Ann. Bot. Fennici 45: 186–194 ISSN 0003-3847 (print) ISSN 1797-2442 (online)
Helsinki 27 June 2008

Low genetic variation in subpopulations of an endangered clonal plant *Iris sibirica* in southern Poland

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Received 2 May 2007, revised version received 5 June 2007, accepted 31 July 2007

Kostrakiewicz, K. & Wróblewska, A. 2008: Low genetic variation in subpopulations of an endangered clonal plant *Iris sibirica* in southern Poland. — *Ann. Bot. Fennici* 45: 186–194.

The spatial genetic structure in three subpopulations of the endangered clonal plant *Iris sibirica* from southern Poland was investigated. The subpopulations occurred in different habitats, i.e. in a *Molinietum caeruleae* community, a *Phragmites australis* patch and in a willow brushwood. Using 13 enzymatic systems, sixteen loci were evaluated. The very low genetic diversity (P = 0%–18.7%, A = 1.0%–1.19%, H_O = 0.000–0.009) observed within the subpopulations is probably due to lack of recruitment, habitat fragmentation and/or historical causes. Five distinct multilocus genotypes, detected from 148 collected samples in the subpopulations, supported this observation. This fact illustrated that only clonal growth could maintain the present low genetic variation through the domination of a single or a few clones within these sites. Moderate genetic differentiation (F_{ST} = 0.077, P < 0.001) that varies strongly between pairs of subpopulations, was observed, thereby suggesting substantial gene flow between populations.

Key words: clonal growth, conservation, genotypic variation, Iridaceae, population subdivision

Introduction

Knowledge of spatial genetic structure is of enormous significance when concerning rare or endangered species. In these cases, determination of the genetic resources in the study group is essential from the viewpoint of their conservation, both on a local scale and throughout their whole geographical range. Undoubtedly, the evolutionary potential of a species depends upon the level of genetic variability retained in its natural populations.

The persistence of such populations depends on their genetic and genotypic variability as well. Recent reviews of plant allozyme literature have revealed the relationships between life history traits and the patterns of genetic and genotypic variability (Loveless & Hamrick 1984, Ellastrand & Roose 1987, Hamrick & Godt 1989, Hamrick *et al*. 1991). Since the early 1960s, the usage of molecular markers (allozymes and DNA), particularly in the population genetics of clonal plants has allowed not only to assess the level of the population genetic variability, but also to determine the affiliations of ramets to specific genotypes (McLellan *et al*. 1997, Brzosko *et al*. 2002). Molecular markers

have then provided answers to previously open questions in the field of demography, concerning especially endangered or vulnerable clonal plants (Krenova & Lěps 1996, Ayres & Ryan 1999, Fischer *et al*. 2000, Hannan & Orick 2000, Xie *et al*. 2001, Brzosko *et al*. 2002, Brzosko & Wróblewska 2003).

One of the rare and strictly protected clonal plants in Poland is *Iris sibirica*. It is listed as a species of the Euro-Siberian subelement, whose distribution ranges from central Europe to central Asia (Hultén & Fries 1986). The individuals of *I. sibirica* consist of numerous leaf rosettes and flowering stems connected by permanent rhizomes with short internodes. The species is characterised by an iterative type of growth resulting from the processes of senility, regeneration and outgrowth, which proceed with different intensities in subsequent phases of development (Harper 1977). Regeneration occurs at the margin of the clone, whereas the senility processes at its centre lead to a gradual expansion of an 'empty centre' or 'disintegration zone'. As a result of advanced senility processes (dying), the divisions occur among the oldest sections of the rhizomes and the groups of their independent fragments emerge in the form of a single individual in the genetic sense (Klimeš *et al*. 1997 and http://www.butbn.cas.cz/klimes).

Iris sibirica also reproduces sexually. While seedlings rarely appear, they usually occur in the vicinity of the parental plants (Kostrakiewicz 2007). The *I. sibirica* populations occur in a range of various communities from tall-herb to shrub and deciduous woods or even coniferous forests communities. However, populations of this species are also found in the community of *Molinietum caeruleae*, characterised by the presence of other rare and protected species, such as *Dianthus superbus*, *Gentiana pneumonanthe*, *Gladiolus imbricatus*, *Trollius europaeus* and *Orchis latifolia* (Kostrakiewicz 2004). Unfortunately, the communities of this type are disappearing, and are listed by The World Conservation Union (IUCN) among the most endangered ones in Europe (Denisiuk 1991).

