Genetic and epigenetic variation in a cosmopolitan grass *Poa annua* from Antarctic and Polish populations

Katarzyna J. CHWEDORZEWSKA[1] and Piotr T. BEDNAREK[2]

[1] *Instytut Biochemii i Biofizyki PAN, Zakład Biologii Antarktyki,
ul. Pawińskiego 5a, 02-106 Warszawa, Polska
<kchwedorzewska@go2.pl>*

[2] *Instytut Hodowli i Aklimatyzacji Roślin, 05-870 Błonie, Radzików, Polska
<p.bednarek@ihar.edu.pl>*

Abstract: *Poa annua* L. is the only non-native vascular plant that was successfully established in the maritime Antarctic. This project aimed to determine the amount of genetic and epigenetic variation within and between two populations of *P. annua*, one from South Shetland Is. (Antarctic) and the other one from Central Europe. We applied two AFLP marker systems, using endonucleases that recognised the same restriction site but differed in their sensitivity towards methylation. The Antarctic population differed from the Polish one both at the genetic and epigenetic levels. Genetic variability in the Antarctic population was lower than in the Polish one. Some loci in the Antarctic population showed signs of selection. The difference between Polish and Antarctic populations might be due to a weak bottleneck effect followed by population expansion. Using only epigenetic markers, the Antarctic population exhibited increased variation level compared to the Polish one. These may have resulted from plastic responses to environmental factors and could be associated with survival in extreme conditions.

Key words: Antarctic, *Poa annua*, invasive species, metAFLP.

Introduction

Environmental factors, such as low-summer temperature, available liquid water, highly seasonal light regime and elevated UV-B radiation, with many seasonally related environmental stresses (Frenot *et al.* 2005) limit the Antarctic biota. Currently, some parts of Antarctica are the fastest warming regions on the planet (Turner *et al.* 2005). In the short term, terrestrial biota are likely to benefit from reduced environmental stresses while, in the long term, the colonisation of the region by lower latitude species with greater competitive capability will become increasingly prominent (Olech 1996, 1998; Convey 2006; Chwedorzewska 2009, 2010).

Dynamic climate changes combined with increased human activities might have modified the status of some introduced species and increased the pressure of exotic organism propagules (Frenot *et al.* 2001; Convey 2006). There were several reports of non-indigenous vascular plants occurring close to Antarctic stations (*e.g.* Lewis-Smith 1996; Chwedorzewska 2009; Hughes *et al.* 2009; Hughes and Convey 2010). However, only *Poa annua* L. has successfully survived, bred and spread under maritime Antarctic conditions for many years (Olech and Chwedorzewska 2011).

Annual bluegrass is native to Eurasia (Tutin 1952, 1957; Darmency and Gasquez 1997; Heide 2001). It became naturalized around the Earth and was found in association with human activity or pioneer zones. *P. annua* adapted to a broad range of weather conditions, from cold polar regions to hot deserts (Darmency and Gasquez 1981; Frenot *et al.* 2001). The wide distribution of *P. annua* resulted from its high colonising ability (Law 1981; Frenot 1997; Heide 2001). Annual bluegrass grew and reproduced rapidly, primarily *via* seeds that might retain their viability for several years, yielding even 20000 seeds in one season (Hutchinson and Seymour 1982). They could grow over a wide range of environmental conditions (Vargas and Turgeon 2004). Moreover, Heide (2001) demonstrated widely differing flowering responses to day length and vernalization in different *P. annua* populations.The species is considered to be allotetraploid with chromosome count $2n = 28$ (Tutin 1957; Johnson 1995; Heide 2001). However, diploid forms have been reported (Hovin 1957; Johnson *et al.* 1993), but plants are dwarf, weak, male-sterile and did not produce developed seeds (Johnson *et al.* 1993). *P. annua* is an autogamous species, with 0–15% outcrossing, depending on environmental conditions (Ellis 1973). The outcrossing could even get as high as 22% under stress conditions (Mengistu *et al.* 2000; Chen *et al.* 2003). Apomixis was also observed (Johnson *et al.* 1993). Antarctica shows few successful introductions compared to the other continents and the majority of the introduced species were human-dependent immigrants (Chwedorzewska 2009). The abundance of *Poa annua* in the vicinity of the *Arctowski* Station seems to indicate that human activity was responsible for the introduction and dissemination of this species (Olech 1996; Olech and Chwedorzewska 2011). Initially *P. annua* was recorded in front of the entrance to the main building of the Polish Antarctic Station during the summer of 1985/86, followed by a gradual increase of the population size and colonising places strongly altered by human activities (Olech 1996, 1998), then the natural habitats (Olech and Chwedorzewska 2011). Nevertheless, the origin of the Antarctic population is not entirely clear, and previous genetic studies indicated a possibility of multiple introductions from different sources (Chwedorzewska 2008). Every year several tons of cargo and more than 20 expeditions with personal luggage and equipment come mainly from Poland to *Arctowski* (Chwedorzewska and Korczak 2010). According to available historical data one can hypothesize that propagules of *P. annua* probably originate from Poland, most likely from soil for

greenhouse transported to the station more than 30 years ago, but other vectors and sources could not be excluded.

The term "epigenetics" generally refers to molecular processes such as DNA methylation, histone modification and RNA interference, that can alter gene function and, ultimately, the phenotype without changes in DNA sequence (Grant-Downton and Dickinson 2005, 2006; Berger 2007; Bird 2007). DNA methylation, the modification that affects cytosine residues of DNA, is one of the best-described epigenetic mechanisms. There are two types of DNA methylation in plants: symmetric and asymmetric. The former affects such sequences as CpG or CpXpG while the latter CpXpX (where X = A, T). DNA methylation and especially CpG sites usually cluster in the regulatory region of genes. Thus, such regions may regulate gene activity or transcriptional silencing (Finnegan 2001, 2010). DNA methylation can be inherited influencing various plant traits (Henderson and Jacobsen 2007; Jablonka 2009; Verhoeven *et al.* 2010). It may also provide some features allowing to survive in novel environments (Lira-Medeiros *et al.* 2010).

The study of such genetic and epigenetic changes became easier with the development of the metAFLP approach. The AFLP technique adapted by Bednarek *et al.* (2007) allows analysis of both genetic and site DNA methylation pattern changes within restriction site sequences recognized by *Acc*65I and *Kpn*I isoschizomers (Fiuk *et al.* 2010). While *Kpn*I is insensitive towards methylation of the restriction site and its nearest vicinity, *Acc*65I is sensitive and cannot cut such sequences. This method provides a way to determine DNA sequence and methylation pattern changes among multiple samples.

The goals of the work were to examine genetic and epigenetic variation among samples originating from the Antarctic population of *Poa annua* and the one from Poland using metAFLP approach.

