

Appendix 2

DESCRIPTION OF SCIENTIFIC ACHIEVEMENTS

NAME: Katarzyna Anna Jadwiszczak

DATE AND PLACE OF BIRTH: 17th February 1970, Białystok

ADDRESS: University of Białystok, Institute of Biology, Świerkowa 20B, 15-950 Białystok;
tel. +48 85 745 72 90; fax +48 85 745 73 01; e-mail: kszalaj@uwb.edu.pl

EDUCATION AND SCIENTIFIC DEGREES:

2002: PhD; Institute of Biology, University of Białystok; dissertation title: "Chromosomal structure of the hybrid zone between Drnholec and Białowieża races of the common shrew, *Sorex araneus* Linnaeus, 1758" (supervisor: prof. dr hab. Stanisław Fedyk)

1994: M.Sc. thesis: "Course and width of the contact zone between chromosomal races of the common shrew (*Sorex araneus* L.) near Olsztynek" (supervisor: prof. dr hab. Marek Gębczyński)

1989-1994: Warsaw University Branch in Białystok, Faculty of Mathematics and Nature, Institute of Biology

1985-1989: Zygmunt August High School no 6 in Białystok

SCIENTIFIC WORK:

1. Since 2003: Department of Genetics and Evolution, Institute of Biology, University of Białystok; assistant professor
2. July 1994 – September 2003: junior scientist at the Department of Zoology and Genetics, later at the Department of Genetics and Evolution, Institute of Biology, University of Białystok (earlier Warsaw University Branch in Białystok)
3. February 1994 – June 1994: trainee-student at the Department of Zoology and Genetics, Institute of Biology, Warsaw University Branch in Białystok; internship carried on during the fifth year of study

SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

I was born in 1970 in Białystok. In 1985-1989, I attended to the Zygmunt August High School in Białystok, to the class of general profile with extended course of the Russian language. In 1989, I passed exams to the Institute of Biology, Warsaw University Branch in Białystok, as I planned to be a teacher of biology. These plans came true partially, because I was working in the Primary School no 24 in Białystok during the fifth year of my study. I resigned from the work in school for an internship at the Department of Zoology and Genetics, Institute of Biology, Warsaw University Branch in Białystok, where I had been preparing research under my M.Sc. study. In 1994, I defended my master's degree thesis entitled "Course and width of the contact zone between the chromosomal races of the common shrew (*Sorex araneus* L.) near Olsztynek" with prof. dr hab. Marek Gębczyński as the supervisor. The issue of my thesis was a natural consequence of my activity in the student scientific club, to which I had signed up during the first year of the study. The members of this club participated and helped in the studies of scientific staff of the Institute of Biology. The study of the common shrew chromosomal variation was in a group of the most significant and awaited at that time. Investigation was supervised by prof. dr hab. Stanisław Fedyk. After a graduation I worked as an assistant at the Department of Zoology and Genetics. In 2002, I defended the Ph.D. thesis "Chromosomal structure of the hybrid zone between Drnholec and Białowieża races of the common shrew, *Sorex araneus* Linnaeus, 1758". Professor Stanisław Fedyk was a supervisor of my thesis. I have been working as an assistant professor at the Department of Genetics and Evolution in the Institute of Biology of University of Białystok since 2003.

I am the first author or co-author of 31 scientific articles: 28 papers are published in journals listed in the Journal Citation Reports, two other ones are out of JCR and one chapter is in the English-language monograph [Appendix 3]. I have also written one popular article. The papers of my authorship have been cited 204 times in total (167 times without self-citations). The h-index (according to the Web of Science, 17.03.2014) reached the value of 8, and the total summarized rate of IF for published articles amounted to 33.142 [Appendix 3]. Most of my papers have emerged as the effect of realization of six national grant projects. I was the principal investigator of the three projects, in the other ones I was a main investigator.

Some of my publications resulted from the cooperation with foreign scientists: dr Agnés Horn from University of Lausanne in Switzerland and dr Oleg V. Sozinov from the Janka Kupala National University of Grodno in Belarus. Cooperation with dr O. Sozinov has

resulted in the publication of three papers in *Plant Systematics and Evolution*, *Tree Genetics and Genomes* and *Plant Ecology & Diversity* as well as in finding a new orchid species in Belarus - *Ophrys insectifera* (<http://sb.by/files/MT/09/N28/06.pdf>). I also cooperated with dr Mladen Vujošević from the Institute for Biological Research „Siniša Stanković” in University of Belgrade in Serbia. In cooperation with dr M. Vujošević, I analyzed the chromosomal variability of Soricomorpha species in Serbia in 1997.

The results of my studies were presented as the oral and poster presentations. I was first author of eight oral presentations [Appendix 3]. My contribution at the 7th International Meeting of the International *Sorex araneus* Cytogenetics Committee (ISACC) in St.-Petersburg in 2005 was possible thanks to a scholarship of the Warsaw Scientific Society and Foundation for Polish Science.

During my work I had lectures, laboratories and seminars with students of general, molecular and environmental biology, protection of environment as well as with listeners of postgraduate study of biology. I was a tutor of seven M.Sc. thesis and three bachelor works. I was also responsible for teaching molecular techniques a Ph.D. student from Białystok University of Technology, within the Operational Programme *Human Capital* [Appendix 3].

I was also involved in activities related to the organization of science: I revised manuscripts for the JCR journals, i.e. *Acta Theriologica* and *Revista Brasileira de Entomologia*, chapters to the English-language monograph of the Polish Botanical Society Branch in Białystok and a grant for the Czech Science Foundation. Twice, I was a member of the Council of the Institute of Biology, once a member of the Council of the Faculty of Biology and Chemistry and once a member of Electoral College of the University of Białystok. For three years I worked as a secretary of recruitment commission at the Institute of Biology. Since 2009, I am a coordinator of the Erasmus program at the Institute of Biology. Research conducted by me in Belarus resulted in establishing contacts with directors of the National Environmental Institution „Berezinskij Biosfernyj Zapovednik”, and later in signing an agreement on mutual cooperation between NEI and University of Białystok. Since 2009, I am a member of the Polish Botanical Society (PBS). In 2010-2013, I was a secretary of the Revision Commission of the PBS Branch in Białystok, now I am a chairman of this commission. Moreover, since 2013, I am a secretary of Dendrology Section of PBS. This section was re-launched on the 56th Congress of PBS in Olsztyn.

