Preslia 97: 129–155, 2025
doi: 10.23855/preslia.2025.129

Preslia

Not as boring as expected: triploids, pentaploids and aneuploids of invasive *Solidago* species revealed by detailed karyological examination in central Europe

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Abstract: Three alien *Solidago* species of North American origin have become naturalized in Europe. While *S. canadensis* and *S. gigantea* are considered among the most aggressive plant invaders, *S. altissima* is rarely found. Here, the detailed karyological variation and cytogeography of alien *Solidago* species in central Europe and the genetic relationships among the cytotypes is elucidated in their native and other invaded areas. Almost 4,800 plants from 800 sites across central Europe were studied using flow cytometry and complementary chromosome counting. A representative subset was sequenced (ITS, cpDNA) and compared with available data. The findings are in accord with previous reports, with the diploids of *S. canadensis* and tetraploids of *S. gigantea* dominating the invaded range. There was up to 10% variation in the genome size among the studied populations of particular species, the relationship between genome size and the geographical location and altitude of the population was statistically confirmed. In addition, for the first time in the invaded range, rare cytotypes, i.e. triploids of *S. canadensis* and pentaploids and aneuploids of *S. gigantea,* were recorded. The record of *S. altissima* in the study area was not confirmed and its previous hexaploid record was refuted based on re-examination. The triploids of *S. canadensis* were represented by scattered plants within diploid populations, and they most likely resulted from an occasional fusion of reduced and unreduced gametes. The origin of the *S. gigantea* aneuploids and especially pentaploids is more obscure as almost three hundreds were recorded, although so far, only one pentaploid has been reported from North America. Samples from the invaded range shared ITS ribotypes with those from the native range; however, ITS data indicate slight genetic differentiation of pentaploids and aneuploids of *S. gigantea*. It was also revealed that two widespread haplotypes of cpDNA in central Europe were shared by all cytotypes of *S. gigantea* and *S. canadensis.* The cytogeographic pattern of invasive *Solidago* species in Europe is more diverse than expected. Because genome duplication can promote invasiveness, the origin, distribution and invasive potential of newly discovered polyploids need to be investigated in order to prevent their negative effect on the native flora.

Received: 11 Jun 2024; Revised: 14 Oct 2024; Accepted: 22 Oct 2024; Published: 26 Feb 2025

Keywords: *Asteraceae*, biological invasions, chromosome numbers, flow cytometry, genome size, polyploidy, rare cytotypes

Introduction

Invasive alien species adversely affect biodiversity and are one of the major threats to native species (Cassey et al. 2018, IPBES 2023). Despite great efforts in terms of both research and management (Hulme et al. 2013, Stricker et al. 2015, Tekiela & Barney 2017), several aspects of invasion, including post-introduction processes and effects on ecosystems are under-investigated and in need of further study (e.g. Vilà et al. 2011).

Many invasive plant species do not show genetic depauperation in the invaded ranges, either due to high genetic variation in the founder populations, multiple or mass introductions, or the development of newly generated genotypes (Sakata et al. 2015). In addition to admixture among populations previously isolated in the native range (Sutherland et al. 2021), a novel variation in invasive populations can stem from hybridization and/or polyploidization. It has been repeatedly shown that these evolutionary processes (often in combination) can result in new, more invasive genotypes (Ellstrand & Schierenbeck 2000, Wendel & Cronn 2003, Bleeker et al. 2007, Hegarty & Hiscock 2007, Currat et al. 2008, Abbott et al. 2013, Soltis et al. 2014). Hybridization with native species provides alien species with genes preadapted for new environments (Ellstrand & Schierenbeck 2000). Consequently, hybrids and introgressed progeny may benefit from a broader range of environmental conditions than their parental taxa (Ellstrand & Schierenbeck 2000, Bleeker et al. 2007, Currat et al. 2008, Abbott et al. 2013). Similarly, polyploidization also expands the gene pool, potentially resulting in genotypes being able to occupy a wider ecological niche (Wendel & Cronn 2003, Hegarty & Hiscock 2007, Soltis et al. 2014). In addition to the effect on genetic diversity, genome duplication is often associated with alterations in sexual/asexual reproduction, life span or other fitness traits in plants (te Beest et al. 2012). Thus, in the case of invasive plants, polyploidization might increase colonization success and subsequent establishment (Mayrose et al. 2011, Rosche et al. 2016) and should be included as an essential trait in invasion models (Sakata et al. 2015). It is generally assumed that polyploidization brings a genetic and adaptive advantage, resulting in the reduced susceptibility of polyploids to inbreeding depression, their higher fitness and survival rates in the earliest establishment phase, as well as an ability to rapidly expand their geographic distribution (Soltis & Soltis 2000, te Beest et al. 2012). Several studies have suggested that polyploids have a higher invasive capacity than diploids (Moura et al. 2021), are more frequent among invasive than non-invasive species (Pandit et al. 2011, Góralski et al. 2014), and that their relative proportion among alien plants increases with progressive stages of invasion (Wani et al. 2018). A study of 81 invasive species of plants and their 2,356 congeners revealed that polyploids are 20% more likely to be invasive than diploids (Pandit et al. 2011).

In addition to the ploidy level, the relationship between genome size and invasiveness is addressed in several studies, with both holoploid (C) and monoploid (Cx) genome sizes generally being smaller in invasive than non-invasive species in most of the groups of angiosperms studied (Chen et al. 2010, Pandit et al. 2014, Suda et al. 2015, Pyšek et al. 2018). Chen et al. (2010) indicate that polyploid invasive plants are favoured, at least

partly, because of the downsizing of basic genome size that is associated with polyploidization. They report that the sizes of holoploid and monoploid genomes decreased significantly with increasing invasiveness based on the categories established by Holm et al. (1979) from non-weeds through common weeds, principal weeds up to serious weeds. However, studies on invasive alien plants that include detailed ploidy level or variation in genome size are still relatively scarce (e.g. Kubátová et al. 2008, Schlaepfer et al. 2008a, Dematteis et al. 2020, Mereďa et al. 2023, Tian et al. 2023).

Currently, three alien species of the genus *Solidago* L. of North American origin, *S. altissima* L., *S. canadensis* L. and *S. gigantea* Aiton [*Solidago* subsect. *Triplinerviae* (Torr. et A. Gray) G. L. Nesom and *Solidago* subsect. *Serotinae* (Rydb.) Semple et J. B. Beck sensu Semple & Beck 2021, Semple 2022], have become naturalized in Europe. While *S. canadensis* and *S. gigantea* are considered to be among the most aggressive plant invaders across almost the whole of Europe (except the southernmost and northernmost parts) (Kowarik 2010, CABI 2021a, b), the records of *S. altissima* in Europe are still rare (Verloove et al. 2017, but see Tian et al. 2023). All three mentioned species are long-lived perennials and obligate outbreeders, pollinated by a wide range of insects. The plants produce numerous small fruits (tens of thousands) with a pappus and are easily dispersed by wind over long distances. They also propagate via underground rhizomes (Werner et al. 1980, Huang et al. 2007, Moravcová et al. 2010, CABI 2021a, b). *Solidago canadensis* was introduced into Europe in 1645 and *S. gigantea* in 1758 as attractive, late-flowering ornamental plants. Their first observations in the wild in Europe date back to 1850s and they still spread in invaded range (Weber 1998, Kowarik 2010). The historical circumstances of the introduction of *S. altissima* into Europe are more obscure (see below).

The basic chromosome number in the genus *Solidago* is x = 9 and the ploidy-level varies from 2x to 14x (Semple & Cook 2009, Semple 2016). Within the genus, diploid-only species are the most common (ca 87 species), the diploid-polyploid species (ca 51) usually have more polyploid cytotypes (Semple 2016, Semple & Beck 2021). Several taxa in the genus *Solidago* (including *S. altissima* and *S. gigantea*) have been the subject of detailed cytogeographical studies, focusing on native (Morton et al. 2019, 2020, Martino et al. 2020, Semple 2022), invaded (Szymura et al. 2015) or both types of distribution (Jakobs 2004, Schlaepfer et al. 2008a, Cheng et al. 2021).

Solidago canadensis, in the strict sense (cf. Semple & Cook 2006, Semple & Beck 2021), includes only diploids (2n = 2x = 18) in both its native (more than 200 records, var. *canadensis* and var. *hargeri* Fernald in the central and north-eastern parts of the United States and the southern regions of eastern Canada) and invaded (less than 100 records, only var. *canadensis* in Asia and Europe) ranges (Semple & Cook 2006, Semple & Chmielewski 2022, Semple 2023) (Supplementary Table S1). However, for some invaded regions, there are almost no karyological data for naturalized plants (e.g. Asia, Australia and New Zealand).

