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Aneuploids in the shrub birch *Betula humilis* populations in Poland

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Abstract

Shrub birch (*Betula humilis* Schrk.) is endangered glacial relict growing in natural and drained fens and transitional mires. At present study we examined karyotypes of 103 individuals of *B. humilis*, collected in six populations from eastern and north-eastern Poland. We found 60% of diploid individuals with 2n = 28. The rest of studied plants were aneuploids with 26, 27, 29, 30 and 31 chromosomes in their karyotypes. High frequencies of aneuploids in Polish populations of *B. humilis* could be a consequence of: (***i***) hybridization with congeneric species, (***ii***) stress resulting from range fragmentation, (***iii***) karyotype instability of individuals with 2n ≠ 28, or (***iv***) vegetative reproduction.

Keywords: aneuploidy, caryology, chromosome, *Betula humilis*

Introduction

Shrub birch *Betula humilis* Schrk. is a glacial relict growing in natural and drained fens and transitional mires, where it is sympatric with *B. pendula* Roth. and *B. pubescens* Ehrh. Although the species is endangered (EN category) in central Europe [1], very little is known about its genetic variation in Poland. Till now chromosome analysis was conducted in one population in Germany only, where hybridization between *B. humilis*, *B. pendula* and *B. pubescens* was found [2]. *B. humilis* and *B. pendula* are diploids with 2n = 28, and *B. pubescens* is allotetraploid with 2n = 56. The aim of this study was to describe the chromosome structure in six Polish localities of the shrub birch.

Material and methods

Populations under study are located in the eastern (BB, UU, MO, TS) and north-eastern Poland (ROS, JEZ; Tab. 1). The collection of material was approved by Polish Ministry of Environment (DOPogiz-4211/I.A-10.3/10674/05/06). In each population samples were collected at arbitrarily implemented distance of at least 20 m from one branch to the next, in order to avoid collection from the same genetic individual. Samples of buds were obtained from 103 specimens in April, 2008 and 2009. *B. humilis* is very variable species in terms of morphology [3], which could be additionally strengthened by hybridization with congeneric taxa [4,5]. Hence, for chromosome studies we chose, in the previous vegetative seasons, plants having glands on the bark and ovate or ovate-orbicular leaves [3].

In general, the preparation of buds for chromosome analyses was conducted according to the method of Anamthawat-Jónsson [6]. However, we made some modifications. First, we added 0.4% colchicine solution for 3-4 hours prior to fixation [7]. Second, after pilot studies, we elongated the hipotonic treatment to 30 min. Protoplast suspensions were centrifuged at 4000 rpm for 5 min every time, which was the last innovation. Anamthawat-Jónsson [6] suggested to use a microfuge at 7000 rpm. Preparations were conventionally stained with Giemsa reagent. Chromosome counts were made using a light microscope at a magnification of 1250×. Ten to 25 metaphase spreads were analysed for each individual (Tab. 1).

Results

Out of 103 sampled plants, 62 individuals (60%) had 2n = 28 (Tab. 1, Fig. 1a). Such individuals were the most abundant in all populations, except of BB, where only 40% of specimens had 28 chromosomes in their karyotypes. All other studied plants were aneuploids. No triploid was found. Aneuploids with 2n = 29-31 chromosomes (Fig. 1b-d) constituted 22.3% of the studied plants. Such individuals were the most frequent karyotypic category in BB site. Aneuploids with less than 28 chromosomes were also observed in all populations, and in total they reached frequency of 17.5%. Out of 18 such aneuploids, three had 2n = 26 (Fig. 1e) and 15 individuals were monosomics with 2n = 27 (Fig. 1f).

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Discussion

Birch chromosomes are extremely small (Woodworth 1931, cited by [8]), which makes them very difficult to count. Hence, cytogenetical studies in natural *Betula* populations brought unsatisfying results for many years. Method using the plant leaf meristems discovered by Anamthawat-Jónsson [6], allows to obtain the chromosome spreads of good quality. At present study the chromosome structure of six *B. humilis* populations located in Poland is described. We found plants with the chromosome number ranged from 26 to 31. The most surprising result was the presence of aneuploids. Such individuals appeared in all populations with the frequency ranging from 19 to 60%. Till now occurrence of aneuploidy was suggested in *B. pubescens* on the basis of analysis of meiotic stages [8]. Moreover, individuals with aneuploid karyotypes were observed in sympatric populations of *B. pendula* and *B. pubescens* (Hagman 1971, cited by [9]; Helms and Jørgensen 1925, cited by [10]). Aneuploids were not found in the mixed population of *B. humilis*, *B. pendula* and *B. pubescens* in Germany [2]. It was supposed that aneuploid spreads in the birches could be a consequence of a loss of some chromosomes during tapping and squashing root-tips [6]. However, we used protoplast dropping method, which usually gave complete spreads [6]. We also noticed that it was a little chance of loss or gain of chromosomes during preparation, as they were surrounded with cytoplasm. Hence, our results confirm the possibility of existence of aneuploid individuals in the genus *Betula*.

In general, aneuploidy has a detrimental effect on phenotype. Experiments showed that aneuploidy influenced gene expression in *Arabidopsis thaliana* [11] and *Zea mays* [12]. However, is was also noticed that aneuploidy did not have to be always deleterious, especially in plants and fungi, which better than mammals tolerate loss or gain of single chromosome [11,13,14]. For example, common aneuploid cytotypes confirm substantial involvement of that aberration in the evolution of mosses [15]. It was also shown that in older sward of *Festuca pratensis* aneuploids had a competitive ability and yielding capacity equal to or the same as euploids [16].