We investigated genetic and genotypic diversity among *I. sibirica* subpopulations in the context of the above-mentioned problems in three distinct communities in southern Poland. Therefore, the aims of our investigations were: (1) to assess the intensity of generative and vegetative reproduction, (2) to determine the level of genetic and genotypic variability in the subpopulations, and (3) to describe the effect of life history traits and habitats on the genetic and genotypic diversity among the subpopulations.

Material and methods

Study sites and characteristics of subpopulations

The field studies were carried out at the Kostrze site, situated in the western part of Kraków, southern Poland (Fig. 1 insert). The patches of *Molinietum caeruleae* community are all that remain of the vast meadows, which once occupied an area along the Vistula river (Zarzycki 1956, 1958). The changes in water regime and the cease of grass cutting has enabled shrub vegetation to colonise the area and has after several decades led to a transformation of the herb layer (Dubiel 1991, 1996, Kostrakiewicz 2001). At present, the vast patches of *Molinietum caeruleae* display partial or even significant disturbance (Dubiel 2005).

Three neighbouring subpopulations of *I. sibirica* were selected for this study (Fig. 1). Spaced some 200 m from one another, they occurred in distinctly different habitats. The first one, numbering 52 ramet clusters, occurred in a patch where *Phragmites australis* was the dominant species (side code PH). The second subpopulation, formed by 174 ramet clusters, occurred in a willow brushwood (*Salix rosmarinifolia* and *S. cinerea*), with a major proportion of sedges (e.g. *Carex gracilis* and *C. nigra*, side code SX). The third subpopulation of 506 ramet clusters was situated in a patch of *Molinietum caeruleae*, characterised by a considerable species diversity (site code MO). In 2006, a single permanent plot was established in each subpopulation for the analyses of life history traits and spatial genetic structure. The distribution of all ramet clusters was determined with 10 of these being marked on a cartogram. The distance between the neighbouring ramet clusters was at least 2 m. In each subpopulation, the average number of ramets in

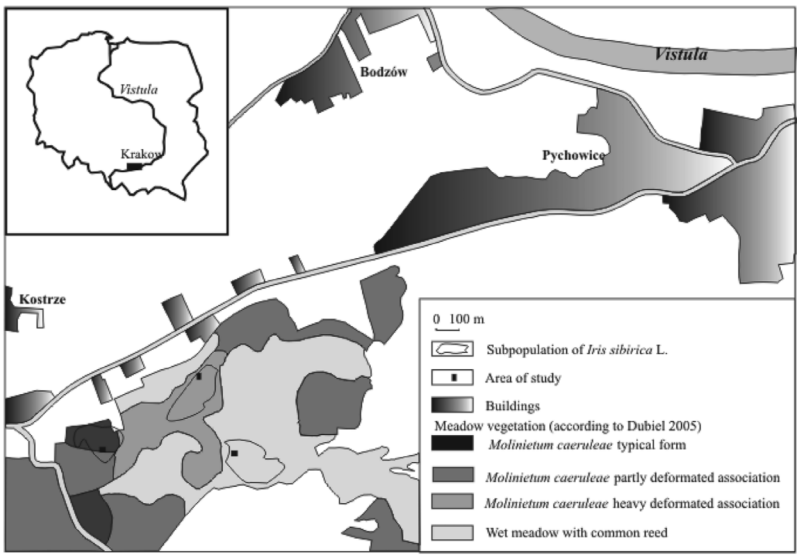

Fig. 1. Locations of the *Iris sibirica* subpopulations analysed in this study.

a cluster was determined, as well as the average proportion of fruits sets in a cluster, and the number of juveniles. The maximum length and width of the disintegration zone was also measured and the differences in the size of the disintegration zones were tested using ANOVA (Tukey test). From each group of ramets in a cluster evidently growing from the same rhizome fragment, a single leaf sample was collected for genetic analyses. In total, 148 samples were obtained, including 62 samples from the patch with a predominance of *Phragmites australis* (PX), 34 samples from willow brushwood (SX), and 52 samples from the patch of *Molinietum caeruleae* (MO) (Table 1).