Methods

Samples of *P. annua* collected during the austral summer season (2008/2009) on the west shore of the Admiralty Bay in the close vicinity of *Arctowski* Station (King George Island, South Shetlands Islands, 62°09'S, 58°28'W) along the transect (150 m long and about 10 wide) covered majority of *P. annua* population established at *Arctowski*. The total number of individuals of Antarctic population reached a couple of thousands on the acreage covering about 12000 m^2 (Olech, personal communication). The sampled individuals were collected at least one meter apart from each other, from separate genet. The same numbers of plants were collected from Poland in May 2009 (at the Botanical Garden in Powsin, 52°07'N, 21°06'E), from the place of origin of soil transported for the greenhouse in *Arctowski* Station more than thirty years ago. Plants in this area were much more spread out, so the transect was 400 m long and 10 m wide. To minimize develop-

mental epigenetic variation that might affect population differentiation plants forming the representative sample were collected at a similar phenological stage (at the beginning of flowering). Fresh shoots of individual plants were placed in plastic bags in the presence of silica gel and immediately frozen at -70°C until DNA extraction. DNA were extracted from 96 individuals of each population (in total 192), by the MagAttract® 96 DNA Plant kit (Qiagen).

The AFLP procedure followed the method proposed by Vos *et al.*(1995) with some modifications (Bednarek *et al.* 2007). In total, 500ng of genomic DNA underwent digestion with *Acc*65I/*Mse*I endonucleases. Another 500ng of the same DNA followed digestion with *Kpn*I/*Mse*I enzymes. Adaptor ligation to both digests followed pre-selective and selective amplification steps. The latter reaction was conducted in the presence of 5'-(^{32}P) labelled selective primer complementary to the adaptor sequence ligated to the *Acc*65I(*Kpn*I) sites. Eight selective primer pair combinations (Table 1) amplified DNA fragments separated on 7% polyacrylamide gel and exposed to X-ray films at -70°C overnight.

Table 1

Arrangement of data generated with the selected primer pairs for both restriction enzymes combinations

Enzyme combination	Selective primer pairs combination	Detected bands (in total)	Detected bands			
			Polish population	Antarctic population	Polymorphic Polish population	Polymorphic Antarctic population
*Acc*65I/*Mse*I	CpXpG-AGC/M-CCA	33	32	28	10	10
*Kpn*I/*Mse*I	CpXpG-AGC/M-CCA		32	29	11	11
*Acc*65I/*Mse*I	CpXpG-GGC/M-CAA	31	26	29	16	15
*Kpn*I/*Mse*I	CpXpG-GGC/M-CAA		27	28	14	11
*Acc*65I/*Mse*I	CpXpG-AGA/M-CCC	28	25	25	11	9
*Kpn*I/*Mse*I	CpXpG-AGA/M-CCC		28	28	9	7
*Acc*65I/*Mse*I	CpXpG-AGG/M-CAG	59	57	50	33	30
*Kpn*I/*Mse*I	CpXpG-AGG/M-CAG		59	55	33	27
*Acc*65I/*Mse*I	CpXpG-TGC/M-CGG	19	19	16	5	5
*Kpn*I/*Mse*I	CpXpG-TGC/M-CGG		18	15	7	5
*Acc*65I/*Mse*I	CpXpG-ACC/M-CCA	78	75	66	43	36
*Kpn*I/*Mse*I	CpXpG-ACC/M-CCA		78	69	43	36
*Acc*65I/*Mse*I	CpG-GGT/M-CCG	13	13	13	0	0
*Kpn*I/*Mse*I	CpG-GGT/M-CCG		13	13	0	0
*Acc*65I/*Mse*I	CpG-AGG/M-CAT	16	16	15	4	4
*Kpn*I/*Mse*I	CpG-AGG/M-CAT		16	15	4	2
*Acc*65I/*Mse*I	Total	277	263	242	122	109
*Kpn*I/*Mse*I			271	252	121	99

Reproducible, clearly distinguishable AFLP fragments were scored in a form of a binary matrix with presence (1) and absence (0), while the absence of a band was estimated by two independent persons. The matrix based on *Kpn*I/*Mse*I digests reflected genetic variation, while the *Acc*65I/*Mse*I matrix reflected genetic and epigenetic variation. Comparison of matrices based on *Acc*65I/*Mse*I and *Kpn*I/*Mse*I digests gave a single matrix containing epigenetic markers only. The AFLPop EXCEL add-in software (Duchesne and Bernatchez 2002) eliminated redundant markers (redundant markers are loci that show identical scores among all individuals across both populations) amplified by the two platforms. The non-redundant data converted to the third matrix reflected epigenetic variation. The rationale for the transformation is so that the markers present in *Kpn*I/*Mse*I and missing in *Acc*65I/*Mse*I digests (and *vice versa*) reflect restriction sites with methylated cytosine residues (epigenetic variation).

The matrices formed the basis for the calculation of the percentage of polymorphic loci (P%), number of unique markers, expected heterozygosity (H_E), Shannon's diversity index (I) (Nei 1978, Shannon and Weaver 1949) and Nei's genetic distance (Nei GD) (Nei 1978) in GenAlEx 5.3 EXCEL add-in (Peakall and Smouse 2001). Calculation of Polymorphic Information Content (PIC) followed formula PIC = Σ 0.25×(1-f_i)× f_i, where f_i is the frequency of the ith allele (Roldán--Ruiz *et al.* 2000; Schönswetter and Tribsch 2005). Neighbour Joining clustering was constructed with PAST (Schönswetter and Tribsch 2005) using Jaccard genetic distances. The robustness of the branches was estimated using 1000 bootstrap replicates. Principal Coordinate Analysis (PCoA) was also performed in PAST (Halliburton 2004; Hammer *et al.* 2001).

Analysis of Molecular Variance – AMOVA (Φ_{PT} index values) was performed with GenAlex (Halliburton 2004). 999 permutations were performed to estimate reliability of the data. Tajima's D, Fu's F_s neutrality tests were evaluated in Arlequin 3.5 (Excoffier and Lischer 2010).

For Bayesian analysis of genetic structure performed with STRUCTURE 2.2.3 (Falush *et al.* 2007; Foll and Gaggiotti 2008), no admixture model and independent allele frequency options with the length of burn-in (10 000) and Markov Chain Monte Carlo (1 000 000) for each iteration were used. The range of possible K_s varied from 1 to 10. Ten independent runs were conducted to quantify the amount of variation of the likelihood for each K. Estimation of the hierarchical level of the genetic structure was made using *ad hoc* statistics DK (Evanno *et al.* 2005). Computations were made thanks to BioPortal project (Kumar *et al.* 2009).

Demographic and historical expansions were tested in Arlequin 3.5 software using mismatch distribution analyses (Fu 1997; Ray *et al.* 2003; Excoffier 2004). The goodness-of-fit of the observed mismatch distribution to that expected under demographic expansion was tested using the sum of squared deviations (SSD) statistics. The raggedness (*r*) statistics was used for quantifying the smoothness of the mismatch distribution (Rogers 1995).

