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Evolution of an Invasive Species
Solidago Canadensis in Europe and the Americas: A Comparison Using Ribosomal ITS and 5S-NTS Sequences

Rachael Dickenson

The genus *Solidago* is recognized as an invasive species around the world. This study aims to identify emerging genetic divergence in the species *Solidago canadensis*, a well-established species in both Europe and North America. Since the introduction of S. canadensis to Europe is far too recent for any changes to be manifested in morphology of the species, ribosomal ITS and 5S-NTS DNA were sequenced from samples collected on both continents and compared to identify genetic variations. Geneious 8.1.8 software was used to align sequences, identify genetic variations, and build phylogenetic trees. Comparison of phylogenetic trees lacked sufficient reliability regarding location of origin and development of populations, therefore no definite conclusions were reached regarding time and manner of introduction or rate of variation. However, an understanding was reached that more research on both ITS and 5S-NTS sequences is necessary before drawing full conclusions regarding origin and development of *S. canadensis* populations.

Keywords: Solidago canadensis, invasive species, rDNA sequencing, phylogenetic analysis, Internal Transcribed Spacer (ITS), 5S-Non-Transcribed Spacer (NTS)

Introduction

The genus *Solidago*, commonly called Goldenrod, contains over 100 species and is considered highly invasive in nearly all areas of the world outside of its native region in Midwestern North America (excepting one species native to Europe) (Sheppard, 2006). Observation of the frequency of *Solidago* and similarly invasive herbaceous plants gives rise to questions regarding how quickly and in what manner such genera change to dominate a previously unintroduced area.

Most simply, invasive species are defined as "any nonnative species that significantly modifies or disrupts the ecosystems it colonizes" (Rafferty, 2015). Human globalization in recent decades has greatly spurred the introduction and encroachment of invasive species on native habitats around the world. Notable consequences of this intrusion include destruction of native keystone species and subsequent ecological imbalances, disruption of natural landscapes, decreased biodiversity, and adverse effects on human food sources (Stace & Crawley, 2015). Current combative measures to eliminate invasive species include physical removal and use of biological controls, but unfortunately reestablishment of balanced ecosystems is often difficult to achieve. Several species of the genus *Solidago*, including *S. canadensis*, are labeled by the Royal Horticulture Society in the British Isles as invasive species commonly sold for ornamentation but can quickly become unmanageable when introduced to a native ecosystem (Stace & Crawley, 2015). Therefore, a question can be posed as to not only how these *Solidago* species affect a given area as an invasive species, but how (if at all) their genetics change to facilitate invasion of an area once introduced.

Emergence and Relevance of Intraspecies Variation

Genetic information of living organisms is known to spontaneously mutate through errors in DNA replication during meiosis and mitosis, resulting in beneficial, detrimental, or neutral impact on organism survival. In other words, organisms with beneficial mutations for their given situation are more likely to survive and those with detrimental mutations are less likely to survive, while those with neutral mutations will continue to exist as before (Hillis, Sadava, Heller, & Price, 2012). For invasive species such as those of the genus *Solidago*, introduction and invasion of new areas means exposure to different environmental factors, thereby affecting whether a given mutation is considered beneficial, detrimental, or neutral upon its spontaneous occurrence. Dong Yu, and He (2015) concluded that "climate and recipient communities explained 71.39% species impact of S. canadensis," indicating that movement into regions with different characteristics likely results in different expression of *Solidago* genes and eventual evolutionary divergence by development of mutations beneficial, detrimental, or neutral to a given *Solidago* species in a new area.

Genetic changes in plants require millions of generations to accumulate and become manifested in morphology (Schaefer, 2015). Therefore ribosomal Internal Transcribed Spacer (ITS) and 5S-Non Transcribed Spacer (NTS) sequences were selected for use in this study. ITS and 5S-NTS are ribosomal noncoding DNA, proven to be one of the most variable sequences and commonly used in phylogenetic comparisons (Alvadhani *et. al.*, 2012 ; Álvarez and Wendel, 2003). Use of these sequences in phylogenetic comparison, while unable to identify full mutations emerging in a given species, allows identification of intraspecies variation and divergence of new populations. Phylogenetic comparison involves comparison of characteristics, in this case genetic characteristics, for the purpose of determining an organism's evolutionary history. Diagrams known as phylogenetic trees display these differences by creating "branches" to show which organisms first developed genetic differences from the most recent common ancestor (see Results for further explanation).

In the context of this study, it is important to understand how such noncoding DNA as the ITS and 5S-NTS sequences are related to coding sequences: In each tandem repeat of ribosomal DNA (rDNA), ITS1 and ITS2 flank the 5.8S subunit of coding DNA while 5S-NTS exists in the Intergenic Spacer (IGS) region between each complete repeat of rDNA (see Fig. 1), filling the unused spacer regions with additional repeats of code.

