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RESEARCH ARTICLE

A Trojan horse inside the gates: Alien–native mating interactions in mixed populations of *Solidago* taxa

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ABSTRACT

- The establishment and possible impacts of alien–native hybrids depend on their formation frequency and ability to spread using generative and vegetative reproduction. Here, we investigated the frequency and pathways of hybrid *Solidago* ×*niederederi* formation and its subsequent mating interactions with parental species in mixed populations of invasive alien *S. canadensis* and native *S. virgaurea*.
- DAPI flow cytometry was used for progeny screening (991 seedlings) of the 15 plants from two mixed populations to document their mating interactions based on previously shown differences in relative genome size of the studied taxa.
- Seedlings of presumed hybrid origin (F1 hybrids) were formed at low frequency (up to 3%) by both *S. canadensis* and *S. virgaurea*. About 46% of germinating seeds produced by *S.* ×*niederederi* plants represented later hybrid generations. Backcrossing of *S.* ×*niederederi* with parental species within the mixed populations was thus intense, but its frequency differed in direction: backcrossing was less frequent with *S. virgaurea* as pollen donor (<8%), while there was a strong asymmetric pattern towards *S.* ×*niederederi* backcrossing with *S. canadensis* as the pollen donor (almost 47%). Furthermore, the formation of tri- and tetrapolyploid seedlings within the progeny of the two hybrid plants was recorded.
- Hybridisation and introgression appear to be established in mixed populations of *S. canadensis*, *S. virgaurea* and *S.* ×*niederederi*. These post-invasion processes might later be mirrored in the spreading success of descendants of the invasive species and genetic erosion of the native species. Therefore, their pathways, extent and consequences require further attention.

INTRODUCTION

Accidentally or intentionally introduced alien plants can encounter their congeners and, unless they establish reproductive isolation due to separate evolutionary histories, this can lead to hybridisation (cf. Huxel 1999; Barrett *et al.* 2008; Harrison & Larson 2014). If alien invasive plants successfully invade natural habitats, their interactions with related native species can be intense. The new genotypes of hybrid origin can have greater fitness and wider ecological tolerances because the native species provide alien species with preadapted genes for new environments (cf. Thompson 1991; Ellstrand & Schierenbeck 2000, 2006; Bleeker *et al.* 2007; Currat *et al.* 2008; Abbott *et al.* 2013). Newly arisen hybrids are treated as alien species (Pyšek *et al.* 2004) and might thus represent an additional direct threat of biological invasions by reducing the diversity of autochthonous communities and/or causing genetic erosion of native flora (Huxel 1999; Baack & Rieseberg 2007; Bailey *et al.* 2007; Sun *et al.* 2015; Morais & Reichard 2018). At the same time, ongoing alien-to-native hybridisations provide an excellent opportunity to investigate evolutionary processes on-site and at contemporary time scales (Ellstrand & Schierenbeck 2006; Barrett *et al.* 2008).

At least 25% of plant species are estimated to be involved in effective hybridisation, characterised as the production of viable offspring (Mallet 2005). Interspecific hybrids frequently suffer from reduced fertility that reflects reproductive isolation between parental lineages (Charron *et al.* 2019). Different mechanisms underlie hybrid infertility, including genetic incompatibilities (nuclear and cytonuclear) and changes in genome architecture (ploidy level or chromosome rearrangements) (Charron *et al.* 2019). In some cases, sterile hybrids can spread successfully via vegetative growth (Huxel 1999) or can recover fertility spontaneously and rapidly through whole-genome duplication (Charron *et al.* 2019; Meeus *et al.* 2020). In both cases, because of the reproductive isolation from parental species, hybridisation may lead to further speciation (Huxel 1999; Baack & Rieseberg 2007). Alternatively, fertility restoration can be achieved by crosses among at least partially fertile hybrid individuals or backcrosses with either parental species, allowing the purging of incompatibilities through recombination. If backcross hybrids are fertile, this process most often leads to the formation of a hybrid swarm consisting of numerous hybrid and introgressed genotypes rather than to a new hybrid species (Huxel 1999; Mallet 2005; Charron *et al.* 2019).

It has been shown that alien–native, as well as alien–alien, hybridisation results in hybrids of variable and unpredictable spreading potential, which can differ even between hybrid combinations of closely related species. For example, *Fallopia* ×*bohemica* (Chrtek et Chrtková) J. P. Bailey is substantially more invasive than *F.* ×*moravica* Hodálová & Mered'a, which currently has a very restricted distribution (Hodálová *et al.* 2022). Similarly, hybrids of *Tamarix chinensis* Lour. and *T. ramosissima* Ledeb. are more common than their hybrid combinations with *T. parviflora* DC. and *T. gallica* L. (Gaskin & Schaal 2002). Further, backcrossing and introgression can generate novel genotypes with different invasive manifestations. For instance, although *Typha* × *glauca* Godr. backcrosses with native *T. latifolia* L. and introduced parent species *T. angustifolia* L., F1 hybrids persist at higher frequencies (Zapfe & Freeland 2015; Bansal *et al.* 2019; Joyee *et al.* 2024). Conversely, *Senecio vulgaris* var. *hibernicus* Syme is an example of a stabilised and invasively successful introgressant (Abbott *et al.* 1992, 2010).

In some cases, polyploidization further increases hybrid invasive potential by modifying key morphological traits and enhancing genetic changes and potential speciation phenotypic plasticity, adaptability and ecological tolerance to novel environments (Mounger *et al.* 2021; Wang *et al.* 2022), as documented for allopolyploids *Spartina anglica* C. E. Hubbard (Ainouche *et al.* 2009; Roberts 2024), *Tragopogon mirus* Ownbey and *T. miscellus* Ownbey (Soltis *et al.* 2012; Matthews *et al.* 2015).

