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Disappearing population of *Betula humilis* Schrk. on the Maliszewskie Lake, NE Poland

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Abstract: *Betula humilis* Schrk. is an endangered glacial relict inhabiting wet meadows, natural and drained fens. One of its declining populations is located on the Maliszewskie Lake (the Wizna swamp, north-eastern Poland). The goal of the present study was to estimate the number of *B. humilis* individuals in this locality. In the Maliszewskie Lake population, 59 ramets, grouped into three clusters, were found. Twelve nuclear microsatellite loci were chosen to genotype 52 ramets. The analysis revealed that all the shoots within the single cluster had the same genotypes at the loci considered. This means that each cluster constituted one genetically distinct individual; thus, there were only three individuals of *B. humilis* in the studied population. The maintenance of the *B. humilis* population in the Maliszewskie Lake area requires urgent active protection involving removal of the shading vegetation. In fact, the entire Maliszewskie Lake is worthy of protection because of its hitherto unexplained origin and the occurrence of many endangered bird species.

Key words: *Betula humilis*, conservation genetics, genet, microsatellite, ramet, wetland conservation

1. Introduction

In land use classification, wetlands are known as wastelands in terms of both agriculture and forestry. However, biologists postulate that they are significant biodiversity units because the combination of aquatic and terrestrial conditions allows the existence of a variety of animal and plant organisms, including many endangered species (Bacon 1997; Fujita *et al*. 2014). Wetlands also play a very important role in water retention, limitation of soil erosion, lowering the risk of flood and in water quality improvement by serving as filters. In addition, wetlands act as resting and feeding places for some birds during their spring and autumn migrations. Unfortunately, the area of the world's swamps has declined by half during recent centuries (Keddy 2000). In Poland, the loss of mires has reached more than 80% (Wolejko *et al*. 2005), which is mainly a consequence of drainage. The overgrowth of drained fens and meadows by reeds, shrubs and trees reduces their areas and initiates the decline of mire habitats. These processes could have a disastrous impact on wetland species, ranging from reductions in the numbers of individuals to a complete disappearance of populations.

Wizna swamp is one of the biggest declining fens in Poland. Until the First World War, the central part of the mire, drained by poor systems of ditches, was used to a small extent by farmers who mowed the grass for cattle and horses (Kołos & Próchnicki 2004). During this time, some parts of the swamp were particularly valuable in terms of natural diversity, as they were populated by numerous specimens representing rare plant species in Poland, including *Herminium monorchis*, *Pedicularis sceptrum-carolinum*, *Schoenus ferrugineus*, *Swertia perennis* and two sedge species, *Carex chordorriza* and *C. limosa* (see Kołos & Próchnicki 2004). The abandonment of mowing after the Second World War caused the overgrowth of the Wizna swamp by bushes. However, drainage conducted in the whole fen from 1962 to 1971 changed this region completely. Previous communities of bushes, dominated by *Betula pubescens*, *Frangula alnus*, *Populus tremula*, *Salix cinerea*, *Viburnum opulus* and two glacial relicts, *Betula humilis* and *Salix lapponum,* were extirpated and replaced by high fodder productivity grasslands (Kołos & Próchnicki 2004). Almost 6,000 hectares of drained mire were included into the State Agricultural Farm "Wizna".

Although the adverse effects of drainage are visible in all parts of the Wizna mire, there are three parts that are relatively undisturbed, namely: "Biel" and "Grzędy" ranges as well as the vicinity of the Maliszewskie Lake (Kołos & Próchnicki 2004). Some authors have suggested that the Maliszewskie Lake could be one of four old glacial water reservoirs in the Północnopodlaska Plain (north-eastern Poland), and the only one in the Narew river valley (Banaszuk 2004; Kołos & Tarasewicz 2005). It is surrounded by topogenic-soligenic mire, where *B. humilis* can still be found. *B. humilis* has been classified in the EN (endangered) category of the International Union for Conservation of Nature (IUCN) in central and western Europe (Załuski *et al*. 2014). The disappearance of its populations is mainly a consequence of the lowering of groundwater levels and a decline in the use of wet meadows. An inventory carried out at the end of the twentieth century revealed that the number of *B. humilis* stands in Poland diminished to approximately 20% of the former number (Załuski *et al*. 2014). The *B. humilis* population located on the Maliszewskie Lake also seems to be threatened because the lake is becoming smaller and shallower, which are both effects of the drainage conducted in the 1960s. Environmental monitoring by the General Inspection of Nature Protection showed that the ecological status of the lake was unsatisfactory (U1 category; Wilk-Woźniak *et al*. 2012). Specifically, the reduction of the lake surface and shallowing caused the overgrowth of trees and scrub vegetation. Consequently, the growth of the light-demanding *B. humilis* is disadvantaged in the shaded stands, and its population has declined in such places (Jabłońska 2012). The goal of this paper is to estimate the number of *B. humilis* individuals in the Maliszewskie Lake population and to propose suitable conservation practices.

