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Does higher ploidy level increase the risk of invasion? A case study with two geo-cytotypes of *Solidago gigantea* Aiton (Asteraceae)

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Abstract

Aims

Understanding the role of genetics in biological invasions has become an important aspect for modern plant ecology. Many studies suggest that increased ploidy level benefits the success of an invasive species, but the basis for this phenomenon is not fully understood. In its native, North American range, *Solidago gigantea* has three geo-cytotypes comprising di-, tetra- and hexaploid populations, while in Europe, where it is highly invasive, *S. gigantea* stands are composed primarily of tetraploid individuals. Our study investigates whether North American hexaploids can induce a greater risk of invasion, due to their higher performance in a non-native range, as compared to the existing tetraploids of that range.

Methods

We performed greenhouse and common garden experiments along with microsatellite analyses to test whether differences in chromosome number and origin of the species mean superior fitness in the introduced range.

Important Findings

Genetic diversity was significantly higher in the native hexaploid populations (A_R = 6.04; H_e = 0.7794), rather than the non-native tetraploid populations (A_R = 4.83; H_e = 0.6869). Furthermore, differentiation between geo-cytotypes was moderate (ρ_{ST} = 0.1838), which was also confirmed by their clear segregation in principal component analysis and structure analyses, proving their different genetic structure. In contrast to genetic diversity, the non-native tetraploid geo-cytotype performed better in the common garden experiment, implying that higher genetic diversity does not always mean better success. Our results suggest that native hexaploids do not present a greater risk, as assessed by their performance in the introduced range, when compared to the non-native tetraploids, as was suggested by previous studies. Nevertheless, their introduction is still undesirable due to their different genetic structure, which, through hybridization, could give a new drive to the invasion of *S. gigantea*.

Keywords: EICA, plant invasion, microsatellite, ecotype, polyploidy

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INTRODUCTION

Transcontinental studies in invasion biology are crucial for modern ecology, since the same species can react in different ways due to its biogeographical origin (Pal *et al.* 2015; Shah *et al.* 2014). It is essential to reveal which factors contribute to the success of non-native, invasive plant populations over their native forms. By investigating changes which have occurred in the nuclei of non-natives, we may provide answers to these questions. Differences in cytogeography appear common amongst invasive plant species in their non-native range (Kubátová *et al.* 2008; Lafuma *et al.* 2003). Higher chromosomal

numbers lead to increased success amongst invasive species (Pandit *et al.* 2014). Polyploids, due to their higher genomic material, have wider ecological variability and plasticity (Soltis *et al.* 2014). The strength of polyploidization lies in their combining effects on genetic diversity and the alteration of sexual/asexual reproduction, longevity or fitness (te Beest *et al.* 2011).

Understanding the genetic background of invaders may provide insight into the process and guide the management of future invasive species (Ellstrand and Schierenbeck 2006). Both genetic drift and selection could constitute the genetic structure, altering the tolerance spectrum and the behavior of a species. Although a decrease in genetic diversity is generally considered detrimental, some studies have shown that widespread ecological success can occur despite genetic bottlenecks (Tsutsui *et al.* 2000). Polyploidization can positively influence genetic diversity and hence, increase colonization success and later establishment (Mayrose *et al.* 2011; Rosche *et al.* 2016). Yet, it is only when these lineages are particularly fit, that they may persist to enjoy longer-term evolutionary success. In contrast, the effect of polyploidy on genetic diversity is still not clear. Even a polyploid can be less diverse if sexual/asexual reproduction rate is skewed towards asexual reproduction (Cosendai *et al.* 2013; Liu *et al.* 2015).

Genetics can influence plasticity and widen the tolerance spectrum of an invader (Strayer 1999; Wolff 2000, but see: Davis and Shaw 2001; Lee 1999). According to Lee (2002), the invasion success of a species depends on the strength of its response to the forces of selection, while plasticity and tolerance are less important. These factors suggest that the genetic and ecological segregation of a population, caused by adaptation to a new environment, plays an important role in invasion ability (Colautti and Lau 2015). Polyploidy can influence this ability, too, by altering the life cycle and vegetative growth while giving this species a broad opportunity to adapt to changing environmental conditions (Monty *et al.* 2010; Treier *et al.* 2009; te Beest *et al.* 2011).

Moreover, the success of an invader can depend on its response to herbivory. As the EICA hypothesis asserts, invasive plants reallocate their resources from defense mechanisms into growth (Blossey and Nötzold 1995). Rogers and Siemann (2005) refined this mechanism and claimed that invasive ecotypes suffer herbivory more than native ecotypes. This reaction is not only influenced by the origin of the invader, but differences in ploidy can also alter this reaction. As Hull-Sanders *et al.* (2009a) suggested, insect herbivores have a dissimilar reaction to the different geo-cytotypes of *Solidago gigantea*.