Solidago canadensis was selected as a well-established species of the genus *Solidago* in North America and Europe. Alternative species considered under the given time and seasonal restrictions of this study were *Solidago gigantea* (also native to North America and invasive to Europe) and *Impatiens glandulifera* (native to the Himalayan region and invasive to Europe), included in the first iteration of sequencing but thereafter discarded when it was determined that *S. canadensis* displayed the most variability. Stability of *S. canadensis* populations in both North America and Europe increases the likelihood of mutations having been developed in Europe since its introduction as a garden plant in the mid-1700s (Sheppard, 2006) or that mutations have emerged in North America since the time of its passage to Europe. Furthermore, identification of differences between varying populations in Europe may suggest whether a single large introduction or several smaller introductions of *S. canadensis* resulted in its eventual establishment as an invasive species. Discovery of which populations were able to subdue native species may also provide insight into which areas of Europe and North America need

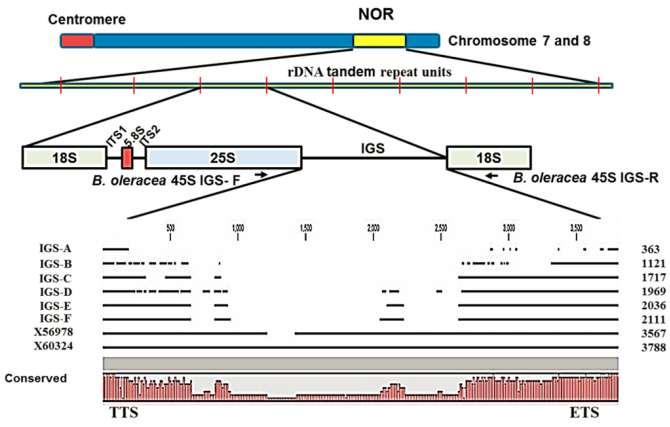


Fig. 1 Arrangement of sequences within rDNA repeats

improved management of *S. canadensis* to prevent further introduction outside the native range of the species.

Literature Review

Previous research has been done regarding changes in *Solidago* DNA sequences and its development following introduction to an environment, particularly by Schaefer (2015) in his examination of morphological and genomic sequence differences between Azorean *Solidago azorica* and other *Solidago* specimens from North America to determine ancestry and optimal nomenclature for *S. azorica*. His use of phylogenetic trees derived from ITS sequence differences to identify *S. azorica* origin prior to its emergence as a distinct species demonstrates the practicality of such methods of comparison for use in this study. Schaefer's use of morphology as a basis of comparison between *S. azorica* and its potential parent species is used effectively to support his conclusion, but is impractical in the context of this study due to my focus on a given species, members of which are morphologically indistinguishable.

A similar method of molecular analysis is used by Laureto and Barkman (2011) to identify origins of the *S. houghtonii* hybrid, including ITS sequences and phylogenetic trees to determine ancestry of the given species. Morphology is not used as major support for conclusions of their study, but multiple other regions of genetic information such as cpDNA intergenic spacers (within IGS region depicted in Figure 1) were used to further support results. Time and monetary restrictions made sequencing of more than two regions impractical in the context of my study; however, use of GenBank as done by both Schaefer and Laureto and Barkman was considered feasible. GenBank is a publicly available database of genetic sequences by researchers around the world, allowing additional data points to be added without the researcher needing to obtain a separate sample from the given location and go through the process of DNA extraction and sequencing. One of the few concerns regarding inclusion of sequences from GenBank is that samples cannot be verified as having been correctly identified and sequenced, meaning that an unreliable sequence could possibly be included as a viable data point and lead to false conclusions or results. Regardless, Laureto and Barkman (2011) describe it as necessary for comparisons to determine parentage, necessitating "at least one species from each section and subsection of the genus… to represent the phylogenetic breadth of the genus" in their own successful study involving *Solidago* species ancestry and phylogenetic development.

Despite extensive use of ITS sequences by Shaefer, Laureto and Barkman, and their predecessors, some concern is expressed regarding the reliability of ITS sequences for phylogenetic trees due to their biparental nature and continual homogenization with unstable pseudogenes (Álvarez & Wendel, 2003). Otherwise stated, ITS sequences may contain unequal amounts of genetic material from maternal and paternal lineages, and constant recombination of repeats due to the repetitive nature of these sequences may result in combination of recent ITS sequences with disused and decaying ones. This combination has been shown to result in lower guanine-cytosine (GC) content, meaning less stable DNA structure and thus less reliable sequencing results (Álvarez & Wendel, 2003). The main focus of the study by Álvarez and Wendel (2003) is a comparison of ITS region sequencing against that of other viable sequences to determine which is most reliable in phylogenies. The ITS region was determined to be the least reliable of those examined due to it being the most homoplasious of the study, meaning that it is the most likely to have gene similarities not resulting from shared ancestry. One possible explanation for these similarities is convergent evolution, an occasion in which organisms of different ancestry develop similar characteristics through evolution under similar living conditions. These similarities result in skewed phylogenetic conclusions regarding species origin and development. Nevertheless, research by Ritland, Ritland, & Straus (1993) aims to distinguish between closely related species of the *Mimulus* family using ITS-based phylogenies and attempts to account for possible misleading results of ITS sequences by including three "clone" samples (plants of the same species, collected from the same location) of each species examined. Multiple clones of each test sample may have limited undue influence of ITS based results, but limited Ritland et al. (1993) to eight samples for comparison. This number of samples was sufficient in his case

for distinguishing between eight given species, but insufficient for identifying differences between populations of varying geographic locations (especially since samples of the same location could be from different populations) as was the goal of my study.