In contrast, *S. altissima* and *S. gigantea* are karyologically more variable. *Solidago gigantea* includes diploid (2n = 2x = 18; var. *gigantea*), tetraploid (2n = 4x = 36; var. *gigantea*) and hexaploid (2n = 6x = 54; var. *shinnersii* Beaudry) populations, along with very rare odd ploidies: triploids (2n = 3x = 27) and pentaploids (2n = 5x = 45) in its native range in southern Canada and the central and eastern parts of the United States (Schlaepfer et al. 2008a, Morton et al. 2019, Semple 2022, 2023) (almost 400 records; Supplementary Table S1). Mixed-ploidy populations are rare in *Solidago gigantea* native

range, with particular cytotypes appearing to be reproductively isolated, and differing in their growth characteristics and ecological requirements (Melville & Morton 1982, Jakobs 2004, Schlaepfer et al. 2008a, 2010, Hull-Sanders et al. 2009, Martino et al. 2020). Only the tetraploid plants of *S. gigantea* have so far been confirmed in the invaded ranges in Europe and East Asia (Weber 1997, Schlaepfer et al. 2008a, Szymura et al. 2015, Semple 2023) (almost 200 records; Supplementary Table S1), but there is no data for the Azores or New Zealand.

Solidago altissima is naturally distributed in central and eastern North America, where there are three varieties [var. *altissima*, var. *gilvocanescens* (Rydb.) Semple and var. *pluricephala* M. C. Johnston] and three ploidies (diploid 2n = 2x = 18, tetraploid 2n = 4x = 36 and hexaploid 2n = 6x = 54), which occur frequently in mixed-ploidy populations (Halverson et al. 2008b, Sakata et al. 2015, Etterson et al. 2016, Morton et al. 2019). *Solidago altissima* is a highly competitive invader in East Asia (only the hexaploid cytotype; Japan, China, Korea and Taiwan; Chen & Semple 2011, Sakata et al. 2015), with a few sites also reported in Oceania (Semple & Uesugi 2017), India (Semple & Sankara Rao 2017) and South Africa (Cheek & Semple 2016). According to Verloove et al. (2017), *S. altissima* (hexaploid cytotype) was first recorded in Europe at a site in Belgium. However, there is an older but overlooked hexaploid chromosome count for *S. altissima* from Slovakia (Bratislava-Kramáre, oak groves near Červený most bridge Májovský et al. 2000, Uhríková & Králik 2000). Currently, the hexaploid *S. altissima* is reported from five more unspecified sites located in European cities (Tian et al. 2023).

Until recently, there was a lack of comprehensive studies on species of *Solidago* in their natural range in North America and the status of morphologically similar diploid plants, currently distinguished as *S. canadensis* (var. *hargeri*) and *S. altissima* (var. *gilvocanescens*), has been unresolved for a long time (Semple et al. 2015). Consequently, *S. canadensis* and *S. altissima* were not recognized as separate species and the diploid plants now identified as *S. altissima* were classified as *S. canadensis* or its variety *S. canadensis* var. *scabra* (Willd.) Torr. et A. Gray (Cronquist 1952, Croat 1972, Scoggan 1979, Werner et al. 1980). Similarly, European invasive diploid plants of the genus *Solidago* are misclassified by some authors as *S. altissima* (i.e. Weber 1997, 2001, Mikoláš 1998, Sell & Murrell 2006, Szymura & Wolski 2011, Szymura & Szymura 2013), while other authors treat all European *Solidago* diploid plants with hairy stems as *S. canadensis* (Semple et al. 2013, 2015, Cheek & Semple 2016). Contrarily, the polyploid plants of *S. altissima* in China are often misidentified as *S. canadensis* (Chen & Semple 2011, Semple et al. 2015; see Supplementary Table S1).

Despite the evidence of a relationship between polyploidy and invasiveness in *Solidago* (Halverson et al. 2008a, Hull-Sanders et al. 2009, Schlaepfer et al. 2010, Richardson & Hanks 2011, Martino et al. 2020, Walczyk & Hersch-Green 2023) and the wide distribution and continued spreading of invasive alien *Solidago* throughout Europe (CABI 2021a, b), knowledge of their karyological variation there is scattered, and records are more-or-less limited to only some parts of Europe (see Supplementary Table S1). Genome size, which is another trait related to the invasive capacity of plants, has been rarely studied in invasive species of *Solidago* (Kubešová et al. 2010, Szymura et al. 2015, Verloove et al. 2017). Therefore, in the present study, the focus is on the poorly studied region of central Europe and adjacent areas, using flow cytometry (FCM), ITS and chloroplast (cp)DNA genetic analyses to answer the following questions: (i) What

are the detailed karyological variation and cytogeography of invasive alien *Solidago* species in the region studied? (ii) Are there any cytotypes different from those previously reported from the invaded range? (iii) Is it possible to confirm the hexaploid chromosome count and the presence of *S. altissima* in central Europe (Májovský et al. 2000, Uhríková & Králik 2000)? (iv) What are the genetic patterns within the species studied in their native and introduced areas? (v) What are the genetic relationships between the revealed cytotypes?

Material and methods

Sampling

In total, 4,782 plants from 791 sites were collected in the wild (no plants from cultivations) between 2017 and 2021 in Slovakia, Austria, Croatia, the Czech Republic, Hungary, Poland and Romania (Supplementary Table S2). The flowering plants were determined in the field using known diagnostic morphological characters: *S. altissima* – produces polycormons (long branched rhizomes up to 40 cm long, above ground without a clear connection between new and old stems), stems usually short-hairy throughout; leaves thick and firm with adaxial faces ± scabrous, involucres 2.5–4.5 mm long, ligules of ray florets 0.7–1.5 mm long; *S. canadensis* – grows in clusters (rhizomes up 10 cm long, new stems arise in a circle near the old ones), stem densely hairy in the middle and upper part, adaxial faces of leaves sparsely hairy, involucres 1.7–3.0 mm long, ligules of ray florets 1.4–1.8 mm long; *S. gigantea* – produces polycormons, stem glabrous in the middle and upper part, adaxial faces of leaves glabrous, involucres 3.5–5.0 mm long, ligules of ray florets 2.5–3.5 mm long (Slavík 2004, Semple & Cook 2006, Verloove et al. 2017, Skokanová 2023). A total of 2,769 plants were identified in 566 populations as *S. canadensis*, and 2,013 plants in 368 populations as *S. gigantea*; at 143 sites, both species were collected (Supplementary Table S2). In the field, no plants were identified as *S. altissima* based on their morphology. Voucher specimens (at least one per population) are deposited in the SAV herbarium (Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Bratislava).

From each plant, fresh leaves or stems with rhizomes were collected and kept in a cool place (4–6 °C) until used in the FCM analysis. From selected plants (representing the area of study and the detected karyological variation), intact fresh leaves were dried in silica gel for molecular analysis. Finally, rhizomes of some plants (representing detected cytotypes) were cultivated in pots at room temperature for determining chromosome number. Distribution maps were created using QGIS 3.24.2 (QGIS Development Team 2022).

Chromosome counting

Chromosome numbers were counted in the root-tip meristems of cultivated plants of *S. canadensis* (six populations, six individuals) and *S. gigantea* (seven populations, 12 individuals) (Table 1; Supplementary Table S2). The same plants were also included in flow cytometry analyses. The root tips were pretreated in a 0.002-M water solution of 8-hydroxyquinoline at 4 °C for about 16 h (overnight), fixed in a 1:3 mixture of 98% acetic acid and 96% ethanol for 1–24 h, washed in distilled water, macerated in 1-N hydrochloric acid at 60 °C for 5 min, and then washed in distilled water. Permanent slides were made

Table 1. DNA ploidy level, relative genome size (RGS), absolute genome size (AGS), and chromosome counts for *Solidago canadensis* and *S. gigantea* (RSS – ratio of the G1 peak of the *Solidago* sample to the G1 peak of the standard *Solanum pseudocapsicum*. Values in cells are number of plants, mean±S.D. and range (min–max), except for ploidy level and chromosome number where the number of plants and respective values are given.

Species	DAPI flow cytometry		PI flow cytometry		Chromosome counts
	Ploidy level	RGS (2 C-values; RSS)	AGS (2 C-values; RSS)	AGS (2 C-values; pg)	
S. canadensis	2,766; 2n ≈ 2x ≈ 18	688; 0.82±0. 01 (0.78–0.86)	20; 0.78±0.01 (0.76–0.80)	20; 2.02±0.02 (1.96–2.06)	5; 2n = 18
	3; 2n ≈ 3x ≈ 27	3; 1.22±0.02 (1.20–1.24)	2; 1.17±0.00	2; 3.03±0.01 (3.02–3.04)	1; 2n = 27
S. gigantea	1,747; 2n ≈ 4x ≈ 36	394; 1.47±0.02 (1.42–1.54)	20; 1.40±0.02 (1.37–1.42)	20; 3.61±0.04 (3.55–3.69)	5; 2n = 36
	2; 2n ≈ 4x+3 ≈ 39	2; 1.60±0.01 (1.59–1.61)	2; 1.52±0.01 (1.52–1.53)	2; 3.94±0.02 (3.93–3.96)	2; 2n = 39
	264; 2n ≈ 5x ≈ 45	40; 1.80±0.01 (1.77–1.83)	6; 1.70±0.01 (1.69–1.71)	6; 4.40±0.03 (4.37–4.44)	5; 2n = 45

using the cellophane square technique (Murín 1960). The slides were stained with a 7% solution of Giemsa Stain–Modified Solution (Fluka Analytical) in Sörensen phosphate buffer. The chromosomes were counted using a Leica DM1000 microscope equipped with an HDCE-X5 camera and ScopeImage ver. 9.0 software.