2. Material and methods

The studied *B. humilis* population is located on the western side of the Maliszewskie Lake (N 53º10´07.8´´, E 22º30´45.5´´), ca. 44 km west of the city of Białystok (Fig. 1). The marginal zone of the lake is dominated by *Thelypteridi-Phragmitetum* and *Phragmitetum australis* (Kołos & Próchnicki 2004). Bulrush vegetation directly surrounding the lake is separated from the adjacent fields and meadows by a narrow belt of forest with *Betula pubescens* and *Salix cinerea*. Three clusters of *B. humilis* shoots were found in this forest. The clusters were designated as follows: A, B and C. In total, 59 ramets were counted in the three clusters, with nine in cluster A, 23 in B and 27 in C. Some ramets were very young and small. Thus, one leaf was taken from every ramet having more than three leaves. Altogether, 52 ramets were sampled, of which eight were in cluster A, 21 – in B and 23 – in C (Table 1). The samples were collected with the permission of the Regional Director of Environmental Protection in Białystok (WPN. 6400.45.2013.AP).

Fig. 1. Location of *Betula humilis* population in NE Poland (a), on the Maliszewskie Lake (b)
Explanations: A, B and C – location of genetic clusters of *B. humilis* on the Maliszewskie Lake; 1 – limit of the ice-sheet in the Vistulian Glaciation, 2 – limit of the ice-sheet in the Odranian Glaciation, 3 – road, 4 – ditch

The leaf material was transferred to the laboratory in a car refrigerator and stored at – 80ºC until analysis. Before DNA extraction, leaves were dried at room temperature for one day. Next, they were homogenised with the TissueLyser mill (Qiagen) using steel balls. Total genomic DNA was extracted using an AX Plant Kit (A&A Biotechnology), according to the manufacturer's procedure. To determine the genotype of each ramet, 12 nuclear microsatellite primer pairs, originally designed for *B. pendula* (L1.10, L2.7, L13.1, L5.4, L4.4, L5.1, L3.1, L2.3, L022; Kulju *et al*. 2004) and *B. pubescens* ssp. *tortuosa* (Bo.G182, Bo.F394, L021; Truong *et al*. 2005), were chosen. The combinations of primers into four multiplex PCRs, the proportions of the PCR reaction components and the PCR profile for each multiplex were as previously described by Jadwiszczak *et al*. (2011a). The separation of fluorescently labelled amplified fragments was conducted on an ABI PRISM 3130 sequencer (Applied Biosystems) and scored using GeneMapper 4.0 (Applied Biosystems) analysis software.

3. Results and discussion

Successful amplification was obtained for all nuclear microsatellite loci of the *B. humilis* ramets studied, except for the L4.4 locus in the cluster B (Table 1). The lack of amplification in B ramets at the L4.4 locus likely resulted from mutations occurring at primer sites, leading to the appearance of null alleles (van Oosterhout *et al*. 2004). This result strongly suggests that the B ramets had the same homozygous genotype at this locus. Further analysis revealed that all shoots belonging to the same cluster had identical alleles at all the microsatellite loci considered. This means that each cluster constituted one genetically distinct individual (genet); hence, there were only three specimens of *B. humilis* in the Maliszewskie Lake population. A similar result was previously obtained in the four populations of endangered *Haloragodendron lucasii* (northern Sydney, New South Wales, Australia), where 53 ramets were sampled and only six multilocus genotypes were observed at allozyme and RAPD loci (Sydes & Peakall 1998).

All individuals in the Maliszewskie Lake population were homozygotes at L13.1 and L2.3 loci. In general, these loci showed a very low level of polymorphism in *B. humilis*, as the previous analysis of 327 specimens from 18 populations from Poland and Belarus revealed four alleles at the L13.1 locus and three at L2.3 (Jadwiszczak *et al*. 2011a). Loci L2.7, L5.4, L5.1, Bo.F394 and L022 were heterozygous in all the analysed genets, and the remaining loci were heterozygous or homozygous, depending on the individual studied. Allele sizes of particular microsatellites detected in the Maliszewskie Lake population were in the size ranges described previously in other *B. humilis* locations (Jadwiszczak *et al*. 2011a, 2011b). We did not find any unique or private allele in the Maliszewskie Lake stand; however, this result was not surprising. The population of *B. humilis* in the Wizna mire underwent a severe reduction in numbers during drainage in 1960s. Low frequency alleles are lost rapidly during bottlenecks because the elimination of any specimen having unique alleles in their genotype results in the disappearance of such alleles (Nei *et al*. 1975; Luikart & Cornuet 2008). Moreover, the effective population size of the bottlenecked populations is significantly reduced, which consequently causes a further reduction in the allele number (Cornuet & Luikart 1996).