Importance and Purpose of Study

Very few studies have been published to compare intraspecies variation between populations and attempt to pinpoint methods of introduction into a given environment. Weber and Schmid (1998) examined morphological variations of *Solidago* species introduced to Europe with a study featuring statistical analysis of shoot growth, leaves, and inflorescence measurements from 24 rhizome system samples gathered from different regions and climates between 44 and 61 degrees latitude on the continent. Their results stated that "only a small and insignificant proportion of variation among field populations could be explained by correlation between the characters and latitude" (Weber and Schmid, 1998), suggesting that not all differences between population could be explained by geographic latitude differences. Differing parent populations prior to introduction into Europe provides a possible explanation of these variations. Gradual morphological change correlating to latitude change of sample collection was observed in controlled garden cultivation over the subsequent two years of the study, but the lack of correlation in field populations was never analyzed as to whether such variations were genetic differences (developed since introduction to Europe or present due to differing parent populations) or simply characteristics of an identical genome expressed differently under varying environmental conditions across Europe (as seems assumed by the authors).

The purpose of this experiment is to draw conclusions about introduction and variability of *S. canadensis* between Europe and North America using ITS and 5S-NTS sequencing and phylogenetic comparison in a manner similar to that of Schaefer (2015) with gel electrophoresis techniques based off those of Ritland et al. (1993). The hypothesis is that if ITS and 5S-NTS rDNA are sequenced, aligned, and examined from both European and North American samples of *S. canadensis*, then variations will be observed in both spacer sequences and use of comparison techniques such as phylogenetic trees will make it possible to determine origins and development of the species since its introduction to Europe.

Materials and Methods

Acquisition of Sample Materials

Samples of *S. canadensis* to be used in this experiment were requested via email from various institutions across the United States and Europe (see acknowledgements), as well as several samples from the herbarium at the Naturkundemuseum Stuttgart. All tissue samples were either silica-gel dried or herbarium specimen leaves, with the exception of a single silica-dried root sample.

Three separate iterations of DNA extraction, Polymerase Chain Reaction (PCR), and sequencing were carried out over the course of this study. The first iteration contained eight tissue samples, four of which were *S. canadensis* (the others being *Solidago gigantea* and *Impatiens glandulifera*), and sequenced ITS, ITS1, ITS2, and 5S-NTS. The second iteration contained nine samples and the third contained eight samples of

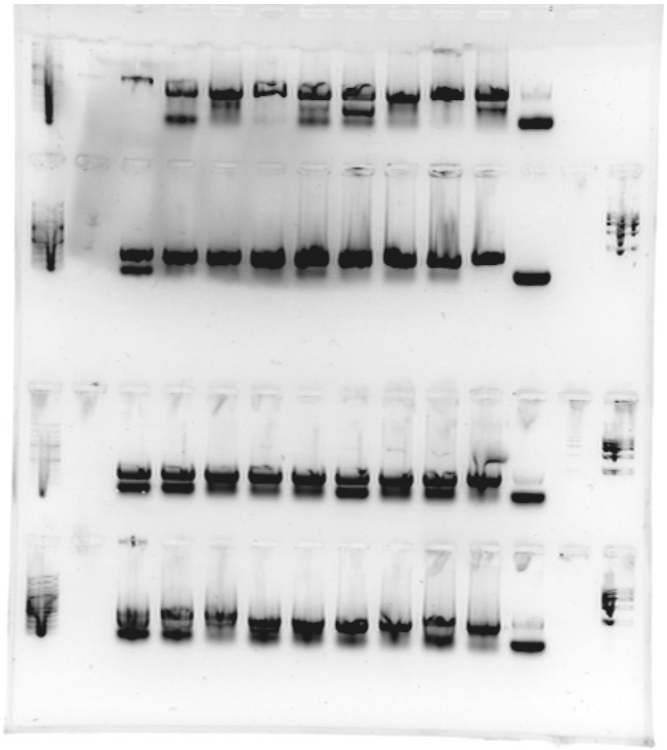


Fig 2: Gel electrophoresis image of first iteration samples

S. canadensis. Second and third iterations sequenced only complete ITS and 5S-NTS due to monetary constraints and expediency of results, since sequencing of ITS forward and reverse was both cheaper and less time consuming than sequencing ITS1 and ITS2 separately and combining the two.

Sequences of *S. canadensis* and other *Solidago* species from GenBank for the ITS region were obtained and utilized in an additional phylogenetic tree (see Results) despite concerns expressed in the introduction of this study.

General Procedure

Initial cell lysis and DNA extraction from sample tissue was conducted following Macherey-Nagel protocols for extraction of genomic DNA from plant tissue using Nucleospin® Plant II kit (Macherey-Nagel, 2014). Prior homogenization of samples was completed using metal beads and a high throughput homogenizer. Subsequent DNA was prepared for PCR with the addition of 0.2µl Taq Polymerase (isolated from Thermus aquaticus bacterium), 2.5µl 10X buffer (green, with 20mM $MgCl_2$), 2mM deoxynucleotide triphosphate solution (dNTPs), 1.0µl forward primer, 1.0µl reverse primer, and 16.8µl nuclease-free water for each 1.0µl sample of DNA template. Forward and reverse primers were arranged in four combinations as follows: ITSA/ITSB for entire ITS amplification, ITSA/ITSC for ITS1 amplification, ITSB/ITSD for ITS2 amplification, and 5S-NTSF/5S-NTSR for entire 5S-NTS amplification.

PCR thermocycling program for ITS strands began with 5 minutes at 95°C (for denaturation), followed by 10 cycles of the following: 30 seconds at 95°C, 45 seconds at 60°C (decreasing 0.5°C each cycle), and 1 minute at 72°C. Next was 30 cycles as follows: 30 seconds at 95°C, 45 seconds at 55°C, and 1 minute at 72°C. The program was completed with 5 minutes at 72°C.