My first scientific interests were focused on the chromosomal variability of the common shrew *Sorex araneus*. In the karyotype of the common shrew, there are 40 chromosomal arms, which are labeled from *a* to *u* by the International *Sorex araneus* Cytogenetic Committee (ISACC; Searle et al. 1991, *Mém. Soc. Vaud. Sc. Nat.* 19). These

arms can stay as unfused single-armed chromosomes (acrocentrics) or, as a result of Robertsonian fusion, may form two-armed chromosomes (metacentrics). Co-existence of different chromosomal morphs in a population (metacentric homozygote, simple Robertsonian heterozygote, acrocentric homozygote) is called a polymorphism. Group of adjacent or recently separated populations, having the same set of metacentrics and acrocentrics, is known as a chromosomal race (Hausser et al. 1994, *Folia Zool.* 43). Till now, more than 70 chromosomal races are described within the range of the common shrew (Wójcik et al. 2003, *Mammalia* 68; White et al. 2010, *Syst. Biol.* 59). Due to the polymorphism and racial differentiation, *Sorex araneus* is a model species to study evolutionary consequences of karyotypic variability.

Firstly, as a M.Sc. student, and later as an assistant at the Department of Zoology and Genetics, from which Department of Genetics and Evolution was arisen in 1996, I was a member of team investigating impact of chromosomal variation on the speciation process, studying the relationship between the complication of karyotype and fertility and morphology of individuals as well as analyzing modifications of the chromosomal structure of hybrid zones between *S. araneus* chromosomal races. The analysis of processes ongoing in the hybrid zones is not possible without previous description of races and their ranges. Carrying out research in the area between Wisła and Nogat rivers, we found a new chromosomal race of the common shrew, which was called Nogat. This race is characterized by the highest frequency of acrocentrics among *S. araneus* races described in Poland [Appendix 3, publication 7]. The presence of this race is consistent with a hypothesis claimed that the acrocentric chromosomes were dominant in the marginal populations of the common shrew. We did not find any genetic differentiation at the allozyme loci in four populations of the Nogat race. In turn, based on meiotic configurations of chromosomes in the common shrew hybrids we verified ranges of the Stobnica and Drnholec races in Poland [11]. These two races are very similar in respect of their karyotypes, having only one small metacentric different. Analysis of meiotic preparations was a strong evidence that the range of Drnholec race was much greater than was previously thought. Common shrews of the Drnholec race occur in southern, south-western and central parts of the country, and they come into contacts with the Białowieża in the east, and Popielno, Guzowy Młyn and Laska races in the north. We were unable to confirm an existence of the Stobnica race. If this race exists, its range has to be very limited.

Till now 11 chromosomal races of *S. araneus* are found in Poland. They belong to the two karyotypic groups: East-European Karyotypic Group (EEKG) and West-European Karyotypic Group (WEKG; Wójcik 1993, *Acta Theriol.* 38), which results in numerous hybrid

zones formation throughout the country. The hybrid zones serve as “natural laboratories” because they offer possibility to study various evolutionary phenomena. The first step of this study is description of chromosomal structures of contact zones between the common shrew races. The objective of my master’s degree research was to define a course and a width of the hybrid zone between the Stobnica (presently Drnholec) and Łęgucki Młyn races near Olsztynek town. These results were extended after M.Sc. defence and published in the paper 6 [Appendix 3]. In this work, we revealed an increase of frequency of recombinant individuals in the centre of the hybrid zone. Maximization of the frequency of recombinants is due to decrease of the frequency of interracial hybrids. We accepted the high frequency of recombinants as a mechanism enhancing fertility of hybrid populations; hence, improving the gene flow between the races coming into the contact.

The next hybrid zone described by our team was the Drnholec/Białowieża near Dęblin town. That area was a study field within my Ph.D. thesis. Preliminary results of that research were published in the paper 8. The Drnholec/Białowieża hybrid zone is interesting because of the two reasons. First, 10-element meiotic complex of chromosomes is formed in the Drnholec/Białowieża hybrids. It is one of the longest complexes known in the common shrew; hence, a substantial selection pressure against the hybrids is expected. Second, the Drnholec/Białowieża hybrid zone is stabilized on the high railway embankment. Rapid decrease of a frequency of the diagnostic metacentrics across the embankment suggests that it is a strong barrier for migration of individuals. In this contact zone we did not reveal any mechanism facilitating gene flow between the races.

It is supposed that severity of meiotic problems depends on the complication of hybrid complexes. The larger karyotypic differences exist between contacting races of *S. araneus*, the more complicated meiotic complexes in the shape of ring or chain are formed in the hybrids. Irregularities in conjugation and segregation of chromosomes involved in hybrid complexes may result in a death of generative cells or/and formation of aneuploid gametes and embryos, which in consequence may result in lowered fertility of individuals. As the lowered fertility can act as a barrier to gene flow, prompting the speciation, a next aim of our study was to estimate a fertility of *S. araneus* interracial hybrids in the different contact zones. Analysis of spermatogenesis in males having 4-element ring, 4-element chain and 5-element chain configurations, coming from the Drnholec/Łęgucki Młyn and Guzowy Młyn/Łęgucki Młyn hybrid zones, revealed that the complication of karyotype had a significant impact on germ cell death [10]. It seems, however, that the mortality level of gametes in the male hybrids in two contact zones was not high enough to reduce fertility of these individuals. Our further studies showed that lowered fertility of the male hybrids with 5-

element chain configuration might be an effect of meiotic non-disjunction, that results in formation of chromosomally unbalanced gametes and embryos [14]. In the Drnholec/Łęgucki Młyn and Guzowy Młyn/Łęgucki Młyn hybrid zones, a selection acts also against females having a hybrid karyotype. We evidenced that levels of pre- and post-implantation mortalities were significantly higher in populations with substantial contribution of hybrids forming 5-element chain (>10%) compared to the other populations [12]. We hypothesized that substantial mortality of embryos was not only a result of karyotype complication, but partly was a consequence of unfavourable environmental conditions. We also analyzed the course of meiosis and spermatogenesis in the common shrews from the Drnholec/Białowieża hybrid zone. It was shown that males forming one long (10- or 9-element) or two shorter (7- and 4-element as well as 6- and 5-element) chain configurations suffered from substantial infertility, but they were not sterile [19]. The reasons of reduced fertility of the male hybrids were both a significant level of chromosome non-disjunction and an inordinate germ cell death. It is likely that the last phenomenon could reduce the testis mass. Studies of meiosis and spermatogenesis in the Drnholec/Białowieża hybrid zone of the common shrew were conducted within my Ph.D. thesis.

