Hybridogenic activity of *Solidago* L. in North-Eastern Europe

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Two invasive species of goldenrod of North American origin, *Solidago canadensis* and *S. gigantea*, widely spread in Europe, are known to be able to form hybrids with the native goldenrod *S. virgaurea*. In the Kaliningrad Region of Russia and in Lithuania, we found plants morphologically classified as the hybrids *S. × snarskisii* (= *S. gigantea* × *S. virgaurea*) and *S. × niedereideri* (= *S. canadensis* × *S. virgaurea*). Analysis of the nuclear ribosomal internal transcribed spacer 1–2 (ITS 1–2) showed the ambiguity of the origin of the alleged hybrids. Thus, in *S. × niedereideri*, the nucleotide substitutions that differentiate the parent species *S. virgaurea* and *S. canadensis* had ambiguous readings, indicating heterozygosity, which confirmed the hybridogenic origin of the individuals. However, for many specimens of *S. × snarskisii*, the hybridogenic origin was not confirmed. Occasionally, hybrids do occur, but appear to be soon absorbed by the maternal species. An analysis of the chloroplast non-coding intergenic spacer *rpl32–trnL* showed that in these rare cases, the maternal species is *S. virgaurea*, and the paternal one is *S. gigantea*.

Keywords: *Solidago*, invasive species, hybridization, *Solidago* × *niedereideri*, *Solidago* × *snarskisii*, ITS 1–2, *rpl32–trnL*.

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Introduction

The North American species *Solidago canadensis* Linnaeus, 1753 and *S. gigantea* (Aiton, 1789) are among the top 100 most aggressive species in Russia (Dgebuadze et al., 2018; Vinogradova et al., 2015). Indeed, during the naturalization of alien species, they interact with native species, which can lead to the formation of hybrids. In European Russia, the proportion of hybrids reaches 10% of the total number of invasive plant species (Vinogradova and Mayorov, 2015). It is worth noting that one of the hypotheses explaining the success of plants in the new homeland is the assumption of increased hybridization in the secondary range (Ellstrand and Schierenbeck, 2000; Elton, 1958).

Populations of the hybrid *S. × niedereideri* Khokhlov, 1905, whose parent species are *S. canadensis* and

S. virgaurea Linnaeus, 1753, are widespread in Europe. It is widespread in Austria, Great Britain, Lithuania, and Poland (Pagitz, 2016), and in recent decades, the expansion of its range has continued intensively (Karpavičienė and Radušienė, 2016; Pliszko and Zajączko, 2016). Since most records of *S. × niedereideri* are from Northeastern Europe, we also decided to conduct research in this region. Earlier, we studied the *S. × niedereideri* population in the Pskov Region (Galkina and Vinogradova, 2019) and confirmed the hybridogenic origin of these plants, however, data on a single population are insufficient for a reliable result.

Regarding characteristic morphological characters, *S. × niedereideri* has paniced inflorescence, pubescent shoots and small heads, like *S. canadensis*, and large leaves at the base of the shoot, like *S. virgaurea*.

rea. The second hybrid, *S. × snarskii* Gudžinskas & Žalneravičius, 2016, differs from *S. × niedereideri* in unshaven shoots and larger heads (Fig. 1). The inflorescences of this hybrid are also paniculate, while actively branching, with characteristic erect branches (Gudžinskas and Žalneravičius, 2016). *S. × snarskii* is much less common. It is described on the basis of sampling in Lithuania (Gudžinskas and Žalneravičius, 2016), although earlier one specimen of this taxon was discovered by S.R. Mayorov in the Moscow Region in the Losiny Ostrov National Park (2011) and two specimens were found on the outskirts of Kaluga (2012). An ISSR analysis of the DNA of these plants and parent taxa does not refute their hybridogenic origin (Vinogradova and Galkina, 2019). We

decided to collect plants with morphological characters of *S. × snarskii* in the population described by Z. Gudžinskas and E. Žalneravičius (2016), as well as in the populations closest to this point (Zabarauskai, Lithuania) to confirm the hybridogenic origin of the data plants by molecular genetic methods.

Materials and methods

DNA was isolated from silica gel dried leaves of various *Solidago* taxa (27 samples in total) using the Extran kit (manufactured by Synthol CJSC). Plant samples were collected in the Kaliningrad region of Russia and in Lithuania in August – September 2019 (Table 1). We also analyzed data on plants from the Pskov Region obtained in 2018. Polymerase chain

Fig. 1. Hybrids and parental species within the genus *Solidago*.