The mutation-drift equilibrium model versus bottleneck hypothesis was tested using the Bottleneck software (http://www.ensam.inra.fr/URDC; Cornuet and Luikart 1997; Luikart *et al.* 1998). The Infinite Allele Model (IAM), model implemented in the Bottleneck software was tested using Sing, Standardized and Wilcoxon tests. The probability distribution was established using 1000 simulations.

The presence of outliers – putative loci under positive and balancing selection was evaluated with Mcheza (http://popgen.eu/soft/mcheza/, Antao and Beaumont 2011). The "neutral" mean F_{ST}, forced mean F_{ST} in parallel with Infinite Allele Model were used for calculations consisting of 95 000 simulations.

Results

DNA profiling of the plant samples collected from Poland (P) allowed for the identification of 263 (*Acc*65I/*Mse*I) and 271 (*Kpn*I/*Mse*I) markers amplified by eight primer pair combinations. There were 242 by AFLPs amplified by the former and 252 the latter platform, respectively (Table 1) in case of individuals from the Station (S).

After elimination of the redundant markers, there were 112 and 95 markers amplified for individuals from (P) and from (S) for *Acc*65I/*Mse*I AFLP platform. The *Kpn*I/*Mse*I based profiling resulted in 118 (P) and 101 (S) AFLPs. Comparison of the *Acc*65I/*Mse*I and *Kpn*I/*Mse*I yielded 36 AFLP signals associated with epigenetic changes. Among them, 22 were in the Polish population and 36 in the Antarctic one. Most of the bands (more than 87% in each case) were present with a frequency higher than 5%. There were as many as 19 private bands present among individuals from the Polish population and only 2 detected in case of the samples from the Station for *Kpn*I/*Mse*I platform. In epigenetic markers there were 7 private bands in samples from Polish and 13 from Antarctic population. The markers shared among individuals of the Polish population were highly polymorphic (87%) for *Kpn*I/*Mse*I. The samples from the Antarctic population exhibited lower polymorphisms (62%) for this platform (Table 2). However, it appeared that the number of polymorphic markers was greater among individuals from the Antarctic population (53%) than from Poland (44%) with epigenetic markers. Moreover, the Polish population was more heterozygous (0.258) than the one from the Station (0.203) based on genetic markers. However, the Polish population (0.083) was less heterozygous than the Antarctic one (0.125) based on epigenetic markers (Table 2).

Shannon's Information Index (Table 2) as well as Polymorphic Information Content values were higher for the *Kpn*I/*Mse*I than for the *Acc*65I/*Mse*I derived markers. Moreover, these indices were higher for the Polish than for the Antarctic population in case of the *Kpn*I/*Mse*I AFLP platform. The opposite situation was observed in case of epigenetic markers.

Table 2

Genetic diversity of populations from Poland and from Antarctic Station. P% – the percentage of polymorphic loci, I – Shannon's diversity index , PIC – Polymorphic Information Content, H_E – expected heterozygosity, UH_E – unbiased expected heterozygosity, SE – standard error

Population	Sample size	P%	I±SE	PIC± SE	H_E± SE	UH_E± SE
*Kpn*I/*Mse*I						
P	96	87.39	0.393±0.023	0.033	0.258±0.017	0.261±0.017
S	96	62.18	0.305±0.026	0.027	0.203±0.018	0.204±0.018
Mean			0.356±0.014	0.036	0.233±0.010	0.235±0.010
Epigenetic markers						
P	96	44.44	0.136±0.034	0.012	0.083±0.022	0.083±0.022
S	96	52.78	0.195±0.042	0.018	0.125±0.029	0.125±0.029
Mean			0.167±0.032	0.015	0.104±0.018	0.104±0. 018

Table 3

Genetic distance between analyzed populations

	Nei's genetic distance	Φ_{PT}
*Kpn*I/*Mse*I platform	0.147	0. 267 (p = 0.000)
Epigenetic markers	0.119	0.501 (p = 0.001)

Neighbour Joining Clustering revealed two separate clusters representing individuals from Poland and the Antarctic. The same results were obtained based on markers amplified by both AFLP platforms and epigenetic markers (Fig. 1).

The first two principal coordinates of the PCoA encompassing *c*. 19% of the cumulative variance based on *Kpn*I/*Mse*I AFLPs grouped the samples into two groups. The data grouped according to the origin of the samples. Similar analyses based on epigenetic markers also demonstrated that the samples grouped according to their origin. In this case, the two main axes explained 39.5% of the total variance. Dispersed *Kpn*I/*Mse*I cloud of the Polish population reflected the presence of higher variation level than the Antarctic one. In case of epigenetic markers, the level of variation within each population was comparable (Fig. 2).

Nei's genetic distance between Polish and Antarctic populations reached 0.147 for *Kpn*I/*Mse*I AFLPs and 0.119 for epigenetic markers (Table 3).

Analysis of Molecular Variance revealed the presence of data structuring based on genetic and epigenetic markers. The *Kpn*I/*Mse*I derived markers differentiated populations from Poland and Antarctic ($\Phi_{PT} = 0.267$). However, stronger differences were evaluated for epigenetic markers ($\Phi_{PT} = 0.501$) (Table 3).

