

SELF-REVIEW AND PRESENTATION

Diplomas, scientific degrees

- 07.07.1999 **Master of Science in biology**
University of Białystok, Faculty of Biology and Chemistry, Institute of Biology
Supervisor: Prof. dr hab. Emilia Brzosko
Title of Master's thesis: Floristic diversity of mineral islands in the Brzeziny Kapickie Range
- 04.07.2006 **Doctor of biological sciences in the field of biology**
University of Białystok, Faculty of Biology and Chemistry, Institute of Biology
Supervisor: Prof. dr hab. Emilia Brzosko
Title of doctoral dissertation: Genetic diversity within geographical range and colonization history of *Iris aphylla* L.

Information about employment in research units

- 01.10.1999 University of Białystok, Faculty of Biology and Chemistry, Institute of Biology, Department of Botany, assistant
- 01.10.2007 – to date, University of Białystok, Faculty of Biology and Chemistry, Institute of Biology, Department of Botany, assistant professor

**ACHIEVEMENT PROPOSED AS THE BASIS FOR
OBTAINING THE RANK OF DOKTOR HABILITOWANY**

A series of four original papers is proposed as an achievement in the sense of Art.16 Par. 2 of the Law of March 14, 2003 on scientific degrees and scientific titles as well as degrees and titles in the arts (Dz. U. nr 65, poz. 595 ze zm.).

**Genetic diversity patterns of plants
with the circumboreal distribution**

1. **Wróblewska A.** 2014. Genetic diversity and spatial genetic structure of *Chamaedaphne calyculata* (Ericaceae) at the western periphery in relation to its main continuous range in Eurasia. *Folia Geobotanica* 49: 193-208.
IF: 1.565, MSHE: 25

2. **Wróblewska A.** 2013. High genetic diversity within island-like peripheral populations of *Pedicularis sceptrum-carolinum*, a species with a northern geographic distribution. *Annales Botanici Fennici* 50: 289-299.
IF: 0.657, MSHE: 15

3. **Wróblewska A.** 2013. The phylogeographical and population genetic approach to the investigation of the genetic diversity patterns in self-incompatible clonal and polyploid *Linnaea borealis* subsp. *borealis*. *Botanical Journal of the Linnean Society* 173: 64-76.
IF: 2.589, MSHE: 30

4. **Wróblewska A.** 2012. The role of disjunction and postglacial population expansion on phylogeographical history and genetic diversity of the circumboreal plant *Chamaedaphne calyculata*. *Biological Journal of the Linnean Society* 105: 761-775.
IF: 2.193, MSHE: 20

**PRESENTATION OF SCIENTIFIC AIMS OF THE FOREGOING PAPERS
AND ACHEIVED RESULTS**

The usage of molecular markers and advance molecular techniques in the field of biogeography have revolutionized this discipline and help to complete the knowledge about, i.e. the origin of species, locations of their refugia and postglacial colonization routes. Phylogeography as the sub-discipline of biogeography, is the study of the processes that may be responsible for the contemporary geographic distributions of gene lineages. Additionally, phylogeography provides the empirical and conceptual bridge between population genetics, demography and/or phylogenetic. Unfortunately, the phylogeography of circumboreal plants has been not described in detail, despite the fact that distribution area of these taxa covers ca. 13% of the Northern Hemisphere (Schultz 2005). Moreover, the surveys of genetic diversity patterns of this group are still scarcer than those of circumpolar one (arctic/arctic-alpine). This fact is surprising, because the circumboreal plants have important contribution in global species diversity. The preservation of genetic diversity within geographical ranges of circumboreal taxa is also important in the context of climate changes (Alsos et al. 2012). The climate warming might affect the predicted loss of genetic diversity, and thereby the susceptibility of species to genetic depauperation under range reduction. Because genetic diversity is important for species' persistence and evolutionary potential and also for community structure may affect all levels of biodiversity.

In the recent year, with the most comprehensive surveys using a few molecular markers, the genetic diversity patterns within northern species have been interpreted with greater precision. In this way, the investigated species were found to have more complex evolutionary history, which a single molecular marker would not necessarily have revealed (Abbott et al. 2000; Holderegger & Abbott 2003; Alsos et al. 2005, Schönswetter et al. 2007; Eidesen et al. 2007; Ehrich, Alsos & Brochmann 2008; Keller et al. 2010; Westergaard et al. 2010).