PCR thermocycling for 5S-NTS began with 5 minutes at 95°C (for denaturation), followed by 10 cycles of the following: 30 seconds at 95°C, 45 seconds at 64°C (decreasing 0.5°C each cycle), and 1 minute at 72°C. Next was 30 cycles as follows: 30 seconds at 95°C, 45 seconds at 59°C, and 1 minute at 72°C. The program was completed with 5 minutes at 72°C.

Once PCR had been completed, agarose gel electrophoresis was performed and examined in inverted color as shown in Fig 2 , with the inclusion of a positive and negative control to reveal possible contaminations and determine which samples had enough replications of the desired noncoding region to be sent for full sequencing. Sufficient samples of the first and third iterations were sent as part of a 96-well plate (along with samples of unrelated projects by the Naturkundemuseum), and thus did not require manual purification by the researcher. In the second iteration, preparation for sequencing of selected samples was performed in accordance with procedures listed in PCR clean-up manual by Macherey-Nagel (2014). Following clean-up (when necessary), samples were labeled and sent to LGC Genomics for their "Flexi Run" service, yielding complete forward and reverse ITS and 5S-NTS sequences.

After receiving raw ITS, ITS1, ITS2, and 5S-NTS data for samples of the first, second, and third iterations, a bioinformatics software called Geneious 8.1.8 (created by Biomatters Ltd.) was used to align forward and reverse strands into full DNA sequences. In the case of the first iteration, ITS1 and ITS2 sequences were combined to yield full ITS sequence for comparison with those of the second and third iterations. Forward strands of 5S-NTS in all three iterations were thoroughly garbled by sequencing errors, forcing the researcher to use only reverse sequences in 5S-NTS comparisons. Furthermore, 5S-NTS reverse direction of second iteration samples of required resequencing due to unreadability of sequences. The Geneious 8.1.8 program was then used to create phylogenetic trees of the ITS and 5S-NTS regions (see results), as well as a phylogenetic tree based on ITS region sequences along with *Solidago* ITS sample sequences obtained from GenBank.

Results

Unclear portions containing errors in sequencing, generally located at the start and end of the sequences, were removed along with sequences of primer DNA so only true differences would appear when DNA was compared between samples. These differences could then be viewed in alignments by the Geneious 8.1.8 program, as indicated in Figure 3 (below) by differences between rows of sequencing.

Clear differences in sequences between samples are indicated by different color box and letter (indicating name of nitrogenous nucleotide base), such as that in position 5 of Figure 3, where thymine is clearly indicated while all other samples at that position displayed cytosine. Less certain differences are displayed as gray boxes where the sequencing was unclear or detected two base pairs at that position. One explanation for this uncertainty is the possible existence of two variations of code within the several tandem repeats of a given specimen's sequence, both of which would then be amplified by the PCR and detected by sequencing software.

Furthermore, the Geneious 8.1.8 software was used to produce phylogenetic trees of both ITS and 5S-NTS sequences (see Fig. 4 and 5). Numbers listed at the divisions in the tree are known as "bootstrap values" and indicate how many out of the 100 possible phylogenetic trees the program creates that particular division was present in. Thus, higher bootstrap values indicate higher certainty that a given division or dividing variation occurred at the indicated point. The number at the bottom of each image (not attached to any particular division) is the changes per base pair for each phylogenetic tree. Samples which appear diverge on the farther left side of each image (earlier in the tree) are those with the most differences from the consensus alignment of the sequences, therefore calculated to be the most genetically different in comparison to the others in the tree.

Sample titles as listed in Figures 4 and 5 are formatted as follows: uppercase letter R followed by iteration number, underscore followed by sample number within the previously specified iteration, underscore followed by name of region sequenced (ITS for combined ITS forward and reverse primers or 5SNTSR for 5S-NTS reverse primer), then underscore followed by abbreviation for country of origin.

Notable areas of the ITS tree depicted in Figure 4 include the three lowermost samples, four samples to the left and below the bootstrap value 56, and the sample R3_7_ITS_US located in the upper right.

Notable areas of the 5S-NTS tree depicted in Figure 5 include the two lowermost samples, as well as the location of all samples in the tree relative to their locations in the ITS tree (Fig. 4).