The present study focuses on the relatively well-studied hybridisation of invasive *Solidago canadensis* L. of North American origin, which has widely naturalised and spread across Europe, with the European native *S. virgaurea* L., leading to the formation of the spontaneous hybrid *S.* ×*niederederi* Khek (Musiał *et al.* 2020; Skokanová *et al.* 2022). Since the hybrid was discovered at the very end of the 19th century, the number of sites where it has been reported has exponentially increased to more than 400 in 17 European countries (Skokanová, Mered'a Jr., et al. 2020; Skokanová, Šingliarová, *et al.* 2020). Interestingly, analysis of the ITS region of nrDNA suggests that *S.* ×*niederederi* plants are F1 hybrids that arose independently from multiple hybridisation events; however, the spreading of the hybrid itself has not been observed (Pliszko & Zalewska-Gałosz 2016; Galkina & Vinogradova 2019; Skokanová *et al.* 2022). Therefore, hybrid establishment seems to be fully dependent on mating interactions in mixed populations of *S. canadensis* and *S. virgaurea*. Chloroplast DNA analysis indicates that hybridisation between *S. canadensis* and *S. virgaurea* can occur in both directions (Pliszko & Zalewska-Gałosz 2016; Skokanová *et al.* 2022). In populations already including hybrid plants, mating interactions might be more complex. *Solidago* ×*niederederi* plants are known to have high production of viable pollen (Pliszko & Kostrakiewicz-Gierałt 2019), and a significant proportion of well-developed fruits with high germination rates demonstrates their successful sexual reproduction (Migdałek *et al.* 2014; Karpavičienė & Radušienė 2016; Pagitz 2016; Pliszko & Kostrakiewicz-Gierałt 2017; Pliszko & Kostrakiewicz-Gierałt 2020). However, whether their progeny included later hybrid generation (F2) or also backcrossed plants and at what frequency remains unknown. Backcrossing in the system has been previously indicated based on nuclear or chloroplast sequences, as well as discrepancies between morphology, ITS polymorphisms, and DNA content values found in field-collected plants (Pliszko & Zalewska-Gałosz 2016; Galkina & Vinogradova 2019; Skokanová *et al.* 2022; Lysenkov & Galkina 2023).

Previously, we showed that unless *S. canadensis*, *S.* ×*niederederi* and *S. virgaurea* are of the same diploid (2n = 2× = 18) ploidy level, they differ in their relative DNA content values (by ca. 9%) with no overlaps, and that flow cytometry is a reliable and efficient tool for their detection (Skokanová *et al.* 2022; see also Material and Methods). Here, we used DAPI flow cytometry to screen the progeny of plants from mixed populations of *S. canadensis*, *S. virgaurea* and their hybrid *S.* ×*niederederi* to document mating interactions of co-occurring *Solidago* taxa in the wild. The specific aims were to: (1) detect the frequency and direction of F1 (*S.* ×*niederederi*) hybrid formation; and (2) analyse further mating interactions of the hybrid and parental species, specifically, the extent and direction of backcrossing of *S.* ×*niederederi* with parental species.

MATERIAL AND METHODS

Plant material

Because this study primarily focuses on elucidating the pathways and mechanisms of introgression in mixed populations of *Solidago* taxa—rather than determining its frequency at a broader scale—we designed our study to generate a robust dataset of analysed seedlings derived from a limited number of mother plants in two well-studied populations (Skokanová *et al.* 2022). The material was collected on 9 September 2021 from two mixed populations of *S. canadensis* and *S. virgaurea* in Slovakia (JEL: Jelšava, 48.64° N, 20.24° E, 294 m a.s.l. with significant prevalence of *S. canadensis*; VPA: Hrhov, Veľký Paklán hill, 48.58° N, 20.73° E, 196 m a.s.l. with balanced occurrence of both parental species). In a previous study (Skokanová *et al.* 2022), we morphologically identified the presence of hybrid plants of *S.* ×*niederederi* in these populations (JEL: only one hybrid plant; VPA: four hybrid plants, but only two flowering in 2021) but no flowering introgressants. The hybrid origin of all plants of *S.* ×*niederederi* included in this study was confirmed by genome size and additive patterns in ITS sequences in the previous study (Skokanová *et al.* 2022). Near the VPA population, *S. gigantea* Aiton also grows, this species however does not cross with *S. virgaurea* at this site (nor other mixed sites in Slovakia) and was therefore not included in the study (Skokanová *et al.* 2022, 2025). The studied *Solidago* taxa are self-incompatible (Nilsson 1976) and insect-pollinated, with Diptera (particularly hoverflies) being the dominant group (Pliszko & Kostrakiewicz-Gierałt 2018; Pliszko *et al.* 2025). Their flowering periods overlap, they share the same pollinators, and pollen transfer patterns within mixed populations appear to be largely random (cf. Pliszko *et al.* 2025). Because the pollen exchange among the nearest neighbouring plants is most likely, we collected fresh leaf material and mature seeds from hybrid plants and the closest plants of parental species (i.e. two mother plants of *S. canadensis* and two plants of *S.*

virgaurea) (Fig. S1). The exact position of each sampled plant was mapped using a GNSS receiver (Emlid RS) with submeter (usually up to 5 cm) precision. Fresh leaf material was used for subsequent DAPI flow cytometry analyses. The inflorescences with ripe seeds were dried at room temperature and placed in a refrigerator for cold stratification during winter. Seeds were sown in soil in February 2022 with three repetitions per mother plant. Seedlings in the two-primary-leaf stage (Fig. S1) were analysed continuously as the seeds germinated over a 3-month period. We analysed all emerging seedlings, except for mother plants JEL-C1, VPA-C2, VPA-C3 and VPA-C4 in which a high number of seeds germinated, for which we stopped at about 130 seedlings to have a balanced dataset.

Flow cytometry

Flow cytometry (FCM) was used to measure the relative DNA content of 15 mother *Solidago* plants and their progeny. This method was previously shown to be accurate and particularly useful for detecting minor differences in the genome size of the studied *Solidago* taxa (Skokanová *et al.* 2022). To ensure the accuracy of relative DNA content estimates, each mother plant and seedling were analysed separately using fluorochrome 4′, 6-diamidino-2-phenylindole (DAPI). The protocol was the same as in our previous study (Skokanová *et al.* 2022) following a simplified two-step protocol (Doležel *et al.* 2007) and using *Solanum pseudocapsicum* L. (2.59 pg DNA/2C; Temsch *et al.* 2010) as an internal standard. Measurements were performed using a Cyflow ML instrument or Cyflow Space instrument (Partec, Münster, Germany) equipped with a UV-LED as an excitation source. Flow cytometric histograms were evaluated using FloMax software v. 2.7d (Partec). The analyses of mother plants and seedlings were performed in a similar way. However, according to the amount of plant material available, in analyses of mother plants, 1 mL of Otto I and Otto II buffers was added, and the fluorescence of at least 5000 particles was recorded, while in the case of seedlings, only 0.5 mL of Otto I and Otto II buffers was added and the fluorescence of 3000 particles was recorded.