Population structure. — The *Kpn*I/*Mse*I markers used to study population structure via Bayesian statistics revealed the presence of three, instead of two, groups of samples (as indicated by K = 3 value). Polish population was subdivided
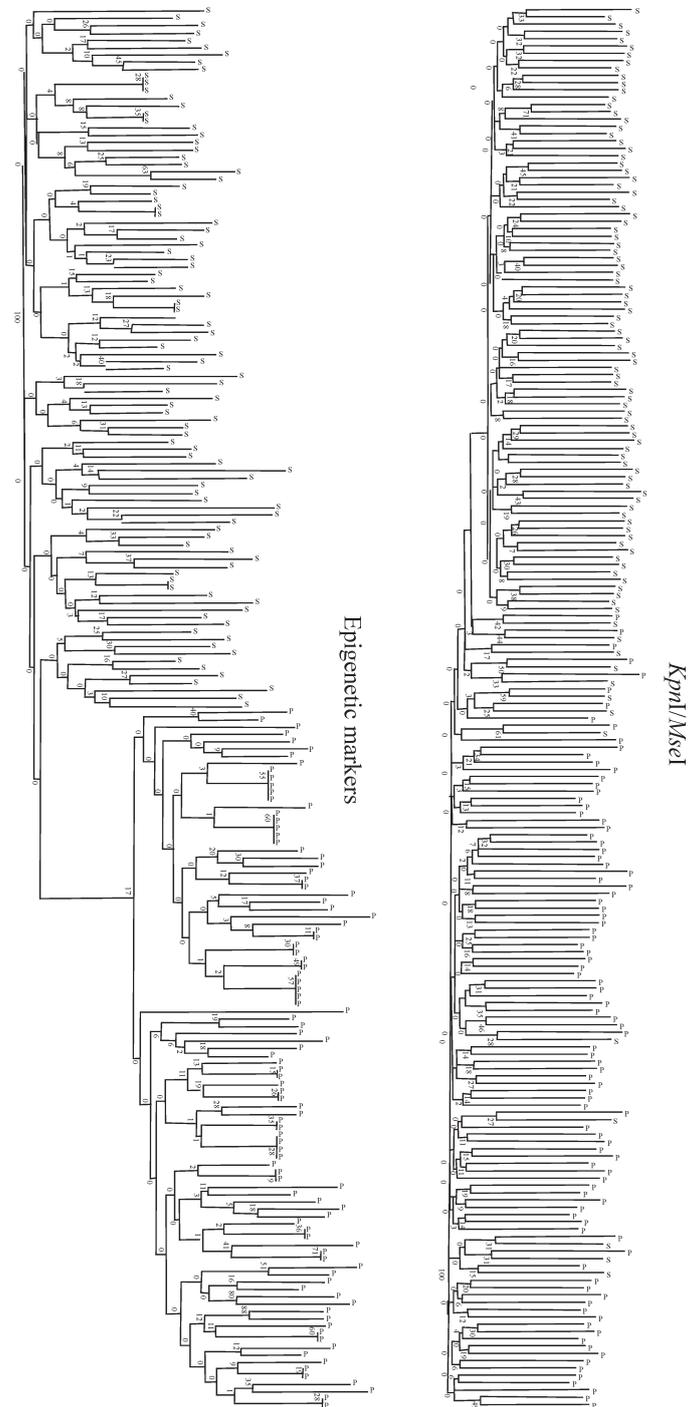
Fig. 1. Neighbour-Joining tree based on both genetic and epigenetic markers for all individuals of *Poa annua* from both populations.
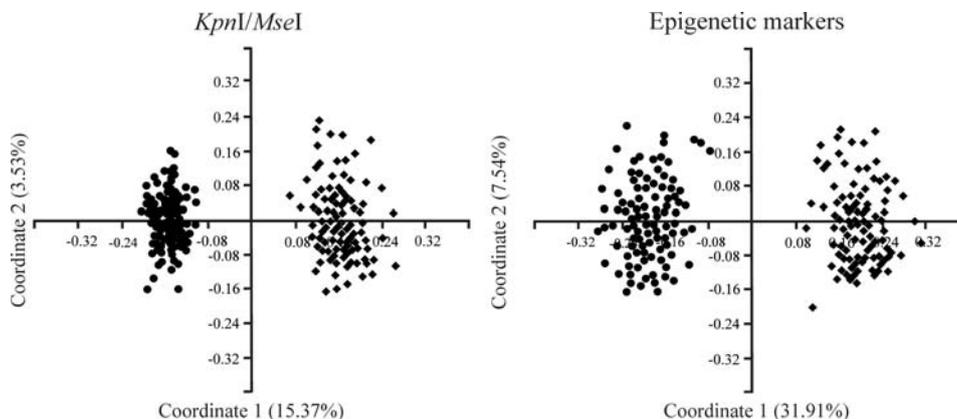
Fig. 2. Principal Component Analysis based on *Kpn*I/*Mse*I platform and epigenetic markers.

into two groups but structuring was weak as indicated by ΔK value equal to 100 at K = 3. The study on epigenetic markers revealed only two distinct groups of samples (K = 2, ΔK = 1200) reflecting the two origins.

Population differentiation. — Tajima's D neutrality test was positive but insignificant, while Fu's F_S negative and significant for both populations in case of *Kpn*I/*Mse*I markers. Epigenetic markers gave comparable results (Table 4).

Demographic expansion. — Based on DNA sequence markers evaluated with *Kpn*I/*Mse*I derived digests, the SSD statistics were insignificant for the Polish population ($p = 0.39$). Similarly, raggedness index was also not significant ($p =$

Table 4

Arrangements of neutrality tests based on *Kpn*I/*Mse*I AFLP's and epigenetic markers for both populations Polish (P) and from the *Arctowski* Station (S)

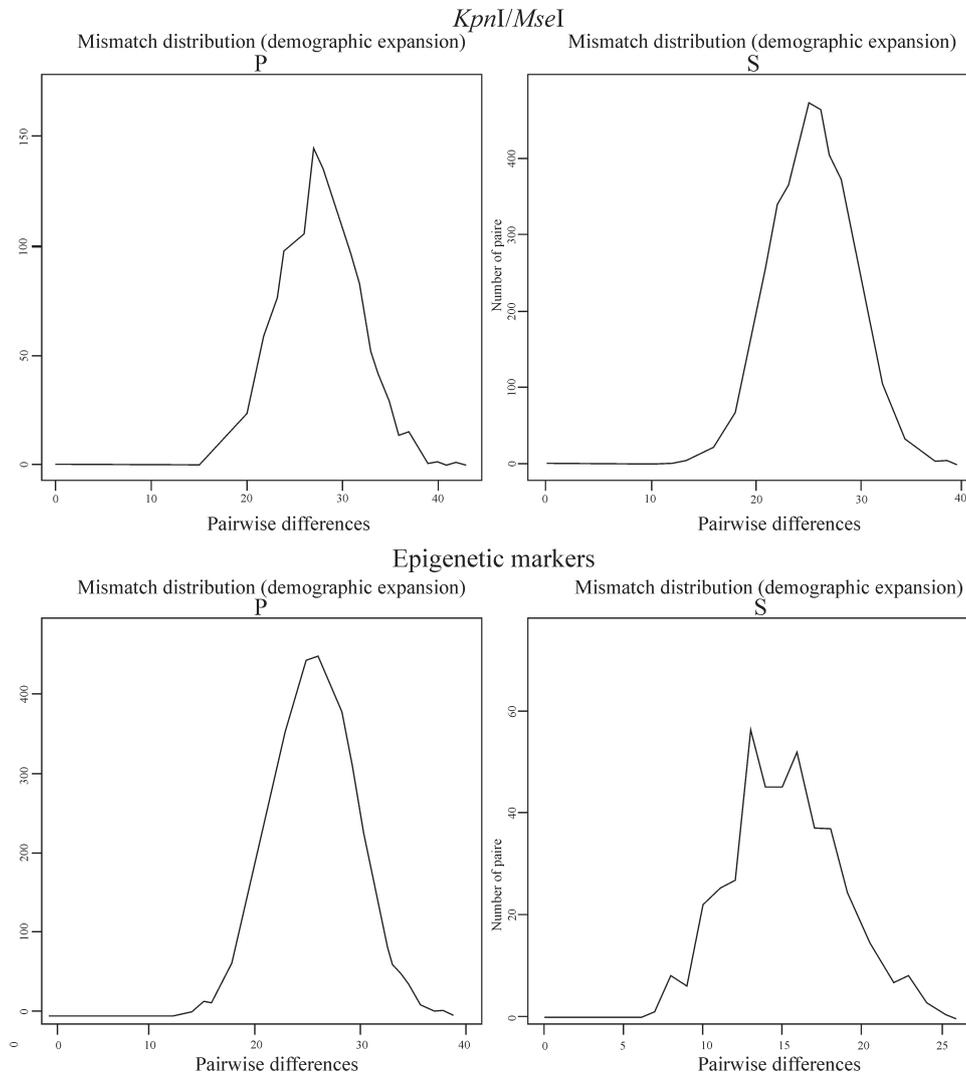|	*Kpn*I/*Mse*I			Epigenetic markers		
	P	S	Average	P	S	Average
Neutrality test						
Number of individuals	96	96	96	96	96	96
S	104.0	74.0	93.7	16.0	19.0	17.5
π	32.943	25.614	29.344	3.539	5.160	4.350
Tajima's D	1.345	2.539	1.646	0.385	0.141	0.762
Tajima's D p-value	0.917	0.993	0.923	0.715	0.896	0.806
Fu's F_s test						
Number of individuals	96	96	96	96	96	96
Θ−π	32.94	25.61	28.34	3.54	5.16	4.35
Exp. no. of alleles	27.80	40.30	32.92	12.32	15.85	14.08
F_s	-19.99	-23.96	-22.70	-26.13	-25.44	-25.79
F_s p-value	0.000	0.000	0.001	0.000	0.000	0.000

