

## Evolution caught ‘red-handed’ – the transformation of plants in industrial areas (microevolution)

Olga BEMOWSKA-KAŁABUN<sup>1</sup>, Dorota PANUFNIK-MĘDRZYCKA<sup>2</sup>,  
Małgorzata WIERZBICKA<sup>3\*</sup>

<sup>1,2,3</sup>*Department of Ecotoxicology, Institute of Environmental Biology, Faculty of Biology, University of Warsaw Ilji Miecznikowa 1 St., 02-096 Warsaw*

<sup>1</sup>*e-mail: olga.bemowska@biol.uw.edu.pl, ORCID: 0000-0003-3716-4538*

<sup>2</sup>*e-mail: dmedrzycka@uw.edu.pl, ORCID: 0000-0002-5499-793X*

<sup>3</sup>*e-mail: wierzbicka@biol.uw.edu.pl, ORCID: 0000-0002-0328-6521*

### What is microevolution?

Evolution can be considered at three levels of organization of organisms: population (microevolution), race and species (speciation), and higher systematic units (macroevolution) (Grant 1981, Levin 2000). Microevolution concerns the change in the allele<sup>1</sup> frequency within a specific population<sup>2</sup> over a short period of time (from a few years to decades). These changes appear as a result of mutation, natural selection, genetic drift<sup>3</sup>, gene flow between populations, non-random interbreeding and recombination<sup>4</sup>, as

well as epigenetic mechanisms<sup>5</sup>. Microevolution leads to variation (differences between individuals) within the population of a particular taxon (Stace 1993, Hendry and Kinnison 2001, Krzanowska et al. 2002, Wierzbicka and Rostański 2002, Ashley et al. 2003, Ernst 2006, Freeland 2008, Vandegehuchte and Janssen 2011, Babst-Kostecka 2015). New traits that arise during the process, adaptations to new environmental conditions, may be hereditary and increase the probability of survival and reproduction of these individuals. In many cases, these adaptations can form within a short time frame that is comparable to that of man-made changes in the environment (Ashley et al. 2003, Mitchell and Whitney 2018, Gorné and Díaz 2019). The study of microevolutionary processes occurring in anthropogenically altered environments

<sup>1</sup> Alleles – different versions of the same gene.

<sup>2</sup> Population – a group of individuals of one species living in the same area.

<sup>3</sup> Genetic drift – changes in the frequency of genes resulting from random phenomena.

<sup>4</sup> Recombination – the process of exchanging genetic material. As a result, new genotypes arise.

\* Corresponding author

<sup>5</sup> Epigenetic mechanisms – changes in gene expression which not related to changes in the nucleotide sequence in DNA.

is now one of the primary issues of ecotoxicology (Bickham 2011, Coutellec and Barata 2011, Vandegehuchte and Janssen 2011, van Straalen et al. 2011). In the age of progressing environmental pollution, plant microevolution processes in chemically degraded habitats become particularly important, as over time they may lead to the formation of new subspecies (Medina et al. 2007).

Factors that may contribute to an acceleration of plant microevolution include environmental pollution (e.g. heavy metals, herbicides) as well as pathogens, the impact of invasive alien species, habitat fragmentation, or global changes (e.g. global warming, or the increase in CO<sub>2</sub> content in the atmosphere). All these may affect plants, resulting in changes in morphological features, physiological and biochemical processes, reduced fertility, and increased tolerance to stress. The rapid evolution of these traits may occur in both annual and perennial plants, and even long-living trees (Bone and Farres 2001, Ashley et al. 2003). In Table 1, based on the work by Bone and Farres (2001), examples of different characteristics of plants that have changed (after the specified amount of time) due to microevolutionary processes in response to selected environmental abiotic factors are presented.

The emergence of increased tolerance of plants to heavy metals is a model process for researching the evolution of adaptations to extremely unfavorable environments. This feature evolved independently in various plant species, as a result of microevolutionary processes acting on individuals of non-metallicolous populations (from clean areas) that colonized the contaminated area. Due to the action of toxic metals, the selection of individuals takes place and those that created defense mechanisms (e.g. the ability to detoxify metals, the system for reducing oxidative stress) (Wierzbicka 1999, 2015, Ernst 2006, Wierzbicka et al. 2017) exhibit increased survival.

Figure 1 shows how a deficit or excess of each chemical element in the environment can affect the direction of evolution, and the arising of the selection process.

In areas contaminated with heavy metals, such as zinc-lead waste heaps, there are many plants which tolerate high concentrations of metals, called metallophytes (Bemowska-Kałabun et al. – Chapter 6 of this volume). Metallophytes of interest include *Biscutella laevigata* L., *Dianthus carthusianorum* L., *Armeria maritima* (Mill.) Willd., *Silene vulgaris* (Moench) Garcke, *Viola lutea* var. *westfalica* A. A. H. Schulz, *V. lutea* subsp. *calaminaria* (Ging.) Nauenb., or *Arabidopsis arenosa* (L.) Lawalrée. These species have a number of adaptations which allow them to survive the unfavorable conditions of these habitats. This includes the ability to detoxify metals in the cell (in a vacuole or cell wall, for example), as well as the development of r-type life strategy which involves generating forms with a shortened life cycle which enter the generative phase quickly (Bemowska-Kałabun et al. – Chapter 6 of this volume). Numerous studies show significant differences in characteristics between individuals in populations in areas contaminated with heavy metals and those in clean areas, which suggests the emergence of new forms, varieties and even subspecies (Baker and Proctor 1990, Lefebvre and Vernet 1990, Wierzbicka and Rostański 2002, Baumbach and Hellwig 2003, Ernst 2006, Hildbrandt et al. 2006, Słomka et al. 2011c, Abratowska et al. 2012, Godé et al. 2012, Migdałek et al. 2013, Wójcik et al. 2013, Kuta et al. 2014, Wąsowicz et al. 2014, Wierzbicka 2015, Wierzbicka et al. 2017).

In regard to the tolerance of excess of heavy metals, the rate of evolution depends on the strength of selection pressure (e.g. the amount of the element available in the soil) and the flow of genes between metallicolous and non-metallicolous populations. When the flow of genes between the metallicolous population

Table 1. Examples of the occurrence of microevolution processes in selected plant species in response to high concentration of heavy metals in environment (after Bone and Farres 2001, modified)

Tabela 1. Przykłady zajścia procesów mikroewolucyjnych u wybranych gatunków roślin w odpowiedzi na wysokie stężenia metali ciężkich w środowisku (za Bone i Farres 2001, zmieniłone)

Species Gatunek	Metal Metal	Time in years, which was needed to change a given trait Czas w latach, który był potrzebny na zajście zmiany danej cechy	The examined trait – measured parameter Badana cecha – mierzony parametr	Authors Autorzy
<i>Anthoxanthum odoratum</i>		100	Zinc tolerance – tolerance index <sup>1</sup> Tolerancja cynku – indeks tolerancji <sup>1</sup>	Bone and Farres (2001)
<i>Anthoxanthum odoratum</i>	Zinc Cynk	68	Zinc tolerance – tolerance index, plant height Tolerancja cynku – indeks tolerancji, wysokość roślin	Bone and Farres (2001)
<i>Funaria hygrometrica</i> (moss)		60	Zinc tolerance – percentage of spore germination, protonemal growth Tolerancja cynku – procent kiełkujących zarodników, wielkość strzępek	Shaw et al. (1987)
<i>Agrostis tenuis</i>		200	Cu tolerance – tolerance index Tolerancja miedzi – indeks tolerancji	Bone and Farres (2001)
<i>Lupinus bicolor</i>		130	Cu tolerance – tolerance index Tolerancja miedzi – indeks tolerancji	Wu and Kruckeberg (1985)
<i>Lotus purlbianus</i>	Copper Miedź	130	Cu tolerance – tolerance index Tolerancja miedzi – indeks tolerancji	
<i>Funaria hygrometrica</i> (moss)		60	Cu tolerance – percentage of spore germination, protonemal growth Tolerancja miedzi – procent kiełkujących zarodników, wielkość strzępek	Shaw et al. (1987)
<i>Mimulus guttatus</i>		124	Cooper tolerance – variation in root length Tolerancja miedzi – zmienność długości korzeni	Macnair et al. (1993)
<i>Agrostis tenuis</i>	Lead Ołów	1000	Lead tolerance – tolerance index Tolerancja ołowiu – indeks tolerancji	Bone and Farres (2001)
<i>Plantago lanceolata</i>		40	Lead tolerance – tolerance index Tolerancja ołowiu – indeks tolerancji	Wu and Antonovics (1976)