Another aspect of the investigation of fitness of different karyotypic categories of shrews was morphometric analysis. We looked for a relationship between complication of karyotypes and the morphology of individuals. Studies were carried out in the three hybrid zones. Unfortunately, obtained results were not clear. In the Drnholec/Łęgucki Młyn zone, adult hybrids had the significantly lower body mass compared to the one of the parental races [9]. In turn, the same observation was made in the other hybrid zone, but only in the class of juvenile specimens. In the Drnholec/Białowieża hybrid zone, hybrids were characterized by significantly lower hindfoot length in relation to the parental race. Analysis conducted in the Guzowy Młyn/Łęgucki Młyn hybrid zone revealed no relationship between karyotype and both the size and shape of mandible in the young shrews; however, in the mature specimens there was a statistically significant relationship between karyotype and the mandible shape [16]. Immature shrews from the centre of the Guzowy Młyn/Łęgucki Młyn hybrid zone were smaller than those from the marginal populations. We suggested that a lack of such difference in the group of adult individuals could be a consequence of the overmuch mortality of shrews inhabiting the centre of the hybrid zone, where unfavourable habitat conditions existed.

In all common shrew hybrid zones under our study, we found individuals with lowered fertility parameters. Searle (1993; *Hybrid zones and evolutionary process*, Oxford Univ. Press) as well as Searle and Wójcik (1998; *Evolution of shrews, MRI PAS*) postulated that in

the case when reduced fertility of *S. araneus* interracial hybrids resulted from karyotype heterozygosity, a premating reproductive barrier could evolve, and in consequence, a speciation by reinforcement is possible. According to the model of speciation by reinforcement, the natural selection favours specimens who show a tendency to mate with partners from the same population (Barton and Hewitt 1981, *Evolution and speciation, Cambridge Univ. Press*). This prevents the gene flow throughout a hybrid zone and finally may result in the end of the speciation process. To answer a question, if chromosomal speciation is likely in the common shrew, we estimated the level of gene exchange in some hybrid zones. First attempts were done using allozyme loci [13, 17]. In two hybrid zones a gene flow, expressed as a number of migrants between populations, seems to be substantial. Despite the high level of gene exchange, the genetic differentiation was low, although statistically significant. This strongly suggests an existence of a barrier limiting migration of shrews. In the Guzowy Młyn/Łęgucki Młyn hybrid zone, Paślęk town could be a such barrier [17]. May reduction of gene flow result also from interracial hybrid infertility, being an effect of chromosomal incompatibility, in the studied areas? We did not reveal any relationship between the chromosomal and genetic variation in the two zones [13, 17]. The likely explanation may be a low level of variation of allozyme loci.

In further studies we used highly polymorphic nuclear microsatellites [18]. However, analysis of these markers conducted in the Drnholec/Białowieża showed no genetic differentiation between races coming into contact. It was surprising because a rapid decrease of the frequency clines of diagnostic metacentrics, that was observed within a distance of about 40 m [8], may suggest a presence of a strong barrier to gene flow. The reduced gene exchange between the races Drnholec and Białowieża could result from lowered fertility of male hybrids [19]. This hypothesis was supported by about 4-fold lower male-mediated gene flow compared to biparentally-mediated autosomal markers [18]. We found that lack of genetic differentiation between the Drnholec and Białowieża races was an effect of their common ancestry. Moreover, a genetic differentiation of races and a chance for chromosomal speciation in this area are obliterated by an extensive gene flow from areas adjacent to the hybrid zone.

It was supposed that lack of evidences of genetic differentiation between races of the common shrew was a result of unified analysis of microsatellites linked and unlinked with chromosomes involved in meiotic complexes of hybrids. Thus, a next step in the investigation of the impact of chromosomal rearrangements on a gene flow in *S. araneus* hybrid zones was the comparison of a genetic differentiation at microsatellite loci linked and unlinked to chromosomes involved in meiotic configurations [26]. Analyses were conducted in five hybrid

zones, the one of them was the Drnholec/Białowieża zone near Dęblin. However, no evidence for limitation of a gene exchange was detected in any of the analyzed areas. One explanation is the role of chromosomal rearrangements might be less important in reducing fertility of the common shrew hybrids than in other species. Second, karyotype evolution of the common shrew could be a recent and fast phenomenon; hence, the length of time was insufficient for accumulation of genetic differences among the races. Third, as a recombination rate depends on both a location of locus and a structure of chromosome, only molecular markers with known position could be helpful in detecting impact of chromosomal rearrangements on fertility of shrews.

The hypothesis of the recent origin of *S. araneus* chromosomal races is enhanced by lack of genetic differences in mitochondrial gene *cyt b*, which was investigated in five races belonging to the two karyotypic groups WEKG and EEKG [15]. The substantial karyotypic variability, along with estimated coalescence time of *cyt b* gene and star-like phylogenetic tree, suggest a recent demographic expansion of the species. It is likely that racial differentiation of the common shrew have appeared during expansion; thus, genetic similarity of races may reflect ancestral polymorphism of the species.

In the common shrew, both the polymorphism and racial differentiation involve the basic complement of chromosomes called the A-chromosomes. Another example of chromosomal polymorphism is polymorphism of B-chromosomes. These chromosomes are extra elements in karyotype, independent from the set A, and are not necessary for survival of an individual. To investigate a mechanism responsible for the maintenance of Bs in the population of yellow-necked mouse *Apodemus flavicollis*, we analyzed meiosis in male mice [20]. We found some loss of B-chromosomes (*meiotic drag*) in the studied specimens. The elimination of chromosomes from population should be compensated through either meiotic drive in females, which is congruent with parasitic model, or they should be beneficial for their carriers, which corresponds to heterotic model. In our study, parasitic model was additionally supported by increasing number of chiasmata on A-chromosomes with increasing number of Bs in the karyotype. We suggested that Bs maintenance in the yellow-necked mouse resulted from changes of selection pressure around the year or in different environmental conditions.