Tab. 1 Number of individuals with chromosome count in the studied populations of *B. humilis* in Poland.

Location name	Abbrev	Position	N	2n					
				26	27	28	29	30	31
Bagno Bubnów	BB	N 51°22.30 E 23°16.35	20	0	2	8	5	3	2
Jezioro Moszne	MO	N 51°27.29 E 23°07.28	18	1	2	11	2	2	0
Uroczysko Uściwierskie	UU	N 51°21.72 E 23°03.72	18	1	4	13	0	0	0
Torfowisko Sobowice	TS	N 51°07.02 E 23°23.33	16	0	2	9	3	1	1
Dolina Rospudy	ROS	N 53°54.34 E 22°56.72	15	1	3	8	1	2	0
Jeziorko k/Drozdowa	JEZ	N 53°84.22 E 21°81.29	16	0	2	13	1	0	0
Total			103	3	15	62	12	8	3

Abbrev – abbreviation of location name; N – number of individuals studied; 2n – chromosome number.

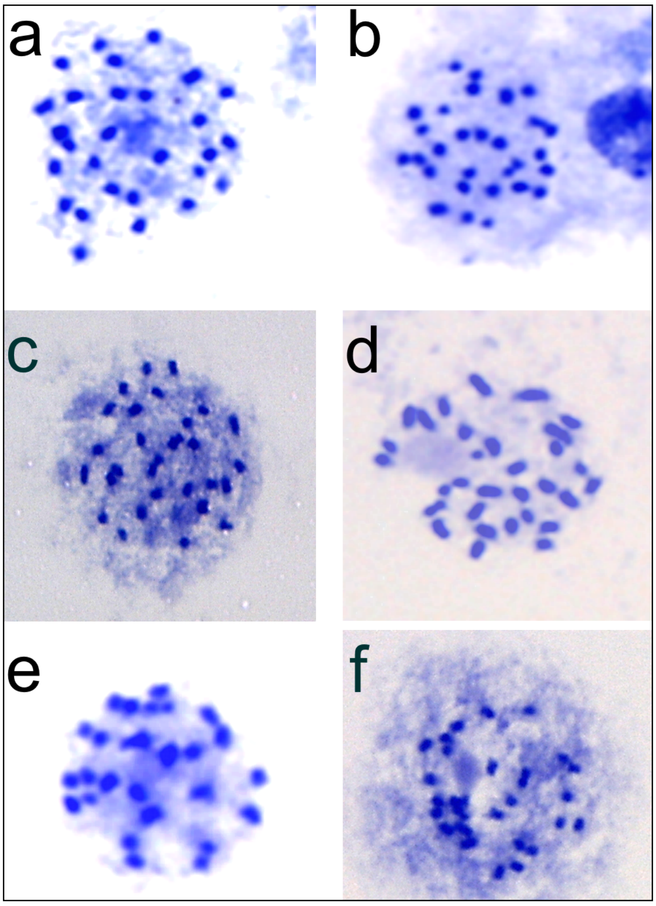

Fig. 1 Metaphase cells of individuals from *B. humilis* populations located in Poland. **a** Diploid with 2n = 28. **b** Aneuploid with 2n = 9. **c** Aneuploid with 2n = 30. **d** Aneuploid with 2n = 31. **e** Aneuploid with 2n = 26. **f** Aneuploid with 2n = 27.

There are four probable explanations for the occurrence of high frequencies of aneuploids in Polish populations of the shrub birch. First, it is possible that individuals with 2n ≠ 28 are an effect of hybridization process. Hybridization between *B. humilis* and other congeners was previously supposed on the basis of morphological measurements conducted in several Polish localities [4,5]. Staszkiewicz et al. [5] found 45% of hybrids and introgressive forms, which is comparable with our study. Moreover, variety of numerous aneuploid cytotypes of hybrid origin was described in *Carex sociata* [17], *Rutidosis leptorrhynchoides* [18] and *Eleocharis kamtschatica* [19].

Second, the high frequency of aneuploid plants in *B. humilis* populations can be generated by environmental conditions. For example, aneuploidy and other chromosome aberrations were found in *Abies sibirica* growing under extreme conditions of lowland swamp in Russia [20]. Murcia [21] noticed that the environmental conditions can be modified by habitat fragmentation, which could result in a change of selection pressure against atypical genotypes [22]. Habitat fragmentation is one of the main threats for the current biodiversity. Habitat loss, resulting from the drainage of peatlands and invasion of brushwood and forest plant competitors, is also the main reason of disappearing of *B. humilis* from its stands [1]. Hence, high frequency of aneuploids in the shrub birch populations could be a consequence of a stress caused by the range fragmentation.

Third, it was discovered that primary trisomics can promote subsequent trisomies and other chromosomal aberrations (see [13]). Moreover, the experiments showed that aneuploid cells were about 3-fold more likely to display changes in chromosome copy number than diploid ones in fungal pathogen *Candida albicans* [23]. Hence, substantial level of aneuploidy in *B. humilis* may also be a consequence of karyotype instability of individuals with 2n ≠ 28.

Fourth, Lavania [24] stated that polyploidy and aneuploidy are frequent in the populations of plants with dominance of vegetative reproduction. However, that explanation seems to be little possible, because nuclear microsatellite analysis conducted in six Polish localities of *B. humilis* showed very high genotype differentiation [25], which is typical for the population reproducing sexually.

Future detailed cytogenetic and physiological studies, including molecular cytogenetic approach such as FISH and analyses of plant fertility, are necessary to understand the possible causes and effects of aneuploidy in the endangered shrub birch.

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