The four original papers, which are achievement proposed as the basis for obtaining the rank of “doktor habilitowany”, focus on the surveys of genetic diversity patterns within three circumboreal plants (*Chamedaphne calyculata* (L.) Moench, *Linnaea borealis* subsp. *borealis* L. and *Pedicularis sceptrum-carolinum* subsp. *sceptrum-carolinum* L.). Furthermore, these papers belong to the first ones in the literature, considering the entire geographical range of this plant group and the factors (e. g. life-history traits, biological and population properties), which are frequently reported as the influencing genetic diversity at the population and species level. In these studies, both cpDNA and nDNA and two molecular techniques (Sangers DNA sequencing and amplified fragment length polymorphism, AFLP) were applied. The investigations of the genetic diversity patterns were considered at the different scales of geographical ranges, i.e. at the population as well as groups of populations existing in the continuous and at the periphery of range. This hierarchical approach yielded to describe more precise the genetic diversity within this plant group.

The results of my studies showed that circumboreal plants were characterized by different phylogeographical structure. First, the clear phylogeographical structure, i.e. the distinct geographical distribution of the genetic lineages, reflected the barriers of gene flow. On the other hand, the shallow phylogeographical structure in the other taxa, can explain by the lack of any barriers and multi-directional gene flow among populations. The cpDNA and AFLP data indicated putative refugia in Beringia and south-eastern North America similar to arctic-alpine plant species as well as the areas in central Asia and in Alps. The Alps were also often recognized as the areas of persistence of populations as the glacial relict during the Last Glacial Maximum. At this stage of my investigation, I do not exclude these two hypothesis, because of a few Alpine populations have only survived to the present day. On the other hand, I have also pointed out that both private and common cpDNA haplotypes, which belonged to one genetic lineage, were observed within Alpine populations. This phenomenon can suggest that isolation of these populations was relatively recent and they could be enriched by immigrants in postglacial colonization processes.

Additionally, AFLP data have revealed that the similar genetic diversity patterns (e.g. relatively low genetic diversity in populations at the margin and in continuous range) within the these studied taxa, which differed by life-history, biological and population properties, was observed. The lack of genetic diversity gradient from the center to the margin of species' range (ACH hypothesis, *the abundant centre*

hypothesis, Sagarin & Gaines 2002) did not confirm the leading-edge colonization as well as marginal-central population model. The isolated populations at the edge of range occurred relatively recent and was rather result of fragmentation of habitats under the natural and anthropogenic disturbances.

The first of my paper revealed the genetic diversity patterns of *C. calyculata*, the plant with the widest geographical range from the three investigated circumboreal taxa, with distribution in the Eurasia and North America (Wróblewska A., Biol. J. Linn. Soc. 2012, 105: 761-775). The second paper showed the more complex microevolutionary processes in populations of this species only in Eurasian geographical range (Wróblewska A., Folia Geobot. 2014, 49: 193-208). Unquestionably, the cpDNA (*trnL* (UAA), *trnL-F*) as well as ITS sequences revealed that there are two genetic lineages i.e. north-eastern North America and Eurasia/north-western North America and showed that north-eastern North America harboured more haplotypes than Eurasia/north-western North America and these haplotypes within two groups were almost entirely disjunct (gene flow between regions was rather absent). The clear disjunction between north-eastern North American and Eurasian/north-western North American geographical range and distinct phylogeographical history between populations between these geographical regions were effect of spatial and temporal differences in the formation of peatlands after the last glaciation. Halsey *et al.* (2000) gave evidence that the occurrence of *Sphagnum*-dominated habitats for *C. calyculata* was restricted to two distinct regions: Alaska and the east coast of North America (narrow band east of 90°W between 33° and 37°N) until 12 000 years BP. I assumed that, the observed pattern of genetic structuring can be explained in terms of two putative glacial refugial areas (Beringia and south-eastern North America), and further suggested that the corresponding populations have distinct evolutionary histories. My investigations also pointed out that the present-day population structure is the result of rapid expansion, probably since the last glacial maximum but only in north-eastern North America this colonization took place by stepping stone model. The present studies have also shown that despite the previous geographical isolation of the two groups, a secondary narrow contact zone has arisen in north-western North America through homogenization of nuclear genomes through pollen-mediated gene flow.

Furthermore, they verified the status of populations considered as relicts especially in Central Europe (Poland; Kruszelnicki, 2001). In central and north-eastern Europe, macrofossils of *C. calyculata* were dated to ca. 8500-2800 years BP,

suggesting that earlier this species may have been absent there. A succession of studies has highlighted high genetic differentiation between relict populations and those from the main distribution areas. My study of *C. calyculata* found weak and nonsignificant genetic differentiation between its outermost Polish populations and those from the continuous range. Other evidence supporting the postglacial occurrence of these populations comes from palaeobotanical data from a peat bog in western Poland, which dated the first expansion of this species to only 100–150 years ago (Latałowa 1999). This statement also concerned the two other taxa, which were established in Central Europe during postglacial colonization and in the present day, their often occurrence is still observed in this area.