For few years my scientific interests focus on the conservation genetics and phylogeography of endangered species: shrub birch *Betula humilis*, dwarf birch *B. nana* and common hamster *Cricetus cricetus*. Conservation genetics is a discipline that aims to apply molecular markers to the preservation and restoration of genetic resources of population and species. In the cases of birches and hamster, these are very urgent tasks. Fragmentation of

natural habitats resulting from more and more anthropogenic pressure has led to 5-fold decrease in the number of *B. humilis* populations in the twentieth century (Zaluski et al. 2001, *Polish plant red book, IB PAS*). The range of the common hamster in Poland dwindled about 75% during the last thirty years (Ziomek and Banaszek 2007, *Folia Zool.* 56). In turn, phylogeography is a discipline dealing with the knowledge of principles and processes responsible for geographic distribution of phylogenetic lineages (Avice 2000, *Phylogeography: the history and formation of species, Harvard Univ. Press*). Realization of research topics related to the analysis of genetic variation of populations, description of glacial and postglacial histories of birches and common hamster was able thanks to the grants obtained by me and dr hab. Agata Banaszek from the Ministry of Science and Higher Education and the National Science Centre.

Sequencing of the control region (*ctr*) of mitochondrial DNA (mtDNA) revealed very low level of genetic variation in the Polish populations of the common hamster [21]. Most populations were fixed for one haplotype and single haplotypes were distributed throughout large areas. Geographic pattern of haplotype distribution strongly suggests that reduction in numbers (*bottleneck effect*) during isolation in glacial refugia and postglacial recolonization is responsible for a low genetic variation rather than a recent fragmentation of habitats. Analysis of three molecular markers: control region (*ctr*), cytochrome b (*cyt b*) and 16S ribosomal RNA (*16S*) showed that Polish populations of *C. cricetus* belonged to two phylogeographic lineages [22]. Lineage E1 was discovered in the present study, lineage P3 comprises haplotypes characteristic for the Pannonia group, described in the territory of the Carpathians (Neumann et al. 2005, *Mol. Ecol.* 14). It is likely that representatives of the Pannonia group occurred in southern Poland ca. 53 000 years ago. Settlement of south-eastern Poland by the hamsters from E1 lineage took place after retreat of glacier. Phylogeographic groups E1 and P3, co-existing in the Małopolska Upland, do not come into a contact [27]. This view is supported by the lack of mixed populations within a 20 km wide belt, which separates the marginal populations of both groups. We suppose that the lack of gene flow between phylogeographic lineages may result from unfavourable habitat conditions.

The estimation of genetic variation of microsatellite loci in the Polish populations of the common hamster revealed low number of alleles per locus and relatively high average heterozygosity [23]. Such disproportion between these two measures of genetic variation is characteristic for populations with recent decline of genetic variability. The substantial genetic differentiation between populations of the P3 lineage and moderate differentiation within the E1 group suggest that gene flow between hamster populations is reduced to some extent,

which was also revealed by identification of migrants. As populations of *C. cricetus* form very distinct genetic clusters, it might imply that species functions in a metapopulation structure. In favourable habitat conditions, local populations grow in number, conversely, rapid environmental changes cause population extinction. Hence, ongoing fragmentation of agriculture habitats is a threat for the existence of species.

The comparative morphometric investigation of the common hamster phylogeographic lineages revealed that individuals of P3 group were significantly smaller with longer feet and tails compared to the specimens of E1 lineage [29]. This difference is likely to be a consequence of the accumulation and fixation of genetic differences during isolation in glacial refugia and postglacial recolonization. Lack of color variants suggests that declines of the species range and number of individuals may cause reduction of genetic variation. Based on the pronounced genetic and ecological differentiation, it was postulated to define the Polish phylogeographic lineages of *C. cricetus* as evolutionary significant units (ESU) and management units (MU).

Perfect models to study an impact of habitat fragmentation on genetic diversity of populations are bush birches – shrub birch and dwarf birch. In Poland, these two species represent different numbers of individuals per population and different level of geographic isolation. *B. humilis* forms abundant populations in Pomerania, Masurian Lake District, Podlasie and Lublin regions (Załuski et al. 2001, *Polish plant red book, IB PAS*). Number of individuals in particular populations is differentiated. In some localities hundreds of bushes occur, e.g. populations in the Biebrza National Park. In turn, among 52 ramets found in the vicinity of Maliszewskie Lake, I have defined the three genotypic individuals (genets) only (unpubl. data). Dwarf birch appears in three Polish reserves only – “Linje” in the Chełmno Lake District and “Torfowiska Doliny Izery” and “Torfowisko pod Zieleńcem” in the Sudety Mts. (Kruszelnicki and Fabiszewski 2001, *Polish plant red book, IB PAS*). The shortest distance between populations is ca. 90 km.

Results of genetic analyses conducted in the populations of both birches were compiled as original papers [1-3, 5, 24, 25, 28], one review article [4] and one chapter of the English-language monograph [31]. I have described the morphology and biology of particular European *Betula* species in a popular paper [30]. Articles from 1 to 5 are included into the scientific achievement submitted for habilitation procedure and are fully discussed below.

To estimate the genetic resources of endangered birch species, different kinds of molecular markers were used: chromosomes, nuclear microsatellites, nuclear gene alcohol dehydrogenase (*ADH*) and non-coding sequences of chloroplast DNA (cpDNA). The karyotypic studies were carried out in six populations of the shrub birch [25]. In each

population, diploid individuals with $2n=28$ as well as aneuploids with $2n\neq 28$ were noted. The presence of non-balanced set of chromosomes usually negatively influences the fitness of an individual; thus, four hypotheses were proposed to explain the causes of the maintenance of aneuploid karyotypes in populations. First, aneuploidy could be a result of hybridization between the shrub birch and its close relatives. Second, stress resulting from fragmentation of environment could cause a change of selection pressure against atypical karyotypes. Third, individuals with $2n\neq 28$ have instable karyotype that generates next aneuploid cases. Fourth, the high frequency of aneuploid specimens is maintained because of domination of vegetative reproductive system. Further studies are required to verify those hypotheses.