Table 1. Samples of various *Solidago* taxa (purported hybrids and parental species) used for molecular genetic analysis.

Sample no.	Sequence number in Genbank		Taxon	Collection point and habitat
	ITS1–2	<i>rp/32–trnL</i>		
Sc_1B	MT376752	MT385316	<i>S. canadensis</i>	Lithuania, meadow along the highway Aukstadvaris – Trakai N 54.59° E 24.77°
Sc_4T	MT376753	MT385317		Lithuania, meadow along the highway Trakai – Vilnius N 54.63° E 25.13°
Sc_5T	MT376754	MT385318		
Sc_17Z	MT376755	MT385319		Lithuania, vicinity of the village of Zarabauskai, sparse pine forest N 54.55° E 24.51°
Sc_W	MT376756	MT385320		Russia, Kaliningrad region, village of Shatrovo, roadside N 54.85° E 20.52°
Sc_P_20a	MK491854	MK474085		Russia, Pskov Region, unused field in the vicinity of Pskov N 57.80° E 28.25°
Sc_P_20b	MK491855	MK474086		
Sc_P_20c	MK491856	MK474087		
Sn_3B	MT376757	MT385321	<i>S. × niedereideri</i>	Lithuania, meadow along the highway Aukstadvaris – Trakai N 54.59° E 24.77°
Sn_1T	–	MT385322		Lithuania, meadow along the highway Trakai – Vilnius N 54.63° E 25.13°
Sn_6T	MT376758	MT385323		
Sn_W	MT376759	MT385324		Russia, Kaliningrad region, village of Shatrovo, roadside N 54.85° E 20.52°
Sn_P_21a	MK491852	MK474082		Russia, Pskov Region, unused field in the vicinity of Pskov N 57.80° E 28.25°
Sn_P_21b	MK491853	MK474083		
Sn_P_21c	–	MK474084		
Sv_2T	MT376760	–	<i>S. virgaurea</i>	Lithuania, meadow along the highway Trakai – Vilnius N 54.63° E 25.13°
Sv_10T	MT376761	MT385328		
Sv_2Z	MT376762	–		Lithuania, vicinity of the village of Zarabauskai, sparse pine forest N 54.55° E 24.51°
Sv_1-2C	MT376763	MT385325		Russia, Kaliningrad region, vicinity of the village of Tsvetnoe, sparse pine forest N 54.77° E 20.07°
Sv_2-2C	MT376764	MT385326		
Sv_3-2C	MT376765	MT385327		
Sv_W	MT376766	MT385329		Russia, Kaliningrad region, village of Shatrovo, roadside N 54.85° E 20.52°
Sv_P_19a	MK491849	MK474079		Russia, Pskov Region, unused field in the vicinity of Pskov N 57.80° E 28.25°
Sv_P_19b	MK491850	MK474080		
Sv_P_19c	MK491851	MK474081		

Sample no.	Sequence number in Genbank		Taxon	Collection point and habitat
	ITS1–2	<i>rpl32–trnL</i>		
Ssn_5Z	–	MT385332	<i>S. × snarskisii</i>	Lithuania, vicinity of the village of Zarabauskai, sparse pine forest N 54.55° E 24.51°
Ssn_6Z	MT376767	MT385333		
Ssn_10Z	MT376768	MT385334		
Ssn_12Z	MT376769	MT385335		
Ssn_13Z	MT376770	–		
Ssn_1-2C	–	MT385330	<i>S. gigantea</i>	Russia, Kaliningrad region, vicinity of the village of Tsvetnoe, sparse pine forest N 54.77° E 20.07°
Ssn_2-2C	MT376771	MT385331		
Ssn_3-2C	MT376772	–		
Sg_1-2C	MT376773	MT385336		
Sg_2-2C	MT376774	MT385337		
Sg_3-2C	MT376775	MT385338		

reaction (PCR) was run in a DNA Engine Dyad Pel-lier Thermal Cycler amplifier (Biorad, USA). For the nuclear ribosomal internal transcribed spacer 1–2 (ITS 1–2), we used primers nnc18s10 (forward) and c26A (reverse) at an annealing temperature of 58° C. For the chloroplast highly variable non-coding inter-genic spacer *rpl32–trnL*, the primers *rpl32F* (forward) and *trnL* UAG (reverse) were used at the annealing temperature from 0.3 to 65° C according to J. Shaw's method (Shaw et al., 2007). The PCR product for sequencing was purified in a mixture of ammonium acetate with ethanol. The DNA was sequenced using an automatic sequencer at CJSC Syntol. Further processing of the nucleotide sequences was performed in the BioEdit program. The data obtained were placed in the GenBank database (NCBI), in which these nucleotide sequences can be found by the additional numbers assigned to them (Table 1). Further data processing and the construction of haplotype networks were carried out in the TCS 1.21 program.

