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Cytotaxonomic analysis of Austrian *Polygonatum* species *(Ruscaceae)*

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Abstract: Chromosome numbers and karyotypes of three populations of two species of *Polygonatum (Ruscaceae)* from Austria are presented. *P odoratum* and *P latifolium* have $2n = 20$ chromosomes with asymmetric karyotypes. Polymorphism concerning the length and morphology of two pairs of homologous chromosomes was observed in *P odoratum* from a population near Mödling. These changes were found to be associated with the presence/absence of chromosomal regions containing rDNA loci (secondary constrictions). Individuals from the other population of *P odoratum* (Dürnstein) did not exhibit heterogeneity in homologous chromosome length, however, their karyotypes differed significantly from the karyotype of the Mödling population by having different number of satellites and/or secondary constrictions.

Key words: *Polygonatum*, *Ruscaceae*, *Convallariaceae*, *Liliaceae*, *Asparagaceae*, chromosome numbers, karyotype, chromosome length polymophism.

Zusammenfassung: Zytotaxonomische Untersuchungen an österreichischen *Polygonatum*-Arten *(Ruscaceae)*. – Englisch mit deutscher Zusammenfassung.

Chromosomenzahlen und Karyotypen von drei Populationen österreichischer *Polygonatum*-Arten *(Ruscaceae)* wurden bestimmt. *P odoratum* und *P latifolium* haben $2n = 20$ Chromosomen und asymmetrische Karyotypen. In einer Population von *P. odoratum* aus dem Raum Mödling wurde ein Polymorphismus betreffend Länge und Morphologie zweier homologer Chromosomen gefunden. Diese Unterschiede stehen mit dem Vorhandensein bzw. Fehlen von chromosomalen Regionen mit sekundären Einschnürungen zusammen. Eine zweite Population von *P odoratum* aus der Umgebung von Dürnstein zeigte keinen derartigen Polymorphismus, unterschied sich aber von der Mödlinger Population deutlich durch das Vorhandensein von Satelliten und sekundären Einschürungen.

Introduction

Polygonatum (Ruscaceae [syn. *Convallariaceae*: see RUDALL & al. 2000]) comprises 50 to 60 species widely distributed in the northern hemisphere (BENTHAM & HOOKER 1883, DAHLGREN & al. 1985, JANG 1998). It has been divided into 3 sections: *sect. Polygonatum*, *sect. Oppositifolia* and *sect. Verticillata*, based on phyllotaxis, size of perianth, the presence or absence of bracts and basic chromosome numbers (BAKER 1875, ABRAMOVA 1975, TANG 1978, JEFFREY 1980, TAMURA 1990, JANG 1998). Only four species of the genus occur in Europe, all of them also in Austria: *P. odoratum* (syn. *P. officinale*), *P. latifolium*, *P. multiflorum* of *sect. Polygonatum* and *P verticillatum* of *sect. Verticillata* (JANCHEN 1956–1960).

The aim of the present study was to analyse the chromosome numbers of two Austrian *Polygonatum* species and examine their karyotypes in detail. Species of former *Lilia-*

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ceae (s. latiss.) often exhibit polymorphism in length and/or morphology of homologous chromosomes (e.g., GREILHUBER 1995). Such polymorphism concerning two chromosome pairs was observed in the karyotype of one individual of *P. odoratum* from a population near Mödling (Lower Austria). In order to check the frequency and fixation of this type of rearrangement in the genome of this species, a second population of *Polygonatum odoratum* from near Dürnstein (Wachau, Lower Austria) was also analysed.

Materials and Methods

Plants of two species of *Polygonatum* were analysed karyologically (Tab. 1). The plants are cultivated in the Botanical Garden of the University of Vienna, Austria (HBV), vouchers are deposited in the herbarium of the Institute of Botany, University of Vienna (WU).

Tab. 1: Plant material investigated. Vouchers in herbarium WU.
Tab. 1: Untersuchtes Material. Herbarbelege in WU.