Main reasons of decline of *B. humilis* populations are: drainage and too intensive use of fens and competition of brushwood and forest plants (Zaluski et al. 2001, *Polish plant red book, IB PAS*). Preliminary analysis revealed that, in spite of increasing habitat fragmentation at the south-western margin of the species range, genetic variation at the nuclear microsatellite loci in populations situated in north- and south-eastern Poland is still quite considerable [24]. It is likely a result of too short time that has elapsed for genetic variation to be reduced following a decrease in population size and spatial isolation. Shrub birch can reach 20 years of age; hence, a decrease of genetic diversity in a population is much slower than in an organism with a generation time of one year. Another explanation of substantial genetic variation is predominance of outcrossing breeding system in *B. humilis* populations. In the locations under study, particular ramet had a distinct genotype, and more than 90% of the total genetic variation was found at the intrapopulation level. These both facts indicate that generative reproduction is frequent in the shrub birch populations.

Jabłońska's results (2012; *Phytocoenologia* 42) and our field observation indicate that the shrub birch is the same abundant in the wet habitats in north-eastern Poland and Belarus as well as in the drained fens in south-eastern Poland and central Belarus [28]. Both kinds of habitats are represented by high variation of nuclear microsatellites [1], but we observed higher diversity of cpDNA markers in north-eastern Poland compared to the south-eastern part of the country. This probably results from the mixing of phylogenetic lineages in north-eastern Poland [2, 5]. Next aim of our study was to test whether population history is the only factor determining the level of genetic variation in *B. humilis* populations. We scrutinized relationships between parameters describing diversity of both nuclear microsatellites and *ADH* gene and physicochemical parameters of the environment [28]. Negatively significant relationship between concentration of Ca^{2+} and heterozygosity of microsatellite loci was found. Additionally, we revealed no genetic differences between populations located in wet and drained fens. Expression of *ADH* gene is related to the oxidative stress; thus, lack of

differences at this locus between birches inhabiting wet and dry habitats suggests that *B. humilis* is tolerant to an excess of water. An analysis of phylogenetic network, constructed on the basis of *ADH* haplotype differentiation, supports population history of the shrub birch previously inferred from cpDNA markers [2]. I have summarized data on genetic resources, phylogeography and palaeobotany from the Polish populations of *B. humilis* in the chapter of monograph [31].

At present, I analyze the results obtained during the study of reproduction mode in the populations of *B. humilis* and *B. nana* (grant no 2011/01/B/NZ8/01756). In marginal (Polish) and central (Belarusian and Finnish) populations of both species, I have collected data on flowering and fructification of birch specimens. Reproductive parameters will be related to genotypes of particular individuals at AFLP loci (*Amplified Fragment Length Polymorphism*). I am interested in to what extent severity of generative reproduction depends on birch genotype and location of population. It should answer the question generative or vegetative mode of reproduction predominates in the *B. humilis* and *B. nana* populations. In the future, I would like to study an impact of interspecific hybridization on the genetic and morphometric diversities of European birches. Birches are among taxa characterized by the highest frequency of interspecific hybridization (Barrington 2011, *J. Torrey Bot. Soc.* 138). The study of cpDNA haplotypes revealed that sympatric birch species were more similar to one another than individuals of the same species growing in allopatric populations (Palmé et al. 2004, *Mol. Ecol.* 13). A substantial gene flow between birch populations is responsible for a lack of knowledge about number of species in the *Betula* genus. Using the next generation sequencing technology, I plan to identify diagnostic loci for particular European birch species, and later, to estimate an extent and consequences of interspecific gene flow between rare (*B. humilis* and *B. nana*) and widespread congeners (*B. pendula* and *B. pubescens*). To achieve these goals, I have applied for a grant to the National Science Centre (OPUS 6 funding scheme).

SUMMARY OF PUBLICATIONS THAT MAKE UP SELECTED SCIENTIFIC ACHIEVEMENT

The scientific achievement is monothematic set of papers entitled:

„Genetic structure of *Betula humilis* Schrk. and *B. nana* L. populations as an effect of postglacial history of birches”

Papers included into the achievement:

- [1] **Jadwiszczak K.A.**, Banaszek A., Jabłońska E., Sozinov O.V. 2011. Could *Betula humilis* have survived the last glaciation at a current margin of its distribution? Testing the hypothesis of a glacial refugium using nuclear microsatellites. *Plant Systematics and Evolution* 297: 147-156.
- [2] **Jadwiszczak K.A.**, Banaszek A., Jabłońska E., Sozinov O.V. 2012. Chloroplast DNA variation of *Betula humilis* Schrk. in Poland and Belarus. *Tree Genetics and Genomes* 8: 1017-1030.
- [3] **Jadwiszczak K.A.**, Drzymulska D., Banaszek A., Jadwiszczak P. 2012. Population history, genetic variation and conservation status of the endangered birch species *Betula nana* L. in Poland. *Silva Fennica* 4: 465-477.
- [4] **Jadwiszczak K.A.** 2012. What can molecular markers tell us about the glacial and postglacial histories of European birches? *Silva Fennica* 5: 733-745.
- [5] **Jadwiszczak K.A.**, Banaszek A., Chrzanowska A., Kłosowski S., Sozinov O.V. 2014. The admixture zone of *Betula humilis* Schrk. phylogenetic lineages follows the eastern central European suture zone. *Plant Ecology & Diversity*, DOI: 10.1080/17550874.2014.893593.

INTRODUCTION

One of the main threats for the world biodiversity is the extinction of species that results in decline of gene pools (i.e. genetic death). Besides the climatic changes, that always have occurred in the Earth, humans are responsible for contemporary disappearance of many populations and species. Anthropoppression leads, first of all, to fragmentation of natural habitats resulting in reduction of number of individuals and an increase of spatial isolation between adjacent populations. Theoretically, such changes could cause: (1) decline of genetic variation in particular stands, and (2) increase of genetic differentiation between populations (Young et al. 1996, *Trends Ecol. Evol.* 11). Consequences of genetic diversity reduction could be discussed in both short- and long-term scales. In short-term scale, a decline of heterozygosity in a population is followed by lowered fitness of individuals (Ellstrand and Elam 1993, *Annu. Rev. Ecol. Syst.* 24). It is an effect of fixation of deleterious mutations by strongly operating genetic drift. In long-term scale, increasing homozygosity level is accompanied by decreasing capability of species to interact with changing environment; hence, such species is threatened by extinction (Falconer and Mackay 1996, *Introduction to quantitative genetics, Longman*).