In the *C. calyculata* study, the AFLP data indicated surprisingly low and similar genetic diversity characteristic within its postglacial populations over the whole range. Unlike in north-eastern North America, there was evidence of only slightly reduced levels of gene diversity within the populations located in Eurasia/north-western North America. The genetic diversity of this species did not support 'leading edge' colonization or the 'central-marginal' model in which genetic diversity should be depleted within populations at the margins of the species range in relation to refugial and/or populations from continuous range. My survey indicated that reduced genetic diversity within both refugia of *C. calyculata* shaped its low within-population genetic diversity inside the continuous geographical range, possible refugia, and at the periphery. However, the life-history traits of this plant might also play a large role in its low genetic variation. Flowering and fruit set were observed to be high only in a few populations in the continuous geographical range, and it was low in populations at the margin of species range. *Chamaedaphne calyculata* populations at the margin were generally small, but there was evidence that inbreeding was a significant influence only in the Eurasian/north-western North.

In the case of circumboreal plant, the important factor is the dispersal potential, particularly long-distance dispersal of seeds, despite the lack of morphological adaptation to this transport. The possibility to concurrency of multi-directional gene flow and admixture of different gene pools within populations of these plants throughout geographical range were in contrast to genetic diversity patterns within arctic-alpine and/or alpine taxa. The barriers of gene flow such as mountains and/or habitat fragmentations displayed less important role in circumboreal than in circumpolar plants.

Linnaea borealis subsp. *borealis* was the second circumboreal taxon in which the genetic diversity patterns was described. My survey has revealed that weak phylogeographical structure, inferred from plastid DNA, and moderate genome-wide diversity, estimated from AFLP markers, are general characteristics of self-incompatible clonal and tetraploid taxon in Eurasia. A shallow evolutionary history (six closely related haplotypes) and/or rapid population expansion as well as life history traits, i.e. clonality and breeding systems, may explain the genetic diversity patterns of this species across its range. The distribution of cpDNA haplotype diversity showed a pattern of 'northern purity' (at high latitudes, two haplotypes) vs. 'southern richness' (at low latitudes, six haplotypes) within the geographical range. There were also pronounced inconsistencies between cpDNA and AFLP within populations from eastern Ural Mountains, which contained more rare AFLP fragments than did the southern populations from Europe. This may indicate that the putative refugial areas of this circumboreal plant species were present both in southern Europe (i.e. the Alps) and/or in central Asia. The Alps are often recognized as areas of persistence of populations as relicts or *in situ* refugia during the Last Glacial Maximum. Although, the populations and individuals from the Alps characterized by distinct plastid haplotypes, were not strongly genetically differentiated by AFLP markers comparing to the other Eurasian populations. These patterns can be explained by the recent plastid DNA sequence divergence in this taxon, rapid population expansion and/or episodes of multidirectional nuclear long-distant gene flow among populations.

The weak phylogeographical structure was in agreement to the genetic diversity pattern in Eurasian geographical range of this taxon. The existence of two genetic pools and the high admixture of them within populations showed the multi-directional postglacial colonization and lack of strong genetic barriers of gene flow among populations. The interesting conclusions arouse from comparison of genetic structure of its populations and biology as well as life history traits (self-incompatibility and vegetative reproduction). In Scottish populations, higher genetic diversity was observed in Balmoral (EBA) relative to Curr Wood (ECW) (Scobie & Wilcock 2009), in agreement with the present study (populations EBA and ECW, respectively; $Fragpoly = 33.7\%$, $H = 0.104$ vs. $Fragpoly = 17.5\%$, $H = 0.093$). Higher genetic diversity in Balmoral coincides with higher variability of flower colour (i.e. the high number of genets) and high natural fruit set, indicating high clonal diversity. The different situation

was observed in populations characterized by low level genetic diversity, where the low number of genets were noted.

In the case of the last of studied circumboreal taxon, *Pedicularis sceptrum-carolinum* subsp. *sceptrum-carolinum* the detailed genetic structure was investigated in four island-like, peripheral populations. Similar to the studies of above-mentioned circumboreal taxa, my researches confirmed that populations of this plant in Central Europe should be considered as postglacial than relict units. Additionally, in this populations the admixture of gene pools, relatively low genetic differentiation and lack of private alleles confirmed this statement.