Taxon	Collection details	Voucher number	No. of individuals
P latifolium	Vienna, 3rd district, in the Botanical Garden of the Institute of Botany of the University of Vienna, 180 m s. m.; [7864/1]; 24.5.2002; origin unknown, possibly spontaneous and autochthoneous.	Jang L30	1
P odoratum	Lower Austria, Mödling, Schweinzerberg SW Hinterbrühl, 300 m s. m.; [7963/1]; 20.5.2002	Jang L28	2
P odoratum	Lower Austria, Dürnstein, Loibenberg, 200 m s. m.; [7659/1]; 17.3.2002	Jang L29	4

Actively growing root tips were pretreated with 0.1% colchicine overnight at 4°C in darkness. Subsequently, material was fixed in 3:1 ethanol glacial acetic acid for 24–48 hours at room temperature and stored at –20°C until use. Feulgen staining with Schiff's reagent followed the standard method. Briefly, the material was hydrolysed in 5N HCl (SIGMA, Germany) for 20 min at room temperature, washed with tap water, and stained with Schiff's reagent (SIGMA, Germany) in darkness for 1 hour. Squash preparations were made in a drop of 45% acetic acid (SIGMA, Germany). After coverslip removal on dry ice, preparations were dried for 24 hours at 37°C and mounted in DPX (SIGMA, Germany). Number of individuals analysed for each population is given in Tab. 1. Preparations with minimum ten well-spread metaphases were analysed with light microscope (Polyvar, Reichert-Jung). Photographs were taken on Technical Pan 100 ASA film (Kodak) and karyotypes cut-out from photographs. The photos were scanned and images were processed with Corel Photo-Paint 10 and Corel Draw 10 using only functions that apply to the whole image.

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Results

***P. latifolium*: 2*n* = 20** (Fig. 1A)

This is the first report of chromosome number obtained for the species from Austrian material. The chromosomes of *P. latifolium* are mostly meta- and submetacentric with two chromosome pairs (2 and 4) possessing secondary constrictions in long arms. No polymorphism in length and/or morphology of homologues in individual chromosome pairs has been observed.

***P. odoratum*: 2*n* = 20**

Chromosome numbers of all individuals of the species analysed in the present study were constant and equal $2n = 20$. However, the karyotypes of the two *P. odoratum* accessions (populations) differed significantly in presence and position of rRNA genes (satellites and secondary constrictions).

Population Dürnstein (Fig. 1B)
The chromosomes of *P. odoratum* from this population are mostly meta- and submetacentric with two chromosome pairs (5 and 8) possessing terminal satellites and two other pairs having secondary constrictions in short (pair 9) and long arm (pair 2). Heterozygocity in the length of long arm of two homologue chromosomes of pair 6 has been observed.

Population Mödling (Fig. 1C)
The chromosomes of *P. odoratum* from this population are mostly meta- and submetacentric with no clearly visible terminal satellites. Two pairs of chromosomes have secondary constrictions in short (pair 1) and long arm (pair 3). Heterozygocity in the length of homologous chromosomes of these two NOR-bearing pairs has been observed and is associated with loss of chromosome segments distal to secondary constrictions.

Discussion

The chromosome number of *P. latifolium* ($2n = 20$), both the somatic chromosome number and the karyotype presented in this paper, agree well with the data presented in the earlier study of THERMAN (1953). Austrian accessions of *Polygonatum odoratum* have previously been analysed karyologically. The chromosome numbers ($2n = 20$) reported by DOBEŠ & HAHN in STACE (1997) and VITEK & al. (1992) are congruent with the numbers found in the present study. Unfortunately, the two other studies did not include microphotographs of the chromosomes, therefore it is not possible to make conclusions about the frequency of chromosomal changes (translocations, deletions etc.) in the genome of *P. odoratum*. The karyotype of *P odoratum* from Dürnstein is very similar to that reported by THERMAN (1953). It differs, however, in the presence of two additional satellites located terminally on the short arms of chromosome pairs 5 and 8. The karyotype of *P. odoratum* from Mödling differs significantly from both of these karyotypes by the presence of secondary constrictions in chromosomes of pairs 1 and 3.

Some pairs of homologues chromosomes in *Polygonatum odoratum* are clearly heterozygous concerning presence/absence of the whole chromosome segments. In case of one population of *P. odoratum* (Mödling) heterozygocity of two chromosome pairs concerns