<sup>1</sup> Tolerance index – in the most studies measured as root elongation rate in contaminated medium divided by root elongation rate in uncontaminated medium

<sup>1</sup> Indeks tolerancji – w większości badań mierzone jako tempo elongacji korzeni w zanieczyszczonym podłożu podzielone przez tempo elongacji korzeni w niezanieczyszczonym podłożu

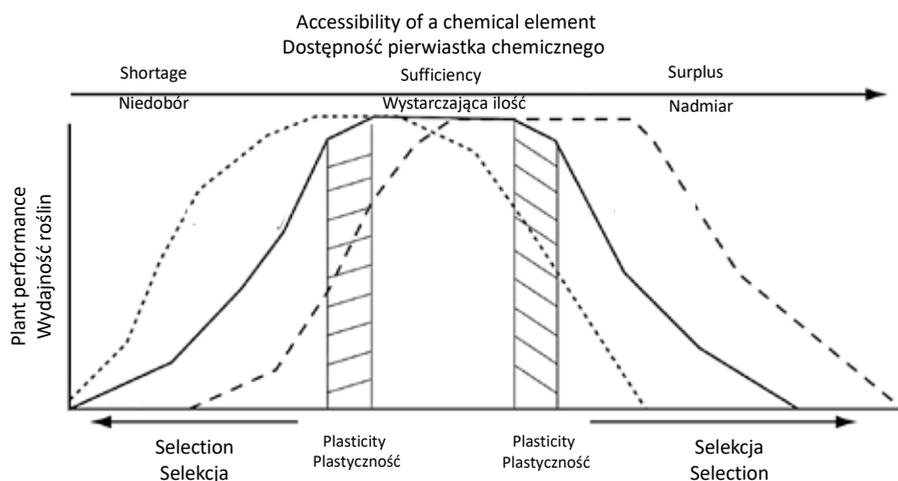


Fig. 1. Diagram of plant evolution trends depending on the presence of a deficit or excess of a chemical element in the environment (after Ernst 2006, modified). In the scheme of hatched zones, the phenotypic plasticity range (the ability of the genotype to produce different phenotypes in response to changes in environmental conditions) of a given group of individuals was determined. The continuous line indicates the state of ecological optimum, when the amount of the chemical element is sufficient and the plants are characterized by optimal physiological performance. In such a situation, the population has both individuals who are exceptionally tolerant to a deficiency of a given element, as well as individuals that are particularly tolerant to the excess of a chemical element. Most, however, are individuals with medium tolerance for both excess and deficiency of the chemical element. The dotted line shows the conditions when, due to a shortage of a given element in the environment, the selection of individuals takes place and the population begins to be dominated by those characterised by tolerance to a deficiency of the element, and intolerant individuals gradually disappear. Whereas, the dashed line illustrates the circumstances when selection occurs due to an excess of chemical element and in the population, individuals with increased tolerance to excess of the element begin to dominate, and those who do not tolerate it are dying out

Ryc. 1. Schemat kierunków ewolucji roślin w zależności od wystąpienia deficytu lub nadmiaru pierwiastka chemicznego w środowisku (za Ernst 2006, zmienione). Na schemacie zakreśkowanymi strefami oznaczono zakres plastyczności fenotypowej (zdolności genotypu do wytwarzania różnych fenotypów w odpowiedzi na zmianę warunków środowiskowych) danej grupy osobników. Ciągłą linią oznaczono stan optimum ekologicznego, gdy ilość pierwiastka chemicznego jest wystarczająca, a rośliny charakteryzuje optymalna wydajność fizjologiczna. W takiej sytuacji w populacji występują zarówno osobniki, które są wyjątkowo tolerancyjne na niedobór danego pierwiastka, jak i osobniki, które są szczególnie tolerancyjne na nadmiar pierwiastka chemicznego. Najwięcej jednak jest osobników o średniej tolerancji zarówno na nadmiar, jak i niedobór pierwiastka chemicznego. Wykropkowana linia pokazuje warunki, gdy na skutek niedoboru danego pierwiastka w środowisku następuje selekcja osobników i w populacji zaczynają dominować te, które charakteryzuje tolerancja na niedobór pierwiastka, a stopniowo wymierają osobniki nietolerancyjne. Przerywana linia obrazuje natomiast okoliczności, gdy selekcja następuje z powodu nadmiaru pierwiastka chemicznego i w populacji zaczynają dominować osobniki o podwyższonej tolerancji na nadmiar pierwiastka, a wymierają te, które tego nie tolerują

and non-metallicolous populations is unlimited, survival of adaptive changes in the newly evolving metallicolous population is only possible if selection pressure exceeds the rate of migration (e.g. when spreading via diaspores) between populations. However, if the flow of genes is impeded between the metallicolous

population and non-metallicolous populations, and there is a low rate of migration, adaptations to metalliferous habitats can be established in the new population after only several generations. If there is also geographical isolation (lack of contact between populations), then the genetic distance between isolated

populations will gradually increase. Over time (relatively short, of a few years to decades), new intraspecific taxonomic units may appear on the area contaminated with metals (Baker 1987, Lefèbvre and Vernet 1990, Stace 1993, Zink and Barrowclough 2008, Babst-Kostecka 2015). A form is considered to be a group of individuals sporadically occurring in populations of a given species, differing in one or several features. Its occurrence is usually a result of minor genetic modifications. A sub-form is the level lower taxonomic category. A variety is a group of individuals establishing a local variant of the species, usually shaped under specific environmental influences (e.g. pH changes, temperature, humidity, etc.), deviating from optimal conditions for a given species, but not so far as to exclude its occurrence completely. The lower taxonomic category is a subvariety. Varieties of a given species can cross between themselves freely. A subspecies is defined as a group of individuals that establish a regional variant of the species. One cause of the differentiation of a subspecies is its geographical variability, which allows adaptation to climatic and environmental differences over a wide range of habitats (Stace 1993, Judd et al. 2008, Stuessy 2009, Turland et al. 2018).

The spatial isolation of the population, together with new environmental conditions (high content of heavy metals in the substrate) for a sufficiently long period of time, can lead to endemism. Endemism is the emergence of completely different forms (endemics) in an isolated population, compared to a non-isolated population. The term endemism is only used to describe cases where the individuals in question occur in a significantly limited area. Endemics are divided into neoendemites (young evolutionary taxa, not yet able to spread to new areas) and plaeoendemites (taxa with a very limited range, which was once much wider). For example, endemics from metalliferous

areas are called absolute metallophytes, because these species' range is limited only to sites containing high concentrations of heavy metals in soil (Kruckeberg and Rabinowitz 1985, Stace 1993, Babst-Kostecka 2015).

As mentioned earlier, microevolution leads to variation within the population of a particular taxon. Genetic variation is subject to natural selection, and the major sources of genetic variation are mutations and genetic recombination. Mutations are changes in the recording of genetic information, such as gene mutations (within one gene), chromosomal mutations (structural chromosome aberrations) and genomic mutations (changes in the number of chromosomes). Where organisms are exposed to toxic compounds, mutations can appear with greater frequency. For example, heavy metals disturb the cell cycle and may cause mutations which affect changes in chromosome number (aneuploidy<sup>6</sup>, polyploidy<sup>7</sup>), chromosome structure, and ontogeny (endomitosis<sup>8</sup>, endoreduplication<sup>9</sup>). Those mutations are usually easy to catch with cytological analyses and DNA content measurements. Inter- and intrapopulation (inter- and intra-individual) variability in chromosome number is a crucial step toward speciation<sup>10</sup>. Another potentially huge source of genetic variation is recombination. Genetic recombinations in eukaryotic organisms are associated with the

<sup>6</sup> Aneuploidy – an unusual (for its species) number of chromosomes in the cells of an organism. This excludes cases of a multiple of one chromosome set.

<sup>7</sup> Polyploidy – possession of more than two complete sets of chromosomes in an organism.

<sup>8</sup> Endomitosis – duplication of genetic material in the cell nucleus followed by no cell division.