Allozyme analysis

For each ramet, a 3-cm-long leaf fragment was extracted in a 30 μl of PVP buffer (Mitton *et al*. 1979). The majority of enzyme systems were separated on 11% horizontal starch gels. A lithium-borate/Tris-citrate buffer system at pH 8.3 was used to separate aconitase (*Aco*), glutamate oxaloacetate transaminase (*Got-1, Got-2*) and glutamate dehydrogenase (*Gdh*). A histidine-citrate buffer at pH 7.0 was applied to alcohol dehydrogenase (*Adh*), isocitric dehydrogenase (*Idh-1, Idh-2*), malate dehydrogenase NAD^+ (*Mdh-1, Mdh-2*), shikimate dehydrogenase (*Skd*), superoxide dismutase (*Sod*) and triose-phosphate isomerase (*Tpi*). The other allozymes such as malic enzyme (*Me*), 6-phosphogluconate dehydrogenase (*6Pgd*), phosphoglucomutase (*Pgm*), and phosphoglucose isomerase (*Pgi*) were screened using cellulose acetate plates (Titan III Zip Zone, Helena Laboratories). Enzyme staining with minor modifications followed the protocols developed by Richardson *et al*. (1986), Soltis and Soltis (1989), and Szweykowski and Odrzykowski (1990).

Genetic and genotypic variation

The proportion of polymorphic loci (*P*), the mean number of alleles per locus (*A*), the mean observed (H_O) and expected (H_E) heterozygosity were calculated using TFPGA (Miller 1997). Deviations from the Hardy-Weinberg equilibrium (HWE) were tested using an exact test of HWE with a Markov chain algorithm with GENEPOP (Raymond & Rousset 1995). *F*-statistics according to Weir and Cockerham (1984) were estimated for each polymorphic locus. The levels of inbreeding in the subpopulations were measured, using the inbreeding coefficient F_{IS} tested by permutation using FSTAT (Goudet 2002). Tests for linkage disequilibrium (LD) between loci for each subpopulation were performed by generating exact probabilities of a

type-I error, for the null hypothesis that a pair of loci is unlinked. The test was performed for all locus pairs within each subpopulation and over all populations with GENEPOP.

All sampled ramets were sorted by a multilocus genotype based on the polymorphic loci. Each of the detected distinct multilocus genotypes were assumed to be a distinct genet. Different measures of the clonal diversity were used in the study. First, we used the ratio of the proportion of the distinct genotypes (G) to the number of ramets sampled (N) (G/N; Ellstrand & Roose 1987). G/N is the probability that the next ramet sampled will be a different genotype. The second measure of genet diversity is the Simpson's index corrected for the finite sample size, $D = 1 - \Sigma[\{n_i(n_i - 1)]/[N(N - 1)\}]$, where n_i is the number of ramets of the ith genet and N is the total number of ramets sampled (Pielou 1969).

The fixation index F_{ST} was calculated to assess the genetic differentiation among subpopulations. The theoretical number of migrants entering every subpopulation per generation (N_m) was estimated using the formula $N_m = (1 - F_{ST})/4F_{ST}$ (Wright 1951). Differences in allele frequencies between each pair of subpopulations were analysed, using a homogeneity test computed as an exact test.

Results

Life-history traits

The ramet clusters of *I. sibirica* in the studied subpopulations differed significantly in respect to the mean number of ramets and the size of the disintegration zone (Table 1). The greatest mean number of ramets (78.8) and the largest disintegration zone (14.1/17.3 cm), characterised the ramet clusters in the PX subpopulation, whereas the lowest number of ramets (28.0) and the smallest disintegration zone (6.8/7.3 cm) was found in the SX subpopulation. Statistically significant differences in the size of disintegration zone were only found between PH and SX (Tukey test: $P <$ 0.01). The percentage of fruit sets was high in all subpopulations, ranging from 71% in the SX to 86% in the MO subpopulation. However, no juveniles were found in any of the plots (Table 1).

Table 1. Characteristics of the *Iris sibirica* subpopulations in southern Poland. R_A = the mean number of ramets in the cluster, D_A = the size of disintegration zone in the ramet cluster, L = the mean length of disintegration zone in the ramet cluster (cm), W = the mean width of disintegration zone within the ramet cluster (cm), N_F = proportion of fruit set in the ramet cluster, N_J = number of juveniles, N = number of ramets sampled for genetic analysis, P (%) = percent of polymorphic loci, A = mean number of alleles per locus, H_O = observed, H_E = expected heterozygosity, F_{IS} = the inbreeding coefficient and the exact test probability associated with the Hardy-Weinberg test (*** $p < 0.001$), G = number of genotypes, G/N = clonal diversity, D = Simpson's diversity index.