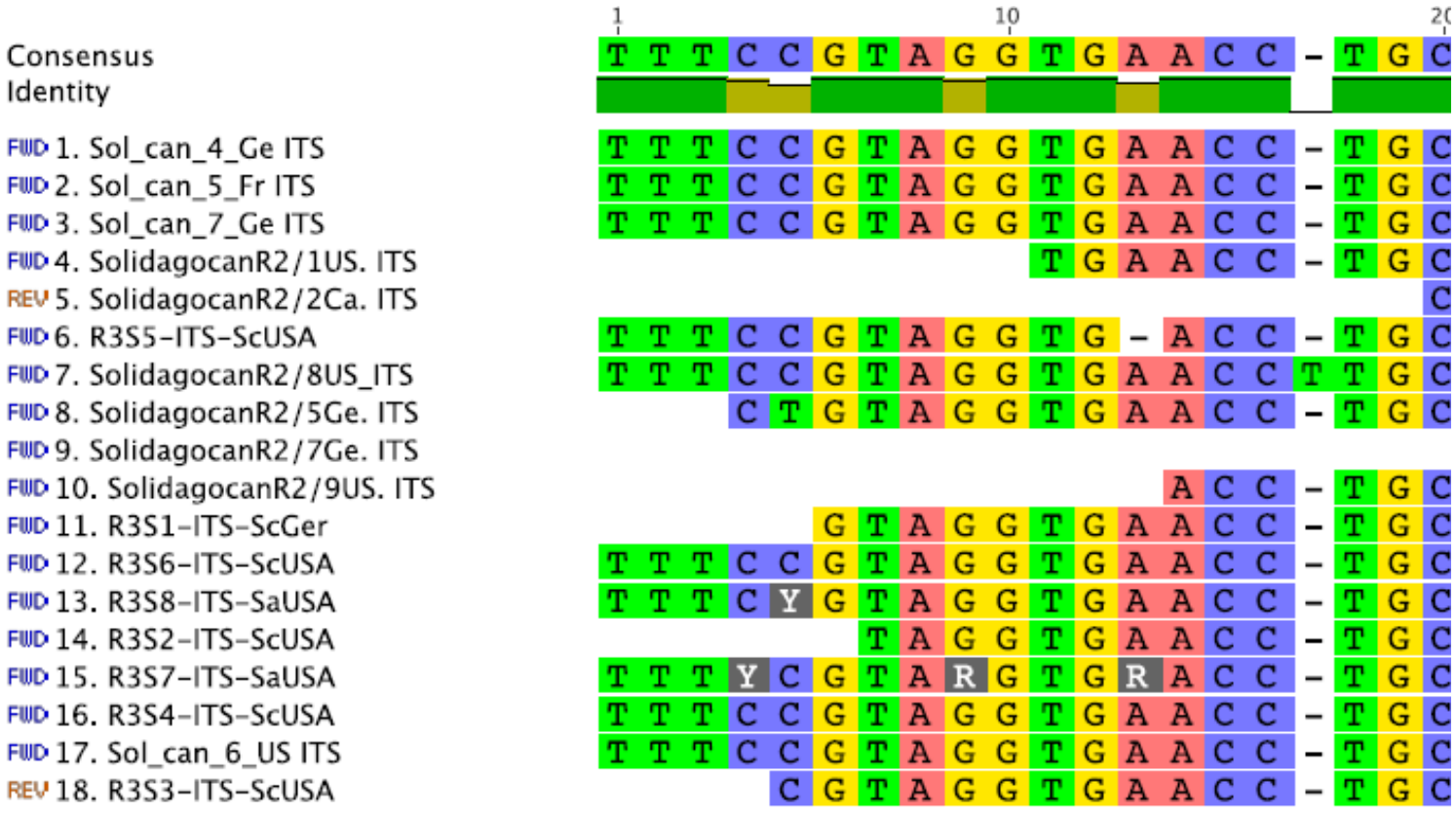


Fig 3: Initial 30 base pairs in ITS alignment of all samples

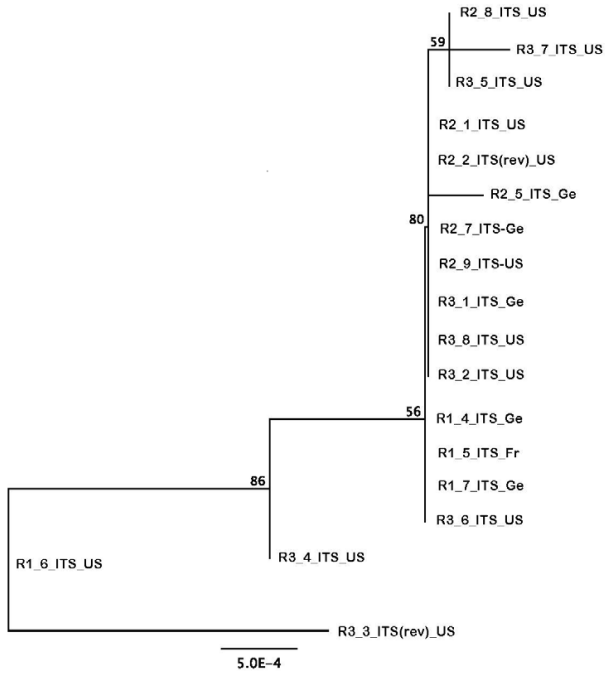


Fig 4: Phylogenetic tree for ITS sequences

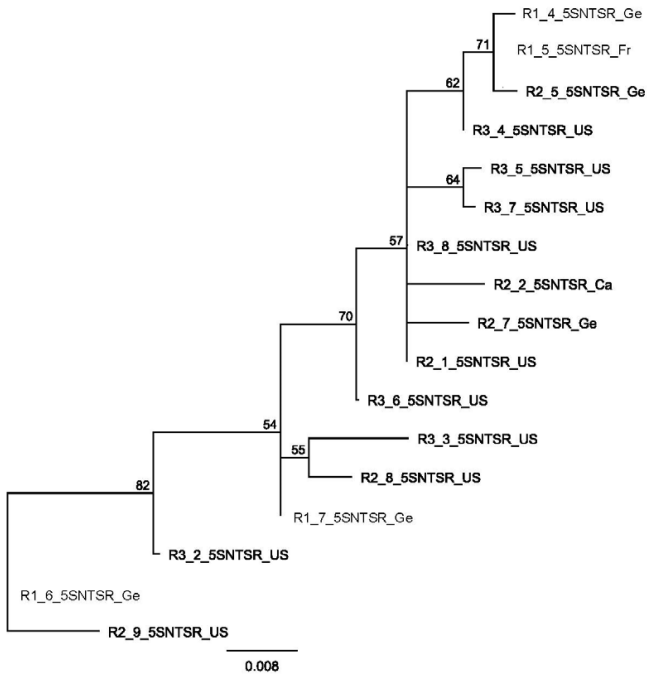
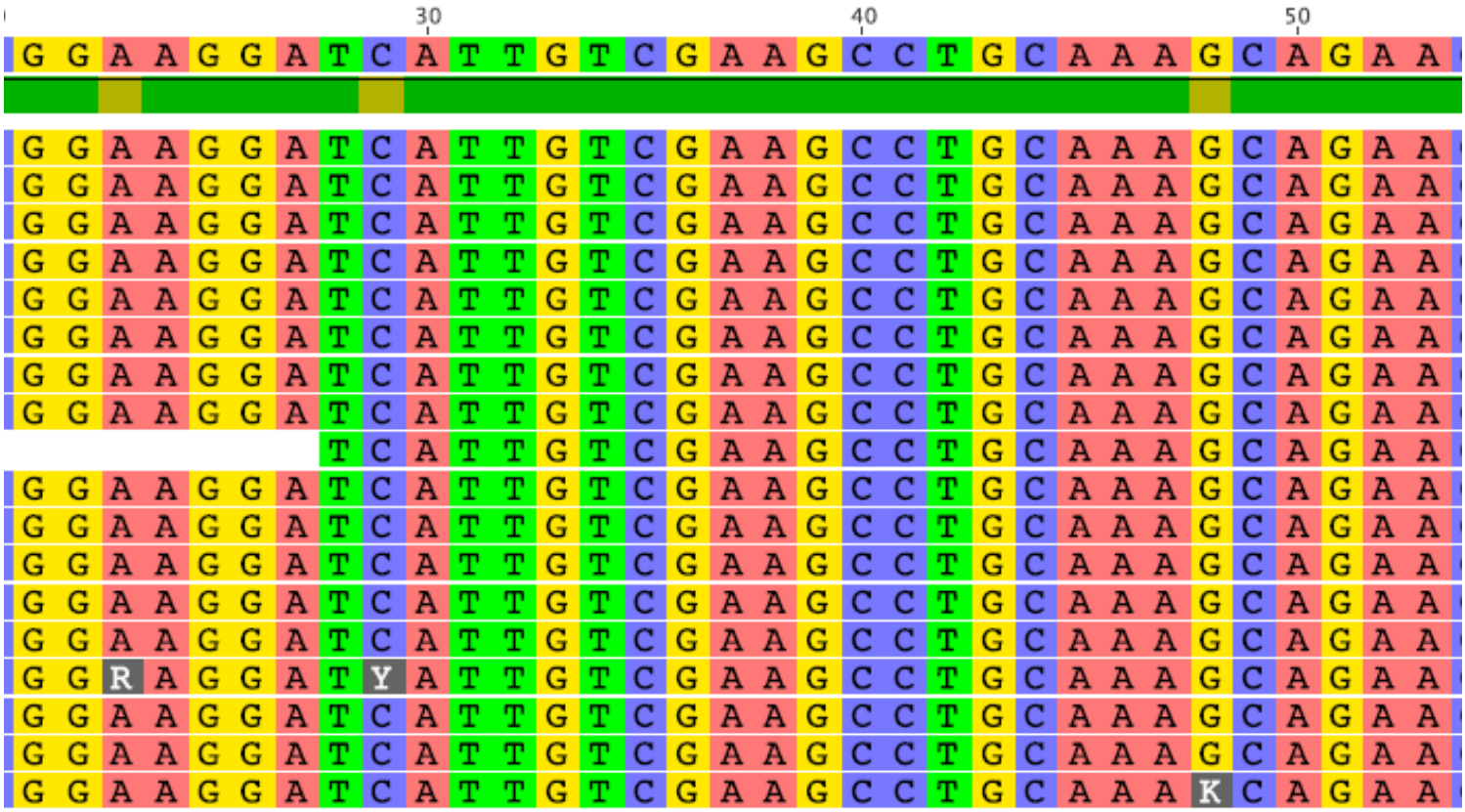


Fig 5: Phylogenetic tree for 5S-NTS sequences

As considered necessary for comparisons to determine ancestry by Laureto and Barkman (2011), ITS sequences from GenBank were used in compilation with the results seen in Figure 4 (5S-NTS sequences being too short and unreliable) to create the phylogenetic tree in Figure 6 (below).



