methods: A – routes firmly established in the literature; B – additional hypothetic routes, proposed in few sources; C – widely accepted connecting routes; D – additional possible connecting routes (after: (13), supplemented)

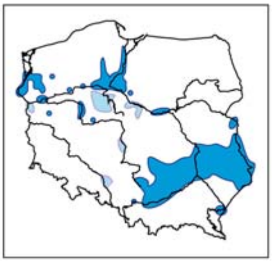
on total DNA AFLP fingerprinting as well as direct sequencing of selected non-coding chloroplast DNA regions. Selection of the relatively closely related species as model organisms should have an additional practical significance that molecular analyses planned may likely be successfully conducted using the same DNA primers across species, thus lowering the cost and workload during the initial phase.

CHARACTERISTICS OF THE SPECIES CHOSEN FOR STUDY

Autecological characteristics of the studied species are synthetically shown on Figures 2–5, column C, based on the ecological indicator values from (24). The varied degree of association of particular species to xerothermic habitat may be observed also on the distribution maps, both in their Polish and general ranges (Figs. 2–5, columns A and B respectively). Phytosociological decriptions are based on (9) and (12).

Campanula sibirica L. (Fig. 2) is the species with the strongest ecological connection to the extremely xeric and polythermal habitats among the Polish representatives of the genus. It is regarded as a characteristic species of the *Festucetalia valesiacae* order (*Festuco-Brometea* class). *C. sibirica* may occur even on initial, low-density, open heliophilous swards growing on very shallow calcareous

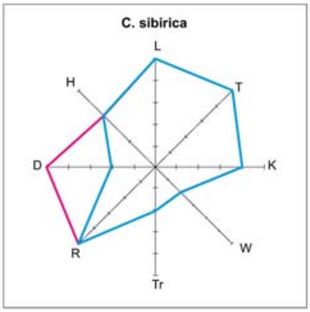
A



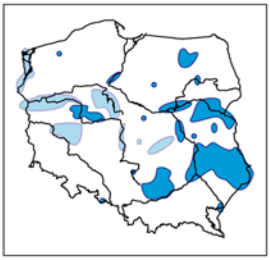
B

Fig. 2.
C. sibirica

C



A



B

Fig. 3.
C. bononiensis

C

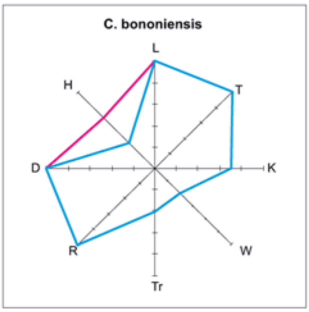
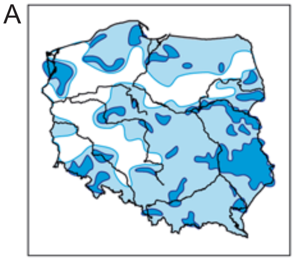


Fig. 4.
C. cervicaria



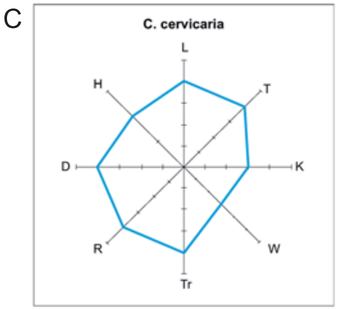
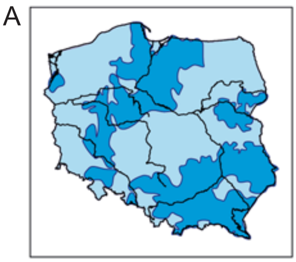
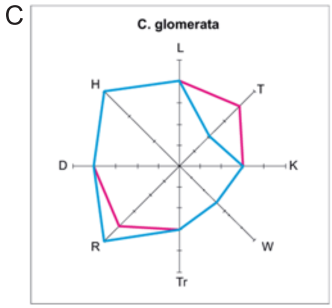
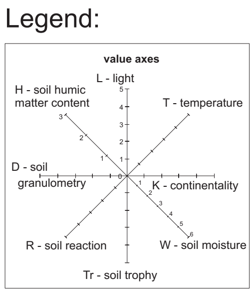