Flow cytometry

Two different fluorochromes were used, depending on the purpose of the analysis: AT-selective 4', 6-diamidino-2-phenylindole (DAPI) for the estimation of DNA ploidy level and relative genome size (RGS), and intercalating propidium iodide (PI) for the estimation of absolute genome size (AGS) (cf. Doležel et al. 2007, Sliwinska et al. 2022).

The DNA ploidy level (estimated using DAPI FCM) was determined for 4,782 specimens of *S. canadensis* and *S. gigantea*. Of these, 4,505 were new estimations and 277 were published reports (Skokanová et al. 2022). To reduce the number of analyses, up to five plants from the same population were analysed together (the CV values of the measurements of the pooled samples were comparably low to those of individual samples, see Results). Depending on population size (see Skokanová et al. 2024) and the density of sampling in a particular area, one to 24 plants were analysed per population, except for population ZST (247 plants), which was sampled in greater detail because a chromosome count (2n = 56) for *S. altissima* is available for this population (Májovský et al. 2000, Uhríková & Králik 2000). The other exceptions were three populations for which new cytotypes were revealed during the present study (M30 – 54 plants, KRL – 195 plants and DUP – 266 plants). Next, the RGS was estimated from a subset of 1,127 plants (including 277 that were previously published in Skokanová et al. 2022). For this, each plant was analysed separately using DAPI fluorochrome. Finally, the AGS was determined using PI for a subset of 50 plants that were analysed separately.

The DAPI measurements were done at the Institute of Botany, Plant Science and Biodiversity Centre, Slovak Academy of Sciences in Banská Bystrica on a Partec CyFlow® ML flow cytometer equipped with a UV-LED excitation source, and in

Bratislava on a Partec CyFlow® ML flow cytometer equipped with an HBO-100 mercury arc lamp (both Partec GmbH, Münster, Germany). The PI measurements were done in Košice at the Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University on a Partec CyFlow® ML flow cytometer equipped with a green solid-state laser excitation source. The data were processed using Partec FloMax® software ver. 2.70 (Partec GmbH, Münster, Germany). *Solanum pseudocapsicum* L. (*Solanaceae*) 2C = 2.59 pg (Temsch et al. 2010) was used as an internal reference standard in all FCM analyses. The isolation of nuclei and staining procedures for the DAPI and PI flow analyses followed a simplified two-step protocol (Doležel et al. 2007) using Otto I + II buffers (Otto 1990), which was used in the DAPI flow analyses with some modifications (see Skokanová et al. 2022) and a general-purpose buffer (Loureiro et al. 2007) and sample preparation as described in Bruňáková et al. (2021) in the PI analyses. At least three independent PI measurements were made on different days for each plant. If the between-day variation exceeded 2%, the value showing the greatest deviation was discarded and the sample was re-analysed. In all the analyses, the fluorescence intensity of at least 5,000 particles was recorded. Only histograms with symmetrical peaks and a low coefficient of variance (CV) of the standard and sample G1 peaks (below 4% for DNA ploidy, 3% for RGS and 5% for AGS) were considered.

The ploidy level and 2 C-values of the RGS and AGS were estimated based on the ratio of the mean of the G1 peak of the fluorescence intensity of the sample and the mean of the G1 peak of the fluorescence intensity of the standard; hence forth, this ratio of sample/standard is referred to as the RSS. The 2 C-values are presented unless otherwise stated. The results of FCM analyses were interpreted based on the chromosome counts (Table 1) (cf. Sliwinska et al. 2022). The Cx-values of the AGS and the equivalent of the Cx-values of the RGS were calculated as the 2 C-values of the AGS and RGS divided by the number of chromosome sets x (cf. Greilhuber et al. 2005).

Box-and-whisker and scatter plots carried out using STATISTICA 12 (StatSoft Inc. 2013) were used to depict variations in RGS and AGS of the species and their cytotypes. A linear mixed-effects model was used (LMMs; Pinheiro & Bates 2000) to test for differences in RGS and AGS among the cytotypes nested within species. As several plants were measured in each population the population identity was incorporated as a random intercept in the LMMs to account for autocorrelation among plants. The performance of each model was evaluated using randomized quantile residuals (Dunn & Smyth 1996). Model based on 1,000 simulations was used and normality and homoscedasticity determined using standard diagnostic plots. Genome size data were logarithmically transformed to meet the assumptions of LMMs. Due to the spatially structured sampling design, spline correlograms were constructed to examine autocorrelation in the residuals (Bjørnstad & Falck 2001); no significant spatial autocorrelation was detected. The statistical significance of the mixed models was evaluated using F-tests with Kenward-Roger adjusted degrees of freedom (Kenward & Roger 1997). Pairwise comparisons following significant overall tests were performed using estimated marginal means with Tukey's adjustments (Lenth 2016). Mixed models were also used, with the same settings as mentioned above, to evaluate the relationship between the RSS 2 C-values of the AGS (PI-based) and RGS (DAPI-based). No transformation of the variables was needed for this analysis. Finally, the geographic patterns in mean RGS 2 C-values (averaged per population) were determined for *S. canadensis* and *S. gigantea* using generalized additive models

(GAMs; Hastie & Tibshirani 1990). Given the absence of a priori expectations regarding the shape of the RGS spatial distribution, a flexible semi-parametric approach was used, which allowed the modelling of a broad range of responses. GAMs with thin plate regression splines (Wood 2003) were used as a smoother basis, which incorporates three spatial predictors in each model: altitude, latitude and longitude, with the latter two linearized to northing and easting. The levels of smoothness was constrained by setting the upper limit on the degrees of freedom to ten. Moreover, an extra penalty to perform variable selection by shrinking the effective degrees of freedom towards zero (Marra & Wood 2011) was implemented. The same regression diagnostic tools as those mentioned above were used to assess the performance of the GAMs. The significance of smooth terms was evaluated using Wald tests (Wood 2013). The analyses were performed in R ver. 4.2.2 (R Core Team 2022) using the libraries DHARMa (Hartig 2022), emmeans (Lenth 2023), ggplot2 (Wickham 2016), lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017), mgcv (Wood 2017) and ncf (Bjørnstad 2022).

Molecular analysis (ITS of nrDNA and rpS15-ycf1 of cpDNA)

Patterns in genetic differentiation between and within the species studied were examined by sequencing two genomic regions: the ITS of the nrDNA (ITS1-5.8S-ITS2) and the *rpS15-ycf1* intergenic spacer of the chloroplast DNA (cpDNA). The latter region is one of the most polymorphic spacers of cpDNA (Prince 2015) and was investigated in a previous study on the hybridization between *S. canadensis* and *S. virgaurea* (Skokanová et al. 2022), where this spacer provided sufficient resolution, with multiple haplotypes recognized within the species. Thus, it was also used in the present study. The genomic DNA was isolated using the Qiagen DNeasy Plant Mini Kit, and the target regions were amplified following the protocols described in Skokanová et al. (2022).

Here, 46 ITS sequences (16 individuals of *S. canadensis* and 30 of *S. gigantea*) (Table 1) were generated, which were complemented by 99 sequences from a previous study (Skokanová et al. 2022), including *S. canadensis*, *S. gigantea* and *S. virgaurea* (Supplementary Table S3). In addition, 113 ITS sequences from GenBank derived from accessions of *S. canadensis* and *S. gigantea* from both native and introduced ranges, as well as from their closest relatives (inferred from ITS sequence similarity) (Supplementary Table S3) were downloaded. The sequences were aligned in Geneious v. R10 (Kearse et al. 2012), and the alignment was analysed using maximum likelihood (ML) phylogenetic inference in Garli ver. 2.01 software (Zwickl 2006). The best-fit model for nucleotide substitutions was determined using jModelTest ver. 2.1.10 software (Darriba et al. 2012) using the Akaike information criterion. Branch support was assessed using bootstrap replicates. Representatives of the closely related genus *Chrysothamnus* Nutt. (cf. Urbatsch et al. 2003, Semple et al. 2023; *C. depressus* Nutt. and *C. viscidiflorus* Nutt.) were used as an outgroup. In addition, ITS sequences from the target clade (comprising *S. canadensis* and *S. gigantea*), resolved in the ML tree, were analysed using Neighbour-Net (SplitsTree4, ver. 4.14.4; Huson & Bryant 2006) based on uncorrected P-distances and ambiguous state codes treated with the 'average' option. Because the strict diploid concept of *S. canadensis* was followed in the present study (cf. Semple & Cook 2006, Semple & Beck 2021), and because *S. altissima* is not usually recognized as a species distinct from *S. canadensis* in Asia (cf. Chen & Semple 2011, Kato-Noguchi & Kato 2022), the Chinese samples of

Wu & Hu (unpublished data) and Xu et al. (2018), as well as samples from China, the United States and Canada of the tetraploid and hexaploid levels reported by Cheng et al. (2021), were treated as being *S. altissima* (for details, see Supplementary Table S3). The *rpS15-ycf1* spacer in 44 individuals (14 individuals of *S. canadensis* and 30 of *S. gigantea*) (Table 1) was amplified and sequenced and aligned with previous data on these two species (63 sequences) from Skokanová et al. (2022) (Supplementary Table S3). The indels present in the alignment were coded as binary characters based on the simple indel coding approach (Simmons & Ochoterena 2000), using GapCoder (Young & Healy 2003), and appended to the nucleotide dataset. The statistical parsimony network was inferred using TCS ver. 1.21 (Clement et al. 2000). All newly generated sequences were deposited in the GenBank nucleotide database (Supplementary Table S3).