Localization of subpopulations	Site code	R_A	D_A		N_F	N_J	N	P (%)	A	H_O	H_E	F_{IS}	G	G/N	D
			L (cm)	W (cm)											
Phragmites australis dominated patch	PH	78.8	14.1[a]	17.3[a]	78%	0	62	18.7	1.19	0.009	0.023	0.782***	5	0.08	0.98
Willow brushwood	SX	28.0	6.8[a]	7.3[a]	71%	0	34	0	1.06	0.001	0.001	1.000	2	0.06	0.99
Molinietum caeruleae community	MO	53.4	11.8	11.9	86%	0	52	6.2	1.00	0	0	1.000	1	0.01	1.00

[a] differences statistically significant between PH and SX at the level of $p < 0.01$ (ANOVA, Tukey test).

Level and patterns of genetic and genotypic variation

Sixteen loci were evaluated from the 13 enzymatic systems. Only three loci (*Adh*, *6Pgd*, *Got-1*) were polymorphic for at least one subpopulation (two alleles per locus, Table 1), resulting in alleles per locus (*A*) ranging from 1.06 in the SX to 1.19 in the PH subpopulation.

In the PH subpopulation a high percentage of polymorphic loci (18.7%) and observed heterozygosity (0.009) was found, while in the MO subpopulation no polymorphism was observed (Table 1).

The mean observed heterozygosity was lower than expected in the PH subpopulation. Taking into account only the PH subpopulation, a large deviation from Hardy-Weinberg equilibrium exists. According to the fixation indices (F_{IS}), these deviations were due to an excess of homozygotes in the two loci *6Pgd* and *Got-1*. We found significant LD in the PH subpopulations only between the pair of loci *6Pgd* and *Got-1*.

Using three polymorphic loci, we separated only five distinct multilocus genotypes in all *I. sibirica* subpopulations. All of these multilocus genotypes were present in the PH and only one of them in the MO subpopulation. Therefore, the genotypic diversity parameter based on *G*/*N* maintained at low level, ranging from 0.01 in MO to 0.08 in PH. On the other hand, the mean Simpson's diversity index (*D*) was 1.0 in the MO subpopulation, because all the studied ramets had a single genotype. In the other two subpopulations, the PH and SX values of this parameter ranged from 0.98 to 0.99, respectively (Table 1). In the PH subpopulation, within eight of the ten studied clumps, we could distinguish two and/or three genotypes, while in the SX subpopulation, only one clump consisted of two genotypes. However, the most important variable is the frequency of each multilocus genotype. The genotype characterized by all homozygous loci was more frequent (75.8%), and the proportion of four other genotypes ranged from between 1.6-12.9% (data not shown).

The values of F_{ST} among all investigated *I. sibirica* subpopulations were moderate (0.077, $P < 0.001$) but varied strongly between pairs of subpopulations. The PH subpopulations were the most strongly genetically differentiated from each other, in particularly from the MO ($F_{ST} = 0.126$, $P < 0.001$) as well as from the SX subpopulation ($F_{ST} = 0.009$, $P < 0.001$), whilst the gene flow between them was moderate (1.7 and 4.3, respectively). On the other hand, genetic differentiation between SX and MO subpopulations was low and not statistically significant ($F_{ST} = 0.006$, $P > 0.05$).

Discussion

Ellstrand and Roose (1987), Hamrick and Godt (1989) as well as Frankham (1996) stressed that endangered plant species tend to maintain a lower genetic diversity than common species. Many endangered plant species i.e. *Howellia aquatilis* (Lesica *et al*. 1988) and *Lactoris fernandeziana* (Brauner *et al*. 1992), exhibit no genetic variation. Also *Pinus resinosa* provides an example of plants with very low allozyme diversity (Mosseler *et al*. 1992). The afore-mentioned author's proposed scenario is accurately confirmed by the extremely low level of genetic variation in *I. sibirica*, in three closely situated subpopulations. Moreover, in the case of *I. sibirica*, geographical range does not predict the level of genetic diversity. The species has a wide distribution range from central Europe to central Asia (Hultén & Fries 1986). All Polish *I. sibirica* populations are located in the centre of the species' geographical range. Therefore we would expect a high genetic variation.

On the other hand, many authors (Zarzycki 1956, Tumidajowicz & Zubel 1978, Denisiuk 1987, Dubiel 1991, 1996) stressed that strong destruction and fragmentation of its natural wet meadow habitats has contributed to decrease the population sizes. In the present day it is possible to observe the small patches of wet meadows as a patchwork in agricultural landscape, only with a few and/or some dozens of *I. sibirica* clumps. Therefore, the species was classified as vulnerable in the Polish Red Data List (Mirek *et al*. 2006). Finally, the isolated populations that are currently expressed in *I. sibirica,* may have resulted in the erosion of genetic diversity.