The relative DNA content (2C value) was calculated as the ratio of the G1 peak of the standard and the G1 peak of the *Solidago* sample (standard/sample ratio is hereinafter referred to as RSS). Chromosome counts reported by Skokanová *et al.* (2022), in which only the diploid number 2n = 18 was confirmed for *S. canadensis*, *S. ×niederederi* and *S. virgaurea*, were used for the correct interpretation of flow cytometry results. The recorded values of relative DNA content were compared with those of mother plants and the results of the previous study (including plants from the JEL and VPA populations) to estimate whether the seedlings belonged to the parental species or were produced by hybridisation or backcrossing (Skokanová *et al.* 2022; RSS for 171 *S. canadensis* plants from 36 populations varied from 0.793 to 0.838; RSS for 50 *S. ×niederederi* plants from 16 populations varied from 0.847 to 0.881; RSS for 161 *S. virgaurea* plants from 36 populations varied from 0.897 to 0.938). Results were visualised using STATISTICA 12 (StatSoft Inc. 2013) and the R packages *dplyr* (Wickham *et al.* 2023) and *ggplot2* (Wickham 2016).

RESULTS

The mean CV values of the G1 peaks of the internal standard *Solanum pseudocapsicum* and *Solidago* estimates were 2.4% ± 0.31% and 2.67% ± 0.30%, respectively.

All investigated mother plants were diploid (Tables 1 and Table S1) and their RSS values of relative DNA content were in the range found for *S. canadensis*, *S. ×niederederi* or *S.*

Table 1. The relative DNA content (RSS, 2C value) of mother plants of *Solidago canadensis*, *S. ×niederederi* and *S. virgaurea* and their progeny.

population code	mother plants			seedlings					
				composition of hybrids and backcrosses			n, RSS (min-max)		
	Code	Taxon	RSS	H	IntC	IntV	2× (2n ~ 2× ~ 18)	3× (2n ~ 3× ~ 27)	4× (2n ~ 4× ~ 36)
JEL	JEL-C1	*S. canadensis*	0.824		5 (3.9%)		128; 0.801–0.845		
	JEL-C2	*S. canadensis*	0.812	3 (3.1%)			97; 0.779–0.869		
	JEL-N1	*S. ×niederederi*	0.867	14 (36.8%)	13 (34.2%)	11 (28.9%)	38; 0.828–0.916		
	JEL-V1	*S. virgaurea*	0.910	1 (3.6%)		3 (10.7%)	28; 0.864–0.932		
	JEL-V2	*S. virgaurea*	0.910	2 (3.6%)		1 (1.8%)	56; 0.865–0.934		
VPA	VPA-C2	*S. canadensis*	0.810	11 (8.3%)			133; 0.792–0.875		
	VPA-C3	*S. canadensis*	0.813	1 (0.8%)	2 (2.5%)		127; 0784–0.860		
	VPA-C4	*S. canadensis*	0.820				123; 0.791–0.836	1; 1.162	
	VPA-C5	*S. canadensis*	0.812	1 (2.6%)	1 (2.6%)		38; 0.794–0.863		
	VPA-N2	*S. ×niederederi*	0.855	67 (58.8%)	45 (39.5%)	2 (1.8%)	114; 0.812–0.932	10; 1.230–1.277	3; 1.630–1.721
	VPA-N3	*S. ×niederederi*	0.858	7 (17.1%)	32 (78%)	2 (4.9%)	41; 0794–0.894	1; 1.225	
	VPA-V2	*S. virgaurea*	0.900			1 (12.5%)	8; 0888–0.915		
	VPA-V3	*S. virgaurea*	0.902				7; 0.906–0.923		
	VPA-V4	*S. virgaurea*	0.914			1 (12.5%)	8; 0.896–0.915		
	VPA-V5	*S. virgaurea*	0.905	1 (3.3%)		2 (6.7%)	30; 0.869–0.924		

Number of seedlings of specific ploidy levels (n) and number of seedlings whose relative DNA content corresponds to *S. ×niederederi* (H) and its backcrosses with *S. canadensis* (IntC) or with *S. virgaurea* (IntV).

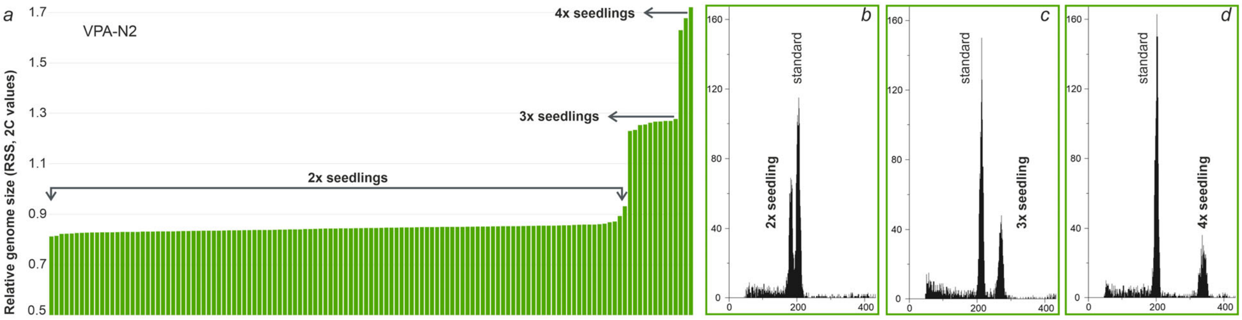

Fig. 1. Bar plot depicting the relative genome size (RSS, 2C values) of all seedlings from the progeny of the *Solidago ×niederederi* plant VPA-N-2 (a), and histograms of DAPI flow cytometry analyses of three selected seedlings: a seedling with a relative DNA content corresponding to the diploid level (b); a seedling with a relative DNA content corresponding to the triploid level (c); a seedling with a relative DNA content corresponding to the tetraploid level (d).

virgaurea in the study of Skokanová *et al.* (2022), which is consistent with our morphological determination in the field.

In total, 991 seedlings were analysed by DAPI flow cytometry (Table S1). The majority of *S. canadensis*, *S. ×niederederi* and *S. virgaurea* seedlings were diploid with 2n = 2× = 18 (Table 1). In addition, for population VPA, we recorded 12 triploid seedlings originating from two *S. ×niederederi* mother plants (VPA-N2 and VPA-N3, 7.3% and 2.4% of their progeny, respectively) and one *S. canadensis* mother plant (VPA-C4, 0.8%). Three tetraploid seedlings were also recorded within the progeny that originated from the *S. ×niederederi* mother plant VPA-N2 (2.2%) (Fig. 1, Table 1).