Results

Chromosome numbers

Five plants of *S. canadensis* (populations BNL, HLM, M30, M133 and VSE) were diploid, with 2n = 2x = 18 (Fig. 1A), one plant of *S. canadensis* in population KRL was triploid, with 2n = 3x = 27 (Fig. 1B). The tetraploid number 2n = 4x = 36 was recorded for five *S. gigantea* plants (populations ILS, HLC, M8, VSE and ZEG) (Fig. 1C). Interestingly, the pentaploid chromosome number 2n = 5x = 45 was recorded for five plants of *S. gigantea* in populations DUP and SRP (Fig. 1E). Two plants of *S. gigantea* in population

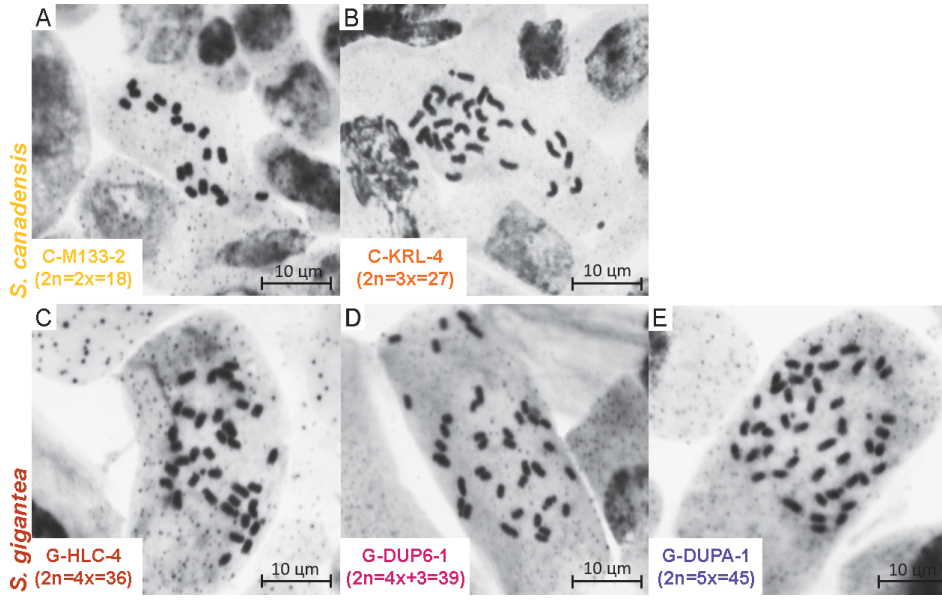


Fig. 1. Microphotographs of the chromosome metaphase plates of *Solidago canadensis* (A, B) and *S. gigantea* (C–E).

DUP were aneuploids with the chromosome number 2n = 4x+3 = 39 (Fig. 1D) (Table 1; Supplementary Table S2).

DNA ploidy levels

The CVs of the analyses for DNA ploidy level estimates were 1.13±0.47 and 1.07±0.45 for the samples and the internal standard *Solanum pseudocapsicum*, respectively. The DNA ploidy estimation for 2,766 plants of *S. canadensis* revealed a diploid level with 2n ≈ 2x ≈ 18. In addition, one plant of *S. canadensis* in population KRL and two in population M30 were triploids with 2n ≈ 3x ≈ 27 (Fig. 2, Table 1; Supplementary Table S2).

The tetraploid level 2n ≈ 4x ≈ 36 was recorded for 1,747 plants of *S. gigantea*, whereas 253 plants of *S. gigantea* in the DUP population and 11 plants of *S. gigantea* in the SRP population were pentaploid, with 2n ≈ 5x ≈ 45. DNA ploidy estimation revealed two aneuploids of *S. gigantea* (2n ≈ 4x+3 ≈ 39) in the DUP population (Fig. 2, Table 1; Supplementary Table S2).

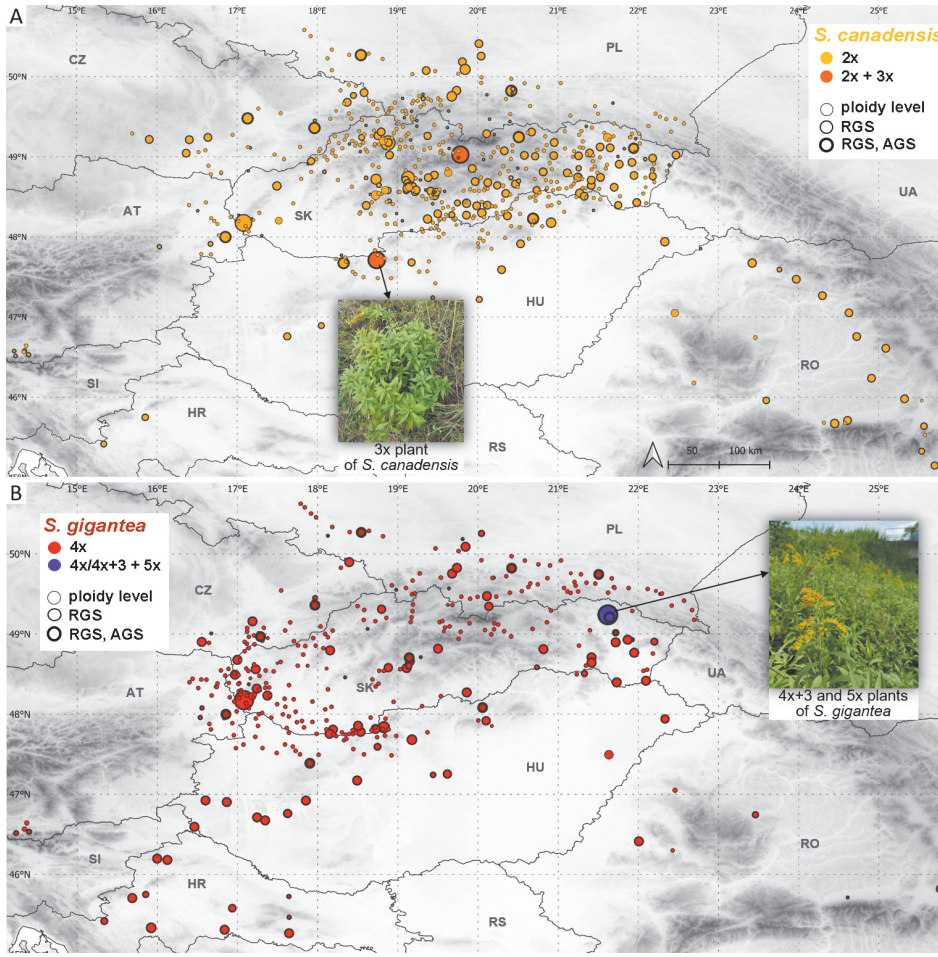


Fig. 2. Map showing the sites at which *Solidago canadensis* (A) and *S. gigantea* (B) were collected. Dot size is directly proportional to the number of plants analysed using DAPI flow cytometry.

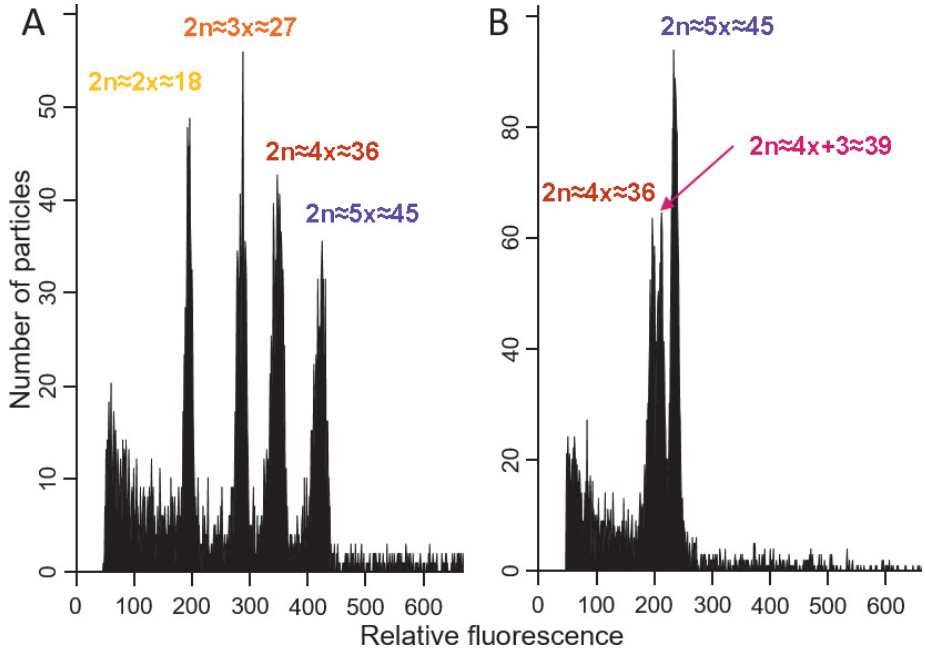


Fig. 3. Histograms of simultaneous flow cytometry analyses of *Solidago canadensis* (yellow and orange) and *S. gigantea* euploid plants (red and violet) (A) and all detected cytotypes of *S. gigantea* (red, magenta and violet) (B).