Unfortunately, genetic erosion, which occurs characteristically in small populations, can dramatically influence their resistance to diseases and parasites, as well as their ability to cope with environmental changes (Ellstrand & Elam 1993; Lacy 1997). In general, *B. humilis* shows a wide spectrum of ecological tolerance. Jabłońska (2012) distinguished the following seven types of habitats populated by the species: *Sphagnum* moss-small sedge poor fens with a high contribution of bog species from the *Oxycocco-Sphagnetea* class,

Table 1. Genotypes at the nuclear microsatellite loci of *Betula humilis* ramets collected in the three clusters on the Maliszewskie Lake

Cluster	No of ramets	Microsatellite loci											
		L 1.10	L 2.7	L 13.1	L 5.4	L 4.4	L 5.1	Bo.G 182	Bo.F 394	L 3.1	L 2.3	L 021	L 022
A	8	177	175	080	247	279	286	129	140	215	198	190	171
		177	179	080	253	279	300	129	150	217	198	192	195
B	21	187	175	080	235	na	298	127	134	217	198	194	181
		191	179	080	247	na	326	133	148	217	198	200	197
C	23	175	173	080	241	263	288	129	148	217	198	200	179
		187	183	080	257	271	300	133	170	217	198	200	203

Explanation: na – no amplification

brown moss-small sedge subneutral fens with the highest number of mesotrophic subneutral fen species from the Scheuchzerio-Caricetea nigrae class, brown moss-small sedge alkaline fens with species from the Caricion davallianae alliance, strongly degraded fens dominated by *Urtica dioica* and *Galium aparine,* spring mires with water rich in Mg^{2+} and Ca^{2+} ions and alkaline fen meadows. It was found that the maintenance of *B. humilis* in its habitats depended on calcium concentration and water level. Both high Ca^{2+} concentrations and high water levels prevented the spread of other plants and enabled the growth of light-demanding *B. humilis* (Jabłońska 2006). In the Maliszewskie Lake population, the concentration of calcium ions is rather average, compared to other *B. humilis* localities (Jabłońska 2009; Jadwiszczak *et al*. 2015), which could have weakened the competitive ability of this species. However, the water table in the Maliszewskie Lake seems to be advantageous for the species, as it is around the peat surface (Jabłońska 2009, 2012). In addition to the relatively high water table, *B. humilis* in the Maliszewskie Lake clearly suffers due to shading by other shrub and tree species. It is likely that the dominance of shading vegetation results from year to year variations in the water level in this locality, which depends on rainfall. In dry years, brushwood and forest species might spread increasingly and displace *B. humilis*. In the undisturbed mires, e.g., the Rospuda mire in north-eastern Poland, stable hydrologic conditions allow the existence of a stable and long-lasting *B. humilis* population (Jabłońska *et al*. 2011).

Populations of *B. humilis* located in north-eastern Poland are located mainly in national parks, landscape parks or reserves. However, active conservation practices are not used in these locations (Matowicka & Jabłońska 2008). It is obvious that the maintenance of *B. humilis* on the Maliszewskie Lake requires urgent active protection, such as the removal of brushwood and forest plants, at least around the existing clusters of the species. This should be followed by water retention enhancement, which should stop the succession of other species. In the present habitat conditions, the transplantation of *B. humilis* individuals from the adjacent populations is not recommended because there is little chance for their acclimation.

The Maliszewskie Lake is a unique place not only because of the presence of the endangered glacial relict *B. humilis*. In addition, its unexplained origin and huge richness of birds make it extremely valuable for conservation. The results of palynological investigations suggest that the Maliszewskie Lake arose as a result of melting of ground ice blocks (Stasiak 1979; Żurek *et al*. 2002; Banaszuk 2004). However, that part of Poland was not covered by ice sheets during the last glaciation (Fig. 1). Could the lake be dated back to the third of the Middle Polish Glaciations, the Odranian Glaciation (210-130 ka BP)? This hypothesis seems to be confirmed by a relatively small area of the lake, its shallowness (the maximum depth is 80 cm) and its very thick layer of sediments (22.5 m; Stasiak 1979). However, the beginning of biogenic layer formation was previously dated to the Alleröd interstadial of the Late Vistulian (see Żurek *et al*. 2002).

The Maliszewskie Lake is a paradise for ornithologists. Among others, such endangered bird species as: *Philomachus pugnax* and *Acrocephalus paludicola* can be observed here (Zakrzewska 2010). As such, the conservation of the Maliszewskie Lake habitat and increasing publicity could help to relieve and protect the adjacent Biebrza National Park, which is particularly important during the spring migration of birds, when large numbers of tourists and bird-watchers visit the park (Zakrzewska 2010).

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