Results and discussion

Analysis of the nuclear ITS 1–2 showed the ambiguity of the origin of hybrids within the genus *Solidago*.

For most samples of *S. × niedereideri*, alignment positions corresponding to nucleotide substitutions that differentiate the parent species *S. virgaurea* and *S. canadensis* had ambiguous readings indicating heterozygosity (Table 2). This ambiguity in the chromatograms represents double peaks that may indicate the hybridogenic origin of individuals (Fig. 2). In addition, this is also indicated by the fact that in some cases (in the absence of ambiguous readings), some of the samples have at a certain position (for example, at 579) nucleotides as in one of the parent species, and part as in the other (Table 2). Analysis of the *rpl32–trnL* loci did not provide sufficient evi-

dence as to which of the parental species is maternal and which is the paternal. Our samples showed the variability of this chloroplast fragment within all three taxa: *S. × niedereideri*, *S. virgaurea*, and *S. canadensis* (Table 3). A similar situation was observed for the *S. × niedereideri* population in an unused field in the vicinity of Pskov (Galkina and Vinogradova, 2019).

Regarding the second hybrid, it is possible to state that an individual of *S. × snarskisii* from the vicinity of the village of Tsvetnoe in the Kaliningrad Region (Ssn_2-2C) has ambiguous readings of the sequence at the sites of nucleotide substitutions of the ITS 1–2 locus, differentiating the parent species (Table 2).

Other specimens identified based on morphological characters as *S. × snarskisii*, have ITS 1–2 nucleotide sequences identical to those of indigenous goldenrod *S. virgaurea* (Table 2). It is likely that these plants are a sustainable ecological form of *S. virgaurea*. The *rpl32–trnL* chloroplast sequences are identical in *S. virgaurea* and putative *S. × snarskisii* (Table 3). For rare hybrids of *S. × snarskisii*, this means that *S. virgaurea* is the maternal plant and *S. gigantea* is the paternal plant. Apparently, hybrids are very unstable and are quickly absorbed by the maternal species. We can also assume that not all forms of *S. virgaurea* can occasionally interbreed with *S. gigantea*, but only a form with a broad paniculate inflorescence with elongated and multi-flowered branches (listed as *f. genuina Fiori* (Flora SSSR, 1959)).

We built haplotype networks on the nuclear and chloroplast DNA sections of the studied plants (Fig. 3) taking into account the previously studied *S. × niedereideri*, *S. virgaurea*, and *S. canadensis* from the vicinity of Pskov. As for the ITS 1–2 site, all hybrids entered the same haplotype with *S. virgaurea* (Fig. 3A), with the exception of sample Sc_P_20c.

Fig. 2. Fragments of chromatograms of sequences of specimens of *Solidago canadensis* (Sc_4T), *S. × niedereideri* (Sn_6T) and *S. virgaurea* (Sv_2T) from Lithuania.

Table 2. Polymorphism of nucleotide sequences ITS 1–2 in putative hybrids of *Solidago* (in comparison with parent taxa). The symbols of nucleotides are given according to the IUPAC nomenclature.

Sample no.	Alignment position												
	230	358	405	417	438	465	482	490	523	552	579	580	581
Sc_1B	T	C	C	A	C	T	T	C	G	G	G	T	G
Sc_4T	T	C	C	A	C	T	T	C	G	G	G	T	G
Sc_5T	T	C	C	A	C	T	T	C	G	G	G	T	G
Sc_17Z	T	C	C	A	C	T	T	C	G	G	G	T	G
Sc_W	T	C	C	A	C	T	T	C	G	G	G	T	G
Sn_3B	T	Y	M	A	C	T	Y	C	R	G	T	C	R
Sn_6T	T	T	M	A	C	T	Y	C	R	G	G	C	G
Sn_W	T	Y	M	A	Y	Y	Y	C	A	G	T	C	R
Sv_2T	T	T	A	A	C	T	C	C	A	G	T	C	A
Sv_10T	T	T	A	A	C	T	C	C	A	G	T	C	A
Sv_2Z	T	T	A	A	C	T	C	C	A	G	T	C	A
Sv_1-2C	T	T	A	A	C	T	C	C	A	G	T	C	A
Sv_2-2C	T	T	A	A	C	T	C	C	A	G	T	C	A
Sv_3-2C	T	T	A	A	C	T	C	C	A	G	T	C	A
Sv_W	T	T	A	A	C	T	C	C	A	G	T	C	A
Ssn_6Z	W	T	A	A	C	T	C	C	A	G	T	C	A
Ssn_10Z	T	T	A	M	C	T	C	Y	A	K	T	C	A
Ssn_12Z	T	T	A	A	C	T	C	Y	A	G	T	C	A
Ssn_13Z	T	T	A	A	C	T	C	Y	A	G	T	C	A
Ssn_2-2C	T	Y	M	A	C	T	C	C	A	G	T	C	A
Ssn_3-2C	T	T	A	A	C	T	C	C	A	G	T	C	A
Sg_1-2C	T	C	C	A	T	Y	C	C	G	G	G	T	G
Sg_2-2C	T	C	C	A	Y	T	C	C	G	G	G	T	G
Sg_3-2C	T	C	C	A	Y	T	C	C	R	G	G	T	G