Despite the fact that the investigation of genetic studies including the populations from the wider part of geographical range are under scientific description (manuscript in preparation), this study based only on four peripheral populations revealed that these patterns differed from other circumboreal plants. Despite the strong isolations between the four populations (ca. 35-350 km), and irrespective of the small population size (14-50 individuals) and areas (6-100 m²), they preserved relatively high genetic diversity in comparisons with other perennial and outcrossing taxa. The genetic diversity was also higher than in other *Pedicularis* species studied so far. Among the factors generating such high genetic diversity was the polyploidy origin of *P. sceptrum-carolinum* subsp. *sceptrum-carolinum*, domination of sexual reproduction and mixed mating strategy.

The practical application of my researches is multi-faceted. The analysis can focus on genetic resources of investigated taxa and the hotspots of genetic diversity, which are important for evolutionary potential (their ability to adapt to environmental changes) and conservation strategy of biodiversity view. The knowledge of level genetic diversity is important in conservation action (e.g. introduction) at the periphery of range in Central Europe (Poland). The protection of genetic resources is also important for long-term survival of taxa particularly under climate changes. The investigation revealed that plant taxa can response in different ways in relation to the prediction of change within geographic range and genetic diversity under climate warming in the future. Undoubtedly, plants have potential to significant lost their geographic ranges in the nearest future, while the genetic diversity could be still shape on the similar level or slightly lower to the present day (Alsos et al. 2012). Subsequently, the results might be used to analysis of natural selection on the genome

level (AFLP markers) and describe the tendency of these changes under climatic changes.

Summary of achievements and scientific activity (a detailed description is presented in Appendix no. 4) and a discussion of other scientific and research achievements

I have been developing my interests related to the natural sciences from the first year of studies in biology at the Institute of Biology, University of Białystok (IoB, UoB). In 1994-1997, as a member of the Biology Students Scientific Circle operating at IoB, I participated in many national and international research projects focused on different topics, such as botany, ornithology and herpetology. From the first year of studies I have also been actively participating in projects executed at the Department of Botany, supervised by Prof. E. Brzosko, who is researching the life history of species from the family *Orchidaceae* in the Biebrza Valley. In 1999, at the same department, under the supervision of Prof. E. Brzosko, I defended my Master's thesis on the floristic diversity on mineral islands in the Biebrza Valley. The thesis was awarded 2nd prize in a contest organized by the Provincial Council of the Polish Federation of Engineering Associations (SNT NOT) in Białystok for the best Master's degree thesis in ecology and environmental protection in 1999 and a distinction from the Minister of the Environment in the contest "Science for Environmental Protection and Nature" for the best Master's and Bachelor's theses prepared at Polish and Ukrainian universities in 2000. After graduation I pursued my further scientific and professional career, which I continue to date, with the Department of Botany, IoB, UoB, and various research projects on ecology and evolutionary biology executed there. During that period, working in the research group led by Prof. E. Brzosko and developing my own research methodology, I participated in three scholarships in Poland (A. Mickiewicz University, Department of Genetics, Poznań; International Institute of Molecular and Cellular Biology UNESCO/PAS, Warsaw; Institute of Plant Genetics and Acclimatization, Radzików, Błonie), and one scholarship abroad (Joseph Fourier University, Laboratory of Ecology, Grenoble, France). All of them have led to scientific cooperation with researchers from these centres, and papers were published in scientific journals, including those from the ISI Master Journal List. Because of the experience I have gained in Poland and abroad, and scientific cooperation with Polish and foreign centres

on various research projects resulting in published papers, I received a scholarship in 2004, with prolongation to 2005, from the Foundation for Polish Science, and five awards from Rectors of the University of Białystok. Through the acquisition of financing for two projects from the Committee for Scientific Research (CSR), in 2001-2003 (6PO4F 091 21) and 2003-2006 (3PO4F 009 25), I was able to carry out research on the patterns of genetic diversity and phylogeography of *Iris aphylla*, a species of the Pontic flora. This research became the topic of my doctoral dissertation, which I defended in 2006 under the supervision of Prof. E. Brzosko.

My interests are focused on many areas, and therefore, after receiving my PhD, I continued parallel research merging the evolutionary biology, ecology and population genetics of protected and rare plants, mainly Orchidaceae in Poland, in cooperation with Prof. E. Brzosko, I. Tałałaj, PhD., B. Ostrowiecka, MSc, E. Jermakowicz, MSc, and P. Mirski, MSc (IoB, UoB). I also analyse the phylogeographical patterns and evolutionary history of different groups of plants (with K. Kostrakiewicz, PhD, IoB, PAS), including taxa with the northern geographical range, and those in mountain ranges, mainly the Carpathians, as well as taxonomic problems associated with the genus *Aconitum* (Dr. hab. J. Mitka, Botanical Garden, Jagiellonian University).