Fig. 3. Mismatch distribution, demographic expansion of both populations for *Kpn*I/*Mse*I and epigenetic markers.

0.27). When the Antarctic population was analyzed, then SSD statistics were significant ($p = 0.00$), however, raggedness index was not ($p = 0.08$). Analyses performed on epigenetic markers revealed significant values for SSD and raggedness indices in Polish and Antarctic populations (Fig. 3).

Spatial expansion analyses showed insignificant values for the SSD statistics and raggedness index evaluated on sequence based markers (*Kpn*I/*Mse*I platform) in the Polish population. In case of the Antarctic population, the SSD statistics were significant while raggedness index not significant. Epigenetic markers ex-
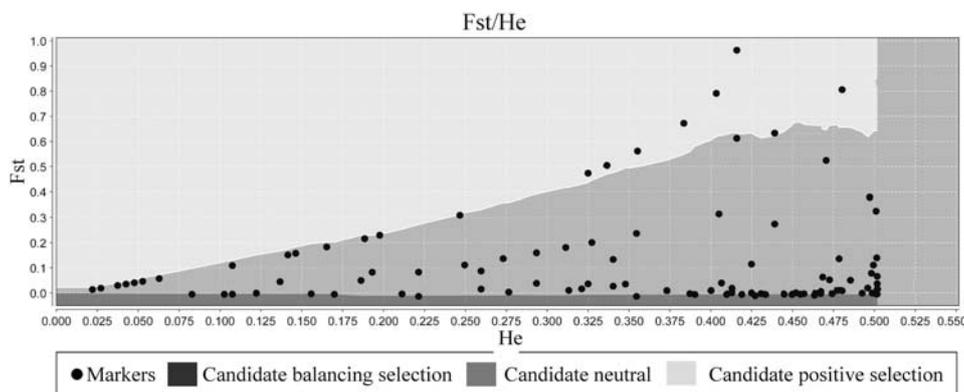
Fig. 4. *Kpn*I/*Mse*I based outliers as indicators of putative positive and balancing selection. Loci under positive and balancing selection are labeled with their names.

hibited significant values for the SSD statistics and raggedness index in the Polish population. Nearly the same data were evaluated for the Antarctic population. However, raggedness index *p*-value slightly exceeded 5% (Fig. 3).

Mutation-drift model versus bottleneck hypothesis. — The three tests for excess heterozygosity implemented in the bottleneck software produced significant p-values based on the IAM model with genetic and epigenetic markers for the Polish and Antarctic populations. Sing, Standardized and Wilcoxon tests rejected the mutation-drift model for the Antarctic population using both *Kpn*I/*Mse*I derived and epigenetic markers, suggesting the bottleneck effect.

Positive and balancing selection. — Analysis of putative loci under selection identified 28 outliers amplified in *Kpn*I/*Mse*I digests with 9 under positive and 19 under balancing selection (Fig. 4).

Discussion

Recent studies have stressed limited information on epigenetic variation in natural plant populations (Kalisz and Purugganan 2004; Rapp and Wendel 2005; Bossdorf *et al.* 2008). There are few papers exploiting properties of isoschizomers in identifying epigenetic alternations in a population survey on wild species, for example: *Deschampsia antarctica* (Chwedorzewska and Bednarek 2011), *Viola cazorlensis* (Herrera and Bazaga 2010) and *Brassica oleracea* (Salmon *et al.* 2008). Nevertheless, the commonly used isoschizomers (*Hpa*II and *Msp*I) combined either with RFLP (Jaligot *et al.* 2002) or AFLP (Herrera and Bazaga 2010; Lira-Medeiros *et al.* 2010) approaches can identify only changes in methylation pattern but do not support data concerning exclusively genetic variation. Recently introduced metAFLP approach involves *Acc65*I and *Kpn*I isoschizomers that dif-

fer in sensitivity towards site DNA methylation (Bednarek *et al.* 2007). While *Acc65*I is sensitive to the methylation of the restriction site and its vicinity the *Kpn*I is not. Such a property could be used to identify markers related to genetic variation (*Kpn*I/*Mse*I AFLPs) or epigenetic marker. The latter could be easily derived *via* extracting epigenetic markers from *Acc65*I/*Mse*I platform. The marker present in *Kpn*I/*Mse*I and absent in *Acc65*I/*Mse*I digest originates from a DNA fragment with methylated site. Similarly, marker amplified in *Acc65*I/*Mse*I platform and missing in *Kpn*I/*Mse*I should also be related to methylated site. However, such a profile could be explained if a fragment with two successive restriction sites is recognized by the isoschizomers surrounded by a single *Mse*I site. Moreover, the inner site needs to be methylated. Thus, using the metAFLP approach, one may compare the same loci both for genetic and epigenetic variation. To our best knowledge, this is the first time when such an approach was used.

The differentiation ability of the marker platform may be evaluated using numerous information indices such as Shannon's Information Index (I) as well as Polymorphic Information Content (PIC) index. The *Kpn*I/*Mse*I platform was highly efficient in differentiating the Polish and Antarctic populations since I value exceeded 0.3 (Table 2). When epigenetic markers were used I value decreased but the markers were still informative enough. Thus, the two marker types evaluated based on metAFLP platform could be applied for population studies.

Based on analyses of molecular markers amplified by metAFLP approach the Polish population exhibited more rare markers, higher level of polymorphisms and heterozygosity than the Antarctic one based on genetic markers (*Kpn*I/*Mse*I platform) (Table 2). This may suggest that the Antarctic population underwent some demographic processes while the one from Poland is in a stationary phase.

The metAFLP approach was capable of differentiating samples from the Polish and Antarctic locations. This was clearly demonstrated with clustering analysis (Fig. 1), PCoA (Fig. 2), coefficients of Nei's genetic distance and AMOVA (Table 3). All those analyses showed that the two populations differed from each other and grouped according to their origin independently whether genetic or epigenetic markers were used.