Two hypotheses have been proposed to explain the distribution of genetic diversity within the species ranges. According to “centre–periphery” model, central populations show a higher genetic variation compared to the marginal ones (Eckert et al. 2008, *Mol. Ecol.* 17). Dobzhansky (1951, *Genetics and the origin of species*, Columbia Univ. Press) stated that most beneficial habitats for individuals of the species existed in the middle part of the range, where differentiated niches enable co-occurrence of many genotypes. In turn, the margins of the species range are often characterized by unfavourable environmental conditions; thus, a strong selection eliminates a number of genotypes. The marginal populations are usually small; therefore, a genetic drift is another mechanism reducing genetic diversity at the species range edge.

Results of phylogeographical studies indicate that distribution of genetic variation within the species ranges is likely an effect of range expansions and retractions during glacial and interglacial periods of the Quaternary rather than the local demographic fluctuations (Hewitt 2004, *Philos. T. Roy. Soc.* 359; Hampe and Petit 2005, *Ecol. Lett.* 8). Changes of species range resulted in a high genetic diversity in areas of glacial refugia (‘stable’ rear edge) and gradual decrease of genetic variation with distance from the former glacial isolates (leading edge; Hampe and Petit 2005, *Ecol. Lett.* 8). It is revealed that populations located within contact zones of different phylogenetic lineages showed very often higher genetic variation compared to the refugial stands (Petit et al. 2003, *Science* 300).

In Poland, the shrub birch (*Betula humilis* Schrank, 1789) and dwarf birch (*Betula nana* Linnaeus, 1753) are recognized as endangered species [EN category according to the International Union for Conservation of Nature (IUCN)]. These birches are glacial relicts in western, central and partially eastern Europe. Before my investigation has started, there was no data on the level and distribution of genetic diversity in marginal populations of both birches, except for RAPD (*Random Amplified Polymorphic DNA*) analysis conducted in one Polish population of *B. nana* (Dąbrowska et al. 2006, *Dendrobiology* 55) and chromosomal study carried out in one German locality of *B. humilis* (Nato 1959, *Feddes Repert.* 61). Evaluation of genetic resources is very important in the conservation of endangered species.

The objectives of my studies included into the scientific achievement were:

1. To test hypothesis of *B. humilis* survival in a refugium located in the Lublin Polesie region
2. To test hypothesis of “centre–periphery” model of genetic diversity distribution in the south-western and subcentral parts of *B. humilis* range

3. To identify reasons of high genetic diversity of the shrub birch in north-eastern Poland – testing hypotheses of periglacial isolate and contact zone of phylogenetic lineages
4. To estimate level of genetic variation within and between populations of *B. nana* in Poland
5. To compare patterns of genetic diversity distribution within the ranges of European *Betula* species

[1] Jadwiszczak K.A., Banaszek A., Jabłońska E., Sozinov O.V. 2011. Could *Betula humilis* have survived the last glaciation at a current margin of its distribution? Testing the hypothesis of a glacial refugium using nuclear microsatellites. *Plant Systematics and Evolution* 297: 147-156.

Main goals of this study were to estimate level of genetic variation in *B. humilis* populations and to test hypothesis of *B. humilis* refugium located in the Lublin Polesie region (Środoń 1979, *Birches*, PWN). We assumed that in the case of the shrub birch survival in south-eastern Poland, populations situated in this area should exhibit a higher level of genetic diversity than populations located in areas covered by glacier, as well as populations from the centre of the species range. We studied 10 nuclear microsatellite loci in 327 individuals from 15 marginal (Poland) and three subcentral (Belarus) populations. Six populations under study were located in glaciated area. Average number of alleles per locus (A) was significantly higher in unglaciated area compared to glaciated territory (ANOVA; $F_{17}=6.435$, $P=0,021$). This parameter was also significantly lower in northern and south-eastern Poland in relation to north-eastern Poland and Belarus ($F_{17}=3.666$, $P=0.037$). Number of private alleles (N_{PA}) was not statistically different neither between geographical regions ($F_{17}=0,712$, $P=0,548$) nor glaciated/unglaciated areas ($F_{17}=0.011$, $P=1,000$). Highest values of observed (H_O) and expected (H_E) heterozygosities were noted in subcentral populations in Belarus. Obtained results do not support hypothesis of *B. humilis* glacial survival in south-eastern Poland. Moreover, hierarchical analysis of molecular variance (AMOVA) revealed that more than 95% of the total genetic diversity was found at the population level and 4% only resided between populations, regardless of whether glaciated/unglaciated areas or four geographical regions were considered. These results are additional proofs against hypothesis of glacial refugium of the shrub birch in the Lublin Polesie region.

We also rejected hypotheses of genetic diversity distribution according to the “centre–periphery” model in the part of *B. humilis* range studied. It was revealed that marginal and subcentral populations are not genetically differentiated (AMOVA; $F_{CT}=-0.00095$, $P=0.483$). Most surprising result of our investigation was high values of genetic variation parameters in

the shrub birch populations situated in north-eastern Poland. Some of these populations are located in the area covered by Scandinavian glacier, and some in the periglacial belt. We hypothesized that a substantial genetic variation of *B. humilis* in north-eastern Poland could result from either a periglacial refugium or mixing of phylogenetic lineages derived from distinct glacial isolates. Lack of isolation by distance (IBD) suggests that *B. humilis* could have survived the maximum of the last glaciation at higher latitudes (Mantel test; $r=0.1375$, $P=0.219$). However, hypothesis of periglacial refugium is weakened by low frequency of private alleles (0.99-4.44%) in populations situated in the Suwałki and Podlasie regions.