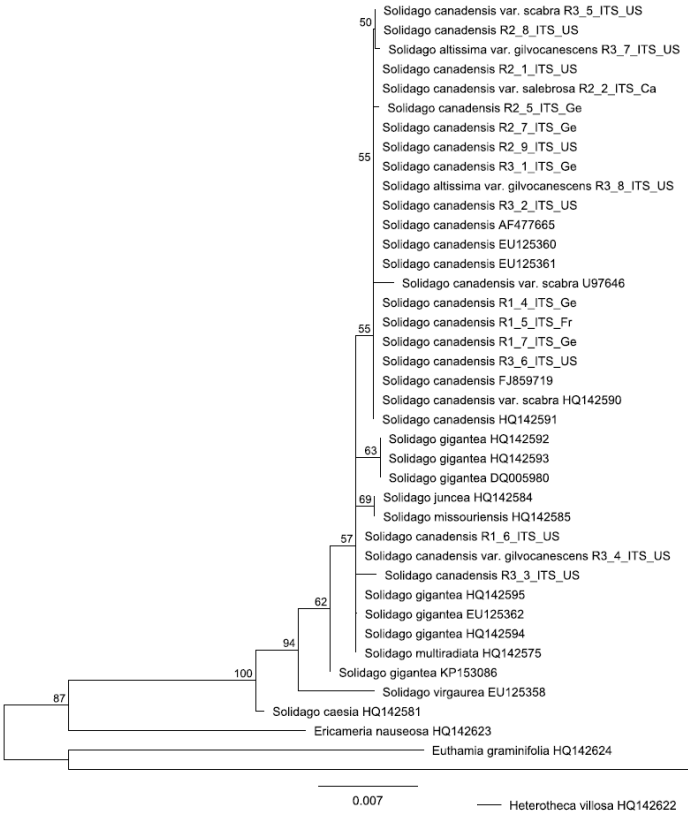


Fig 6: Phylogenetic tree for ITS with GenBank samples

Inclusion of three non-*Solidago* plants allowed for what is referred to as "rooting": a visual representation of how different populations are when compared to much less closely related species such as the bottom three specimen included in Figure 6. Formation of an outgroup by use of 1-3 samples of a differing genus occurred also in studies by Schaefer (2015), Laureto and Barkman (2011), and Ritland et al. (1993). The amount of difference between given samples of *S. canadensis* and outgroup species also provides clear indication of directionality of evolutionary change (Kinene, Wainaina, Maina, & Boykin, 2016) thus clarifying which variations likely occurred earlier than others, due to their similarity to a species which diverged from the last common ancestor of the tree much earlier. Inclusion of this phylogenetic tree confirms that all *S. canadensis* samples used in the study are closely related as populations within a given species should be, thus ruling out possibilities of incorrect identification by the researcher in sample acquisition.

Analysis and Discussion

Analysis of ITS phylogenetic tree (Fig. 4) reveals that ITS samples R3_3, R3_4, and R3_6, collected from U.S. states Colorado, Wyoming, and New York, respectively, are significant due to their high amounts of variation from the ITS consensus sequence (see Fig. 3). The lowest two samples of the ITS tree (R1_6 and R3_3, of Vermont and Colorado respectively) are grouped together because they have an identical variation of cytosine rather than guanine in position 546 of ITS. Sample R3_4_ITS_US of Wyoming is located between the two lowermost samples and the rest of the samples because it is unsure at the same position (Fig. 7). The lowermost sample, R3_3_ITS_US from Colorado, is farthest removed because it has another definite difference in a different position not shared by any other samples in the alignment. Laureto and Barkman (2011) utilized a similar method involving analysis of sequence alignment and comparison of specific base pairs to supplement explanations of phylogenetic differences displayed in the constructed trees.

Based off these differences, there is a possibility that sample R1_6_ITS_US from the state Vermont may have been introduced to its area of collection from Colorado, where sample R3_3_ITS_US was collected and within the native range of *S. canadensis*, before developing an additional variation. Furthermore, sample R3_4_ITS_US (collected in Wyoming) indicates uncertainty possibly due to its collection location in the native region of the species, where populations and gene pools are larger, causing greater variation and possibly resulting in the presence of two different DNA sequence repeats within the same sample.

A grouping of four samples (titles beginning with R1_4, R1_5, R1_7, and R3_6) are grouped in the same clade of the ITS tree, indicating an identical amount of difference from the ITS consensus sequence and thus the possibility of a shared origin. The sample R3_6_ITS_US was collected in the US state of New York, meaning that it would be the parent population of the group. The other three samples of the group were collected in France and Germany, close enough together for the possibility that their populations could have developed following a single introduction from the