A hybrid origin was assumed for seedlings from mother plants of *S. canadensis* and *S. virgaurea*, which had a relative DNA content within the range found for *S. ×niederederi* in the study of Skokanová *et al.* (2022). Seedlings of probable hybrid origin (F1 generation) were found in the progeny of both parental species—*S. canadensis* (JEL: 1 mother plant, 3.1%; VPA: 3 mother plants, 2.6%–8.3%) and *S. virgaurea* (JEL: 2 mother plants, 3.6%; VPA: 1 mother plant, 3.3%). The variable part of the progeny of three investigated *S. ×niederederi* mother plants (17.1%, 36.8% and 58.8%) can be attributed to the later hybrid generation (F2) according to their relative DNA content (Figs. 2 and 3, Table 1).

Seedlings from the offspring of *S. ×niederederi* mother plants were considered to be the result of backcrossing if their relative DNA content was lower (backcross with *S. canadensis*) or higher (backcross with *S. virgaurea*) than the hybrid range (Skokanová *et al.* 2022). Within the offspring of all three analysed *S. ×niederederi* plants, we found seedlings whose relative DNA content indicated backcrossing with both parental species. Within the progeny of JEL-N1, backcrossing with *S. canadensis* and *S. virgaurea* occurred at a similar frequency (34.2% vs. 28.9%), while within the progeny of VPA-N2 and VPA-N3, backcrossing with *S. canadensis* prevailed (39.5% and 78% vs. 1.8% and 4.9%; Figs. 2 and 3, Table 1). In addition, in the progeny of both parental species, *S. canadensis* (JEL: 1 mother plant, 3.9%; VPA: 2 mother plants, 2.5% and 2.6%) and *S. virgaurea* (JEL: 2 mother plants, 1.8% and 10.7%; VPA: 3 mother plants, 6.7%–12.5%) we found seedlings with intermediate RSS values, which might be attributed to their introgression with *S. ×niederederi* (Figs. 2 and 3, Table 1).

In summary, the analysed seedlings of *S. canadensis* and *S. virgaurea* mother plants mostly originated from the reception of pollen of the same species (96.3% and 91.3%, respectively), and the production of hybrid (2.5% and 2.9%, respectively) and backcrossed (1.2% and 5.8%, respectively) seedlings was only marginal (Fig. 4). Of the seedlings produced by mother plants of *S. ×niederederi*, only 45.6% resulted in later hybrid generation (*S. ×niederederi* was the pollen donor and recipient). About the same percentage (46.6%) of seedlings of *S. ×niederederi* mother plants resulted from backcrossing with *S. canadensis* and 7.8% from backcrossing with *S. virgaurea* (Fig. 4).

DISCUSSION

As long as the genome sizes of the parental species differ sufficiently (by at least 7%), flow cytometry has repeatedly been proven to be effective in identifying their homoploid hybridisation (e.g. Loureiro et al. 2010; Čertner et al. 2015; Pellicer et al. 2021; Urfus et al. 2025). This approach has also been successfully applied in the system involving *S. canadensis, S. ×niederederi*, and *S. virgaurea* (Skokanová *et al.* 2022; this study). In the present study, we extend the application of flow cytometry to a new frontier: identifying backcrossing in a homoploid hybrid system. Because of the minute differences in genome size between parental and hybrid taxa of the same ploidy, flow cytometric analyses must be extremely precise, with protocols optimised to minimise potential interference from secondary metabolites and technical limitations related to sample preparation and analysis (Walker et al. 2006; Doležel et al. 1998). In this study, we ensured maximum analytical precision by using the same instrument and plant as the internal standard across all measurements, maintaining a slow particle flow to achieve the lowest possible coefficient of variation (CV). Furthermore, the reliability of our protocol is supported by extensive prior experience, including the analysis of thousands of *Solidago* individuals (cf. Skokanová *et al.* 2022, 2025). When interpreting genome size values in hybrid zones at the homoploid level, it is essential to consider potential confounding factors such as aneuploidy and the presence of B chromosomes (Bennetzen et al. 2005; Petrov 2001; Šmarda & Bureš 2010). In the studied mixed *Solidago* populations, aneuploidy can likely be excluded, as only a single case of aneuploidy has been reported in the genus *Solidago* —an aneuploid *S. gigantea* (2n = 4× + 3 = 39), presumably originating from meiotic disruption of pentaploid plants (2n = 5× = 45) growing at the same site (Skokanová