<sup>9</sup> Endoreduplication – phenomenon of multiplication of the DNA content bypassing mitosis. During endoreduplication, the number of chromatids is doubled, but they are not segregated (thus the number of chromosomes remains unchanged). Also, neither the loss or reconstruction of the nuclear envelope, nor the condensation or decondensation of chromatin occurs.

<sup>10</sup> Speciation – a process that creates new species.

random combination of gametes during sexual reproduction, and also result from gene rearrangement during meiosis (crossing over) (Kwiatkowska and Kłosowska – Chapter 4 of this volume). Thanks to recombination events, genes or their fragments (in parental organisms) meet in new systems, creating new genotypes (offspring) (Krzanowska et al. 2002, Rogalska et al. 2005, Medina et al. 2007, Freeland 2008, Słomka et al. 2011b, Brown 2018).

Epigenetic inheritance can play an important role in microevolution. The study of epigenetics looks at the changes in gene expression that do not involve changes in the DNA sequence. Instead, the epigenome is a set of chemical modifications of DNA and of histone proteins that regulates chromatin<sup>11</sup> structure and genome functions (Krzanowska et al. 2002, Rapp and Wendel 2005, Perrone and Martinelli 2019, Singroha and Sharma 2019). The variability of epigenetic information is a common phenomenon in plants, many studies document this inter-individual variability in various groups of plants (e.g. Ashikawa 2001, Knox and Ellis 2001, Cervera et al. 2002, Riddle and Richards 2002, Liu and Wendel 2003, Wang et al. 2004, Baulcombe and Dean 2014, Singroha and Sharma 2019, Thiebaut et al. 2019). It has been shown that the interaction of some metals (e.g. lead, cadmium, mercury, copper, and nickel) and organic substances (e.g. benzo[a]pyrene, methoxychlor) may induce epigenetic

changes in individuals exposed to them (Vandegheuchte and Janssen 2011).

Epigenetic inheritance is the passing on of a feature onto progeny without introducing changes in the DNA sequence. The carrier of epigenetic memory is the chromatin structure and the proteins interacting with it (Jaenisch and Bird 2003, Wierzbicki 2004, Rapp and Wendel 2005, Chinnusamy and Zhu 2009, Grativol et al. 2012, Iwasaki and Paszkowski 2014, Puy et al. 2018). An important feature of epigenetic inheritance is the potential for its regulation in response to environmental conditions. This means that in unchanged conditions, the maintenance of a constant, optimized set of subject characteristics (phenotype) is preferred. However, when environmental conditions change, it may be desirable to increase phenotypic variability, which becomes the basis for adaptation to new conditions (Nicotra et al. 2010, Baulcombe and Dean 2014, Perrone and Martinelli 2019, Singroha and Sharma 2019, Thiebaut et al. 2019). Therefore, it is possible that as a response to a change in the environment, a specific pattern of gene expression (decoding the information contained in them) will be established, and will be responsible for certain physiological or developmental adaptations of organisms. Such a gene expression pattern can then be epigenetically fixed in subsequent generations (Wierzbicki 2004, Rapp and Wendel 2005). Thus, characteristics relevant to natural selection can be epigenetically inherited, and there is the possibility of the consolidation of features acquired in response to environmental changes (Rapp and Wendel 2005). Numerous studies show that under stressful conditions there may be a significant increase in phenotypic variability in the population, which in turn speeds up the process of adaptation (Finnegan 2002, Rapp and Wendel 2005, Chinnusamy and Zhu 2009, Nicotra et al. 2010, Grativol et al. 2012, Thiebaut

---

<sup>11</sup> Chromosomes – made of chromatin, a substance made of DNA, histones, non-histone proteins and a small amount of RNA. They can transfer genetic information from generation to generation in the form of a sequence of bases in DNA, and also in the form of instructions contained in the structure and conformation (spatial layout) of chromatin. These epigenetic instructions reflect the functional changes that chromatin undergoes in individual development and during the generation of reproductive cells, for example under the influence of environmental factors. This information may affect gene activity in subsequent generations and play an important role in the process of speciation (formation of new species).

et al. 2019). Given that epigenetic changes can occur much faster than changes based on DNA sequences, and because they can occur due to different environmental stresses, they play an important role in the quick adaptation of plants to the changing environment. New combinations of spatial and temporal gene expression patterns can be achieved using a set of epigenetic mechanisms, even with a complete lack of genetic variation. Thus, upon contact of the organism with a stress-inducing factor, epigenetic responses can trigger huge amounts of latent variation in gene expression that can be tested by natural selection (Rapp and Wendel 2005, Nicotra et al. 2010, Baulcombe and Dean 2014, Perrone and Martinelli 2019, Singroha and Sharma 2019, Thiebaut et al. 2019).

## Microevolution in areas contaminated with heavy metals – examples

In this subsection we will present a few selected examples of plants where microevolutionary processes and adaptation to the increased content of heavy metals in the substrate were observed.

One of the species capable of colonizing areas with a high content of heavy metals is *A. maritima* (sea thrift) (Abratowska et al. 2012, 2015, Holeksa et al. 2015, Rostański et al. 2015). The research conducted has shown that *A. maritima* individuals growing in zinc-lead areas differ in morphological features (e.g. fixed dwarfing of plants from the heap population has been demonstrated), tolerance level and the development of immune mechanisms for heavy metals (lead, cadmium and zinc), compared to individuals of the same species growing on soils without metal contamination. These features were recorded in

the next generation (F1 – the first generation of descendants). It was shown that microevolutionary processes in the population of *A. maritima* in the zinc-lead areas of the Olkusz region led to the development of a separate, unique subspecies with increased resistance to heavy metals – *A. maritima* subsp. *boleslaviensis* subsp. nov. (Abratowska et al. 2012, 2015). Previous studies on the adaptations of *A. maritima* for survival in areas enriched with heavy metals have shown that this species has a complex network of defense mechanisms. The most important strategy for the resistance of *A. maritima* at the level of the individual organism is the storage of metals in the oldest leaves, which dry and are discarded by the plant. Its adaptation at the physiological and biochemical level for growth under conditions of excess toxic heavy metals was also investigated. An increase in the content of glutathione<sup>12</sup> as well as changes in the content and proportions of organic acids such as malic acid was observed (Olko et al. 2008, Abratowska et al. 2012, 2015). It was also shown that in the *A. maritima* plants growing on the substrate enriched with zinc and copper, as well as plants cultivated in medium enriched with lead, cadmium and zinc, the metal accumulated in tannin cells<sup>13</sup>. Another physiological-biochemical adaptation to the excess of heavy metals found in the *A. maritima* plants is the presence of HSP17 small-molecule stress proteins in the cytoplasm, which belong to the heat shock protein family<sup>14</sup>. It is believed that these proteins are involved in the repair of

---

<sup>12</sup> Glutathione – an organic chemical compound with antioxidant properties, made up of amino acid residues: glutamic acid, cysteine, and glycine.

<sup>13</sup> Tannin cells – produce tannins, i.e. organic chemical compounds that are defense compounds against pathogens, herbivores, and adverse environmental conditions.

<sup>14</sup> Heat shock proteins – a group of proteins whose expression increases when cells are exposed to stress factors, e.g. high or low temperature, heavy metals.

intracellular injuries caused from the activity of metals. Defense mechanisms in the *A. maritima* plants in the conditions of excess heavy metals are also associated with the functioning of the products of the leaf epidermis (skin) – trichomes and salt glands. They can accumulate excess metals, which helps to reduce their concentration in leaf mesophyll cells, and thus protects sensitive physiological processes. It was shown that heavy metals, such as lead, cadmium, and zinc, accumulated in simple trichomes and in the salt glands of the *A. maritima* epidermis leaf (Olko et al. 2008, Abratowska et al. 2012, 2015). A particularly interesting feature is the secretion of heavy metals by the salt glands of this species. Salt glands are specialized secretory structures, which play a role in regulating salt concentration in halophytes, plants that grow on saline substrates (e.g. at the seaside). On the leaves of *A. maritima*, the salt glands are found in the upper and lower epidermis of the leaves. When the plants are bred in presence of a substrate containing lead, cadmium and zinc, their leaves are covered by the characteristic clods, formed by the crystallization of salt. In the salt gland cells and in the salt secretions on the surface of the leaves, were those three metals that the plants were subjected to – lead, cadmium and zinc. They were secreted to the surface of the leaf by the epidermal salt glands. The secreted solution also contained other elements that are mineral components of the culture medium: calcium, sulfur, chlorine, magnesium, and potassium. Thus, the regulation of the concentration of metals in the mesophyll of the leaf was dependent on salt glands. This mechanism occurred both in plants from metalliferous areas (e.g. zinc-lead heaps) and from non-metalliferous areas (Wierzbicka and Słysz 2005, Abratowska et al. 2012, 2015). Other researchers (Neumann et al. 1995, Heumann 2002) have also observed the contribution of

salt glands in the detoxification of *A. maritima* plants exposed to excess of heavy metals.