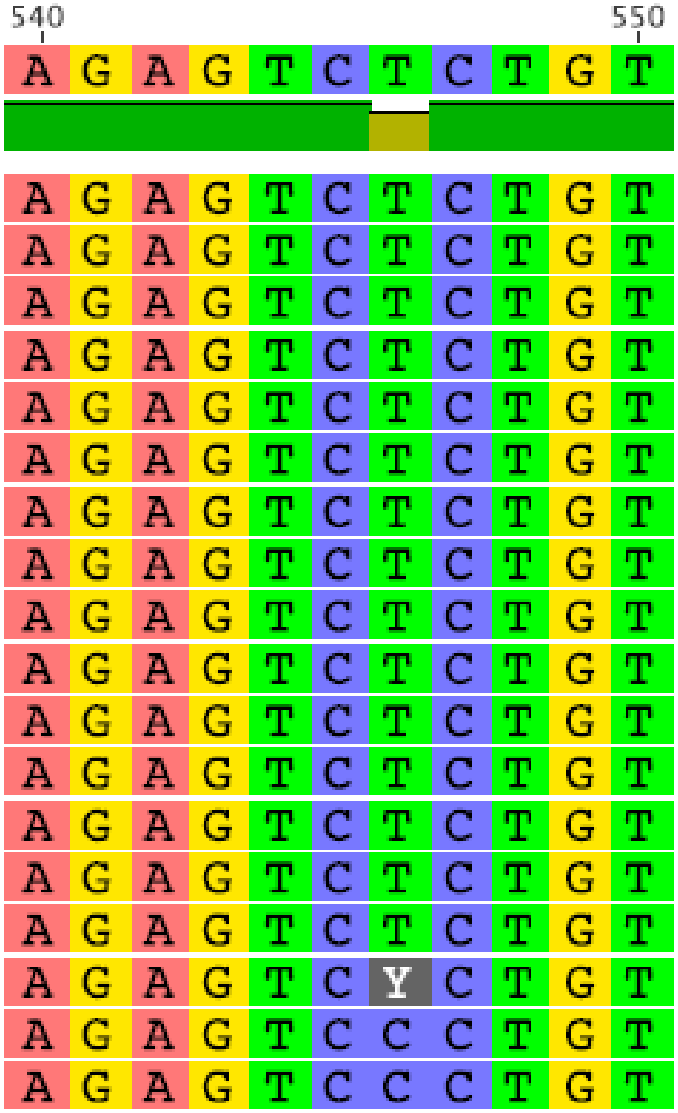


Fig 7: Position 546 of ITS alignment

New York population of the species into Europe. Similar groupings were identified by Schaefer (2015) and supported by the multiple regions he sequenced, thus allowing him to make practical conclusions regarding parent populations of *S. azorica*, the species whose ancestry he was trying to determine.

The sample R3_7_ITS_US was collected from the US state of Colorado, within the native range of the species. Its significant difference from other samples of the ITS tree (see Fig 4) could be due to a lack of sequence clarity. Many base pairs in this sample were noted as uncertain, the sheer amount (as can be observed in Fig 3) of which suggests sequencing errors unrelated to the sample or research methodology, rather than the presence of two base pairs at one position in a single sample's set of DNA repeats (as would typically be responsible for an unclear base interpretation). Such possible sequencing errors are also noted by Laureto and Barkman (2011) in agreement with earlier statements by Álvarez and Wendel (2003) regarding PCR errors and false variable sequences due to the recombinant nature of tandem repeat regions such as ITS.

Due to the unclear forward primers of the 5S-NTS sequences and the shorter nature of that noncoding region, ITS results obtained by the researcher can be considered more reliable and more likely to be accurate than those of the 5S-NTS region. Additionally, many areas of the reverse 5S-NTS sequences were unclear (similar to the forward primer but less severe) and thus were cut out by the researcher in editing prior to alignment, further shortening the number of positions available for comparison. Conflicting phylogenies between the two regions were noted immediately in the two trees (Fig 4 and 5), including most obviously the difference in the two lowermost samples from the ITS sequence results. Sample R2_9_5SNTS_US, collected from the US state of North Carolina, was depicted the sequence most variable from the consensus, suggesting that it may have also originated from the same R3_6 sample population from Colorado. However, this same suggestion regarding the R2_ 9 sample is not reflected in the more reliable ITS tree, leading the researcher to discard this evidence. Laureto and Backman (2011) avoided this conflict in their study involving sequencing of multiple regions by determining percent variation for regions such as chloroplast DNA rather than focusing on individual placement of samples within a phylogenetic tree. This was made possible by their study's goal of differentiating between species, which can be indicated by overall variance rather than specific base pair polymorphisms. Unfortunately, this study's goal of determining emergence of populations (that is, when and in what manner populations came to differ within a given species), as indicated by emergence of polymorphisms, means that such avoidance was not possible.

Conclusion

ITS and 5S-NTS rDNA were successfully sequenced, aligned, and examined from both European and North American samples of *S. canadensis* with use

of comparison techniques such as phylogenetic trees. Previous studies in the field were used as models for methodology and analysis, but key differences exist when compared to conclusions by Shaefer (2015) and Laureto and Barkman (2011) in that the purpose of their studies was simple ancestry and nomenclature, while my study aimed to identify emerging variations in a species and their geographic locations of origin. The hypothesis for this study was partially proven in that variations were observed in both ITS and 5S-NTS sequences. However, due to sequence unreliability and insufficient evidence, the researcher was unable to determine with certainty the origins and development of *S. canadensis* populations since its introduction to Europe.

Future Directions

Given more time and financial resources, full re-sequencing of the 5S-NTS region would benefit this research with the inclusion of clear and reliable data for meaningful comparison to ITS results. Also, inclusion of other *S. canadensis* genetic information, such as the ETS region of nrDNA recommended for use in conjunction with ITS by Laureto and Barkman (2011) or single-copy nuclear genes suggested by Álvarez and Wendel (2003) as a more stable alternative to ITS sequencing for creation of phylogenetic trees, could prove useful in confirming conclusions made from ITS and 5S-NTS results. Additional samples from the same and more varied locations across Europe and the Americas would allow for more comparisons between a larger number of potential populations and introductions.

On a larger scale, it has been made clear through my research that more exploration should be done to determine the origins and development of invasive species *S. canadensis* as was done by Schaefer (2015) regarding the origins of *S. azorica*. As pointed out by Stace and Crawford in their documentation of species invasive to the British Isles (2016), "this topic [development of *S. canadensis* and *S. gigantea* since introduction to Europe] has been almost totally neglected by researchers in Europe… DNA technology might change that", meaning that many advances in this field of study are yet to come with the refinement of existing sequencing and comparison technology. Perhaps such sequencing errors as were an obstacle in this study can be prevented in the future through development of more advanced DNA. Although no definite conclusions regarding *S. canadensis* variation were determined through my study, the lack of present research in this area, demonstrated by studies such as that by Weber and Schmid (1998), continues to stand as a reason for additional exploration into how invasive species are changed by the communities which they come to dominate.

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