Analysis of nuclear microsatellites showed that genetic diversity of the shrub birch at the south-western edge of the species range was still substantial, besides the disappearance of its habitats. However, populations with low number of individuals and being geographically isolated exhibit first symptoms of genetic variation decline and start to differentiate, that is seen in the stands from northern Poland.

[2] Jadwiszczak K.A., Banaszek A., Jabłońska E., Sozinov O.V. 2012. Chloroplast DNA variation of *Betula humilis* Schrk. in Poland and Belarus. *Tree Genetics and Genomes* 8: 1017-1030.

The objectives of this study were: (1) to evaluate distribution of chloroplast DNA (cpDNA) markers in marginal (Poland) and subcentral (Belarus) populations of the shrub birch, (2) to test if *B. humilis* populations from north-eastern Poland represent a high level of genetic diversity, as it was previously described using nuclear microsatellites, (3) to estimate extent of hybridization between *B. humilis* and its close relatives - *B. pendula* and *B. pubescens*.

Using the PCR-RFLP (*Polymerase Chain Reaction – Restriction Fragment Length Polymorphism*) method, we studied 365 individuals of shrub birch in 19 populations, 36 specimens of *B. pendula* and 31 of *B. pubescens*. In the studied material, 21 haplotypes were described (labeled from I to XXI). Among 17 haplotypes found in *B. humilis* populations, haplotypes I and II were the most frequent and widespread (57.0 and 28.5%, respectively). The presence of two haplotypes only in the Lublin Polesie region undermines hypothesis of *B. humilis* glacial isolate in that region.

The examination of cpDNA markers supports the high genetic diversity of some populations of the shrub birch in north-eastern Poland inferred previously from microsatellite analysis [1]. The substantial genetic variation could be explained either by a presence of Pleistocene refugium or by mixing of phylogenetic lineages. The lack of relationship between genetic diversity and geographical distance between populations (lack of isolation by

distance) confirms the result of microsatellite research that *B. humilis* could have survived the maximum of the last glaciation at higher latitudes. Remaining results of cpDNA studies support hypothesis of the contact zone between different phylogenetic lineages. First, single private haplotypes occurred in the area under consideration, and such observation is not typical for a former glacial isolate. Second, high haplotypic diversity in Belarus, north and north-eastern Poland was accompanied by higher value of v_T (diversity of haplotypes estimated based on their frequencies) than h_T (diversity of haplotypes estimated based on both their frequencies and similarities), which is result of co-occurrence of haplotypes deriving from two different haplogroups in the studied geographical regions. Refugial areas should be characterized by haplotypes of one phylogenetic lineage. Above results indicate that the most likely explanation of substantial genetic variation in some populations of the shrub birch in north-eastern Poland as well as in Belarus is an admixture of migration waves coming from distinct glacial refugia. This hypothesis is supported by the presence of phylogeographic structure in the area under study. This structure is inferred from statistically significant higher value of N_{ST} (genetic differentiation estimated based on both the frequency of haplotypes and number of mutation between particular haplotypes) than G_{ST} (differentiation among populations taking into account the frequency of particular haplotypes only). Distribution of haplotypes in the studied range of *B. humilis*, location of contemporary populations and data of palaeobotanical studies suggest that the shrub birch could have survived the last glaciation in eastern Europe, another refugium could be localized in a foreland of the Alps or/and the Carpathians.

Introgression ratio (IG) between *B. humilis* and closely related tree birches *B. pendula* and *B. pubescens* – reached a value of 0.71. This indicates that frequency of the common haplotypes is substantial. Geographical pattern of the common haplotype distribution in all birches is similar, that is likely to be an effect of substantial gene flow between species. The extensive exchange of genes is supported by the result of AMOVA test, which revealed the lack of genetic differences between the shrub birch and tree congeners ($F_{CT}=-0.031$, $P=0.563$).

[3] Jadwiszczak K.A., Drzymulska D., Banaszek A., Jadwiszczak P. 2012. Population history, genetic variation and conservation status of the endangered birch species *Betula nana* L. in Poland. *Silva Fennica* 4: 465-477.

Main goals of our study were: (1) to estimate genetic diversity resources in the Polish populations of *B. nana* and (2) to discover their phylogenetic origin. Two kinds of molecular

markers were chosen to realize these aims: 11 nuclear microsatellite loci and three fragments of non-coding cpDNA.

In general, parameters of genetic variation, such as a mean number of alleles per locus (A), observed (H_O) and expected (H_E) heterozygosities, of microsatellites were lower compared to the *B. humilis* populations [1]. The lowest values of calculated parameters were observed in the two localities situated in the Sudety Mts. A decrease of genetic diversity in the Polish stands of *B. nana* could have occurred as a result of cooperation of some factors. First, populations of the dwarf birch in Poland are small; therefore, a main factor reducing genetic variability resources is genetic drift that fixes some alleles and eliminates others. A strong support for the impact of genetic drift on *B. nana* localities are numerous private alleles, some of them reached frequencies higher than 0.05. Second, using the program M_P_Val we revealed that all Polish stands underwent a substantial reduction in numbers, most likely during the postglacial recolonization. Moreover, “Linje” population situated in the Chelĳno Lake District and “Torfowiska Doliny Izery” in the Sudety Mts. experienced significant population reductions quite recently (BOTTLENECK program). Decline of genetic variation was a consequence of reduction in numbers in these localities. Selection pressure might be a third factor influencing genetic structure of the Polish stands of dwarf birch. We postulated that overgrowing of *B. nana* populations by competitive species might be responsible for deterioration of habitat conditions and lack of sexual reproduction in at least some growing seasons. Fourth, geographic isolation of all localities prevents a gene flow between them and enhances effects of genetic drift and natural selection pressure. It is supported by both the significant values of genetic differentiation (F_{ST}) of the microsatellite loci between all sample pairs and the analysis of genetic structure (STRUCTURE software) evidencing that all Polish populations are clearly distinct from one another.

The chloroplast DNA study revealed two haplotypes only in the dwarf birch populations in Poland: haplotype I was found in the “Linje” and “Torfowisko pod Zieleńcem” reserves, haplotype II in the “Torfowiska Doliny Izery”. These haplotypes are the most common in all birch species. In general, Europe was recolonized from two main glacial refugia – western and eastern ones. Waves of migration came into a contact in the territory of Poland among others; thus, a likely explanation of the cpDNA haplotype distribution in the Polish populations of *B. nana* is a contact zone of different phylogenetic lineages. However, at the present stage of study we could not exclude that cpDNA haplotype variation was higher previously in the dwarf birch stands in Poland, but most of haplotypes were eliminated by the genetic drift.