Fig. 5.
C. glomerata







Figs. 2–5. The studied *Campanula* species: distribution ranges in Poland (A), general ranges (B), and synthetic autecological characteristics based on ecological numbers (C). Legend for A: 1 – continuous range; 2 – areas of dispersed locations. Legend for C: the axes used for display of six chosen characteristics; two colours were used for parameters having diffuse values. Sources: for A: (23); for B: (11) (Figs. 2 to 5) and (4) (Figs. 4 and 5); for C: (24)

soils. This is readily visible in its distribution pattern in Poland (Fig. 2A): the species occupies only the areas with the highest density of xerothermic habitats, mostly on the Lubelska Upland, the southern part of the Małopolska Upland as well as bigger rivers' valleys, especially in their gap fragments with numerous steep, strongly insolated cliffs. When compared with *C. bononiensis* (see below), *C. sibirica* is lacking dispersed stations in central parallel belt of Poland (the Vistula river valley between the Lublin region and the mouth of the Bug river together with the Bug valley) and in the north-eastern part of the country. In the consequence, the southern and northern stations are more strongly isolated, thus showing clear disjunction in the regional range. It seems particularly important, therefore, to test whether it may be an effect of the varied biogeographical history (different migration routes/times). The general range, easternsubmediterranean-pontic-pannonian-central Siberian (Fig. 2B) is of similar character, but somewhat wider than in the following species, with a conspicuous (albeit not absolute) disjunction in the zone of the Ukrainian steppes.

Campanula bononiensis L. (Fig. 3) is distributed in Poland similarly as the above-mentioned species but it occurs less frequently on the main areas occupied by *C. sibirica*, which may indicate its higher microhabitat demands (Fig. 3A). According to literature data and preliminary author's observations, the species prefers edges of thermophilous woods and thickets as well as the closed xerothermic tall-grass meadows, not encroaching into the open swards on shallow initial soils. Phytosociologically these ecological preferences are expressed by considering *C. bononiensis* as characteristic of two alliances: *Cirsio-Brachypodion pinnati* grasslands of *Festuco-Brometea* and *Geranion sanguinei* – fringe communities of thermophilous herbs of *Trifolio-Geranietea* class. Local disjunctions within the Polish part of the range are less conspicuous than in the case of *C. sibirica*, thanks to the occurrence of numerous dispersed "connection" stations, but this does not preclude this pattern being the result of different migration routes. In this context, the distribution "island" on the Masurian Lakeland (NE Poland) seems especially interesting. It takes advantage of the suitable habitats on the last-glacial moraine hills. The general range of *C. bononiensis* (Fig. 3B), middlesubmediterranean-pontic-pannonian-sarmatian-western Siberian, with a leg reaching Caucasian foothills, is the smallest of the species considered here, without any conspicuous disjunctions (apart from the narrow Carpathian barrier, not shown on the generalized map here).

Campanula cervicaria L. (Fig. 4), in spite of notably higher density of localities in the main Polish regions of xerothermic habitats (the Lublin region, the southern part of the Małopolska Upland, Vistula, Bug and Oder river valleys), occurs on dispersed lowland stations much more frequently than the species described before. Additionally, it has many mountain localities: mostly in the Sudetes and, somewhat less, in the Carpathians, here with concentration in the Dunajec river valley (Fig. 4A). As regards habitats, this species prefers thermophilous shrubs and light forests; to the lesser degree open, grassland communities. Phytosociological position of the *C. cervicaria* is poorly defined; it is most often included into the species set of the thermophilous fringes from the *Geranion sanguinei* alliance, thermophilous light deciduous forests of the *Quercetalia pubescentis* order, but also of the warmer varieties of *Molinion* meadows or *Carpinion* forests. The general range (Fig. 4B), a little wider than in previous species, middle submediterranean-subatlantic-southern Scandinavian-middle Siberian, reaching central France in the West, southern Scandinavia in the North, and Baikal lake in the East, basically does not encroach to the South farther than the forest-steppe zone. This suggests a weaker association of *C. cervicaria* with the warmer varieties of the continental climate and avoiding the extremely xeric and polythermal habitats.