It should be stressed that the above mentioned statistics showed that only small part of variation is responsible for the differentiation and that AMOVA was most discriminative (especially in case of epigenetic markers). The most evident confirmation of the differentiation came from the analyses of population structure. It was revealed that the population from Poland may be subdivided into two subpopulations. However, such grouping was weak.

Moreover, agglomeration analysis showed that the Antarctic population was the subpopulation of the Polish one based on *Kpn*I/*Mse*I digests. This is in agreement with previous data stating that *P. annua* might have originated from Poland (Olech 1996; Olech and Chwedorzewska 2011). The rationale for the notion is that the soil transported to the Station for greenhouse originated from Botanic Garden.

Additionally, the Antarctic population appeared less variable than the Polish one (based on *Kpn*I/*Mse*I markers) supporting historical data (Fig. 1) and indicating that the Antarctic population might have undergone some demographic and/or selection processes due to its settlement in a new environment. Thus, the time that passed from its founding, limited gene flow between analyzed populations and putative demographic or selection processes affecting the Antarctic population may explain data structuring supporting the hypothesis that the Antarctic population originated from Poland. However, we cannot exclude that the Antarctic population was founded by multiple introductions from different sources (Chwedorzewska 2008); this hypothesis seems to be supported by still high level of genetic variability in this population.

It is interesting that the level of variation (Table 2) was higher for the Antarctic than for Polish population when epigenetic markers were used (this is opposite to the data obtained with *Kpn*I/*Mse*I AFLPs). Thus, we tend to think, that although the population from *Arctowski* Station is less variable at the genetic level (possibly due to founder or bottleneck effects), the increase of epigenetic variation in the Antarctic population may point out a response to external stress factors. It could be induced by environmental stimuli (Chinnusamy and Zhu 2009; Boyko and Kovalchuk 2011; Uthup *et al.* 2011) such as *e.g.* cold (Stewards *et al.* 2002), water (Labra *et al.* 2002), and/or osmotic (Tan 2010) stresses (Bruce *et al.* 2007), commonly experienced by organisms under polar condition. This result seems to agree with Lira-Medeiros *et al.* (2010) research on mangrove plants occurring in contrasting natural environments. The authors suggested that the epigenetic components of a genome played a crucial role in long-term survival in unfavorable conditions. Within-population epigenetic variance was a critical pre-requisite to have some micro-evolutionary potential (Kalisz and Purugganan 2004; Herrera and Bazaga 2010).

The data presented above (low number of rare markers, decreased number of polymorphic markers and decreased heterozygosity) suggested that at least the population from the Station should be affected by some demographic or selection processes. Thus, demographic expansion following initial bottleneck (or founder effect) was suspected. On the other hand the Polish population was supposed to be in a stationary stage. However, negative values of Fu's F_s statistics obtained based on genetic and epigenetic markers indicated that both populations might have experienced demographic expansion. Analysis of mismatch distribution confirmed the expected demographic expansion for the Antarctic but not for the Polish population using both types of markers.Thus, the time that passed from founding Antarctic population and limited gene flow between analyzed population indicated that spatial expansion could be also possible. Only weak evidences were obtained supporting such a hypothesis. Probably, in the given study, the demographic expansion is strong enough and masks spatial expansion. It is interesting that epigenetic markers suggested spatial expansion for the Polish population and gave weak evidences of it in case of the

Antarctic one. Possibly such results reflect the nature of the epigenetic markers. When the population is in mutation equilibrium epigenetic markers may fluctuate randomly in contrast to the population affected by demographic processes which could be recognized by statistical tests as spatial expansion. Thus, we tend to think that the presented data support the notion of demographic expansion in the Antarctic population and mutation drift model for the Polish one.

If the initial population size is small, one may expect founder effect (Dlugosch and Hays 2008; Alacs *et al.* 2010). However, if the initial size is quite large but then it decreases due to *i.e.* selection, the bottleneck is likely (Dlugosch and Parker 2008a, b). To study such effects using dominant markers infinite allele model can test mutation-drift versus bottleneck hypothesis (Tero *et al.* 2003). As expected, the Polish population followed the mutation-drift model while the Antarctic one fulfilled the bottleneck hypothesis or founder effect. Unfortunately, using dominant markers we can hardly discriminate between bottleneck and founder effects but the latter seems to be more realistic assuming historical data.

In parallel to demographic processes the Antarctic population may be affected by some kind of selection processes (selection sweep or hitchhiking). If any selection process takes place one should expect to identify markers under positive selection pressure. Such markers could be identified as outliers. Within the Antarctic population there were several *Kpn*I/*Mse*I based markers that could be classified as outliers (Fig. 4). Thus, our data may indicate that at least to some extent the Antarctic population underwent selection processes.

Summing up, metAFLP represents an efficient and reliable method for the detection of genetic changes and site DNA methylation alternations among individuals of the populations under study. The variation evaluated by the approach could be easily adopted for population genetic studies delivering valuable information on genetic and epigenetic differences between them. Thus, the metAFLP approach filled the existing gap delivering marker system for simultaneous genetic and epigenetic population studies. Based on molecular data we tend to think that the population from Poland is under mutation-drift model while the Antarctic one under demographic expansion. The Antarctic population is less variable than the Polish one due to founder and/or bottleneck effect. Moreover, it may undergo adaptation to new conditions as indicated by outliers.

This research suggests that *Poa annua* may pose a serious threat to sensitive and lacking competitive abilities indigenous species in the Antarctic terrestrial ecosystem. So, the intensive efforts should be taken to stop new introductions and exterminate established species, especially by strict biosecurity program.

References

ALACS E.A., P.B.S., DE TORES P.J. and KRAUSS S.L. 2010. Population genetic structure of island and mainland populations of the quokka, *Setonix brachyurus* (Macropodidae): a comparison of AFLP and microsatellite markers. *Conservation Genetics* 17: 297–309.

ANTAO T. and BEAUMONT M.A. 2011. Mcheza: A workbench to detect selection using dominant markers. *Bioinformatics* 1010.1093/bioinformatics/btr253.

BEDNAREK P.T., ORŁOWSKA R., KOEBNER R.M.D. and ZIMNY J. 2007. Quantification of the tissue-culture induced variation in barley (*Hordeum vulgare* L.). *BMC Plant Biology* http://www.biomedcentral.com/1471-2229/7/10

BERGER S.L. 2007. The complex language of chromatin regulation during transcription. *Nature* 447: 407–412.

BIRD A. 2007. Perceptions of epigenetics. *Nature* 447: 396–398.

BOSSDORF O., RICHARDS C.L. and PIGLIUCCI M. 2008. Epigenetics for ecologists. *Ecology Letters* 11: 106–115.

BOYKO A. and KOVALCHUK I. 2011. Genome instability and epigenetic modification – heritable responses to environmental stress? *Current Opinion in Plant Biology* 14: 260–266.

BRUCE T.A., MATTHES M.C., NAPIER J.A. and PICKETT J.A. 2007. Stressful "memories" of plants: evidence and possible mechanisms. *Plant Science* 173: 603–608.