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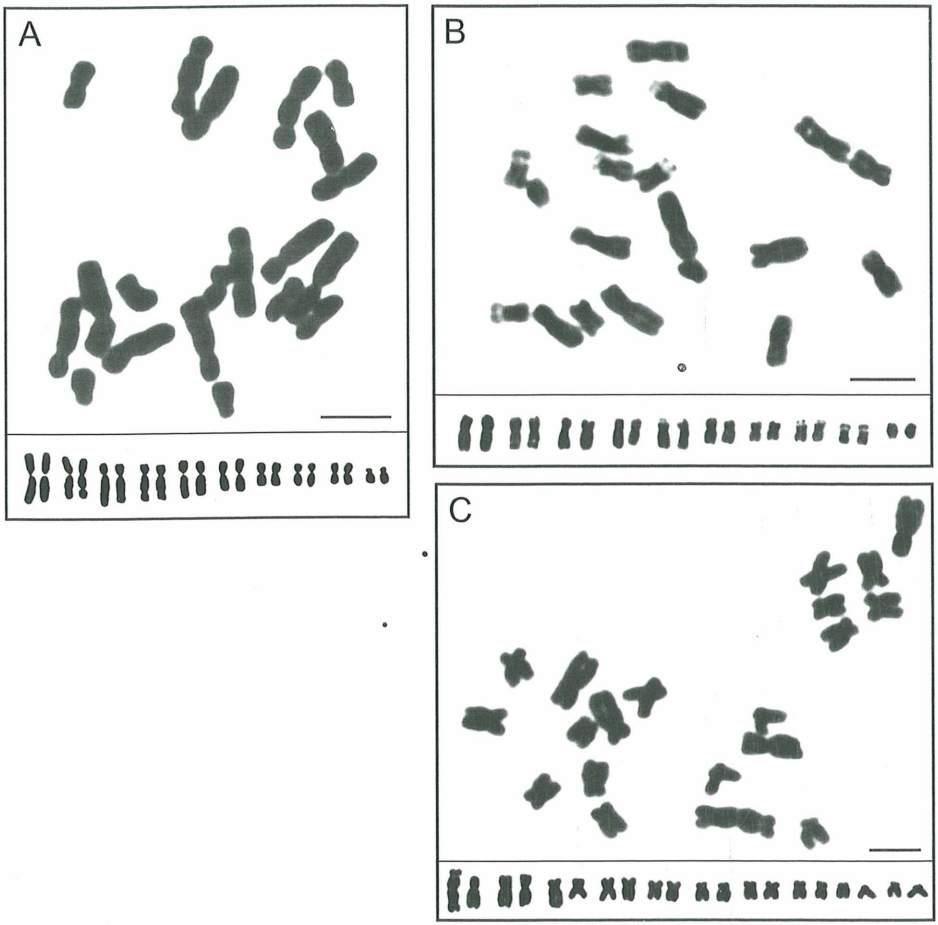


Fig. 1: Mitotic chromosomes and karyotypes of **(A)** *Polygonatum latifolium*, **(B)** *P. odoratum* from Dürnstein, and **(C)** *P. odoratum* from Mödling. – Scale bar 5 µm.

Abb. 1: Mitotische Chromosomen und Karyotypen von **(A)** *Polygonatum latifolium*, **(B)** *P. odoratum* aus dem Raum Dürnstein und **(C)** *P. odoratum* aus dem Raum Mödling. – Maßstrich 5 µm.

the loss of the region containing rDNA and adjacent sequences located in distal part of the chromosome arms. The changes, however, are not fixed within the species since the other *P. odoratum* population analysed (Dürnstein) does not exhibit any such heterozygocity in the karyotype. Despite a relatively stable chromosome number, karyotype changes are frequent in these two species and have already been reported in numerous publications from accessions collected world-wide. They occur probably due to translocations and/or chromosome segments loss (THERMAN 1953).

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The significant differences in karyotypes of two populations of *Polygonatum odoratum* clearly involve interchromosomal changes of 18S-25S rDNA loci. These differences are likely to be the result of change of position of segments containing rRNA genes (satellites and/or secondary constrictions). These loci were shown to behave as "mobile elements" of the genome (SCHUBERT & WOBUS 1985). Changes of number and/or location of 18S-5.8S-25S rDNA loci have frequently been observed in various species (e.g., *Allium*, SCHUBERT & WOBUS 1985; *Arabidopsis*, WEISS & MAŁUSZYNSKA 2000; *Brachyscome*, ADACHI & al. 1997; *Paeonia*, ZHANG & SANG 1998). Usually these changes are occurring on the intraspecific level. However, chromosomal polymorphism concerning homologous chromosomes has been frequently reported in many monocotyledonous species, being associated either with various types of heterochromatin or rDNA loci (e.g., GREILHUBER 1995). It is also known that the presence of terminally located satellites may stimulate chromosomal change, promoting translocations, deletions and inversions (e.g., SCHUBERT & WOBUS 1985, HALL & PARKER 1995). It is possible, therefore, that the change in localization of some loci of rDNA in population of *P. odoratum* near Mödling have arose via translocations of chromosome segments carrying NORs.

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