A detailed survey of population ZST did not reveal any hexaploids in *S. altissima*; instead, only 56 diploid plants of *S. canadensis* and 191 tetraploid plants of *S. gigantea* were recorded (Supplementary Fig. S1).

Relative genome size

The mean CV values of the G1 peaks of the *Solidago* samples and the internal standard *Solanum pseudocapsicum* were 1.07±0.44 and 1.15±0.42, respectively. Histograms of the simultaneous FCM measurements of the analysed cytotypes are shown in Fig. 3.

The RSS values of the RGS of the diploids (2n ≈ 2x ≈ 18) of *S. canadensis* (688 plants) varied from 0.78 to 0.86. The RSS values for the three triploids (2n ≈ 3x ≈ 27) of *S. canadensis* were 1.20 to 1.24 (Table 1; Supplementary Table S2, Fig. S2). The variation in the RGS within particular diploid populations of *S. canadensis* was up to 4.2%, with the general variation within the diploids and triploids being 9.89% and 2.68%, respectively (Supplementary Fig. S2).

The RSS values for the tetraploids (2n ≈ 4x ≈ 36) of *S. gigantea* (394 plants) varied from 1.42 to 1.54, for the pentaploids (2n ≈ 5x ≈ 45, 40 plants) from 1.77 to 1.83, and for the RSS values for the two aneuploids (2n ≈ 4x+3 ≈ 39) from 1.54 and 1.59 (Table 1; Supplementary Table S2, Fig. S2). The overall variation within the cytotypes of *S. gigantea* was 8.86% for the tetraploids and 3.35% for the pentaploid, with the variation within particular populations up to 4.63% and 3.35% for the tetraploids and pentaploids, respectively (Supplementary Fig. S2).

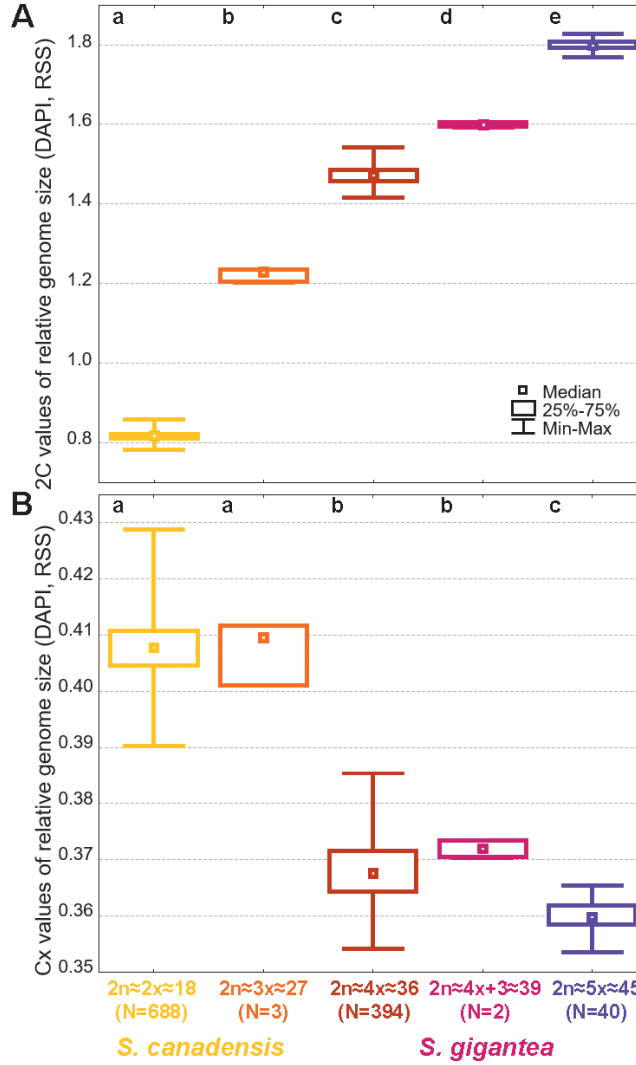


Fig. 4. Boxplots depicting the relative genome size equivalent of 2 C-values (A) and Cx-values (B) of *Solidago canadensis* (yellow and orange) and *S. gigantea* plants (red, magenta and violet). Different lowercase letters indicate groups that are significantly different at the 5% level, as determined by Tukey's pairwise comparison. RSS – ratio of the G1 peak of the *Solidago* sample to the G1 peak of the standard *Solanum pseudocapsicum*; N – number of plants.

Significant differences were recorded in the 2 C-values of the RGS ($F_{4, 929.7} = 115669$, $P < 0.0001$), the post-hoc comparison revealed that all the cytotypes studied were statistically distinct in the 2 C-values of the RGS from each other (Fig. 4A). Significant differences among cytotypes were also recorded in Cx-values of the RGS ($F_{4, 929.7} = 3{,}494$, $P < 0.0001$). Both cytotypes of *S. canadensis* significantly differed from the cytotypes of *S. gigantea* in the equivalent Cx-values of the RSS of the RGS (Fig. 4B). However, within the species, the diploid and triploid cytotypes of *S. canadensis*, as well as the aneuploids

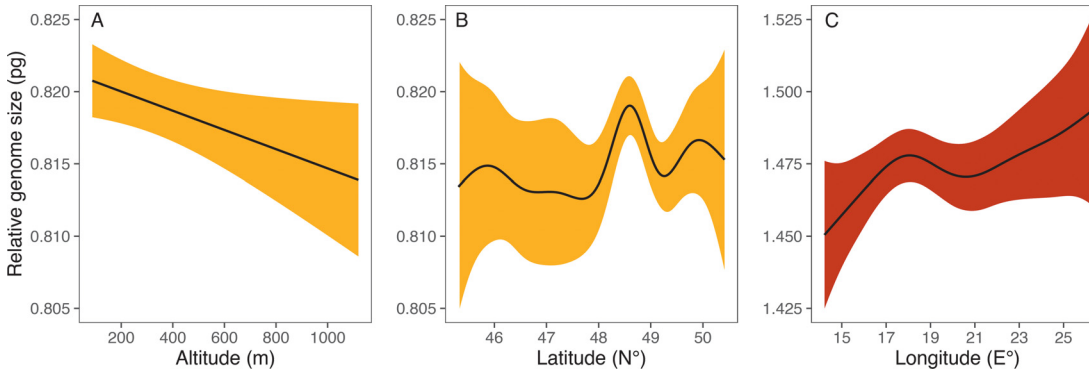


Fig. 5. Significant effects of altitude (A), latitude (B), and longitude (C) on the relative genome size of *Solidago canadensis* (orange) and *S. gigantea* (red). GAM-based predictions (lines) are depicted with 95% confidence bands. Note that easting and northing were back-transformed to longitude and latitude for graphical presentation.

(2n ≈ 4x+3 ≈ 39) and tetraploids of *S. gigantea*, were statistically indistinguishable in the equivalent Cx-values of the RSS of the RGS.

The GAMs revealed a significant altitudinal (effective degrees of freedom [edf] = 0.9, F = 0.51, P = 0.021) and latitudinal (edf = 6.2, F = 1.68, P = 0.019) patterns in the RGS of *S. canadensis*, while the longitudinal changes were non-significant (edf < 0.1, F < 0.01, P = 0.767). The relative genome size of *S. canadensis* decreased with increasing altitude (Fig. 5A) and showed a distinct peak at latitudes around 48.6°N (Fig. 5B). In contrast, the relative genome size of *S. gigantea* changed significantly with longitude (edf = 4.2, F = 1.03, P = 0.043), but remained statistically similar along altitudinal (edf = 3.0, F = 0.40, P = 0.262) and latitudinal gradients (edf = 4.7, F = 0.69, P = 0.226). The RGS of *S. gigantea* increased non-linearly in the eastward direction (Fig. 5C).

Absolute genome size

The mean CV values of the G1 peaks of the *Solidago* samples and the internal standard estimated using PI fluorochrome for the AGS were 3.89±0.40 and 4.15±0.54, respectively. The AGS of the diploids (2n ≈ 2x ≈ 18) of *S. canadensis* (20 plants) varied by 5.15%, from 1.96 to 2.06 pg, the AGS of the triploid plants (2n ≈ 3x ≈ 27, two plants) of *S. canadensis* varied by 0.57%, from 3.02 to 3.04 pg. The AGS of *S. gigantea* varied by 3.94%, from 3.55 to 3.69 pg for the tetraploids (2n ≈ 4x ≈ 36, 20 plants) and by 1.16% from 4.37 to 4.44 for the pentaploids (2n ≈ 5x ≈ 45, six plants). The values of the AGS for the two aneuploids of *S. gigantea* analysed (2n ≈ 4x+3 ≈ 39) were 3.93 pg and 3.96 pg (Table 1; Supplementary Table S2).