CHEN X.Y., LI N., SHEN L. and LI Y.Y. 2003. Genetic structure along a gaseous organic pollution gradient: a case study with *Poa annua* L. *Environmental Pollution* 124: 449–455.

CHINNUSAMY V. and ZHU J. K. 2009. Epigenetic regulation of stress response in plants. *Current Opinion in Plant Biology* 12: 133–139.

CHWEDORZEWSKA K.J. 2008. *Poa annua* L. in Antarctic – searching for the source of introduction. *Polar Biology* 31: 263–268.

CHWEDORZEWSKA K.J. 2009. Terrestrial Antarctic Ecosystems at the Changing World – an overview. *Polish Polar Research* 30: 263–273.

CHWEDORZEWSKA K.J. 2010. Recent rapid regional climate changes in Antarctic and their influence on a low diversity ecosystems. *Papers on Global Change IGBP* 17: 17–30.

CHWEDORZEWSKA K.J. and BEDNAREK P.T. 2011. Genetic and epigenetic studies on populations of *Deschampsia antarctica* Desv. from contrasting environments at King George Islands. *Polish Polar Research* 32:15–26.

CHWEDORZEWSKA K.J. and KORCZAK M. 2010. Human impact upon the environment in the vicinity of *Arctowski* Station, King George Island, Antarctica. *Polish Polar Research* 31: 45–60.

CONVEY P. 2006. Antarctic climate change and its influences on terrestrial ecosystems *In*: D.M. Bergstrom, P. Convey and A.H.L. Huiskes (eds) *Trends in Antarctic Terrestrial and Limnetic Ecosystems: Antarctica as a Global Indicator*. Springer, Dordrecht: 253–272.

CORNUET J.M. and LUIKART G. 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144: 2001–2014.

DARMENCY H. and GASQUEZ J. 1981. Inheritance of triazine resistance in *P. annua*: consequences for population dynamics. *New Phytolgist* 89: 487–493.

DARMENCY H. and GASQUEZ J. 1997. Spontaneous hybridization of the putative ancestors of the allotetraploid *Poa annua*. *New Phytolgist* 136: 497–501.

DLUGOSCH K.M. and HYSE C G. 2008. Genotypes on the move: some things old and some things new shape the genetics of colonization during species invasions. *Molecular Ecology* 17: 4583–4585.

DLUGOSCH K.M. and PARKER M. 2008a. Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecology Letters* 11: 701–709.

DLUGOSCH K.M. and PARKER M. 2008b. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* 17: 431–449.

DUCHESNE P. and BERNATCHEZ L. 2002. AFLPOP: A computer program for simulated and real population allocation based on AFLP data. *Molecular Ecology Notes* 3: 380–383.

ELLIS W.M. 1973. The breeding system and variation in populations of *Poa annua* L. *Evolution* 27: 656–662.

EVANNO G., REGNAUT V.S. and GOUDET J. 2005. Detecting the number of clusters of individuals using the structure: a simulation study. *Molecular Ecology* 14: 2611–2620.

EXCOFFIER L. 2004. Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. *Molecular Ecology* 13: 853–64.

EXCOFFIER L. and LISCHER H. L. 2010. Arlequin suite ver 35: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564–567.

FALUSH D., STEPHENS M. and PRITCHARD J.K. 2007. Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Molecular Ecology Notes* 7: 574–578.

FINNEGAN E.J. 2001. Epialleles – a source of random variation in times of stress. *Current Opinion in Plant Biology* 5: 101–106.

FINNEGAN E.J. 2010. DNA Methylation: a Dynamic Regulator of Genome Organization and Gene Expression in Plants. *In*: E.C. Pau and M.R. Davey (eds) *Plant Developmental Biology – Biotechnological Perspectives*. Springer-Verlag, Dordrecht: 295–323.

FIUK A., BEDNAREK P.T. and RYBCZYŃSKI J.J. 2010. Flow Cytometry, HPLC-RP, and metAFLP Analyses to Assess Genetic Variability in Somatic Embryo-Derived Plantlets of *Gentiana pannonica* Scop. *Plant Molecular Biology Report* 28: 413–420.

FOLL M. and GAGGIOTTI O.A. 2008. Genome scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics* 180: 977–993.

FRENOT Y., CHOWN S.L., WHINAM J., SELKIRK P.M., CONVEY P., SKOTNICKI M. and BERGSTROM D.M. 2005. Biological invasion in the Antarctic: extent, impacts and implications. *Biological Reviews* 80: 45–72.

FRENOT Y., GLOAGUEN J.C., MASSE L. and LEBOUVIER M. 2001. Human activities, ecosystem disturbance and plant invasions in sub-Antarctic Crozet, Kerguelen and Amsterdam Islands. *Biological Conservation* 101: 33–50.

FRENOT Y., GLOAGUEN J.C. and TRÉHEN P. 1997. Climate change in Kerguelen Islands and colonization of recently deglaciated area by *Poa kerguelensis* and *Poa annua*. *In*: D.W.H. Walton (ed.) *Antarctic communities: species, structure and survival*. Cambridge University Press, Cambridge: 358–366.

FU Y.X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. *Genetics* 147: 915–925.

GRANT-DOWNTON R.T. and DICKINSON H.G. 2005. Epigenetics and its implication for plant biology 1. "The epigenetic network in plants". *Annals of Botany* 96: 188–195.

GRANT-DOWNTON R.T. and DICKINSON H.G. 2006. Epigenetics and its implication for plant biology 2. "The Epigenetic epiphany": Epigenetics, evolution and beyond. *Annals of Botany* 97: 11–27.

HALLIBURTON R. 2004. *Introduction to Population Genetics*. Pearson Education Int., Upper Saddle River, New Jersey, USA: 650 pp.

HAMMER Ø., HARPER D.A.T. and RYAN P.D. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica* 4: 1–9 http://palaeo-electronica.org/2001_1/past/issue1_01. htm.

HEIDE O.M. 2001. Flowering responses of contrasting ecotypes of *Poa annua* and their putative ancestors *Poa infirma* and *Poa supina*. *Annals of Botany* 87: 795–804.

HENDERSON I.R. and JACOBSEN S.E. 2007. Review Article. Epigenetic inheritance in plants. *Nature* 447: 418–424.

HERRERA C.M. and BAZAGA P. 2010. Epigenetic differentiation and relationship to adaptive genetic divergence in discrete populations of the violet *Viola cazorlensis*. *New Phytologist* 187: 867–876.

HOVIN A.W. 1957. Seasonal variation of meiotic chromosome pairing in haploid *Poa annua* L. *Genetics* 42: 377–78.

HUGHES K.A. and CONVEY P. 2010. The protection of Antarctic terrestrial ecosystems from inter- and intra-continental transfer of non-indigenous species by human activities: a review of current systems and practices. *International Journal of Climatology* 20: 96–112.