Another species found in zinc-lead waste heaps (calamine waste heaps) in Bolesław is *A. arenosa* (sand rock-cress). Individuals of sand rock-cress from this metalliferous area were compared to those from a non-metalliferous area. It was shown that they differed significantly in all the morphological features analyzed. Plants from the waste heap population were definitely smaller than plants from the unpolluted area. To check whether the observed differences are genetically preserved, biometric studies were carried out in subsequent generations of this species. Once again, significant differences between these populations were shown. The features differentiating the two populations most were: the length of siliques (fruit), the number of trichomes on the leaves, the number of generative shoots, the length and width of the leaves. Visible morphological differences between populations were permanent genetic changes caused by strong, selective environmental pressure on this species. *A. arenosa* plants from the waste heap population were also characterized by a large difference in tolerance to cadmium and zinc, in relation to the reference population. The growth of the *A. arenosa* waste heap population in a metalliferous environment, i.e. in a highly selective environment, led to the development of a highly tolerant group of plants (ecotype<sup>15</sup>) characterized by high induced (acquired) tolerance to zinc and cadmium (Przedpełska and Wierzbicka 2007).

The *D. carthusianorum* (carthusian pink) is one of the important species of grasslands on the calamine waste heaps around Olkusz. Studies on this species have shown that the individuals growing on the calamine waste

---

<sup>15</sup> Ecotype – a form within a species that creates a population or a group of populations that has a set of characteristics for a given environment, arisen as a result of evolution.

heap differed significantly in terms of morphology from those on uncontaminated sites – they were finer, they had fewer leaves in the rosette, and their leaves were shorter and narrower with more stomata. They were also characterized by: longer roots in seedlings and production of long and numerous root-hairs, faster rate of entry into the generative phase and its elongation, production of a larger number of flowers per one individual, production of a larger number of smaller seeds (r-type life strategy). These features were fixed in the first generation of offspring (F1) (Załęcka and Wierzbicka 2002, Wójcik et al. 2013). Genetic separation of the *D. carthusianorum* waste heap population has also been demonstrated. Populations from the calamine waste heap and from land with low heavy metal content were characterized by a similar level of intra-population variation, which means that in the population inhabiting the waste heaps there was no narrowing of the genetic pool. The waste heap population was considered as a calamine ecotype (Wójcik et al. 2013). *D. carthusianorum* from the zinc-lead waste heap also had an elevated tolerance level for lead. It has been proven that efficient lead detoxification in the roots of this plant is one of the key immunity mechanisms that allow the growth of this species on substrates with high lead content (Baranowska-Morek and Wierzbicka 2004, Wójcik and Tukiendorf 2014).

A new calamine ecotype was also recognized in the populations of another species growing on the heap in Bolesław – *S. vulgaris* (bladder campion). This common species is characterized by high intra-species variability. Also, for this species, plants from the waste heaps and natural population (F1 generation) were tested. It was found that in a waste heap population, adaptations to growth under extremely unfavorable conditions are genetically preserved. It was shown, among others, that the plants

from the waste heap population, compared to plants from the natural population, were more resistant to lowering the osmotic water potential (greater resistance to drought). They also had high tolerance to lead and zinc. Plants from the waste heap population were especially abundant in root-hairs compared to plants of the natural population. In addition, they attained lower biomass, had shorter narrower and thicker leaves, and had shortened and creeping shoots. Plants from the heap population developed faster, reached the generative phase earlier, and had greater fertility – they set up more seeds, which were smaller than in plants from the natural population (r-type life strategy) (Wierzbicka and Panufnik 1998, Wierzbicka and Zyska 1999).

Genetic differences between populations from metalliferous areas (including waste heaps from ores mining and processing) and non-metalliferous ones was also demonstrated for the *V. tricolor* (heartsease plugs). In Poland, this species is a common taxon, and shows great morphological diversity. It has been shown that phenotypic plasticity and genetic diversity enable this species to colonize areas contaminated with heavy metals. Genetic differences between populations from the metalliferous areas and areas uncontaminated by metals show the ongoing microevolutionary processes, leading to the creation of new, better adapted local ecotypes. It was also observed that populations from the metalliferous area are characterized by higher genetic polymorphism and genetic differentiation than those from the area uncontaminated by metals. However, in this case, it was shown that this species (individuals from metalliferous and non-metalliferous populations) has a high tolerance to heavy metals, similar to other species from the *Melanium* section (pansies) of the genus *Viola* L. (Słomka et al. 2008, 2010, 2011a, b, c, 2012, Słomka and Kuta 2015).

However, there are not always such clear differences between populations from metalliferous areas and clean areas, as mentioned in the examples above. The work of Jiménez-Ambriz et al. (2007) presented two ecotypes of *Nocca caerulea* (J. Presl & C. Presl) F. K. Mey. (syn. *Thlaspi caeruleum* J. Presl & C. Presl) (Alpine Penny-cress) from southern France, which despite geographical proximity, showed some variation due to the selection of heavy metals related to soil contamination. The biggest differences between the ecotype from the metal-bearing and non-metal-bearing area concerned the sensitivity to high concentrations of zinc. The individuals from the metalliferous area showed greater tolerance. They also produced smaller seeds and fewer fruits per flower stalk, compared to those from the non-metalliferous area. Genetic differences between populations in contaminated areas were greater than between populations in clean areas (Jiménez-Ambriz et al. 2007).

Antonovics (2006) showed that there are differences in the flowering time between *Anthoxanthum odoratum* L. (sweet vernal grass) populations of zinc and lead mine and clean areas (pasture) from North Wales. Metal-tolerant plants have flourished before. This may have been the result of genetic isolation between these two types of populations, which would be another example of microevolutionary processes in populations exposed to heavy metals (Antonovics 2006).

### **Subspecies *B. laevigata* ‘woycickii’ as endemite on waste heaps – genetic research**

A very good example of clear differences between populations of metal-bearing areas and clean areas as a result of microevolutionary processes is the ‘main character’ of this volume,

namely *B. laevigata*. Previous studies have shown that two populations of *B. laevigata*, from the Tatra Mountains and Bolesław areas (from mine heaps) differ significantly from each other by many genetically fixed morphological traits (Pielichowska and Wierzbicka 2004, Wierzbicka and Pielichowska 2004, Pielichowska 2007, Wąsowicz et al. 2014, Wierzbicka et al. 2015, 2016, 2017, 2020, Bemowska-Kałuża et al. – Chapter 6 of this volume). The first important element that differentiated both populations was the features associated with the adjustment to water deficits – the thickness of the leaf blade and the intensity of epidermal trichomes covering the leaves. In both populations studied, these adaptations developed independently in two different directions. Leaves of plants from the waste heap population were thinner, but covered with more trichomes, thus protecting the plants from excessive water loss (transpiration). The trichomes were alive, but their outer cell wall was thick enough to restrict transpiration. In turn, the leaves of plants from the Tatra population had few trichomes, but their leaf blade was thicker due to the increase in the size (vacuolization) of mesophyll leaf cells (Wierzbicka and Pielichowska 2004). This allows for increased water storage in the leaves, and safeguards against a water deficit (Podbielkowski and Podbielkowska 1992, Wierzbicka and Rostański 2002). The second group of features differentiating the calamine heap population from the Tatra population are the features related to the adaptation of plants to the excess of heavy metals in the substrate. Plants of the waste heap population showed a much higher level of tolerance to heavy metals (lead, zinc, cadmium) than plants of the Tatra population. It was shown that the zinc tolerance of the heap population was so high that it could be included in the group of the ‘zinc-loving’ plants (Wąsowicz et al. 2014, Wierzbicka

et al. 2015, 2017, Bemowska-Kařabun et al. – Chapter 6 of this volume).