There was a significant relationship ($F_{1, 46.2} = 34125$, $P < 0.0001$) between the RSS values of the AGS, estimated by intercalating the PI and RGS, estimated by the AT-selective DAPI dye (marginal $R^2 = 0.999$) (Supplementary Fig. S3). Statistically significant differences in the 2C ($F_{4, 32.6} = 9917$, $P < 0001$) and Cx-values ($F_{4, 32.6} = 334$, $P < 0.0001$) of the AGS of all the cytotypes estimated were very similar to those revealed for the RGS (see Supplementary Fig. S4).

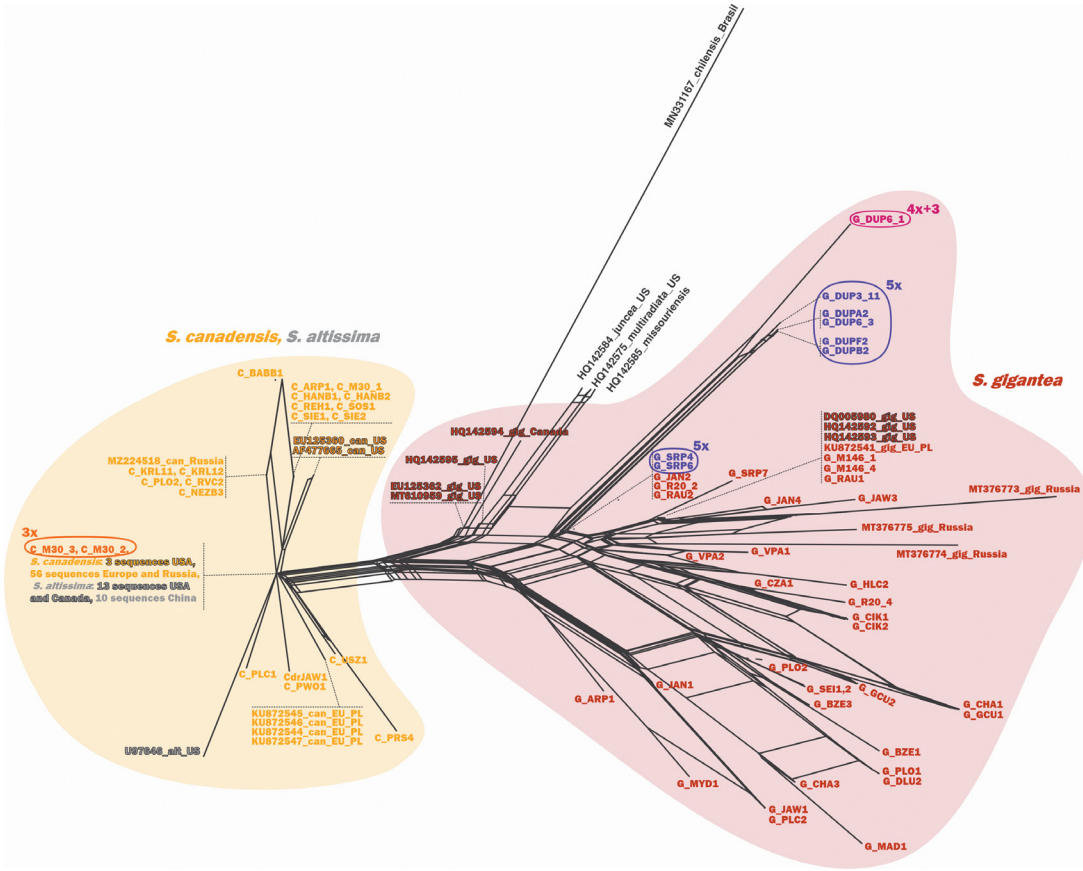


Fig. 6. Neighbour-Net network based on the ITS sequences of the nrDNA of the target clade consisting of *Solidago canadensis*, *S. gigantea* and their closest relatives (see Supplementary Fig. S4). Terminal labels denote individual sequences (see Supplementary Table S3), which are coloured according to the species and cytotype assignment. Labels of samples from the native range appear with black outlined letters.

Molecular analysis (ITS of nrDNA and rpS15-ycf1 of cpDNA)

The final ITS alignment, consisting of 263 sequences, was 596 base pairs (bp) long and contained 37 variable sites. On the ML tree (Supplementary Fig. S4), *S. canadensis* and *S. gigantea* formed a clade that also contained *S. altissima*, *S. chilensis* Meyen, *S. juncea* DC, *S. missouriensis* Nutt. and *S. multiriada* Nutt., but it had little internal structure or support. Two major subclades (bootstrap < 50%) were resolved within this clade, one including only *S. gigantea* sequences (from both native and introduced areas), while the other included all *S. canadensis* and *S. altissima* sequences and some *S. gigantea* sequences from central Europe. In addition, some sequences of *S. gigantea* (from both native and introduced areas) were placed in the basal polytomy of this clade. The Neighbour-Net analysis of the sequences from this target clade partitioned the accessions of *S. gigantea* and *S. canadensis* + *S. altissima* into two species-specific clusters (Fig. 6). Much greater genetic variation was recorded in the *S. gigantea* cluster. In both clusters,

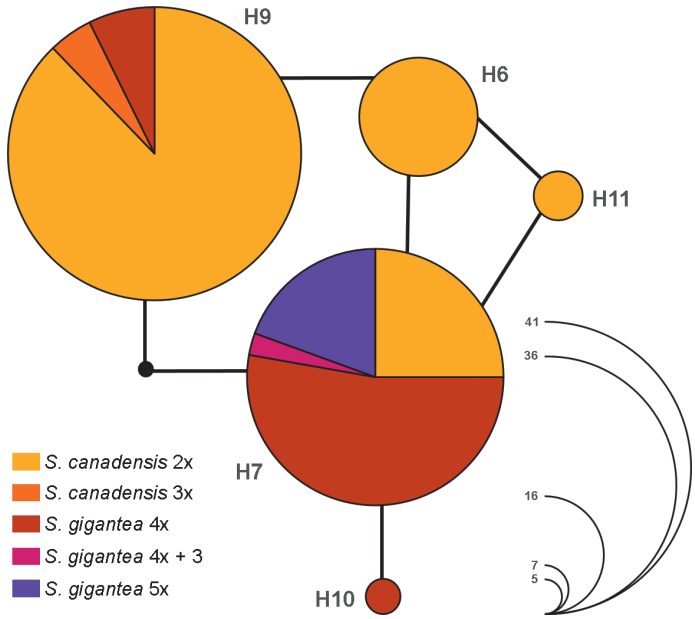


Fig. 7. Maximum parsimony network of the cpDNA haplotypes (the *rpS15-ycf1* spacer) of central-European samples of *Solidago canadensis and S. gigantea*. Circles represent haplotypes (the naming follows Skokanová et al. 2022); black dot missing haplotype; and lines mutational steps. The size of circles is proportional to haplotype frequency (see scale). Colours indicate taxon and cytotype.

no differentiation was recorded between the accessions from native and introduced areas. The triploids of *S. canadensis* had the same ITS sequence as most of the conspecific diploids. The pentaploids of *S. gigantea* (populations SRP and DUP) fell within the variation of the other (tetraploid) samples, but those of population DUP (5x, 4x+3) differ, being placed on long branches.

The *rpS15-ycf1* alignment, consisting of 109 sequences of *S. gigantea* and *S. canadensis*, was 493 bp long and included two single-site polymorphisms and two indels, coded as additional characters. A total of six haplotypes were identified, representing a subset of haplotypes that were identified in a previous study, which also included *S. virgaurea* and its hybrids with *S. canadensis* (Skokanová et al. 2022). *Solidago canadensis* and *S. gigantea* shared two widespread haplotypes H7 and H9. In addition, two haplotypes were *S. canadensis*-specific (H1 and H6), and one was *S. gigantea*-specific (H10). The common H9 haplotype was also found in the triploids of *S. canadensis*, and the common H7 haplotype was present in the pentaploid and aneuploid samples of *S. gigantea* (Fig. 7).

Discussion

New records in the context of previous results

The findings, based on the analysis of almost 4,800 plants of *S. canadensis* and *S. gigantea*, sampled from across under-studied central Europe, predominantly support previous records of diploid plants of *S. canadensis* (2n = 2x = 18) and the tetraploid plants of

S. gigantea (2n = 4x = 36) in their invaded ranges in the broader European region (Supplementary Table S1). However, extensive research also indicates that the karyological variation in *S. canadensis* and *S. gigantea* in the invaded range in Europe is not as boring as expected based on previous records. The presence of a rare pentaploid cytotype of *S. gigantea* was revealed for the first time in the invaded range. Moreover, a new cytotype was recorded for *S. gigantea*: an aneuploid with 2n = 4x+3 = 39, which is the very first aneuploid reported in the genus *Solidago*. In addition, triploids of *S. canadensis*, were recorded for the first time for this species. For comparison, Semple (2016), from a database of 4,466 chromosome numbers published for the genus *Solidago*, only cites three triploid counts and two pentaploid counts. Currently, rare odd ploidy cytotypes are reported mainly for diploid-polyploid species, such as *S. altissima* and *S. gigantea*, in their native range. Therefore, they are interpreted as resulting from crossing within mixed-ploidy populations (Schlaepfer et al. 2008a, Semple 2022). However, this interpretation does not fit with the results presented, with *S. canadensis* being a strictly diploid species and *S. gigantea* not being represented in Europe by mixed-ploidy populations. Therefore, it was surprising to discover these new cytotypes, and especially an abundant pentaploid cytotype.