So far, I have published 29 papers (this does not include conference proceedings). Twenty-one papers are included in the JCR database, such as *Molecular Ecology*, the *Biological Journal of the Linnaean Society* and the *Botanical Journal of the Linnaean Society*. My papers indexed in the Web of Science have, to date, been cited 192 times (145 times without self-citations). The Hirsch Index (HI) is 8 (Web of Science; July 2014). The total IF for my papers is 21.822. The vast majority of my papers were produced under the framework of national grants for which I was the principal investigator (4 projects financed by the National Science Centre (NSC), Ministry of Science and Higher Education/MSHE and CSR) or an key investigator (7 projects financed by MSHE and CSR). The results of studies in which I was the principal investigator or key investigator have been presented in the form of nine lectures and eighteen poster sessions at national or international conferences and workshops. I have organized four nationwide conferences in Poland. I have also been responsible for the organization of education. During my work, I have conducted lectures, lab classes and seminars for BA and MSc students of biology and environmental protection, and for students of post-graduate studies in biology, and under the Erasmus programme. I am an assistant promotor in two PhD dissertation,

and I was a scientific supervisor for 2 students preparing their MSc theses, and for 15 preparing their BA theses. In total I have supervised 8 research internships in the field of analytical methods employing molecular markers for 4 scientists from the Institute of Biology, UoB, and 4 scientists from other scientific centres in Poland. To popularize science, I undertook the preparation of three scientific experts' reports on the protection of plant and animal species.

The further part of this self-review contains a brief description of my interests and research achievements, divided into major topics.

Genetic diversity resources of Orchidaceae and their conservation in Central Europe

One of the first and important topics of my research has been the use of molecular markers to explain a number of problems in demographic studies which could not previously be explored using the standard tools of ecology. In addition, an important aspect of the research has been to identify life history traits responsible for shaping the genetic structure of the studied populations. Over 15-year-long observations carried out by Prof. E. Brzosko (IoB, UoB) in the Biebrza Valley on five orchid species (*Cypripedium calceolus*, *Epipactis helleborine*, *E. atrorubens*, *Neottia ovata* and *Cephalanthera rubra*), and then the use of molecular markers for the analysis of the genetic structure of their populations, have shown how important the integration of demographic and genetic studies is, defined as a trend in molecular ecology dynamically developing in the late 20th and early 21st century. It is worth noting that this approach has become a core element of European research on this rare and highly biologically diversified group of plants, and this is proven by the fact that we were chosen to organize the 6th International Orchid Workshop in 2016.

I was able to master molecular techniques, mainly for the analysis of allozymes, and carry out further genetic analyses at IoB, UoB thanks to an internship completed at the Department of Genetics, Adam Mickiewicz University, at that time headed by Prof. J. Szweykowski. Findings from genetic analyses, together with data from long-term demographic studies, and experiments determining the potential of the reproductive system and seed dispersal strategy in isolated island populations of the above-mentioned orchid species from the Biebrza Valley, demonstrated that in each

of the studied populations of different species of orchids genetic and genotypic diversity are shaped in different ways, and not always in line with the general genetic patterns presented for species with specific life history traits. To summarize the overall research on selected Orchidaceae species on local (Biebrza Valley), regional (north-eastern Poland) and national scales, the key conclusions, and our important contribution to the study of this group of plants include: revealing the fact that the genetic diversity within populations is strongly shaped by such factors as the type of reproduction and reproductive system, the nature of isolation, population size, their post-glacial history, and seed dispersal strategy. The role of these factors varies depending on the population and species level. Collaboration with Prof. Irène Till-Bottraud and Dr. Florence Nicole from Laboratoire d'Ecologie Alpine, Université Joseph Fourier, Grenoble, France, in 2002-2003, under the project Polonium 4229.I (KBN), resulted in a joint publication in which we extended the study area to cover the French Alps, and confirmed the impact of previously indicated factors on the genetic structure of Alpine populations.

Another important achievement was to use demographic and genetic data for the protection of Orchidaceae in Poland. As a result, long-term studies carried out by our group were in 2007 awarded by the Minister of the Environment for the concept of the protection, development and use of the environment and its resources, presented in the work "Ecological and genetic diversity of Orchidaceae species as a measure of their evolutionary potential and background for conservation activities".