Genetic tests were also carried out, which made it possible to learn about the genetic structure of the buckler mustard populations from Bolesław and Tatra Mountains. The research was aimed at determining how the colonization of calamine areas took place and determining whether this process took place

relatively recently or in the more distant past (Wąsowicz et al. 2014, Wierzbicka et al. 2015, 2017). Given that the studied waste heap and Tatra populations are located at a considerable distance from each other (about 100 km in a straight line), and the first mentions about the population of *B. laevigata* developing in metalliferous areas of Olkusz come from 1876 (Uechtritz 1877), it was assumed

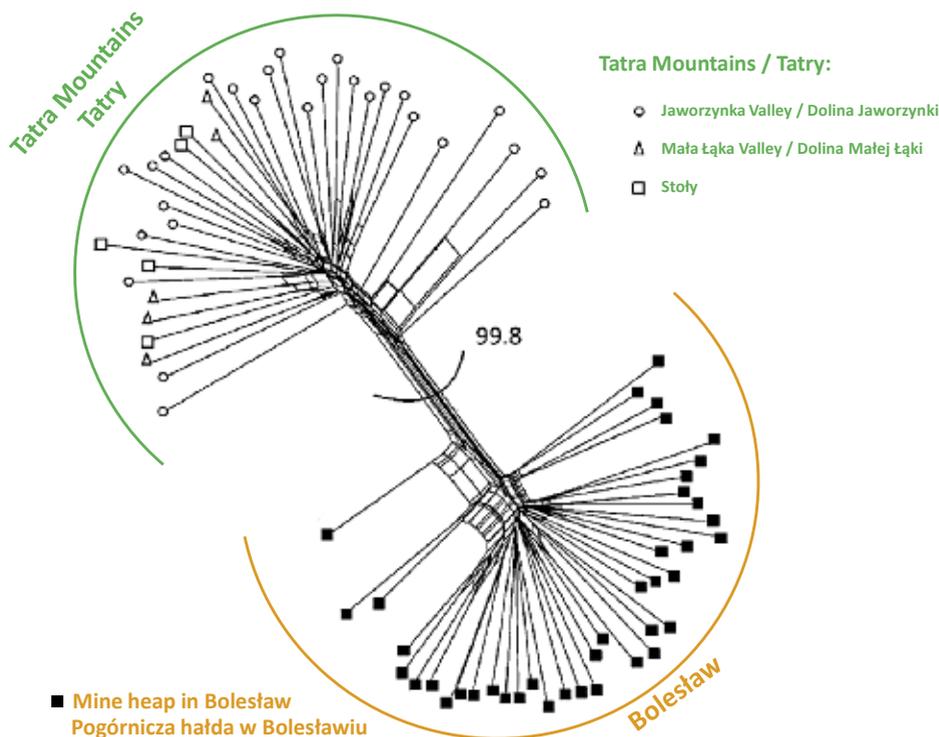


Fig. 2. Neighbour-Joining (NJ) Net showing genetic correlations between *Biscutella laevigata* populations from waste heaps in Bolesław and the Tatra Mountains, developed on the basis of AFLP marker variability data. Various symbols indicate the origin of individuals from different populations in the Tatra Mountains (the Jaworzynka Valley, the Mała Łąka Valley, Stoły). The number above the main branch of the network is the bootstrap value coefficient used to test the ‘reliability’ of the network topology. A factor of up to 100 indicates the high reliability of the obtained image of the genetic structure (after Wąsowicz et al. 2014, Wierzbicka et al. 2015, 2017)

Ryc. 2. Sieć *Neighbor-Joining* (NJ) przedstawiająca zależności genetyczne pomiędzy populacjami *Biscutella laevigata* z hałdy odpadów górniczych w Bolesławiu i z Tatr, opracowana na podstawie danych ze zmienności markerów molekularnych AFLP. Różnymi symbolami oznaczono pochodzenie osobników z różnych populacji w Tatrach (Dolina Jaworzynki, Dolina Małej Łąki, Stoły). Liczba nad główną gałęzią sieci to współczynnik *bootstrap* służący do badania „wiarygodności” topologii sieci. Współczynnik o wartości zbliżającej się do 100 świadczy o wysokiej wiarygodności otrzymanego obrazu struktury genetycznej (za Wąsowicz i in. 2014, Wierzbicka i in. 2015, 2017)

that colonization of the calamine heap by the buckler mustard occurred recently (about 130 years ago) and that this process can be described as ‘long-distance migration’. It was expected that relatively recent colonization of the waste heap and long-distance plant migration contributed to the reduction of the genetic diversity of the waste heap population compared to natural populations in the Tatra Mountains area. Small genetic distances between the studied populations were expected, as well as the lack of genotypes characteristic only for the waste heap population. However, the studies conducted showed that the level of genetic diversity (determined by the AFLP method<sup>16</sup>) was comparable in the two analyzed populations, irrespective of their origin. In addition, genetic distances, which can be read as an indicator of genetic similarity or difference between populations, turned out to be very diverse. The genetic distance measured between the waste heap population and the Tatra populations was more than 30 times greater than the genetic distance between the Tatra populations themselves. The research also showed the presence of genotypes in the waste heap population not found in the Tatra populations. The flow of genes between the populations of the Tatra Mountains and the calamine heap probably ceased at least several thousand years ago, according to the results of genetic research. The results of these tests are presented in Figure 2. It was shown that the population from the calamine heap significantly differs from the Tatra populations in terms of

genetics and forms a separate group. However, individuals from the Tatra populations did not fall into distinct groups (according to their origin from a specific population/place). This may indicate a significant gene flow between individuals from the Tatra populations. The *B. laevigata* population from waste heaps was given the rank of subspecies – *B. laevigata* L. subsp. *woycickii* M. Wierzb., Pielich. & Wasowicz (Wąsowicz et al. 2014, Wierzbicka et al. 2015, 2017, 2020, Bemowska-Kałabun et al. – Chapter 6 of this volume).

The existence of strong genetic diversity between populations of buckler mustard from the Tatra Mountains and the Olkusz region was also shown by Babst-Kostecka et al. (2014), who used microsatellite sequences<sup>17</sup> in their research. They also pointed out that as a result of the microevolutionary processes, the populations dispersed from the area of waste heaps in Bolesław and from the Tatra Mountains. In other studies, Babst-Kostecka et al. (2016) determined the tolerance level for zinc in *B. laevigata* populations from metalliferous and non-metalliferous areas from southern Poland, in order to determine the phenotypic variability of this species in terms of zinc tolerance. Morphological and physiological traits (e.g. biomass, visible symptoms of stress, zinc content in leaves) were examined. Then, the structure of these quantitative traits was compared with neutral molecular markers<sup>18</sup> in order to test whether natural selection caused the *B. laevigata* population to be diversified in terms of zinc tolerance. It turned out that zinc tolerance occurred in the whole species;

---

<sup>16</sup> AFLP method – amplified fragment length polymorphism of DNA, belongs to the group of methods called ‘genetic fingerprint’. AFLP relies on digestion of template DNA with restriction enzymes followed by two amplifications (non-specific and specific) of the DNA fragments. Polymorphism relates to differences in the length of amplified DNA fragments, which arise as a result of nucleotide substitutions within or near restriction sites, or due to deletions or insertions. Most of these polymorphic genes are dominant.

---

<sup>17</sup> Microsatellite sequences – DNA fragments consisting of tandem repeats of a nucleotide motif from 1 to 6 base pairs in length. These sequences are one of the most frequently used molecular markers.

<sup>18</sup> Molecular markers – a genetically controlled phenotypic trait or any genetic difference used to reveal an individual polymorphism that can be identified by analytical methods.

however, higher zinc tolerances were found in plants from metalliferous areas compared to plants from non-metalliferous areas. It has been shown that increased tolerance to zinc in populations of this species from metal-bearing areas results from divergent selection in response to environmental pollution with this metal. In addition, genotype *vs.* environmental interaction analyses indicated that this diversity is hereditary (Babst-Kostecka et al. 2016). Research by Babst-Kostecka et al. (2016) about *B. laevigata* zinc tolerance is consistent with the research of Wierzbicka et al. (2015, 2017), which showed that this species is 'the zinc-loving' plant. It is also worth noting here that the selection process of *B. laevigata* takes place as early as in the production of seeds, as shown by embryological research. Detailed information on this subject is contained in Chapter 4 of this volume.