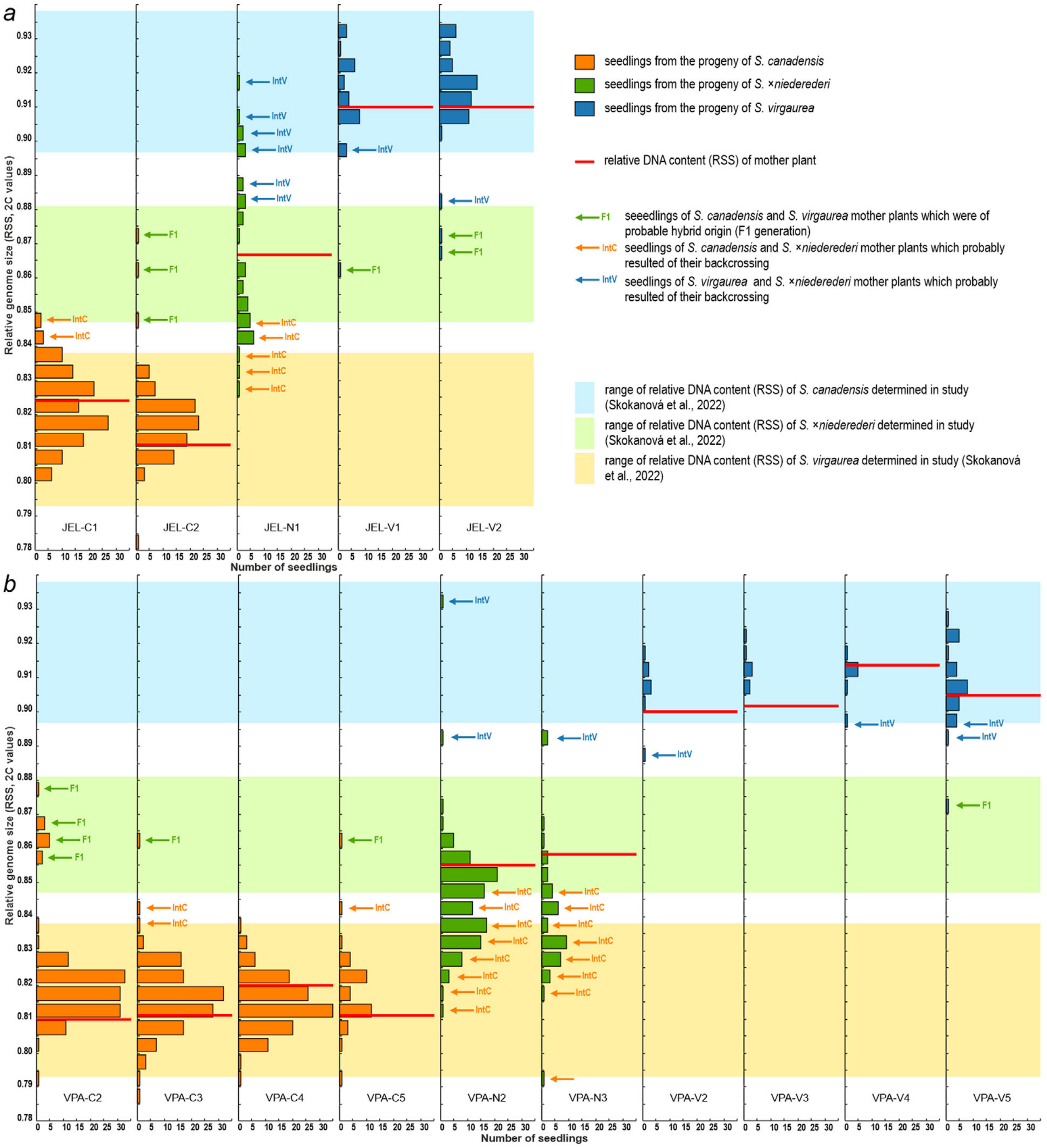

Fig. 2. Histograms depicting the relative genome size (RSS, 2C values) of diploid seedlings from the progeny of *Solidago canadensis*, *S.* ×*niederederi* and *S. virgaurea* from populations JEL (a) and VPA (b).

et al. 2025). The presence of B chromosomes is a low probability; these have been reported only sporadically in *S. virgaurea* and in *S. canadensis* (Rice *et al.* 2015), for *S. canadensis* exclusively from its native North American range but not in the European invasive range, from which at least 29 chromosome counts are currently available (cf. Skokanová *et al.* 2025). Acknowledging the limitations of flow cytometry for studying homoploid hybridisation and introgression, we are already addressing newly arisen questions in an ongoing study employing sensitive molecular approaches based on next-generation sequencing (NGS) techniques.

Formation of hybrids

Reproductive isolation prevents gene flow between taxa, preserving genetic integrity and supporting plant biodiversity (Baack *et al.* 2015). In sympatry, multiple pre- and postzygotic barriers typically limit gene exchange between closely related

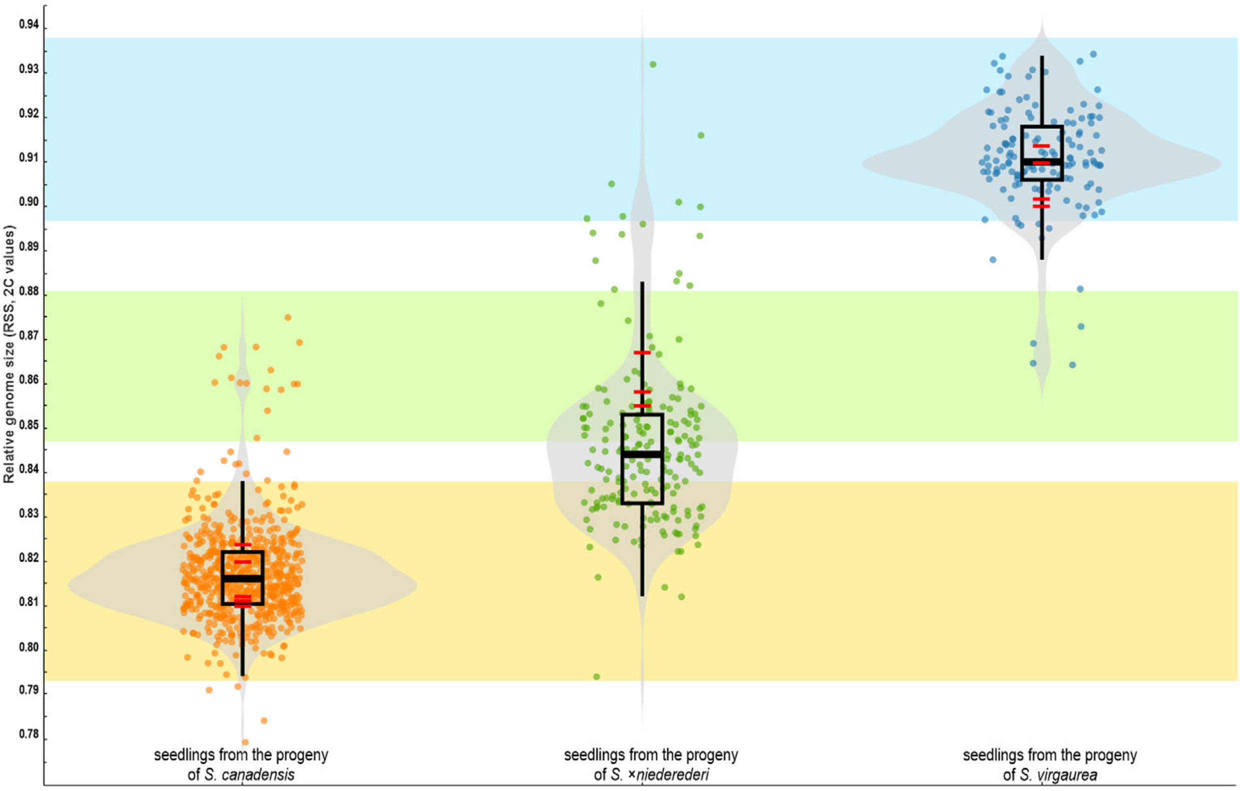

Fig. 3. The combined plot presenting the relative genome size (RSS, 2C values) of diploid seedlings from the progeny of *Solidago canadensis* (n = 646), *S.* ×*niederederi* (n = 193), and *S. virgaurea* (n = 137), pooled from both populations JEL and VPA. Violin plots illustrate the distribution of RSS values. In box-and-whisker plots, boxes show the interquartile range (25%–75%) and the median, while whiskers extend to the smallest and largest values within 1.5× the interquartile range. Colour points represent individual RSS values for each measured seedling, RSS of mother plants is presented as red lines, colour areas represent ranges of relative DNA content of particular taxa (according to Skokanová *et al.* 2022), for colour explanations, see legend in Fig. 2.