We also suggested that not only the fragmentation of wet meadow habitats but also the

reproductive biology of *I. sibirica* influence the patterns of the spatial genetic structure. A survey of the generative reproduction within subpopulations of this animal-pollinated mix-mating plant species showed that the flowers were visited mainly by bees and the level of fruit set was high in all subpopulations (71%–86%), although no recruitments have been noted after a few years of observation. Juveniles were detected only in experimental gaps when vegetative cover and litter were artificially uncovered (K. Kostrakiewicz pers. obs.).

Similarly, in the other *Iris* species and generally in clonal plant populations, a rare establishment of seedlings has been reported (Falińska 1986, Sutherland & Walton 1990, Simonich & Morgan 1994, Hannan & Orick 2000, Lamote *et al*. 2002). This is not surprising because the herb layer in all communities in which *I. sibirica* grows is very compact and the areas were occupied by many other clonal rhizomatous plants, spreading vegetatively by either creating long radially arranged rhizomes (i.e. *Phragmites australis*, *Chamaenerion angustifolium*, *Epilobium hirsutum*, *Filipendula ulmaria*), plants with robust primary root and strongly developed adventitious roots (i.e. *Lythrum salicaria*) or perennials forming large compact tussocks (i.e. *Molinia caerulea*, *Deschampsia cespitosa*, *Juncus conglomeratus* and *Carex gracilis*). Therefore, the probability of establishment of juveniles is low. A previous demographical study of these subpopulations confirmed that the most important type of reproduction was vegetative propagation (growth of rhizomes by the forming of new branches and disintegration of rhizomes; K. Kostrakiewicz unpubl. data).

Many surveys have demonstrated that in mid-successional communities, in which clonal plants are most dominant, generative reproduction was scarce (Brzosko 1999, Falińska 2003). The number of distinct multilocus genotypes found in subpopulations (five genotypes from 148 samples), was partially indicative of this phenomenon. This supported the fact that clonal growth could only maintain the present genetic variation through the domination of a single or a few rare clones within these sites and through the protection of the disappearing genets. Some endangered plant species, particularly those with little or no sexual reproduction such as *Taraxacum obliquum* (Van Oostrum *et al*. 1985) and *Haloragodendron lucasii* (Sydes & Peakall 1998) have also only one or few genets across all populations.

Despite the extremely low level of detected genotypes within the three subpopulations of *I. sibirica* located in various habitats in fairly close proximity (200 m), they differed in the number of genotypes. This phenomenon is connected with habitat fragmentation. Especially one genotype found in the subpopulation occurring in the *Molinietum caeruleae* community, which was the most distant from the subpopulation localized in the *Phragmites australis* patch and characterized by the highest number of genotypes, confirms that isolation leads to a decrease in the numbers of genotypes within the population. The phenomenon has been repeatedly described over the geographical range (Parker & Hamrick 1992, Franks *et al*. 2004, Diekmann *et al*. 2005) as well as at a smaller scale (Albert *et al*. 2004).

On the other hand, the *I. sibirica* subpopulations were not phenologically isolated from each other and the gene flow among them should be frequent. A considerable heterogeneity in allele frequencies among subpopulations was supported by a moderate F_{ST} value (0.077). It should be noted that F_{ST} values were strongly differentiated, particularly between the subpopulations in the *P. australis* dominated area and in the *Molinietum caeruleae* community. The genetic similarity between the subpopulations suggests that historically they belonged to a single big population divided by habitat fragmentation. Tempelton *et al*. (1990) claimed that a further expected consequence of fragmentation of plant populations is an increase in subpopulation genetic divergence due to the random fixation of different populations. The same phenomenon has been observed in numerous species e.g. *Polytrichum commune* (Wilson & Provan 2003), *Maianthemum bifolium* (Arens *et al*. 2005), *Helianthus occidentalis* (Foré & Guttman 1999) and *Eremosparton songoricum* (Lu *et al*. 2007).

A remaining unanswered question is why the *I. sibirica* subpopulations are characterized by the extremely low level of genetic variation. We have no information about a bottleneck in these subpopulations and therefore we cannot

suggest that the present genetic variation results from declining population sizes. Additionally, in a previous study (data not presented) of other *I. sibirica* populations from the northern parts of Kraków (Opatkowice), we detected no polymorphism using allozymes analysis. This would confirm that the historical events connected with the colonization processes (i.e. number of diaspores) could be a primary reason for the observed lack of local-scale genetic variation in *I. sibirica*'s geographical range.