There are examples of the formation of tolerant plants, which show the ability to grow and efficiently reproduce in areas with an increased content of metals in the soil, and this occurs in a relatively short time – from a dozen to several dozen years. However, these are examples of only the physiological adaptation of plants to the elevated concentration of heavy metals (Wu et al. 1975, Bradshaw and McNeilly 1981, Al-Hiyaly et al. 1993, Briggs and Walters 2000). In the case of *B. laevigata* plants from the calamine waste heap in Bolesław, adaptation to high concentrations of heavy metals included morphological and anatomical features on top of physiological traits. Adaptation at these levels requires a longer period of time in which the stress factor acts, and isolation of the population, as it was in case of the waste heap population of buckler mustard (Wąsowicz et al. 2014, Wierzbicka et al. 2015, 2017, 2020).

To summarize the genetic studies of *B. laevigata*, it was shown that the separation

of the population from the calamine heap in relation to the Tatra populations is the result of an evolutionary process whose duration significantly exceeds the time frame set by the first record of this species in mining areas near Olkusz (1876). It seems that natural outcrops of calamine rocks could have become a place of evolution for the present-day *B. laevigata* population from Bolesław much earlier, and after the start of metal ore extraction, the plant spread to opportune mining area habitats. The attempts to date the divergence time of plants from the Tatra Mountains and Bolesław showed that the intersection of genetic bonds (gene flow) between them could have taken place during the Middle-Polish glaciation, that is about 120,000 years ago. The result obtained on the basis of the molecular dating method can be treated as probable, especially as other indirect proofs also support a long period of isolation (Kropf et al. 2009, Uechtritz 1877, Wąsowicz et al. 2014, Wierzbicka et al. 2015, 2017, 2020).

The presence of numerous differences between the natural Tatra populations of *B. laevigata* and its population from the calamine waste heap in Bolesław justifies the hypothesis of the classification of these plants to a separate taxon. The research discussed above (Wąsowicz et al. 2014, Wierzbicka et al. 2015, 2017, 2020) showed that *B. laevigata* from the waste heap in Bolesław has been evolving for a long time in isolation from the population of this species from the Tatra Mountains. The differences between these two populations are so large that the population of Bolesław was given the rank of subspecies – *B. laevigata* subsp. *woycickii* (Wierzbicka et al. 2020, Bemowska-Kałabun et al. – Chapter 6 of this volume). It is a relict subspecies, probably endemic in nature. These plants have numerous features (mentioned previously in the text) which enable them to grow in extremely difficult conditions

of areas heavily contaminated with heavy metals. Thus, this subspecies is particularly valuable for further research into the mechanisms of tolerance to heavy metals in plants. It seems that it can also have practical application for the restoration of brownfield sites (Muszyńska et al. – Chapter 7, Wiszniewska et al. – Chapter 8 of this volume). Given the very limited area in which *B. laevigata* subsp. *woycickii* occurs, to the heaps of the Olkusz region only (Bemowska-Kałabun et al. – Chapter 6 of this volume), and the rapid transformation of this area, which is associated with natural changes in vegetation as well as human activities (Jędrzejczyk-Korycińska and Szarek-Łukaszewska – Chapter 10 of this volume), there is an urgent need to protect these valuable plants.

## References

- Aburatowska A., Wąsowicz P., Bednarek P., Telka J., Wierzbicka M. 2012. Morphological and genetic distinctiveness of the metallicolous and non-metallicolous populations of *Armeria maritima* s. l. (Plumbaginaceae) in Poland. *Plant Biology* 14(4): 586–595.
- Aburatowska A., Wąsowicz P., Wierzbicka M. 2015. Zawciąg nadmorski – *Armeria maritima*. In: M. Wierzbicka (ed.), *Ekotoksykologia. Rośliny, gleby, metale*. Wydawnictwo Uniwersytetu Warszawskiego, Warszawa, pp. 364–376.
- Al-Hiyaly S., McNeill Y. T., Bradshaw A., Mortimer A. 1993. The effect of zinc contamination from electricity pylons. Genetic constraints on selection for zinc tolerance. *Heredity* 70: 22–32.
- Antonovics J. 2006. Evolution in closely adjacent plant populations X: long-term persistence of prereproductive isolation at a mine boundary. *Heredity* 97: 33–37.
- Ashikawa I. 2001. Surveying CpG methylation at 5'-CCGG in the genomes of rice cultivars. *Plant Molecular Biology* 45: 31–39.
- Ashley M., Willson M., Pergams O., O'Dowd D., Gende S., Brown J. 2003. Evolutionarily enlightened management. *Biological Conservation* 111(2): 115–123.
- Babst-Kostecka A. A. 2015. Aspekty ewolucyjne tolerancji roślin na metale ciężkie. In: M. Wierzbicka (ed.), *Ekotoksykologia. Rośliny, gleby, metale*. Wydawnictwo Uniwersytetu Warszawskiego, Warszawa, pp. 117–123.
- Babst-Kostecka A. A., Parisod C., Godé C., Vollenweider P., Pauwels M. 2014. Patterns of genetic divergence among populations of the pseudometallophyte *Biscutella laevigata* from southern Poland. *Plant and Soil* 383(1–2): 245–256.
- Babst-Kostecka A. A., Waldmann P., Frérot H., Vollenweider P. 2016. Plant adaptation to metal polluted environments – physiological, morphological, and evolutionary insights from *Biscutella laevigata*. *Environmental and Experimental Botany* 127: 1–13.
- Baker A. 1987. Metal tolerance. *New Phytologist* 106: 93–111.
- Baker A., Proctor J. 1990. The influence of cadmium, copper, lead, and zinc on the distribution and evolution of metallophytes in the British Isles. *Plant Systematics and Evolution* 173(1–2): 91–108.
- Baranowska-Morek A., Wierzbicka M. 2004. Localization of lead in root tip of *Dianthus carthusianorum*. *Acta Biologica Cracoviensia Ser. Botanica* 46: 45–56.
- Baulcombe D., Dean C. 2014. Epigenetic regulation in plant responses to the environment. *Cold Spring Harbor Perspectives in Biology* 6(9): a019471.
- Baumbach H., Hellwig F. 2003. Genetic variation within and among metal-tolerant and non-tolerant populations of *Armeria maritima* (Mill.) Willd. s.l. (Plumbaginaceae) in Central and Northeast Germany. *Plant Biology* 5(2): 186–193.
- Bickham J. 2011. The four cornerstones of evolutionary toxicology. *Ecotoxicology* 20(3): 497–502.