species (Lowry *et al.* 2008; Widmer *et al.* 2009). In allopatry, spatial separation alone may be sufficient, while non-selective barriers may fail to evolve or be lost over time (Abbott *et al.* 2008). Consequently, upon secondary contact, due to climate change, habitat shifts, or species introductions, the breakdown of (eco)geographic barriers can lead to hybrid formation (Todesco *et al.* 2016). The data presented here indicate that, within the invasive European range of *S. canadensis*, its pre- and postzygotic reproductive barriers with *S. virgaurea* are not sufficient, and it is likely that when they grow nearby, the emergence of hybrids is highly probable because of shared pollinators and (at least partially) overlapping flowering period (Pliszko & Kostrakiewicz-Gierałt 2018; Pliszko *et al.* 2025). *Solidago* ×*niederederi* is an example of relatively rare homoploid hybrid systems when, unless parental species are not systematically related (they belong to distinct *Solidago* subgenera; Semple & Beck 2021), their genetic compatibility is sufficient to produce viable and fertile F1 hybrids.

Our analysis confirmed that seedlings of hybrid origin (F1 generation) appear in the progeny of both parental species, *S. canadensis* and *S. virgaurea*, in mixed populations. We found seedlings with the relative DNA content corresponding to *S.* ×*niederederi* in the progeny of more than half of the examined mother plants, but their number was relatively low (up to 3.6%). The exception was one *S. canadensis* plant (VPA-C2); within its progeny, more than 8% of seedlings had a probable hybrid origin (Table 1). Nilsson (1976), in a crossing experiment with one *S. canadensis* plant and one *S. virgaurea* plant, observed that the hybrid progeny originated solely from *S. virgaurea*, while *S. canadensis* served as the pollen donor. Presented data from *in situ* open pollination indicate that both *S. canadensis* and *S. virgaurea* are mother progenitors of the hybrids, as *S.* ×*niederederi* shared species-specific cpDNA haplotypes with both parental species (Pliszko & Zalewska-Gałosz 2016; Skokanová *et al.* 2022). Since we included only germinated progeny in the study, the proportion of hybrid seeds may be higher because these seedlings could have a low fitness and thus a strong selection against them in early ontogenetic stages must be considered (cf. Rieseberg & Carney 1998). As these do not enter further mating interactions, their ecological and evolutionary importance is rather insignificant. Considering that repetitive hybridisation of different genotypes of parental species increases fitness and the invasion potential (cf. *Fallopia* ×*bohemica*; Bailey *et al.* 2007), the multiple hybridisation events resulting in the origin of F1 hybrids *S.* ×*niederederi* require appropriate management strategies. *Solidago* ×*niederederi* and introgressed progeny have the potential to combine the ecological plasticity of *S. virgaurea* (Turesson 1925; Hirano *et al.* 2017) with the invasiveness of *S. canadensis* (CABI 2025), potentially enabling spread across a broad range of environmental conditions. Management should focus on the eradication of *S. canadensis* and *S.* ×*niederederi* in natural or

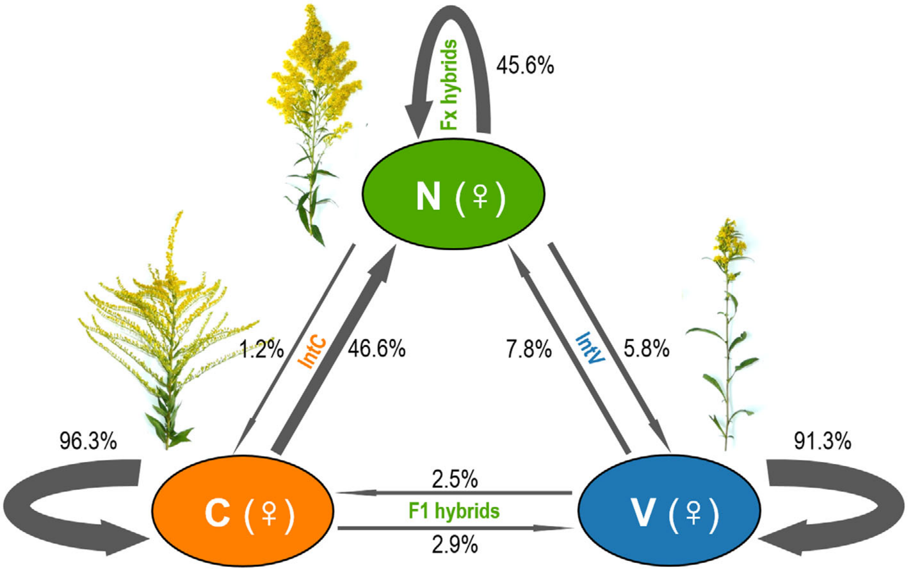

Fig. 4. Mating interactions of *Solidago canadensis* (C), *S.* ×*niederederi* (N) and *S. virgaurea* (V). Summary diagram based on FCM analysis of seedlings from the progeny of mother plants (only diploid seedlings included). Seedlings are classified according to the relative genome size (2C values). For each type of crossing, the proportion of seedlings is indicated by the percentage and thickness of arrows. The arrows point from the expected pollen donor to the expected pollen recipient. Seedlings whose relative DNA content corresponds to *S.* ×*niederederi* backcrosses with *S. canadensis* (IntC) or with *S. virgaurea* (IntV) (Photo P. Mereďa Jr.)

semi-natural areas, and on preventing spread to these areas. The most effective method to limit sexual reproduction and reduce the growth of *Solidago* taxa is to regularly mow before the start of blooming (Botta-Dukát & Dancza 2008; Skokanová *et al.* 2024).