Demographic and genetic patterns in populations of protected plant species

The first studies on the demographic and genetic diversity of selected protected northern and Orchidaceae species were focused mainly on their populations in Poland. Demographic observations and analysis of the genetic structure of these populations were significant from the point of view of conservation genetics, i.e. the use of information on diversity resources at the population and species levels for conservation purposes. The results of these studies have been in part used for conservation measures involving the introduction of individuals from selected populations of the orchids *C. calceolus* and *C. rubra* onto patches of *Tilio-Carpinetum* (mineral islands) in the Biebrza Valley (project CKPŚ, POIŚ.05.01.00-00-374/12, 2012-2014).

In addition to the projects described above, there are two others I consider important parts of my academic achievement. In the first project, I analysed the genetic

diversity patterns of *I. aphylla* within the European part of its geographical range, as well as the colonization routes in Europe. It was the first detailed report on this Pontic species in Europe (Wróblewska A., Plant Syst. Evol. 2008, 272: 49-65; Wróblewska A. et al., Ann. Bot. Fennici 2010, 47: 23-33).

I demonstrated that the genetic diversity within the species *I. aphylla* shaped at a lower level than in other species representing similar biology, which was surprising for this polyploid taxa. I confirmed that the genetic diversity of the species is at a level similar to that of rare species, or those threatened with extinction, and species with a narrow or regional geographical range in size comparable to the range of *I. aphylla*. The results of these studies point out the need for the protection of *I. aphylla*, not only in marginal and isolated populations, but also in the centre of its geographical range. Searching for genetic diversity patterns within this species, I analyzed them on different geographical scales, from single populations to the entire geographical range. I demonstrated that the genetic diversity of *I. aphylla* was at similar levels, both in populations in the centre of the range and in marginal ones. Population size and the level of generative reproduction were not decisive factors for the genetic diversity level. The percentage of unique alleles specific for geobotanical regions confirmed my speculation that the studied areas in Europe were colonized relatively recently, probably during the Younger Dryas and early Holocene, i.e. the post-glacial period most favourable to the migration of steppe plants. Based on the carried out analysis I was able to assume that the post-glacial colonization by *I. aphylla* in Europe originated from a single refugium. The use of AFLP markers allowed for the identification of the hypothetical colonization routes of this species in Europe. The first route ran from areas in the central range towards the north-eastern Hungarian Lowland. The second route probably ran directly towards the Slovak Karst, and the third was associated with spreading towards the areas of Northern Europe above the Carpathian Mountains (Lublin Upland). From the Lublin Upland, one of the colonization routes spread westwards to the Małopolska Upland, and the second towards northern Europe (the Biebrza Valley). I do not exclude the migration of diaspores from south European populations to the populations located northwards of the Carpathians.

The second project, in which I was the key investigator (CSR, 2 P04F 063 28, 2005-2007), concerned the previously discussed problems relevant to demographic and genetic studies on subpopulations of a protected species, *Iris sibirica*. We were the first to describe the genetic diversity of *I. sibirica* subpopulations (Kostrakiewicz K.,

Wróblewska A., Ann. Bot. Fennici 2008, 45: 186-194). We demonstrated that the significantly low genetic variability within subpopulations, or even its monoclonal nature, is associated with expansive vegetative reproduction (aging and growth of rhizomes) and the persistence of a single genet or, in rare cases, the formation of several genets. Thus, even if new individuals occur in the population, they result from inbreeding. However, no recruitments have been noted in these populations in natural habitats. A significant share of juveniles was found only in experimental gaps one year after uncovering the top layer of turf. These studies imply that measures aimed at the conservation of this species, and even the introduction of individuals from these populations to others, should consider the use of at least five genets, to prevent the formation of monoclonal populations. Our findings are also very interesting because the studied subpopulations are in the centre of the geographical range for *I. sibirica*, which contradicts the central-marginal hypothesis.

Phylogeographical structure of other selected plant species in Eurasia

Still expanding my interests in the phylogeography of plants with circumboreal range, I participated in research on *Malaxis monophyllos* (White Adder's Mouth), a rare orchid representing Eurasian flora (MSHE, N N304 606038, principal investigation E. Jermakowicz, MSc, IoB, UoB). Although the geographical range of this species is mainly connected with the boreal zone of the Eurasian continent and North America, a few isolated populations of this species are found in Europe in the mountains, including the Carpathians, the Sudeten Mountains, the Alps, the Bohemian Mountains, and the uplands in Central Europe. The status of the latter populations is extremely interesting, because they are found only in anthropogenic habitats (in southern Poland), which is unique for the entire geographical range of this species. In studies using four cpDNA polymorphic sequences (*trnL*, *trnL-trnF*, *rps16*, *accD-psal*) from 68 Eurasian populations we showed that phylogeographical patterns may differ significantly from those commonly presented for circumboreal plants. This species was characterized by a very high diversity of haplotypes (19 cpDNA haplotypes) and we also distinguished basing on cpDNA three centres of genetic diversity within the European geographical range. The first of them was located in the area of the Central Alps, the second in the northern area, and the third in Eastern Europe. Despite the persistent high genetic diversity there was weak phylogeographical structure. We revealed that within the Eurasian range cpDNA haplotypes occurring in the two genetic