The studied species, *S. gigantea* Aiton (giant goldenrod, Asteraceae) has three different geo-cytotypes (di-, tetra- and hexaploids) in their native range, where all cytotypes occur at similar rates, but are distinct geographically (Schlaepfer *et al.* 2008a). In the non-native range, where two different geo-cytotypes occur, it is most common to find tetraploids with a smaller occurrence of diploids in some populations (Schlaepfer *et al.* 2008a; Weber and Jakobs 2005). Geo-cytotype refers to the geographical range of different ploidy level (cytotype) individuals. Schlaepfer *et al.* (2008b) investigated the phylogeographic aspects of this species, proving the independent, multiple formation of polyploids in the native range and the reduction of genetic diversity in non-native populations. However, their research only focused on North American diploids and tetraploids, and European tetraploids and did not take into account the native hexaploid populations. Hull-Sanders *et al.* (2009b) investigated all geo-cytotypes, with an underrepresentation of hexaploids compared to other geo-cytotypes, and found differences in ecological parameters among the different geo-cytotypes. Yet, they only described the differences between continents, not within them. They suggested, but did not investigate, that besides ploidy, other genetic processes may also take part in their differentiation.

As the previous paragraphs suggest, it is clear that combined analyses of this species are required, including the effects of geo-cytotype on genetic and ecological variations. The results of this research will provide a stronger portrayal of the process of invasion by *S. gigantea*. In our study, we examined the ecological differences between the European tetraploid and North American hexaploid populations of *S. gigantea*. This research was conducted to examine the relationship between the non-native environment, the phenological state between geo-cytotypes, and the genetic background of the two ploidy levels. The reason for choosing tetraploids is the definite dominance of the cytotype in the non-native range. Also, there is a lack of knowledge about hexaploids and an absence of the cytotype in the non-native range. Additionally, this research provided the opportunity to compare the invasive ecotype to the native ecotype which has a higher ploidy level. Thus, we tried to answer the question: do more chromosomes support invasion success and can the possible introduction of native hexaploids present a greater risk of invasion by their performance, as is suggested by Pandit *et al.* (2014)?

The following hypotheses determined our investigations:

(i) Genetic diversity is higher in the native than the introduced geo-cytotypes due to founder effects in the introduced range and the higher ploidy level in the native range.
(ii) The investigated geo-cytotypes differ in genetic structure and ecologically (in growing parameters and reaction to damaging factors), which are influenced by ploidy and/or range of origin.
(iii) In addition, we wanted to investigate how different environmental conditions (green house versus common garden) and duration of time can affect our examined parameters.

MATERIALS AND METHODS

Model species

Solidago gigantea is a rhizomatous perennial herb native to North America with a circumpolar dispersal ranging from Europe to Asia. The species has a negative impact on the plant communities in Central Europe and in the Carpathian

Basin and has even naturalized in Russia, China and in the Japanese islands (Tsuyuzaki 2002; Weber and Jakobs 2005).

Solidago gigantea is self-incompatible and dependent upon insects for pollination (Voser-Huber 1983). The anemochory of the achenes results in long distance dispersal, while established stands propagate mainly vegetatively (Hartnett and Bazzaz 1985). The alloploid or autoploid origin of the species is still unclear.

Solidago gigantea is consumed by both specialist and generalist herbivores in North America, whereas in Europe only a few generalists have been reported to feed on it (Jakobs *et al.* 2004; Weber and Jakobs 2005). Thus, a decline in consumer attack may lead to the evolution of greater growth and higher plasticity to environmental heterogeneity (Blossey and Nötzold 1995). European *S. gigantea* populations appear to be less defended against insect herbivores than North American populations; yet, this decrease in defensive ability in the non-native populations was not found to lead to poor performance after herbivory (Meyer *et al.* 2005). Thus, it is possible that the capacity to tolerate herbivory has increased in European populations since introduction.

Sampling

We collected samples from 11 populations of *S. gigantea* in total. Five of the populations were from its native range in Montana, USA, and six were from the non-native range in Hungary. The collection location was directed by the results of Schlaepfer *et al.* (2008a), who suggested a high probability of occurrence of tetraploids in Europe and hexaploids in Montana. Within each range, the minimum distance among populations was 3 km, whereas the maximum was 150 km. The main consideration for population selection was a similarity in habitat, so as to exclude the factor of adaptation to extremely different environmental conditions. The two collection areas are at the same latitude, but each area is at a different elevation and this, jointly with other factors, creates different climatic conditions. However, we strived to standardize the conditions of sampling. The conditions considered were the climatic region (humid continental type: hot/warm summer subtype) according to Bonan (2008), soil moisture, average precipitation, light conditions (no shading), vegetation type and dominance of the species. Inflorescences with mature seeds were collected randomly in the field from 10 individual shoots per each population (i.e. seed family). Seed families were located at least 10 m apart from another, which reduced the risk of resampling the same clone. To test clonality, all individuals within the populations were examined to check for identical genotype.