Extensive variability in viability and fertility was observed in both F1 and later generation hybrids (Rieseberg & Carney 1998). Therefore, it is not surprising that the F1 generation of alien–native hybrids can exhibit a wide range of outcomes, from being less fit than the parents (e.g. *Oxalis corniculata* L. × *O. dillenii* Jacq.; Fukatsu *et al.* 2019) to potentially outcompeting one parent (e.g. *Rumex obtusifolius* L. × *R. longifolius* DC.; Takahashi & Hanyu 2015; *Nymphaea alba* L. × *N. odorata*; Nierbauer *et al.* 2014) or both parents (e.g. *Fallopia japonica* (Houtt.) Ronse Decr. × *F. sachalinensis* (F. Schmidt) Ronse Decr.); (Mereďa Jr. *et al.* 2023) or even resulting in a new hybrid species (e.g. *Bidens frondosa* L. × *B. cernua* L.; Galkina & Vinogradova 2017) that is reproductively isolated from its parental species. In the case of *S.* ×*niederederi*, F1 hybrids are robust and fertile, which might be related to the heterosis effect (cf. Rieseberg & Carney 1998; Prentis *et al.* 2008; Zapfe & Freeland 2015; Morais & Reichard 2018). A relatively high proportion of the progeny of the three investigated *S.* ×*niederederi* plants (up to 58.8%) can be attributed to the later hybrid generation (F2) according to their relative DNA content (Figs. 2 and 3, Table 1). Previously, it was assumed that all recorded *S.* ×*niederederi* plants in the wild represent F1 generations arising independently from multiple hybridisation events (Pliszko & Zalewska-Gałosz 2016; Galkina & Vinogradova 2019; Skokanová *et al.* 2022); however, Lysenkov & Galkina (2023) proposed that *S.* ×*niederederi* plants result from sexual reproduction of existing hybrids. Here, we show that *S.* ×*niederederi* plants produce numerous germinating seeds of later generations. As nothing is known about the fitness, reproductive behaviour or spreading capacity of later generations of hybrids, everything between the two already known extremes for later hybrid generations should be expected. Later hybrid generations of *S.* ×*niederederi* could be weak or nonviable (cf. Rieseberg & Carney 1998) or, on the contrary, of high fitness including high invasiveness due to the recombination of parental genotypes (cf. Prentis *et al.* 2008).

Formation of introgressants

Homoploid hybrids, such as *S.* × *niederederi*, often establish reproductive barriers with their parental species only after a certain time period (if at all) and thus can facilitate gene flow between the parental species through repeated backcrossing. Consequently, introgression can lead to genetic swamping, ultimately compromising the genetic integrity of native species and potentially driving them to extinction (Abbott *et al.* 1992; Levin *et al.* 1996; Rhymer & Simberloff 1996; Ellstrand & Schierenbeck 2000; Vilá *et al.* 2000). On the other hand, introgression can introduce beneficial alleles into the invasive species, enhancing its adaptability and competitiveness in the new environment. This process, known as adaptive introgression, may accelerate invasion by equipping the invasive species with traits that improve its ability to establish, spread, and dominate in non-native habitats (Pfennig *et al.* 2016; Oziolor *et al.* 2019; Pfennig 2021). Previously, backcrossing of *S.* ×*niederederi* with parental species was considered to be rare, mainly based on genetic data—nuclear or chloroplast sequences and ISSR (Pliszko & Zalewska-Gałosz 2016; Galkina & Vinogradova 2019; Skokanová *et al.* 2022; Lysenkov & Galkina 2023). Mating interactions of hybrid *S.* ×*niederederi* and its parental species have not been studied until now. Here, we show that their mating interaction is very intense.

Solidago ×*niederederi* backcrosses in the wild with both parental species, although the frequency of backcrossing differs within populations and individual plants. In the case of the three hybrid mother plants, within their progeny, we found that 22%, 60.6% and 65.7% of the seedlings had intermediate RSS values which might be attributed to backcrossing (Table 1). In the case of parental species, seedlings with intermediate RSS values were found within the progeny of 10 out of 12 mother plants; the frequency of probable backcrossing was rather low (3.1%–14.3%; Table 1). Further investigations are needed to confirm the backcross origin of seedlings with intermediate RSS values because the variability of RSS values in seedlings might be higher than revealed for plants in the wild because of selection against genotypes with lower fitness. Overall, pathways and the extent of introgression in mixed populations must be further explored using molecular markers because introgression could facilitate the invasiveness of *S. canadensis* or *S.* ×*niederederi* and erode the genome of the native taxon.

We observed that backcrossing of mother plants of *S.* ×*niederederi* was much more likely with *S. canadensis* as a pollen donor than with *S. virgaurea* (in summary, 46.6% vs. 7.8%; Fig. 4). On the other hand, mother plants of *S. virgaurea* were more involved in crossing with *S.* ×*niederederi* as the pollen donor (Fig. 4). In mixed populations, we suppose a strong asymmetric pattern towards backcrossing of *S.* ×*niederederi* with *S. canadensis*. This may be associated with a pollen competitive advantage of *S. canadensis* over the hybrid and *S. virgaurea*, likely resulting from its larger panicles, greater number of capitula, and dense clusters of flowering shoots that increase attractiveness to pollinators (Pliszko *et al.* 2025). Unidirectional introgression is often associated with asymmetry in hybridisation frequencies (Rieseberg & Carney 1998; Kellner *et al.* 2012; Pieper *et al.* 2017), but this was not observed in the present study (see previous section). The direction of introgression could also be affected by the mating patterns of the F1 hybrids and by the relative proportions of parental and hybrid genotypes in the hybrid populations (Rieseberg & Carney 1998). The crossing interactions of *S. canadensis* and *S. virgaurea* with *S.* ×*niederederi* need to be further studied, as the data presented here might be influenced by the distribution of studied plants—*S. virgaurea* plants were collected closer to *S.* ×*niederederi* plants than *S. canadensis* (Fig. S1). Furthermore, we collected a low proportion of seeds of *S. virgaurea* in the VPA population. The seed production of *S. virgaurea* at this site was probably negatively affected by the pollen pressure of *S. gigantea* growing at this site. *Solidago gigantea* and *S. virgaurea* have overlapping flowering, but the taxa are, because of differences in ploidy level, relatively well reproductively isolated (Musiał *et al.* 2020).

The F1 hybrid plants of *S.* ×*niederederi* can be determined relatively reliably based on intermediate morphological traits or by cytometric and ITS analyses (Skokanová *et al.* 2022). Backcrossing typically leads to unequal parental genomic contributions into later hybrid generations (Baack & Rieseberg 2007); therefore, determination of *S.* ×*niederederi* introgressants is much more challenging when the utility of nuclear or chloroplast sequences for detecting introgression is limited (Pliszko & Zalewska-Gałosz 2016; Galkina & Vinogradova 2019; Skokanová *et al.* 2022). Different morphologies were previously observed for plants reported as presumed introgressants; some of them were of intermediate morphology typical for *S.* ×*niederederi*, some were morphologically identical to *S. canadensis* and some were morphologically identical to *S. virgaurea* (Skokanová *et al.* 2022; Lysenkov & Galkina 2023).