The study suggests that management of demographical processes could be effective in long-term preservation of the *I. sibirica* populations. Moreover, the conservation of genetic diversity in *I. sibirica* subpopulations requires activities, especially in the *Phragmites australis* path containing unique alleles. In this case, removal of expansive neighbours could enable free proliferation of *I. sibirica* ramet clusters and their persistence in the habitat. It should be stressed that creating gaps to facilitate seed germination and development of seedlings, and to assure greater genetic diversity, is an effective measure of active protection of the rare and endangered *I. sibirica*.

Acknowledgements

We thank an anonymous reviewer for the valuable suggestions. The study was done within project 2 P04F 063 28 financed by Ministry of Science and Higher Education in Poland.

References

Albert, T., Raspé, O. & Jacquemart, A.-L. 2004: Clonal diversity and genetic structure in *Vaccinium myrtillus* populations from different habitats. — *Belg. J. Bot.* 137: 155–162.

Arens, P., Grashof-Bokdam, C. J., van der Sluis, T. & Smulders, M. J. M. 2005: Clonal diversity and genetic differentiation of *Maianthemum bifolium* among forest fragments of different age. — *Plant Ecol.* 179: 169–180.

Ayres, D. R. & Ryan F. J. 1999: Genetic diversity and structure of the narrow endemic *Wyethia reticulata* and its congener *W. bolanderi* (Asteraceae) using RAPD and allozyme techniques. — *Am. J. Bot.* 86: 344–353.

Brauner, S., Crawford, D. J. & Stuessy, T. F. 1992: Ribosomal DNA variation and RAPD variation in the rare plant family Lactoridaceae. — *Am. J. Bot.* 79: 1436–1439.

Brzosko, E., Wróblewska, A. & Ratkiewicz, M. 2002: Spatial genetic structure and clonal diversity of island populations of lady's slipper (*Cypripedium calceolus*) from the Biebrza National Park (northeast Poland). — *Mol. Ecol.* 11: 2499–2509.

Brzosko, E. & Wróblewska, A. 2003: Genetic variation and clonal diversity in small island populations of *Cephalanthera rubra* in Biebrza National Park (NE Poland). — *Bot. J. Linn. Soc.* 143: 99–108.

Brzosko, E. 1999: The life history of *Carex cespitosa*: consequences for population dynamics and vegetation succession. IV. Population dynamics of *C. cespitosa* in the process of succesion. — *Polish Bot. Stud.* 14: 45–51.

Denisiuk, Z. 1987: Let us safeguard the riverside meadows along the Vistula in Cracow. — *Chrońmy Przyr. Ojcz.* 53: 22–30. [In Polish with English summary].

Denisiuk, Z. 1991: *Interaction between agriculture and nature conservation in Poland*, vol. 12. — ICUN Press, Gland & Cambridge.

Diekmann, O. E., Coyer, J. A., Ferreira, J., Olsen, J. L., Stam, W. T., Pearson, G. A. & Serrão, E. A. 2005: Population genetics of *Zostera noltii* along the west Iberian coast: consequences of small population size, habitat discontinuity and near-shore currents. — *Mar. Ecol. Prog. Ser.* 290: 89–96.

Dubiel, E. 1991: Map of actual vegetation of the city of Cracow. — *Zesz. Nauk. UJ, Prace Botaniczne* 22: 121–133. [In Polish with English summary].

Dubiel, E. 1996: Łąki Krakowa. I. Klasa *Molinio-Arrhenatherea* [Meadows in Cracow. I. *Molinio-Arrhenatherea* class]. — *Studia Ośrodka Dokumentacji Fizjograficznej* 24: 145–171. [In Polish with English summary].

Dubiel, E. 2005: *Map of plant communities of the 3rd Campus of The Jagiellonian University and its surroundings*. — Instytut Botaniki Uniwersytetu Jagiellońskiego, Kraków.

Ellstrand, N. C. & Roose, M. L. 1987: Patterns of genotypic diversity in clonal plant species. — *Am. J. Bot.* 74: 123–131.

Falińska, K. 1986: Demography of *Iris pseudacorus* L. populations in abandoned meadows. — *Ekol. Pol.* 34: 583–613.

Falińska, K. 2003: Alternative pathways of succession: species turnover patterns in meadows abandoned for 30 years. — *Phytocenosis* 15 (N.S.) *Archiv. Geobot.* 9: 1–104.