[4] Jadwiszczak K.A. 2012. What can molecular markers tell us about the glacial and postglacial histories of European birches? *Silva Fennica* 5: 733-745.

In this paper, I reviewed genetic diversity distribution (restriction fragments of cpDNA, nuclear and chloroplast haplotypes) within the ranges of the European birches: *B. pendula*, *B. pubescens*, *B. humilis* and *B. nana* to compare glacial and postglacial histories of the species. Two cpDNA haplotypes clearly predominate within the ranges of all birches. They occupy a central position in the birch phylogenetic trees that indicates ancestral origin of these haplotypes. Most common haplotypes differ from one another by three mutations that suggest they could have appeared before the last glacial maximum.

The distribution of two common haplotypes imply that one birch refugium could have existed in the east, another one in the west of the continent. Lack of relationships between genetic differentiation and geographical distance between populations (lack of isolation by distance) in *B. pendula*, *B. pubescens* and *B. humilis* indicates that these species could have survived the last glacial maximum in refugia situated at higher latitudes. Populations inhabiting former glacial isolates are usually characterized by high genetic variation (Hewitt 2000, *Nature* 405; Hampe and Petit 2005, *Ecol. Lett.* 8). Based on this observation, it is likely that main waves of postglacial migrations of *B. pendula* derived from isolates located in the foreland of the Alps nad Ural Mts. *B. pubescens* could also survived the last glaciation somewhere in the vicinity of the Ural Mts. This assumption is supported by high diversity of cpDNA haplotypes in this part of the species range. It is not known what is the main factor responsible for high genetic diversity in the Scandinavian populations of *B. pendula*, *B. pubescens* and *B. nana*. Among most likely explanations are isolation in microrefugia, local mutations (*B. pendula*), but also in the case of *B. nana* and *B. pubescens* – hybridization. After glacial retreat, western and eastern waves of migration of all European birch species spread throughout the continent and established wide admixture zones in Scandinavia, western and central parts of Europe. Similar distribution of cpDNA haplotypes within the ranges of all birches strongly suggest considerable level of interspecific hybridization. Unfortunately, extensive gene flow between birch species makes the phylogeographical data of European *Betula* intricate to interpret.

[5] Jadwiszczak K.A., Banaszek A., Chrzanowska A., Kłosowski S., Sozinov O.V. 2014. The admixture zone of *Betula humilis* Schrk. phylogenetic lineages follows the eastern central European suture zone. *Plant Ecology & Diversity*, DOI: 10.1080/17550874.2014.893593

Previous studies, based on the nuclear microsatellite [1] and cpDNA markers [2], revealed high level of genetic variation in some populations of *B. humilis* in north-eastern Poland and central Belarus. We suggested that it could result from admixture of phylogenetic lineages. However, small number of populations (3) investigated in the periglacial area were inconclusive in determining if the high genetic diversity of *B. humilis* in north-eastern Poland reflects a heritage derived from a periglacial refugium or is an effect of mixing of migration waves deriving from distinct glacial isolates. Using the PCR-RFLP method, we analyzed cpDNA haplotype variability in eight additional populations of the shrub birch in north-eastern Poland, western Belarus and Latvia. We also estimated values of rarity index (DW) that is a measure of population genetic divergence in 27 *B. humilis* populations studied so far.

We did not find any population with higher gene diversity (H_E) than those described previously [2]. In the eight populations studied, no private cpDNA haplotypes were noted. Values of rarity index estimated in 27 populations were low, from 0.034 (LL sample from Latvia) to 3.363 (SLU from Belarus). In the area of Poland, the highest values of DW were found in JM (3.062) and ROS (3.107) populations, both situated in the territory glaciated during Pleistocene. As value of rarity index depends on the number of private haplotypes that are accumulated during long-lasting isolation (e.g. in refugia), our data allow to definitely reject hypothesis of periglacial survival of the shrub birch in north-eastern Poland. Thus, considerable genetic variation of *B. humilis* in that part of the country resulted from admixture of phylogenetic lineages. This conclusion is supported by the presence of phylogeographic structure (N_{ST} was significantly higher than G_{ST}) in the area spread from north-eastern Poland to central Belarus. Admixture zone of *B. humilis* phylogenetic lineages together with contact and hybrid zones of some other species form suture zone that extends in eastern central Europe.

SUMMARY

Phylogeographic data obtained during my studies in the endangered populations of *B. nana* and *B. humilis* suggest that territory of Poland was recolonized by waves of migration of both birches deriving from different glacial refugia that were presumably situated in eastern and central or western Europe. This assumption coincides with description of Holocene

recolonization of the tree birches. However, the possibility of glacial survival of the shrub birch in refugia located in the Lublin Polesie region and periglacial belt in north-eastern Poland are not confirmed.

Most of marginal populations of the shrub birch exhibit quite substantial genetic variation, comparable to the level of genetic variability observed in subcentral samples in Belarus. Such distribution of genetic diversity is not concordant with the “centre-periphery” model. Among Polish stands, the most genetically variable are those from north-eastern part of the country. My studies imply that this area could be admixture zone of phylogenetic lineages. I have also found populations with declining level of genetic variation. This is a consequence of the low number of individuals and geographical isolation of these stands. Polish populations of *B. nana* are totally isolated from one another as well as from other samples of the species. The lack of gene flow between the dwarf birch localities enhances effects of genetic drift and selection pressure acting in these populations, which results in low values of genetic variation parameters. The substantial number of private alleles at nuclear microsatellite loci and high frequencies of some of them suggest that process of genetic differentiation of particular Polish localities of *B. nana* could be advanced.

Recognition of genetic resources in natural populations and understanding processes responsible for their maintenance or decrease is one of the key factors in planning strategies of the global biodiversity conservation. My studies provide the knowledge that is appropriate not only in conservation of the Polish stands of the endangered birches, but also could be useful in protection of these plants in other parts of their declining ranges.

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Katarzyna Jaschirzaska