Campanula glomerata L. (Fig. 5) in wide comprehension (*sensu lato*) constitutes the most eurytopic species of the group and, at the same time, the most common one. In the Polish range (Fig. 5A) it has a wide coverage but particularly conspicuous concentrations of its populations are located in the regions where thermophilous habitats are common, while lower densities (even voids, not marked due to scale on the generalized map) where moist and mesophilous habitats (both forest and open ones) dominate. *C. glomerata* is regarded as a characteristic species of the whole *Festuco-Brometea* class, however, not rare in fringe communities of the *Origanetalia* order (*Trifolio-Geranietea* class) and even the warmer forms of *Arrhenatheretalia* meadows (*Molinio-Arrhenatheretea* class). The general range of *C. glomerata* (Fig. 5B), European-Siberian-Mongolian-Mandschurian, reaching from the Atlantic to Pacific coast and to the South into the forest-steppe zone, over the Caucasus and (as a separate infraspecific taxon) even to the Middle East, is the largest in the considered group. However, taking into account a high within-species variability of the species, the real picture is most probably much more complex, and particular fragments of that range (and/or various habitat types within it) may be dominated by separate lower-rank taxa (‘small’ species, subspecies or varieties, depending on the taxonomical view). This issue still demands a closer taxonomic and phylogenetic investigation, it is sure nevertheless that within the Central European part of the range particular attention should be paid to *C. glomerata* subsp. *farinosa* (Rochel) Jáv., a taxon connected to thermophilous habitats of the order *Origanetalia*.

PLANNED RESEARCH

1. FIELD STUDIES

Population samples for molecular analyses will be collected for all the studied species from several stations in Poland and neighbouring countries. Because of possibility of ‘noise’ in the general picture of the genetic variability introduced by recent, anthropogenic stations of some of the species (e.g. ergasiophygophytic stations of species treated as ornamental, as *C. glomerata*), for sample collection the localities of stable (semi)natural character will be preferred, situated away from the direct influence of settlements and traffic routes, not being disturbed (as industrial or inundation areas) in a way promoting long-distance diaspore transport. The collection set for every population will comprise samples from 10–20 specimens, according to availability and total population size (collection of altogether more than 800 samples from all the species is anticipated). Basic habitat characteristics will be noted on site, and, especially in the case of the most taxonomically and autecologically variable species, *C. glomerata*, it will be based on phytosociological relevés (Braun-Blanquetian for uniform patches, simple accompanying species lists with abundance estimations for mixed, disturbed or transitory patches). For this species, apart from the usual voucher specimens, more abundant herbarium material (representing the morphological diversity of sampled populations) will be collected to enable potential taxonomic considerations.

2. LABORATORY STUDIES

Molecular diversity studies will be based on DNA analyses using two methods:

1) AFLP – a very useful method (22) both when attempting to answer detailed biogeographical questions (even at a regional scale) and in genetic diversity research and population structure testing. Restriction fragment pattern is derived from the whole genomic DNA, allowing to establish the general genetic diversity level in the studied populations and the degree of their affinity.

2) DNA direct sequencing – establishing nucleotide sequences of the chosen, PCR-amplified fragments of cpDNA from 1–3 samples per particular studied population should allow to unequivocally establish the diversity of the analyzed cpDNA regions over the whole studied range of a given species (see e.g. (19) and (20)). This should enable reconstruction of their range-forming history (colonization dynamics, migration routes). This method has been recently successfully applied to an alpine species of the studied genus – *Campanula alpina* Jacq. (18).

Based on the results yielded by the two methods, patterns of genetic diversity and divergence of populations will be inferred and discussed. At the final step, a comparative analysis of phylogeographies of all studied species will be performed to assess relative similarities between species and potential correlations of their distribution forming history with their ecological requirements.

ACKNOWLEDGMENTS

Research is financed from the national science support funds within a project no. N N304 362738 in the years 2010–2013.

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