Fig. 3. The haplotype network of specimens of *Solidago* from Lithuania and Kaliningrad Region, Russia. **A** – locus ITS 1–2, **B** – locus *rpl32-trnL*.

Table 3. Polymorphism of nucleotide sequences *rp/32–tmL* in putative hybrids of *Solidago* and parent taxa. The symbols of nucleotides are given according to the IUPAC nomenclature.

Sample no.	Alignment position					
	166	184–189	207–228	268–304	378	686–690 843
Sc_1B	C	–	–	–	T	TTTT A
Sc_4T	C	–	TATGCTAAAGAATCTTAATGT	TGCTAAAAGAATAATCTTGTATTTTCTGAATTCTA	C	T A
Sc_5T	C	–	TATGCTAAAGAATCTTAATGT	TGCTAAAAGAATAATCTTGTATTTTCTGAATTCTA	C	T A
Sc_17Z	C	–	–	–	C	TTTT A
Sc_W	C	–	–	–	C	TTTT C
Sn_3B	C	–	–	–	C	T C
Sn_1T	C	–	–	TGCTAAAAGAATAATCTTGTATTTTCTGAATTCTA	C	T C
Sn_6T	C	–	–	TGCTAAAAGAATAATCTTGTATTTTCTGAATTCTA	C	T A
Sn_W	C	–	–	–	C	TTTT C
Sv_10T	A	–	–	TGCTAAAAGAATAATCTTGTATTTTCTA	C	T C
Sv_1-2C	A	–	–	TGCTAAAAGAATAATCTTGTATTTTCTA	C	T C
Sv_2-2C	C	–	–	TGCTAAAAGAATAATCTTGTATTTTCTGAATTCTA	C	T C
Sv_3-2C	C	–	–	TGCTAAAAGAATAATCTTGTATTTTCTA	C	T C
Sv_W	C	–	–	TGCTAAAAGAATAATCTTGTATTTTCTGAATTCTA	C	T C
Ssn_5Z	A	–	–	TGCTAAAAGAATAATCTTGTATTTTCTA	C	T C
Ssn_6Z	C	–	–	TGCTAAAAGAATAATCTTGTATTTTCTGAATTCTA	C	T C
Ssn_10Z	A	–	–	TGCTAAAAGAATAATCTTGTATTTTCTA	C	T C
Ssn_12Z	C	–	–	TGCTAAAAGAATAATCTTGTATTTTCTGAATTCTA	C	T C
Ssn_1-2C	A	–	–	TGCTAAAAGAATAATCTTGTATTTTCTA	C	TT C
Ssn_2-2C	A	–	–	TGCTAAAAGAATAATCTTGTATTTTCTA	C	TT C
Sg_1-2C	C	–	–	–	C	TTTT A
Sg_2-2C	C	–	–	–	C	– A
Sg_3-2C	C	TAATAT	–	–	C	TTTT A

The sample Sc_P_20c, from our point of view, is also a hybrid, but not F1, but the result of introgression (Galkina and Vinogradova, 2019). The *rpl32-trnL* chloroplast intergenic spacer is very variable in almost all studied taxa (except *S. gigantea*), and hybrids are almost evenly distributed across several haplotypes (Fig. 3B).

Conclusions

Thus, *S. × niedereideri* plants in Northeast Europe are true hybrids, while the *S. × snarskii* hybrid is very rare and quickly absorbed by the mother species *S. virgaurea*. Most of the described hybrids of *S. × snarskii* can be called “false”, this is the ecological form of *S. virgaurea*, which probably has weak hybridogenic activity and can rarely form hybrids with invasive *S. gigantea*.

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