This study revealed the values of the AGS to be a bit lower than those previously reported (all available previous records are from Europe). For the diploids of *S. canadensis*, the values in this study are 1.97–2.08 vs 2.03–2.21 pg by Szymura et al. (2015) and 2.04–2.14 pg by Kubešová et al. (2010). For the tetraploids of *S. gigantea*, the values are 3.58–3.72 vs 3.65 pg by Kubešová et al. (2010), 3.7–3.8 pg by Szymura et al. (2015) and 3.81 pg by Verloove et al. (2017) (Supplementary Table S1). In a relatively large number of the populations of a particular species studied, certain variation was recorded in the RGS (diploids of *S. canadensis* up to 9.89% and tetraploids of *S. gigantea* up to 8.86%). This variability seems to be related to the population's geographic location and altitude (Fig. 5). Thus, in the case of genome size, selection has probably occurred in the area invaded in Europe by *S. canadensis* and *S. gigantea*. In the area studied, variability in genome size could be influenced by their distributions and ecological preferences (Skokanová et al. 2024), therefore, this phenomenon and its potential effect on invasiveness needs to be examined in more detail at a larger scale.

Origin of newly revealed cytotypes

The triploids of *S. canadensis* were rarely recorded in the area studied with only one and two plants in two populations (~ 0.1% of all the plants of *S. canadensis* studied). The triploid plants did not karyologically (Cx-values; Fig. 4B) or genetically (Figs 6 and 7; Supplementary Fig. S4) differ from the diploids of *S. canadensis*. Therefore, the triploids are most likely the result of a rare fusion of reduced and unreduced gametes of the same species (Kreiner et al. 2017). The occurrence of triploids could be evidence of unreduced gamete production in natural populations, which, although extremely rare, might play a role (via a triploid bridge) in autopolyploidization (Ramsey & Schemske 1998, Köhler et al. 2010).

The origins of the pentaploids and aneuploids of *S. gigantea* are more obscure. Only one pentaploid plant of *S. gigantea* was recorded in the ploidy-mixed populations in the native range (out of ~400 available records for *S. gigantea*, see Supplementary Table S1). Genetic data (Schlaepfer et al. 2008b) indicates that the populations of *S. gigantea* in

Europe originated from a few introductions. Consequently, the chance of introducing infrequent pentaploids is very low. Therefore, the recording of hundreds of pentaploid (and two aneuploid) plants in two Slovak populations DUP and SRP, where they are abundant in riparian vegetation along a river (Fig. 2B), is astonishing. It is likely that these odd cytotypes may have arisen by in situ polyploidization in the invaded range, but more studies are needed. Correspondingly, higher ploidies of *S. gigantea* and *S. altissima* in the native North American area appear to have formed repeatedly (Halverson et al. 2008b, Schlaepfer et al. 2008a, Martino et al. 2020). Environmental stress (such as changes in temperature and water conditions, pollution, agrochemicals, herbivory and disease) can induce or stimulate the formation of unreduced gametes (De Storme et al. 2012, Mason & Pires 2015, Fuchs et al. 2018). However, in the DUP population, which was sampled in detail (266 plants), no hexaploid plants were recorded, which, because of the formation of 3x gametes, could potentially have led to the formation of pentaploid plants. The genetic data indicate a slight differentiation in the plants in the DUP population due to intragenomic polymorphisms in three neighbouring positions in the ITS2 region. These match those previously reported in the hybrid and introgressant plants that are intermediates between *S. canadensis* and *S. virgaurea* (Skokanová et al. 2022). Nevertheless, it is highly unlikely that the plants in the DUP population are of hybrid origin, considering the significantly higher Cx-values for *S. virgaurea* than for *S. gigantea* (Fig. 4B and Skokanová et al. 2022). Further molecular and cytogenetic analyses are needed to determine the pathway, character and origin of pentaploid and aneuploid plants.

Invasive potential of newly recorded cytotypes

It is likely that the emergence and establishment of new cytotypes in invasive plant groups can affect their invasion potential and behaviour in various ways, e.g. through changes in population fitness, clonal propagation, inter-cytotype gene flow and changes in genetic variation. In the field, only non-flowering triploid plants of *S. canadensis* were recorded whereas plants in pentaploid(-aneuploid) populations of *S. gigantea* flowered (Fig. 1). Despite the recorded non-flowering, it is known that reduced fertility caused by odd ploidy and thus disrupted meiosis, can be compensated for by asexual reproduction (i.e. through increased vegetative propagation; Herben et al. 2017, van Drunen & Husband 2019, Šingliarová et al. 2023) and/or the production of apomictically formed seeds, where meiosis and syngamy are bypassed (Koltunow 1993). Both of the species studied are known for their efficient vegetative spread in established stands (Jakobs 2004, Schlaepfer et al. 2010). However, the second possibility, in this case, can be ruled out because apomixis has not been reported in the genus *Solidago* (Noyes 2007).

The high number of pentaploid plants detected, even if they are so far restricted to two localities, could be a warning signal that they can efficiently spread and colonize nearby sites. Therefore, their potential gene flow with the dominant tetraploids, reproduction biology (reproduction mode, extent of clonal propagation) and invasive potential (reproductive and dispersal success and overall fitness) need to be studied in detail in order to assess the threat they pose in terms of the colonization of natural habitats. Furthermore, previous studies indicate that the tetraploid plants of *S. gigantea* are fitter than the diploid and hexaploid plants. Schlaepfer et al. (2010), based on common garden experiments, report that the diploids grow faster (higher leaf nitrogen and specific leaf area), than the

tetraploids with a 'longer-lived' strategy (more extensive rhizome system and faster accumulation of biomass). Tetraploids from the invaded range, with more stems, taller stems, and an increased number of leaves, which are less affected by herbivory, outcompete hexaploid *S. gigantea* plants from their natural range in greenhouse and common garden experiments (Nagy et al. 2017). Tetraploids are more resistant to pathogens, have better photosynthetic ability and water-use efficiency and generally performed better under high soil nitrogen and phosphorus amendments (Walczyk & Hersch-Green 2023). The study of the ecological tolerances of diploid, tetraploid and hexaploid *S. gigantea* in their native range indicates that genome doubling has resulted in greater ecological amplitude in its polyploid cytotypes (Martino et al. 2020). Polyploidization itself generates novel phenotypes that are adaptive in particular environments (Ramsey 2011), which results in the non-random distribution of cytotypes at macro- and fine-scales (Martino et al. 2020, Mráz et al. 2022). In general, polyploids have a higher invasive capacity (Moura et al. 2021), are more frequent in invasive than among non-invasive species (Góralski et al. 2014), and the relative proportions in alien plants increase with the stage of invasion (Wani et al. 2018). In both *Centaurea stoebe* L. and *Senecio inaequidens* DC, which include diploids and tetraploids in their native ranges, only tetraploid cytotypes became invasive in their introduced range (Treier et al. 2009, Thébault et al. 2011). In contrast, Kubátová et al. (2008) report no relationship between differences in ploidy level and invasiveness in *Lythrum salicaria* L., whereas Grewell et al. (2016) report that invasive diploids outperform congeneric invasive tetraploids in the aquatic macrophyte *Ludwigia* (Grewell et al. 2016).

Hexaploid Solidago altissima in central Europe

A detailed survey of the ZST locality (Bratislava, Kramáre), from where Májovský et al. (2000) and Uhríková & Králik (2000) report hexaploid chromosome counts (2n = 6x = 54) for *S. altissima*, revealed the occurrence of only diploid plants of *S. canadensis* and tetraploid plants of *S. gigantea* (Supplementary Fig. S1). The published chromosome count for *S. altissima* has not been documented by herbarium specimen(s). Based on this study it is likely that the record of hexaploid *S. altissima* from Slovakia is erroneous. This report was probably a misidentification of *Symphyotrichum novii-belgii* (L.) G. L. Nesom, for which the chromosome count 2n = 54 is published for a nearby location (Sedláková 1981). Invasive asters often grow close to *Solidago* plants, and in their sterile stages, their shoots are difficult to distinguish morphologically (Skokanová 2023).

Tian et al. (2023) recently published records of five diploid populations of *S. canadensis* together with five hexaploid populations of *S. altissima* occurring in the European cities Berlin (Germany), Florence, Rome (Italy), Versailles (France) and Vienna (Austria) (treated by those authors as *S. canadensis* s.l.; see Supplementary Table S1). Vienna occurs within the area included in the present study; however, even with more-detailed sampling in the city's surroundings, only diploids of *S. canadensis* and tetraploids of *S. gigantea* were recorded (Fig. 2; Supplementary Table S1). Because Tian et al. (2023) do not specify the source of the seeds obtained from Vienna, it could not be excluded that they originated from cultivated plants. Moreover, it is not clear how the authors detected extraordinary hexaploids, but not the presence of the very common *S. canadensis* or *S. gigantea* (Fig. 2).