lineages are randomly distributed, indicating more likely a multi-directional postglacial colonization, which was rarely observed in Alpine or Arctic-Alpine plants. Multi-directional gene flow could result from the availability of suitable habitats (bog or wet meadows) for this species in the past, which are currently being degraded due to human activity. Therefore, the fragmentation of the geographical range in Europe can certainly be considered a recent process. From the point of view of genetic diversity resources, anthropogenic populations of this species in the uplands of Central Europe play an important role. Although their origin is not fully explained, their emergence in the region is dated to the last decade. These populations, due to the high level of haplotype diversity and the presence of unique cpDNA haplotypes, may become a reservoir for the further colonization of Europe by *Malaxis monophyllos*, especially because the colonization of anthropogenic habitats as alternatives to natural habitats can be associated with certain adaptations at the genome level, which will be tested in the future using AFLP markers (paper under revision at Bot. J. Linn. Soc.).

Continued cooperation with dr. hab. J. Mitka, under the research project led by him and financed by MSHE (N N304 071940, 2011-2014), also concerned the processes being in the scope of interest, i.e. phylogeography. To study phylogeographical patterns using AFLP markers we have selected three species associated with *Tilio-Carpinetum* communities: *Carpinus betulus* (European Hornbeam), *Stellaria holostea* (Stitchwort) and *Galium schultesii* (Schultes' Bedstraw), and the study area has been restricted to the Carpathians. These studies are among the first reported in the European literature on the cryptic refugia of forest flora in the Carpathians, and have not so far been carried out in this important area. Previous comprehensive phylogeographical studies in the Carpathians concerned only alpine plants in terms of their glacial and post-glacial history in relation to the history of Alpine flora. We demonstrated that for *C. betulus* three areas defined as cryptic refugia could be located in the Slovak part of the Western Carpathians, Transcarpathia and Podolia, which indicates their possibly higher number than previously hypothesized, and the great role of these sites in maintaining genetic diversity within the species. We also cannot exclude the existence of refugial areas in the internal parts of the Western Carpathians, namely in the Pieniny Mountains, as suggested by the high number of unique alleles found for the populations of this species. Another interesting finding was the identified multi-directional gene flow between the populations of this species in Podolia and the Bieszczady Mountains, and Transcarpathia and the Bieszczady

Mountains, which potentially indicates two sources of colonization for the populations of the Bieszczady Mountains. We also assume that the Polish part of the Carpathians was a *genetic melting pot*, where many postglacial migration routes of this species running towards northern Europe met.

Because project has been finished in June 2014, the first publication has been prepared and concerned the phylogeographical structure of *C. betulus* in the Northern Carpathians (currently under review in *Quaternary Research*). Analyses and preparation of subsequent publications on *Stellaria holostea* and *Galium schultesii* are in progress.

Phylogeny and taxonomy

Phylogenetic and taxonomic analyses in their strict sense are rare in my research, but the use of DNA markers and cytogenetic analyses in two cases, namely for *I. aphylla* and *Aconitum* sect. *Aconitum* and sect. *Cammarum* genera helped to revise the taxonomic status of the investigated plants.

Cytogenetic and AFLP analyses in populations located in the Slovak Karst, Volhynian, Podolian, Lublin and the Małopolska Uplands, as well as in the Biebrza Valley allowed me to begin research on the verification of the taxonomic status of *I. aphylla* in different parts of its geographical range (Wróblewska A. et al. 2010, *Ann. Bot. Fennici*, 47: 23–33). Controversies that have arisen in relation to the subdivision of *I. aphylla* into lower taxonomic units (subsp. *hungarica* – in Slovakia and Hungary, var. *typica*/subsp. *bohemica* – in Poland, Romania, Ukraine and Russia) concern the identification of different taxa based exclusively on morphological traits. The use of the above-presented research methods confirmed my speculations that division into lower taxonomic units is unjustified. *Iris aphylla* was reported as an autotetraploid, and my research confirmed this. In addition, diploid individuals were identified in one population from the Slovak Karst. It can be concluded based on two karyotype groups identified in the populations from the analysed part of the European geographic range that we are dealing with different chromosomal races, and that polyploidization could have occurred many times during the history of this species. The geographic distribution of certain karyotypes from a single group may be closely related to a specific geographic region or occurred only in one population. These studies have also indicated incongruence between the results of AFLP and cytogenetic analyses for the population from the Podolian, Volhynian and Lubelska Uplands, which can confirm

independent evolution on DNA and chromosome levels, and suggest that the differentiation of chromosomes may be a recent process.