- Bone E., Farres A. 2001. Trends and rates of microevolution in plants. *Genetica* 112–113: 165–182.
- Bradshaw A., McNeilly T. 1981. Evolution and pollution. Arnold, London.
- Briggs D., Walters S. 2000. Plant variation and evolution. Cambridge University Press, Cambridge, pp. 246–250.
- Brown T. 2018. Genomy. Edition 2. Wydawnictwo Naukowe PWN, Warszawa.
- Cervera M., Ruiz-García L., Martínez-Zapater J. 2002. Analysis of DNA methylation in *Arabidopsis thaliana* based on methylation-sensitive AFLP markers. *Molecular Genetics and Genomics* 268(4): 543–552.
- Chinnusamy V., Zhu J. 2009. Epigenetic regulation of stress responses in plants. *Current Opinion in Plant Biology* 12(2): 133–139.
- Coutellec M., Barata C. 2011. An introduction to evolutionary processes in ecotoxicology. *Ecotoxicology* 20(3): 493–496.
- Ernst W. 2006. Evolution of metal tolerance in higher plants. *Forest Snow and Landscape Research* 80(3): 251–274.
- Finnegan E. 2002. Epialleles – a source of random variation in times of stress. *Current Opinion in Plant Biology* 5(2): 101–106.
- Freeland J. 2008. Ekologia molekularna. Wydawnictwo Naukowe PWN, Warszawa.
- Godé C., Decombeix I., Kostecka A., Wasowicz P., Pauwels M., Courseaux A., Saumitou-Laprade P. 2012. Nuclear microsatellite loci for *Arabidopsis halleri* (Brassicaceae), a model species to study plant adaptation to heavy metals. *American Journal of Botany* 99(2): e49–e52.
- Gorné L., Díaz, S. 2019. Meta-analysis shows that rapid phenotypic change in angiosperms in response to environmental change is followed by stasis. *The American Naturalist* 194(6): 840–853.
- Grant V. 1981. Plant speciation. Columbia University Press, New York.
- Grativol C., Hemerly A., Ferreira P. 2012. Genetic and epigenetic regulation of stress responses in natural plant populations. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms* 1819(2): 176–185.
- Hendry A., Kinnison M. 2001. An introduction to microevolution: rate, pattern, process. *Genetica* 112(1): 1–8.
- Heumann H. 2002. Ultrastructural localization of zinc in zinc-tolerant *Armeria maritima* ssp. *halleri* by autometallography. *Journal of Plant Physiology* 159: 191–203.
- Hildebrandt U., Hoef-Emden K., Backhausen S., Bothe H., Božek M., Siuta A., Kuta E. 2006. The rare, endemic zinc violets of Central Europe originate from *Viola lutea* Huds. *Plant Systematics and Evolution* 257(3–4): 205–222.
- Holeksa J., Błońska A., Kompała-Bąba A., Woźniak G., Kurek P., Szarek-Łukaszewska G., Grodzińska K., Żywiec M. 2015. The vegetation of the Olkusz Ore-bearing Region. In: B. Godzik (ed.), Natural and historical values of the Olkusz Ore-bearing Region. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, pp. 105–128.
- Iwasaki M., Paszkowski J. 2014. Epigenetic memory in plants. *The EMBO Journal* 33(18): 1987–1998.
- Jiménez Ambriz G., Petit C., Bourrié I., Dubois S., Olivier I., Ronce O. 2007. Life history variation in the heavy metal tolerant plant *Thlaspi caerulescens* growing in a network of contaminated and noncontaminated sites in southern France: role of gene flow, selection and phenotypic plasticity. *New Phytologist* 173(1): 199–215.
- Judd W., Campbell C., Kellogg E., Stevens P., Donoghue M. 2008. Plant systematics: a phylogenetic approach. Edition 3. Sinauer Associates Incorporated, Sunderland, USA.
- Levin D. 2000. The origin, expansion, and demise of plant species. Oxford University Press, New York, Oxford.
- Lefebvre C., Vernet P. 1990. Microevolutionary processes on contaminated deposits. In: A. Shaw (ed.), Heavy metal tolerance in plants: evolutionary aspects. CRC Press Inc., Boca Raton, Florida, pp. 285–299.

- Jaenisch R., Bird A. 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nature Genetics* 33: 245–254.
- Kiang Y. 1982. Local differentiation of *Anthoxanthum odoratum* L. populations on roadsides. *American Midland Naturalist* 107: 340–350.
- Knox M., Ellis T. 2001. Stability and inheritance of methylation states at PstI sites in *Pisum*. *Molecular Genetics and Genomics* 265: 497–507.
- Kropf M., Comes H., Kadereit J. 2009. An AFLP clock for the absolute dating of shallow-time evolutionary history based on the intraspecific divergence of southwestern European alpine plant species. *Molecular Ecology* 18: 697–708.
- Kruckeberg A., Rabinowitz D. 1985. Biological aspects of endemism in higher plants. *Annual Review of Ecology and Systematics* 16: 447–479.
- Krzanowska H., Łomnicki A., Rafiński J., Szarski H., Szymura J. 2002. *Zarys mechanizmów ewolucji*. Wydawnictwo Naukowe PWN, Warszawa.
- Kuta E., Jędrzejczyk-Korycińska M., Cieślak E., Rostański A., Szczepaniak M., Migdalek G., Wąsowicz P., Suda J., Combik M., Słomka A. 2014. Morphological versus genetic diversity of *Viola reichenbachiana* and *V. riviniana* (sect. *Viola*, Violaceae) from soils differing in heavy metal content. *Plant Biology* 16(5): 924–934.
- Lefebvre C., Vernet P. 1990. Microevolutionary processes on contaminated deposits. In: A. Shaw (ed.), *Heavy metal tolerance in plants: evolutionary aspects*. CRC Press Inc., Boca Raton, Florida, pp. 285–299.
- Liu B., Wendel J. 2003. Epigenetic phenomena and the evolution of plant allopolyploids. *Molecular Phylogenetics and Evolution* 29: 365–379.
- Macnair M., Smith S., Cumbes Q. 1993. Heritability and distribution of variation in degree of copper tolerance in *Mimulus guttatus* at Copperopolis, California. *Heredity* 71: 445–455.
- Medina M., Correa J., Barata C. 2007. Micro-evolution due to pollution: possible consequences for ecosystem responses to toxic stress. *Chemosphere* 67(11): 2105–2114.
- Migdalek G., Woźniak M., Słomka A., Godzik B., Jędrzejczyk-Korycińska M., Rostański A., Bothe H., Kuta E. 2013. Morphological differences between violets growing at heavy metal polluted and non-polluted sites. *Flora-Morphology, Distribution, Functional Ecology of Plants* 208(2): 87–96.
- Mitchell N., Whitney K. 2018. Can plants evolve to meet a changing climate? The potential of field experimental evolution studies. *American Journal of Botany* 105(10): 1613–1616.
- Neumann D., zur Nieden U., Lichtenberger O., Leopold I. 1995. How does *Armeria maritima* tolerate high heavy metal concentrations? *Journal of Plant Physiology* 146: 704–717.
- Nicotra A., Atkin O., Bonser S., Davidson A., Finnegan E., Mathesius U., Poot P., Purugganan M., Richards C., Valladares F., van Kleunen M. 2010. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science* 15(12): 684–692.
- Olko A., Abratowska A., Żyłkowska J., Wierzbicka M., Tukendorf A. 2008. *Armeria maritima* from a calamine heap – initial studies on physiologic – metabolic adaptations to metal enriched soil. *Ecotoxicology and Environmental Safety* 69: 209–218.
- Perrone A., Martinelli F. 2019. Plant stress biology in epigenomic Era. *Plant Science*: 110376.
- Pielichowska M. 2007. Tolerancja na metale ciężkie górskiego gatunku rośliny *Biscutella laevigata*. Wydział Biologii, Uniwersytet Warszawski. PhD thesis.
- Pielichowska M., Wierzbicka M. 2004. Uptake and localization of cadmium by *Biscutella laevigata*, a cadmium hyperaccumulator. *Acta Biologica Cracoviensia. Ser. Botanica* 46: 57–63.
- Podbielkowski Z., Podbielkowska M. 1992. *Przysposobienia roślin do środowiska*. Wydawnictwa Szkolne i Pedagogiczne, Warszawa.
- Przedpętska E., Wierzbicka M. 2007. *Arabidopsis arenosa* (Brassicaceae) from a lead-zinc waste heap in southern Poland – a plant with high tolerance to heavy metals. *Plant and Soil* 299(1–2): 43–53.