Genetic relationships of the detected cytotypes of Solidago canadensis and S. gigantea

The genus *Solidago* includes a surprisingly low level of DNA sequence divergence (Schilling et al. 2008, Semple 2016), which results from its shallow and relatively recent divergence (Beck & Semple 2015, Semple 2016, Beck et al. 2021 and references therein). Recent phylogenetic analyses based on hundreds of nuclear genes (Semple et al. 2023) reports a phylogeny in which there is strong support for species up to the subgenus level; however, the status of polyploid species remains unresolved. The phylogeny presented here was based on ITS sequences and accordingly generated polytomies and clusters with low bootstrap support (< 50%). However, some conclusions can be drawn. Samples of the diploid cytotype of *S. canadensis* and the tetraploid cytotype of *S. gigantea* from the invaded range shared ITS ribotypes with those from the native range. Also, the ribotypes of the newly discovered minority cytotypes did not distinguish them from the others (Fig. 6; Supplementary Fig. S5). It was also possible to confirm that *S. altissima* and *S. canadensis* are genetically very close to *S. gigantea* and are related to other North American species in the same subsection (*Solidago* subsect. *Serotinae*: *S. chilensis* Meyen) and also another subsection [*Solidago* subsect. *Junceae* (Rydb.) Nesom: *S. juncea*, *S. missouriensis*] or even section [*Solidago* sect. *Multiradiatae* (Semple) Semple et J. B. Beck: *S. multiriada*] (Fig. 6; Supplementary Fig. S5). Similarly, Beck & Semple (2015) and Semple et al. (2023), using genotyping by sequencing based on single-nucleotide polymorphisms, report comparable results: *S. gigantea* forming a separate genetic cluster, but with *S. canadensis* being a part of the genetic group with *S. altissima*, *S. lepida* DC. and *S. brendiae* Semple. Molecular analysis has thus repeatedly pointed to a close genetic relationship between *S. canadensis* and *S. altissima*. However, *S. altissima*, is distinct from *S. canadensis*, as it is a diploid-polyploid complex with a distinct morphology (Semple & Cook 2006, Verloove et al. 2017). The morphological differentiation between diploid *S. canadensis* and the hexaploids and tetraploids of *S. altissima* is stable, even in experimental cultivation under similar conditions (Tian et al. 2023). Moreover, the genome size of the hexaploid *S. altissima* is 2.6–2.7 times greater than that of the diploid *S. canadensis* (Verloove et al. 2017, Tian et al. 2023), so species-specific monoploid genome size would rule out a recent polyploidization relationship between them. Furthermore, it is worth noting that, while the above-mentioned taxa fell into polytomies and clusters with low bootstrap support, *S. virgaurea* specimens form a distinct cluster with moderate bootstrap support (88%) and did not include Asian (Iran and China) specimens belonging to the morphologically similar *S. deccurens* Loureiro (Supplementary Fig. S4).

In the genus *Solidago*, the haplotypes of cpDNA are often shared across species (Zhang 1996, Schlaepfer et al. 2008b, Sakata et al. 2015). The cpDNA analyses (Fig. 7) also revealed two widespread haplotypes common to *S. gigantea* and *S. canadensis*. More importantly, the identical haplotypes were also shared by the newly discovered minority cytotypes: triploid plants within a predominantly *S. canadensis* haplotype (H9), pentaploids and an aneuploid within a predominantly *S. gigantea* haplotype (H7) (Fig. 7).

Conclusions

This study has highlighted the importance of in-depth studies on the karyology and genetics of invasive taxa in ranges they have spread into in order to elucidate new genotypes, and thus prevent the overlooking of post-invasion processes and their potential effect on invasiveness. The consequences of post-invasion ploidy-driven or other evolutionary processes on invasiveness have to be, however, based on a study of the fitness parameters of such genotypes. At the same time, it should be emphasized that the correct taxonomic identification of taxa in the genus *Solidago* is important along with that of other taxonomically complicated taxa, before drawing any conclusions because the taxonomic affiliation, and thus the genetic background, is crucial. *Solidago* and other invasive species, cannot be eradicated or even controlled across the board, but, based on data, efforts could be focused not only on endangered biotopes but also on genotypes posing the greatest threat to biodiversity.

Supplementary materials

Fig. S1. Map showing the distribution of plants of *Solidago canadensis* and *S. gigantea* analysed.
Fig. S2. Variation in the RGS of *Solidago canadensis* and *S. gigantea* plants.
Fig. S3. Relationship between RGS and AGS (2 C-values) of *Solidago canadensis* and *S. gigantea* plants.
Fig. S4. Boxplots depicting the absolute genome size (ASS) – 2 C-values (A) and Cx-values (B) of *Solidago canadensis* and *S. gigantea* plants.
Fig. S5. Maximum likelihood phylogram based on ITS sequences.
Table S1. Survey of the published chromosome numbers of *Solidago canadensis* and *S. gigantea.*
Table S2. Locality details, including geographical coordinates, altitude, date and collectors of *Solidago canadensis* and *S. gigantea* and the number of plants included in the karyological analysis.
Table S3. GenBank accession numbers and the origin of the plant material for the ITS and *rpS15-ycf1* sequences of the *Solidago* plants analysed.

Supplementary materials are available at https://www.preslia.cz

Acknowledgements

This study was financially supported by the Scientific Grant Agency, VEGA (Grant no. 2/0024/23) and by the Slovak Research and Development Agency (Grant no. APVV-19-0134).

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Méně nudní než se myslelo – karyologický výzkum odhalil ve střední Evropě triploidní, pentaploidní a aneuploidní rostliny invazních druhů rodu *Solidago*

V Evropě zdomácněly tři nepůvodní druhy rodu *Solidago* původem ze Severní Ameriky. Zatímco *S. canadensis* a *S. gigantea* jsou tu považovány za nejagresivnější invazní druhy, druh *S. altissima* byl nalezen jen vzácně. Zaměřili jsme se na objasnění podrobné karyologické variability a cytogeografie nepůvodních druhů rodu *Solidago* ve střední Evropě a stanovení genetických vztahů mezi odhalenými cytotypy a cytotypy z původních a jiných invazních oblastí. Pomocí průtokové cytometrie a komplementárního počítání chromozomů jsme analyzovali téměř 4800 rostlin z 800 lokalit po celé střední Evropě. Reprezentativní podskupina byla sekvenována (ITS, cpDNA) a dána do kontextu s již dostupnými genetickými daty. Naše zjištění jsou z velké míry v souladu s předchozími poznatky, přičemž ve studované části invadovaného území dominují diploidní rostliny *S. canadensis* a tetraploidní *S. gigantea*. U studovaných populací jsme pozorovali určitou variabilitu v obsahu DNA (do 10 %), přičemž byla statisticky potvrzena souvislost obsahu DNA s geografickou polohou a nadmořskou výškou populace. Vůbec poprvé byly v invadovaném areálu nalezeny triploidní rostliny *S. canadensis* a pentaploidní a aneuploidní rostliny *S. gigantea*. Ve zkoumané oblasti jsme nezaznamenali *S. altissima* a nepotvrdili jsme ani předchozí hexaploidní údaj ze Slovenska. Triploidní cytotypy *S. canadensis* se velice vzácně vyskytovaly v diploidních populacích a pravděpodobně vznikly příležitostnou fúzí redukovaných a neredukovaných gamet. Původ aneuploidů a pentaploidů *S. gigantea* je nejméně zřejmý; na východním Slovensku jsme zaznamenali téměř tři sta pentaploidních rostlin, ačkoli dosud byl udávaný pouze jeden pentaploid ze Severní Ameriky. Rostliny *S. gigantea* z invadovaného areálu měly společné ITS ribotypy s rostlinami z původního severoamerického areálu, nicméně ITS data naznačují mírnou genetickou diferenciaci pentaploidních a aneuploidních rostlin. Ve střední Evropě jsme odhalili dva rozšířené haplotypy cpDNA, které byly společné všem cytotypům *S. gigantea* a *S. canadensis*. Na základě našich výsledků lze konstatovat, že karyologická variabilita invazních druhů rodu *Solidago* v Evropě je rozmanitější, než se předpokládalo. Protože duplikace genomu může podporovat invazivnost, je třeba prozkoumat původ, rozšíření a invazní potenciál nově objevených polyploidů, aby se zabránilo jejich negativnímu dopadu na původní flóru.

How to cite: Skokanová K., Zozomová-Lihová J., Mereďa P. Jr., Španiel S., Hodálová I., Mártonfiová L., Svitok M., Kolarčik V. & Šingliarová B. (2025) Not as boring as expected: triploids, pentaploids and aneuploids of invasive *Solidago* species revealed by detailed karyological examination in central Europe. – Preslia 97: 129–155.

Preslia, a journal of the Czech Botanical Society
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https://www.preslia.cz

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