In 2011 I began my cooperation with dr. hab. Józef Mitka (Botanical Garden, JU) and dr. Piotr Boroń (Faculty of Forestry, University of Agriculture, Krakow) to explain complicated phylogenetic relations within *Aconitum* genus of sect. *Aconitum* and sect. *Cammarum*. The research project in which I participated concerned the use of DNA molecular markers in taxonomic research. So far, results from these studies have been presented in the form of oral presentation (Boroń et al. 2011, PTB, Krakow) and a poster at an international conference (Mitka et al. 2013, First Interdisciplinary Symposium Biogeography of the Carpathians, Krakow). Moreover, results are still supplemented with selected sequences of nuclear and chloroplast DNA from species of *Aconitum* sect. *Aconitum*. By sequencing DNA of *Aconitum* from Europe and Asia and 2 species of subgenus *Lycocotnum* from Asia and America we significantly expanded contemporary research on this genus and clarified the position of European taxa in the context of the diversity of the whole genus *Aconitum*. The most important results of this research include: (1) the confirmation of distinct nature of diploid species of sect. *Cammarum* and tetraploid species of sect. *Aconitum*. The observed partition between these groups almost always corresponded with the classification criteria for taxonomic sections. This may indicate that the ancestors of these lineages colonized the European subcontinent independently. Thus, an alternative hypothesis stating that the tetraploid species in the mountain ranges of Central Europe originated from a hypothetical ancestral diploid form was not supported by our findings; (2) We formulated a hypothesis explaining the evolutionary history of *Aconitum* in Europe, in which the European species, both sect. *Cammarum* and sect. *Aconitum*, originated from a diploid forest species from Western Siberia, similar to *A. pascoi*; (3) The ancestral form of the European tetraploids evolved through recombinant speciation, most likely as a result of allopolyploidisation; (4) Further evolution was independent for diploid and tetraploid lineages, and (5) The contact zones in selected regions of Europe between the European representatives of sect. *Aconitum* and sect. *Cammarum* caused an extensive gene flow, and hence the phylogenetic links between them blurred gradually.

Separate analyses to explain the nature of hybridization for two species from genus *Aconitum*, sect. *Aconitum* (*A. plicatum* and *A. firmum*) in the Carpathians and Sudeten Mts. were carried out using AFLP markers. Using different class markers we

demonstrated again that our studies explain a more detailed scenario of the species' evolutionary history. In this case we confirmed that *A. firmum* is a result of reticular evolution and, in contrast to previous studies of Mitka *et al.* (2007), hybridization between the two species occurred much earlier, probably in the Tertiary. The results of these studies were presented in a paper submitted to Acta Soc. Bot. Pol. and the review process is in progress.

Prospects

My further research plans are fully in line with the currently continued stream, and focus both on evolutionary biology studies, including selected taxa of *Orchidaceae*, and the molecular biogeography of circumboreal plants. The aim of a recently initiated project financed by NSC (2013/09/B/NZ8/03350, 2014-2017) is to explain a problem that has not been explored in detail in the international literature, i.e. whether food deception strategy in *Dactylorhiza* ssp. is beneficial in terms of evolution, particularly for diploid parent species and their polyploid ancestor. An interdisciplinary approach to answer this question, including experiments testing the reproductive system, analysis of fauna and flora in the studied populations, and genetic analyses using different molecular markers, should help to explain many aspects of this problem and identify selective mechanisms at the level of a population.

The second important issue is to extend research on circumboreal plants referring the modelling of their geographical range and genetic diversity resources under climate changes. These analyses I included in my paper (in preparation) indicate that climate warming, and thus the loss and fragmentation of a geographical range in this group of plants (*C. calyculata*, *P. sceptrum-carolinum* subsp. *sceptrum-carolinum* and *L. borealis* subsp. *borealis*) do not necessarily cause depletion of genetic diversity at the level of a population or species. However, for circumboreal plants these changes most certainly might cause the homogenization and lead to the dominance of one gene pool in populations throughout the geographical range. So far this problem has not been discussed in the literature, but considering my findings, a different approach to the conservation of genetic resources and evolutionary potential of various groups of plants should be adopted.

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