- Puy J., Dvořáková H., Carmona C., de Bello F., Hiiesalu I., Latzel V. 2018. Improved demethylation in ecological epigenetic experiments: Testing a simple and harmless foliar demethylation application. *Methods in Ecology and Evolution* 9(3): 744–753.
- Rapp R., Wendel J. 2005. Epigenetics and plant evolution. *New Phytologist* 168(1): 81–91.
- Riddle N., Richards E. 2002. The control of natural variation in cytosine methylation in *Arabidopsis*. *Genetics* 162: 355–363.
- Rogalska S., Małuszyńska J., Olszewska M. 2005. Podstawy cytogenetyki roślin. Wyd. 2. Wydawnictwo Naukowe PWN, Warszawa.
- Rostański A., Nowak T., Jędrzejczyk-Korycińska M. 2015. Metalolubne gatunki roślin naczyniowych we florze Polski. In: M. Wierzbicka (ed.), *Ekotoksykologia. Rośliny, gleby, metale*. Wydawnictwo Uniwersytetu Warszawskiego, Warszawa, pp. 299–322.
- Shaw J., Antonovics J., Anderson L. 1987. Inter- and intra specific variation of mosses in tolerance to copper and zinc. *Evolution* 41: 1312–1325.
- Singroha G., Sharma P. 2019. Epigenetic modifications in plants under abiotic stress. In: R. Maccariello (ed.), *Epigenetics*. IntechOpen, <https://www.intechopen.com/books/epigenetics/epigenetic-modifications-in-plants-under-abiotic-stress>
- Słomka A., Kuta E. 2015. Fiołek trójbarwny – *Viola tricolor*. In: M. Wierzbicka (ed.), *Ekotoksykologia. Rośliny, gleby, metale*. Wydawnictwo Uniwersytetu Warszawskiego, Warszawa, pp. 392–410.
- Słomka A., Libik-Konieczny M., Kuta E., Miszałski Z. 2008. Metalliferous and non-metalliferous populations of *Viola tricolor* represent similar mode of antioxidative response. *Journal of Plant Physiology* 165(15): 1610–1619.
- Słomka A., Kawalec P., Kellner K., Jędrzejczyk-Korycińska M., Rostański A., Kuta E. 2010. Was reduced pollen viability in *Viola tricolor* L. the result of heavy metal pollution or rather the tests applied? *Acta Biologica Cracoviensia Ser. Botanica* 52(1): 123–127.
- Słomka A., Kuta E., Szarek-Łukaszewska G., Godzik B., Kapusta P., Tylko G., Bothe H. 2011a. Violets of the section *Melanium*, their colonization by arbuscular mycorrhizal fungi and their occurrence on heavy metal heaps. *Journal of Plant Physiology* 168(11): 1191–1199.
- Słomka A., Siwińska D., Wolny E., Kellner K., Kuta E. 2011b. Influence of a heavy-metal-polluted environment on *Viola tricolor* genome size and chromosome number. *Acta Biologica Cracoviensia Ser. Botanica* 53(1): 7–15.
- Słomka A., Sutkowska A., Szczepaniak M., Maliec P., Mitka J., Kuta E. 2011c. Increased genetic diversity of *Viola tricolor* L. (Violaceae) in metal-polluted environments. *Chemosphere* 83(4): 435–442.
- Słomka A., Jędrzejczyk-Korycińska M., Rostański A., Karcz J., Kawalec P., Kuta E. 2012. Heavy metals in soil affect reproductive processes more than morphological characters in *Viola tricolor*. *Environmental and Experimental Botany* 75: 204–211.
- Stace C. 1993. *Taksonomia roślin i biosystematyka*. Wydawnictwo Naukowe PWN, Warszawa.
- Stuessy T. 2009. *Plant taxonomy: the systematic evaluation of comparative data*. Columbia University Press, New York.
- Thiebaut F., Hemerly A., Ferreira P. 2019. A role for epigenetic regulation in the adaptation and stress responses of non-model plants. *Frontiers in Plant Science* 10: 246.
- Turland N., Wiersema J., Barrie F., Greuter W., Hawksworth D., Herendeen P., Knapp S., Kusber W., Li D., Marhold K., May T., McNeill J., Monro A., Prado J., Price M., Smith G. (eds). 2018. International code of nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile* 159. Glashütten: Koeltz Botanical Books.
- Uechtritz R. 1877. *Die wichtigeren Ergebnisse der Durchforschung der schlesischen*

- Phanerogammenflora im Jahre 1876. Jahres-Bericht der Schlesische Gesellschaft für vaterländische Cultur 54: 155–195.
- Vandegehuchte M., Janssen C. 2011. Epigenetics and its implications for ecotoxicology. *Ecotoxicology* 20(3): 607–624.
- Van Straalen N., Janssens T., Roelofs D. 2011. Micro-evolution of toxicant tolerance: from single genes to the genome's tangled bank. *Ecotoxicology* 20(3): 574–579.
- Wang Y., Lin X., Dong B., Wang Y., Liu B. 2004. DNA methylation polymorphism in a set of elite rice cultivars and its possible contribution to inter-cultivar differential gene expression. *Cellular and Molecular Biology Letters* 9: 543–556.
- Wąsowicz P., Pieliowska M., Przedpeńska-Wąsowicz E., Bednarek P., Szarek-Łukaszewska G., Abratowska A., Wierzbicka M. 2014. Physiological and genetic differentiation between metalicolous and non-metallicolous diploid populations of Alpine *Biscutella laevigata* (Brassicaceae) in the Tatra Mountains and the Northern Carpathian Foreland. *Annales Botanici Fennici* 51: 227–239.
- Wierzbicka M. 1999. Comparison of lead tolerance in *Allium cepa* with other plant species. *Environmental Pollution* 104(1): 41–52.
- Wierzbicka M. 2015. Obrona roślin przed metalami ciężkimi. In: M. Wierzbicka (ed.), *Ekotoksykologia. Rośliny, gleby, metale*. Wydawnictwo Uniwersytetu Warszawskiego, Warszawa, pp. 83–95.
- Wierzbicka M., Panufnik D. 1998. The adaptation of *Silene vulgaris* to growth on a calamine waste heap (S. Poland). *Environmental Pollution* 101(3): 415–426.
- Wierzbicka M., Pieliowska M. 2004. Adaptation of *Biscutella laevigata* L., a metal hyperaccumulator, to growth on a zinc-lead waste heap in southern Poland: I: Differences between waste-heap and mountain populations. *Chemosphere* 54(11): 1663–1674.
- Wierzbicka M., Rostański A. 2002. Microevolutionary changes in ecotypes of calamine waste heap vegetation near Olkusz, Poland: a review. *Acta Biologica Cracoviensia Ser. Botanica* 44: 7–19.
- Wierzbicka M., Słysz A. 2005. Does *Armeria maritima* subsp. *halleri* (Plumbaginaceae) occur in Poland? *Polish Botanical Studies* 19: 105–117.
- Wierzbicka M., Zyska A. 1999. Zinc tolerance of *Silene vulgaris* population from a calamine waste heap near Olkusz, Poland. In: Trace elements – effect of organism and environment. Uniwersytet Śląski, Katowice, pp: 215–219.
- Wierzbicka M., Pieliowska M., Wąsowicz P. 2015. Pleszczotka górská – *Biscutella laevigata*. In: M. Wierzbicka (ed.), *Ekotoksykologia. Rośliny, gleby, metale*. Wydawnictwo Uniwersytetu Warszawskiego, Warszawa, pp. 411–427.
- Wierzbicka M., Pieliowska M., Abratowska A., Wilkomirski B., Wysocka I., Panufnik-Mędrzycka D., Bulska E. 2016. Thallium hyperaccumulation in Polish populations of *Biscutella laevigata* (Brassicaceae). *Acta Biologica Cracoviensia Ser. Botanica* 58(2): 7–19.
- Wierzbicka M., Pieliowska M., Bemowska-Kałabun O., Wąsowicz P. 2017. Microevolution on anthropogenically changed areas on the example of *Biscutella laevigata* plants from calamine waste heap in Poland. *Journal of Environmental & Analytical Toxicology* 7:471, doi: 10.4172/2161-0525.1000479.
- Wierzbicka M., Pieliowska M., Bemowska-Kałabun O., Rostański A., Wąsowicz P. 2020. A new taxon within *Biscutella laevigata* L. (Brassicaceae) endemic to calamine areas in southern Poland. *PhytoKeys* 160: 123–129.
- Wierzbicka A. 2004. Dziedziczenie epigenetyczne. *Kosmos* 3(53): 271–28.
- Wójcik M., Tukiendorf A. 2014. Accumulation and tolerance of lead in two contrasting ecotypes of *Dianthus carthusianorum*. *Phytochemistry* 100: 60–65.
- Wójcik M., Dresler S., Jawor E., Kowalczyk K., Tukiendorf A. 2013. Morphological, physiological and genetic variation between metalicolous and nonmetallicolous populations of *Dianthus carthusianorum*. *Chemosphere* 90(3): 1249–1257.

- Wu L., Antonovics J. 1976. Experimental genetics of *Plantago*. II: lead tolerance in *P. lanceolata* and *Cynodon dactylon* from a roadside. *Ecology* 37: 205–208.
- Wu L., Kruckeberg A. 1985. Copper tolerance in two legume species from a copper mine habitat. *New Phytologist* 99: 565–570.
- Wu L., Bradshaw A., Thurman D. 1975. The potential for evolution of heavy metal tolerance. III. The rapid evolution of copper tolerance in *Agrostis stolonifera*. *Heredity* 34: 165–187.
- Załęcka R., Wierzbicka M. 2002. The adaptation of *Dianthus carthusianorum* L. (*Caryophyllaceae*) to growth on a zinc-lead heap in southern Poland. *Plant and Soil* 246(2): 249–257.
- Zink R., Barrowclough G. 2008. Mitochondrial DNA under siege in avian phylogeography. *Molecular Ecology* 17: 2107–2121.