HUGHES K.A., CONVEY P., MASLEN N.R. and LEWIS SMITH R.I. 2009. Accidental transfer of non-native soil organisms into Antarctica on construction vehicles. *Biological Invasions* 12: 875–891.

HUTCHINSON C.S. and SEYMOUR G.B. 1982. Biological flora of the British Isles. *Poa annua* L. *Journal of Ecology* 70: 887–901.

JABLONKA E. 2009. Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *The Quarterly Review of Biology* 84: 131–176.

JALIGOT E., BEULÉ T. and RIVAL A. 2002. Methylation-sensitive RFLPs: characterisation of two oil palm markers showing somaclonal variation-associated polymorphism. *Theoretical and Applied Genetics* 104: 1263–1269.

JOHNSON P.G. 1995. Genetics and physiology of flowering in *Poa annua* L. Ph.D. diss. University of Minnesota., St. Paul: 129 pp.

JOHNSON P.G., RUMMELE B.A., VELGUTH P., WHITE D.B. and ASCHER P.D. 1993. An overview of *Poa annua* L. reproductive biology. *International Turfgrass Society Research Journal* 7: 789–804.

KALISZ S. and PURUGGANAN M. D. 2004. Epialleles *via* DNA methylation: consequences for plant evolution. *Trends in Ecology and Evolution* 19: 309–314.

KUMAR S., SKJÆVELAND A., ORR R.J.S., ENGER P., RUDEN T., MEVIK B.H., BURKI F., BOTNEN A. and SHALCHIAN-TABRIZI K. 2009. AIR: A batch-oriented web program package for construction of supermatrices ready for phylogenomic analyses. *BMC Bioinformatics* 10: 357.

LABRA M., GHIANI A., CITTERIO S., SGORBATI S., SALA F., VANNINI C., RUFFINI-CASTIGILIONE M. and BARACALE M. 2002. Analysis of cytosine methylation pattern in response to water deficit in pea root tips. *Plant Biology* 4: 694–699.

LAW R. 1981. The dynamics of colonising population *Poa annua. Ecology* 62: 1267–1277.

LEWIS SMITH R.I. 1996. Introduced plants in Antarctica: potential impacts and conservation issues. *Biological Conservation* 76: 135–146.

LIRA-MEDEIROS C.F., PARISOD C., FERNANDES R.A., MATA C.S., CARDOSO M.A. and FERREIRA P.C. 2010. Epigenetic variation in mangrove plants occurring in contrasting natural environment. *PLoS One* 5: e10326.

LUIKART G., ALLENDORF F.W. and SHERWIN W.B. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity* 89: 238–247.

MENGISTU L.W., MUELLER-WARRANT G.W. and BARKER R.E. 2000. Genetic diversity of *Poa annua* in western Oregon grass seed crop. *Theoretical and Applied Genetics* 101:70–79.

NEI M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.

OLECH M. 1996. Human impact on terrestrial ecosystems in west Antarctica. *Proceedings of the NIPR Symposium on Polar Biology* 9: 299–306.

OLECH M. 1998. Synantropization of the flora of Antarctica: an issue. *In*: J.B. Faliński, W. Adamowski and B. Jackowiak (eds) Synantropization of plant cover in new Polish research. *Phytocoenosis* 10, Supplement *Cartographiae Geobotanicae* 9: 269–273.

OLECH M. and CHWEDORZEWSKA K.J. 2011. The first appearance and establishment of alien vascular plant in natural habitats on the forefield of retreating glacier in Antarctica. *Antarctic Science* 23: 153–154.

PEAKALL R. and SMOUSE P.E. 2001. GenALEx V5: Population genetic software for teaching and research. Australian National University, Canberra. www.anu.edu.au/BoZo/GenALEx/.www.anu.edu.au/BoZo/GenALEx/

RAPP R.A. and WENDEL J.F. 2005. Epigenetics and plant evolution. *New Phytologist* 168: 81–91.

RAY N., CURRAT M. and EXCOFFIER L. 2003. Intra-deme molecular diversity in spatially expanding populations. *Molecular Biology Evolution* 20: 76–86.

ROGERS A.R. 1995. Genetic evidence for a Pleistocene population explosion. *Evolution* 49: 608–615.

ROLDÁN-RUIZ I., DENDAUW J., VAN BOCKSTAELE E., DEPICKER A. and DE LOOSE M. 2000. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). *Molecular Breeding* 6: 125–134.

SALMON A., CLOTAULT J., JENCZEWSKI E., CHABLE V. and MANZANARES-DAULEUX M.J. 2008. *Brassica oleracea* displays a high level of DNA methylation polymorphism. *Plant Science* 174: 61–70.

SCHÖNSWETTER P. and TRIBSCH A. 2005. Vicariance and dispersal in the alpine perennial *Bupleurum stellatum* L. (Apiaceae). *Taxon* 54: 725–732.

SHANNON C.E. and Weaver W. 1949. *The Mathematical Theory of Communication*. University of Illinois Press, Urbana, IL: 117 pp.

STEWARDS N., ITO M., YAMAGUCHI Y., KOIZUMI N. and SANO H. 2002. Periodic DNA methylation in maize nucleosomes and demethylation by environmental stress. *Journal of Biological Chemistry* 277: 37741–37746.

TAN M.P. 2010. Analysis of DNA methylation of maize in response to osmotic and salt stress based on methylation-sensitive amplified polymorphism. *Plant Physiology and Biochemistry* 48: 21–26.

TERO N., ASPI J., SIIKAMÄKI P., JÄKÄLÄNIEMI A. and TUOMI J. 2003. Genetic structure and gene flow in a metapopulation of an endangered plant species, *Silene tatarica*. *Molecular Ecology* 12: 2073–2085.

TUTIN T.G. 1952. Origin of *Poa annua* L. *Nature* 1969: 160.

TUTIN T.G. 1957. A contribution to the experimental taxonomy of *Poa annua* L. *Watsonia* 4: 1–10.

TURNER J., COLWELL S.R., MARSHALL G.J., LACHILAN-COPE T.A., CARLETON A.M., JONES P.D., LAGUN V., REID P.A. and IAGOVKUNA S. 2005. Antarctic climate change during the last 50 years. *International Journal of Climatology* 25: 279–294.

UTHUP T.K., RAVINDRAN M., BINI K. and THAKURDAS S. 2011. Divergent DNA methylation patterns associated with abiotic stress in *Hevea brasiliensis*. *Molecular Plant*: 6: 996–1013.

VARGAS J.M. and TURGEON A.J. 2004. *Poa annua* – physiology, culture, and control of annual bluegrass. Wiley and Sons, Inc., Hoboken, New Jersey: 165 pp.

VERHOEVEN K.J.F, van DIJK P.J. and BIERE A. 2010. Changes in genomic methylation patterns during the formation of triploid asexual dandelion lineages. *Molecular Ecology* 19: 315–324.

VOS P., HOGERS R., BLEEKER M., REIJANS M., VAN DE LEE T., HORNES M., FRIJTERS A., POT J., PELEMAN J., KUIPER M. and ZAUBER M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407–4414.