Formation of polyploids

Unreduced gametes are largely considered to be the primary mechanism of polyploid formation (Ramsey & Schemske 1998; Mason & Pires 2015). They are remarkably widespread among individuals at low frequencies but can be particularly high in some individuals of a population (Kreiner *et al.* 2017). The production of unreduced gametes has previously been proposed for *S. canadensis* as triploid plants (2n = 3× = 27), though rare (~ 0.1%), were recorded in the natural populations and shown that they do not differ genetically or in Cx values of genome size from the diploid plants (Skokanová *et al.* 2025). It has been assumed that, due to non-homologous sets of chromosomes and gametogenesis disorders, unreduced gametes occur more often in hybrids (Mason & Pires 2015; Drazan *et al.* 2021). In the present study, triploid seedlings were found to be relatively frequent in the progeny of the hybrid plants VPA-N2 and VPA-N3 (7.8% and 2.4%, respectively) and rarely in the progeny of the *S. canadensis* plant (VPA-C4, 0.8%). In addition, three tetraploid seedlings were found within the progeny of the VPA-N2 plant (2.4%). These data indicate that *S.* ×*niederederi* plants, likely due to gametogenesis disorders, produce a higher proportion of unreduced gametes, probably both male and female, which could result in rare events, such as the fusion of unreduced gametes leading to the formation of polyploids (cf. Ramsey & Schemske 1998; Mason & Pires 2015; Kreiner *et al.* 2017). Note that polyploids within hybrid progeny were found in population VPA with two flowering plants, not in population JEL with only one plant. Unfortunately, here obtained data do not allow us to draw justified conclusions about their origin. While we can be confident about the number of chromosome sets inherited from the mother plant in tetraploid individuals, this is not the case for triploid seedlings. Furthermore, we lack information about the genome size of the pollen grains produced by the hybrid mother plants as their genome size may vary depending on the relative contributions of the parental genomes. The slightly increased variability in the size of viable pollen grains of *S.* ×*niederederi* plants (but not production of considerably bigger, presumably unreduced, pollen grains) is evident from preliminary results of ongoing research in other mixed populations (Skokanová *et al.* unpubl. data). Although unlikely (not recorded in this or in our previous studies: Skokanová *et al.* 2022, 2025), it cannot be ruled out that triploid individuals of *S. canadensis* exist in the studied populations and may contribute to the production of unreduced pollen.

Many cases of polyploid speciation in flowering plants are thought to be allopolyploids that originate from hybridisation (Otto & Whitton 2000; Mallet 2005), because ploidy changes contribute to the restoration of fertility in partially fertile hybrids (Meeus *et al.* 2020). Different pathways of allopolyploid formation accompany homoploid hybridisation (Tayalé & Parisod 2013; Mason & Pires 2015). Well documented are examples of homoploid hybridisation followed by chromosome doubling, either sexually by fusion of unreduced gametes (e.g. *Tragopogon* ×*mirus* and *T.* ×*miscellus*; Soltis *et al.* 2012; Matthews *et al.* 2015) or asexually via somatic chromosome doubling (e.g. *Primula* ×*kewensis* W.Watson; Pellew & Durham 1916). Another more common pathway is the triploid bridge, in which unreduced (diploid) gametes fuse with normal haploid gametes of other taxa, subsequently allotriploids produce allotetraploids by selfing or backcrossing (e.g. *Spartina* ×*anglica*; Ainouche *et al.* 2009; Roberts 2024). Allopolyploidisation of alien–native or alien–alien hybrids could serve as a primary stimulus in the success of a new invasive species, as documented by cases in genera *Spartina* and *Tragopogon* (Ainouche *et al.* 2009; Soltis *et al.* 2012; Matthews *et al.* 2015; Roberts 2024). Polyploids of *S.* ×*niederederi* have not yet been recorded in the wild (Skokanová *et al.* 2022, unpubl. data), therefore, it can be assumed that polyploid seedlings of the hybrids have a lower fitness and are probably eliminated in the following stages of growth. However, it may be more appropriate to ask when, rather than whether, polyploid seedlings of *S.* ×*niederederi* will establish in the wild, given that viable odd ploidy levels in the genus were documented—beside rare cases of triploids of *S. canadensis*, also pentaploids of *S. gigantea* (2n = 5× = 45) have recently been revealed in invasive populations through extensive karyological research in Central Europe (Skokanová *et al.* 2025).

CONCLUSIONS

The FCM analyses confirmed that hybrid seedlings (F1 generation) appear at low frequencies within the progeny of both parental species, *S. canadensis* and *S. virgaurea*, in mixed populations. *S.* ×*niederederi* plants produce relatively high percentages of germinating seeds of later hybrid generations; therefore, their fitness and invasiveness require further study. FCM analyses of hybrid progeny pointed to two further evolutionary processes which may contribute to the restoration of hybrid fertility and potentially also promote its invasiveness (cf. Ainouche *et al.* 2009). First, we showed that mating interactions within populations of *S. canadensis*, *S. virgaurea* and *S.* ×*niederederi* were very intense. Backcrossing frequency differed in direction, it was less frequent when *S.* ×*niederederi* or *S. virgaurea* were pollen donors, and there was a strong asymmetric pattern towards backcrossing *S.* ×*niederederi* mother plants with *S. canadensis* as pollen donor. Introgression appears to be established in mixed populations, and its pathways and extent are still under ongoing investigation. Furthermore, we observed the formation of tri- and tetra-allopolyploid seedlings within the progeny of some of the hybrid plants, probably related to non-homologous sets of chromosomes associated with higher formation of unreduced gametes. Polyploid seedlings of *S.* ×*niederederi* seem to be of low fitness, and in the wild are likely to be eliminated at subsequent stages of growth.

AUTHOR CONTRIBUTIONS

KS: material and data collection, analysis, visualisation, writing original draft; BŠ: material and data collection, writing; MM: material and data collection; KP: writing.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest in association with this work.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Provenance of the material and location of the studied *Solidago* plants within populations JEL and VPA (Slovakia) (a), examples of analysed seedlings (b).

Table S1. Flow cytometry data of analysed mother plants of *Solidago canadensis*, *S.* ×*niederederi* and *S. virgaurea* and their progeny.

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