Fischer, M., Musi, R., Prati, D., Peintinger, M., van Kleunen, M. & Schmidt, B. 2000: RAPD variation among and within small and large populations of the rare clonal plant *Ranunculus reptans* (Ranunculaceae). — *Am. J. Bot.* 87: 1128–1137.

Foré, S. A. & Guttman, S. I. 1999: Genetic structure of *Helianthus occidentalis* (Asteraceae) in a preserve with fragmented habitat. — *Am. J. Bot.* 86: 988–995.

Frankham, R. 1995: Conservation genetics. — *Ann. Rev. Genet.* 29: 305–327.

Franks, S., Richards, C. L., Gonzales, E., Cousins, J. E. & Hamrick, J. L. 2004: Multi-scale genetic analysis of *Uniola paniculata* (Poaceae): a coastal species with a linear, fragmented distribution. — *Am. J. Bot.* 91:

1345–1351.

Goudet, J. 1995: FSTAT ver. 1.2. A computer program to calculate *F*-statistics. — *J. Hered*. 86: 485–486.

Hamrick, J. L., Godt, M. J. W., Murawski, D. A. & Loveless, M. D. 1991: Correlations between species traits and allozyme diversity; implications for conservation biology. — In: Falk, D. A. & Holsinger, K. E. (eds.), *Genetics and conservation of rare plants*: 75–86. Oxford Univ. Press, New York.

Hamrick, J. L. & Godt, M. J. W. 1989: Allozyme diversity in plant species. — In: Brown, A. H. D., Clegg, M. T., Kahler, A. L. & Weir, B. S. (eds.), *Plant population genetics, breeding, and genetic resources*: 43–63. Sinauer, Sunderland.

Hannan, G. L. & Orick, M. W. 2000: Isozyme diversity in *Iris cristata* and the threatened glacial endemic *I. lacustris* (Iridaceae). — *Am. J. Bot*. 87: 293–301.

Harper, J. L. 1977: *Population biology of plants*. — Acad. Press, London.

Hultén, E. & Fries, M. 1986: *Atlas of North European vascular plants: north of the Tropic of Cancer*, I–III. — Koeltz Scientific Books, Königstein.

Klimeš, L., Klimešowa, J., Hendrics, R. & Van Groenendael, J. 1997: Clonal plant architecture: a comparative analysis of form and function. — In: de Kroon, H. & Van Groenendael, J. (eds.), *The ecology and evolution of clonal plants*: 1–29. Backhuys Publ., Leiden.

Kostrakiewicz, K. 2001: Aktualny stan populacji kosaćca syberyjskiego *Iris sibirica* na wybranych stanowiskach w okolicach Krakowa. — *Chrońmy Przyr. Ojcz*. 57: 95–99.

Kostrakiewicz, K. 2004: The natural stands of *Iris sibirica* in Poland. — *Siberian Iris* 10: 12–15.

Kostrakiewicz, K. 2007: The effect of dominant species on numbers and age structure of *Iris sibirica* L. population on blue moor-grass meadow in southern Poland. — *Acta. Soc. Bot. Pol*. 76:165–173.

Krenova, Z. & Lěps, J. 1996: Regeneration of *Gentiana pneumonanthe* population in an oligotrophic wet meadow. — *J. Veg. Sci*. 7: 107–112.

Lamote, V., Roldán-Ruiz, I., Coart, E., De Loose, M. & Van Bockstaele, E. 2002: A study of genetic variation in *Iris pseudacorus* populations using amplified fragment length polymorphisms (AFLPs). — *Aquatic Bot*. 73: 19–31.

Lesica, P., Leary, R. F., Allendorf, F. R. & Bilderbecl, D. E. 1988: Lack of genetic diversity within and among populations of an endangered plant, *Hawellia aquatilis*. — *Conserv. Biol*. 2: 275–282.

Loveless, M. D. & Hamrick, J. L. 1984: Ecological determinants of genetic structure in plant populations. — *Ann. Rev. Ecol. Syst*. 15: 65–95.

Lu, D., Daoyuan, Z. & Wenbao, M. 2007: Genetic variation and clonal diversity in fragmented populations of the desert plant *Eremosparton songoricum* based on ISSR markers.— *Biodiv. Sci*. 15: 282–291.

Miller, M. P. 1997: *Tools for population genetic analyses (TFPGA) 1.3: a Windows program for the analysis of allozyme and molecular population genetic data*. — Computer software distributed by author.

Mirek, Z., Zarzycki, K., Wojewoda, W. & Szeląg, Z. 2006: *Red list of plant and fungi in Poland*. — Polish Acad. Sci., W. Szafer Inst. Bot., Kraków.

Mitton, J., Linhart, B. Y. B., Sturgeon, K. B. & Hamrick, J. L. 1979: Allozyme polymorphism detected in mature needle tissue of ponderosa pine. — *J. Hered*. 70: 86–89.

Mosseler, A. K., Egger, N. & Hughes, G. A. 1992: Low levels of genetic diversity in red pine confirmed by random amplified polymorphic DNA markers. — *Can. J. For. Res*. 22: 1332–1337.

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

Parker, K. C. & Hamrick, J. L. 1992: Genetic diversity and clonal structure in a columnar cactus, *Lophocereus schottii*.— *Am. J. Bot*. 79: 86–96.

Pielou, E. C. 1969: *An introduction to mathematical ecology*. — Wiley, New York.

Raymond, M. L. & Rousset, F. 1995: GENEPOP (ver. 1.2), a population genetics software for exact tests and ecumenism.— *J. Hered*. 86: 248–249.

Richardson, B. J., Adams, M. & Baverstock, P. R. 1986: *Allozyme electrophoresis*. — Academic Press, UK.

Schmidt, B. & Prati, D. 2000: Genetic differentiation of life-history traits within population of the clonal plant *Ranunculus reptans*. — *Oikos* 90: 442–456.

Simonich, M. T. & Morgan, M. D. 1994: Allozymic uniformity in *Iris lacustris* (dwarf lake iris) in Wisconsin. — *Can. J. Bot*. 72: 1720–1722.

Soltis, D. E. & Soltis, P. S. 1989: *Isozymes in plant biology*. — Dioscorides Press, Portland.

STATISTICA StatSoft Inc. 1997: *STATISTICA user's guide ver. 5*. — StatSoft Inc. Polish ed. by StatSoft Polska, Kraków.

Sutherland, W. J. & Walton, D. 1990: The changes in morphology and demography of *Iris pseudacorus* L. at different heights on a saltmarsh. — *Funct. Ecol*. 4: 655–660.

Sydes, M. A. & Peakall, R. 1998: Extensive clonality in the endangered shrub *Haloragodendron lucasii* (Haloragaceae) revealed by allozymes and RAPDs. — *Mol. Ecol*. 7: 87–93.

Szweykowski, J. & Odrzykowski, I. 1990: Chemical differentiation of *Aneura pinguis* (L.) Dum. (Hepaticae, Aneuraceae) in Poland and some comments on application of enzymatic markers in bryology. — In: Zinsmeister, H. D. & Mues, R. (eds.), *Bryophytes. Their chemistry and taxonomy*: 437–448. Clarendon Press, Oxford.

Tempelton, A. R., Shaw, K., Routman, E. & Davis, S. K. 1990: The genetic consequences of habitat fragmentation. — *Ann. Missouri Bot. Garden* 77: 13–27.

Tumidajowicz, D. & Zubel, E. 1978: The disappearance and changes of wet meadows (*Molinietum coeruleae*) in the Valley of the Vistula river near Czernichow (southern Poland). — *Fragm. Flor. Geobot*. 24: 643–650. [In Polish with English summary].

Van Oostrum, H., Sterk, A. A. & Wusman, H. J. W. 1985: Genetic variation in gamospermous microspecies of *Taraxacum* sect. *Erythrosperma* and sect. *Obliqua*. — *Heredity* 55: 223–228.

Weir, B. & Cockerham, C. 1984: Estimating *F*-statistics for

the analyses of population structure. — *Evolution* 38: 1358–1370.

Wilson, P. J. & Provan, J. 2003: Effect of habitat fragmentation on levels and patterns of genetic diversity in natural populations of the peat moss *Polytrichum commune*. — *Proc. R. Soc. London B* 270: 881–886.

Wright, S. 1951: The genetical structure of populations. — *Ann. Eugen.* 15: 323–354.

Xie, Z., Lu, Y.-Z., Ge, S., Hong, D.-Y. & Li, F.-Z. 2001: Clonality in wild rice (*Oryza rufipogon*, Poaceae) and its implications for conservation management. — *Am. J. Bot.* 88: 1058–1064.

Zarzycki, K. 1956: O zachowanie wilgotnych łąk w Dolinie Górnej Wisły. — *Chrońmy Przyrodę Ojczystą* 12: 11–17.

Zarzycki, K. 1958: Humid meadows in the environs of Czernichów near Cracow deserving protection. — *Ochr. Przyr.* 25: 49–68. [In Polish with English summary].