Twenty seeds per family group were germinated in a Petri dish, and one of them was grown for our experiments (see below). We collected leaf samples in the second year of the experiment from these same individuals and used them for ploidy and microsatellite analysis.

Ploidy analyses

DNA ploidy levels were determined by DAPI flow cytometry (Partec-Sysmex CyFlow Space instrument equipped with the UV diode chip set). We applied the simplified two-step protocol using Otto buffers as detailed in Dolezel *et al.* (2007). Leaf samples from two *Solidago* individuals from the same population were analyzed together with the internal reference standard *Pisum sativum* L. 'Ctirad' (2C = 9.09 pg DNA). Only analyses producing a histogram with coefficient of variation of G0/G1 fluorescence peaks below 5% were retained. Karyologically-counted 4× and 6× *Solidago* plants served as reference points to infer ploidy levels of unknown samples.

Microsatellite analyses and scoring

DNA was extracted from frozen leaves using a ZenoGene40 Plant DNA extracting kit (supplied by Zenon Bio Ltd., Hungary). For the microsatellite analysis, we tested primers developed for other species of the genus. This yielded eight primers, which we optimized for PCR. The markers used were: Salt 1, Salt 4, Salt 8, Salt 17 (Sakata *et al.* 2013), SS4F, SS19D (Wieczorek and Geber 2002), SC45 and SC54 (Zhao *et al.* 2012). The reverse primers for loci with excessive stutter were redesigned with a 5′ PIG-tail (GTTCTT) to facilitate adenylation (Brownstein *et al.* 1996). We used a diluted mixture (1:5 to 1:40) of 4 µl DNA from the concentrate of the originally extracted DNA, 1-1 µl (10 pmol/µl) fluorescence labeled primer, 6.5 µl buffer (0.72 µl $MgCl_2$ (25 mM), 1.25 µl 10 × buffer, 2 × 0.05 µl dNTP (100 mM), 3.93 µl 3 × H_2O), 12.5 µl 3 × H_2O and 0.5 µl U Taq DNA polymerase for PCR reactions. The publications above describe the applied PCR programs. We used ABI 3100 capillary sequencing machines (Applied Biosystems, Inc., Foster City, CA, USA) at the Molecular Taxonomy Laboratory of the Hungarian Natural History Museum using the GeneScan 500 Liz size standard (Applied Biosystems) to perform analysis on the samples. We scored the samples manually with the Peak Scanner v.1.0 software (Applied Biosystems). All peaks were read with at least 1000–1500 intensity. We only allowed the most recurring alleles to advance through analysis to avoid random events of the PCR reactions. From the original 8 markers, 2 were excluded from analysis (SC45 and SC54) because of their unreliability during the scoring. We repeated the reactions to reduce the differential amplification of size-variant alleles or partial nulls if individuals showed incorrect amplifying in one locus (Wattier *et al.* 1998). Finally, three individuals were deleted from the dataset, because of the deficient amplifying and scoring problems with more loci. Effective purification of DNA controlled the quality of extraction (Gagneux *et al.* 1997) and the optimization of PCR reactions was important to reduce null alleles due to technician deficiencies in the amplification process (Flores-Rentería and Krohn 2013). True null alleles (mutations in the primer range) and determination of allele dosage are problematic points in the investigation of polyploid species because the intensity of peaks alone is not enough to estimate them. It is possible to estimate the correct

genotypes of polyploids only if the individual is homozygote or has the exact number of alleles as its ploidy level. In other cases, we could not make use of any allele dosage information (Dufresne *et al.* 2014).

Genetic diversity and genetic structure

Genetic diversity was inferred with SpaGeDi ver. 1.4 (Hardy and Vekemans 2002) to calculate allelic richness (A_R) and expected heterozygosity (H_e; Nei 1978).

Our examination of genetic structure among populations used the Structure ver. 2.3.4. (Pritchard *et al.* 2000), employing a Bayesian assignment analysis. We performed three analyses. The first analysis included all individuals from both geo-cytotypes (i.e. full analysis). Subsequently, we ran two further analyses: one for each geo-cytotype. These data were coded as co-dominant allele matrices. In the full analysis, tetraploids were coded with missing alleles on the fifth and sixth chromosome copy. We tested the number of groups (K) ranging from 1 to 20 with 20 replications for each value of K. We ran each replicate for 100,000 generations preceded by a burn-in period of 100 000 generations, as recommended by Gilbert *et al.* (2012). Admixture was allowed, and allele frequencies were independent in the different populations. To determine the most likely partitioning of clusters, we followed the ΔK approach of Evanno *et al.* (2005) by using Structure Harvester (Earl and vonHoldt 2012). To produce bar plots of the individuals' assignment probabilities (Q), we used CLUMPP ver. 1.1 (Jakobsson and Rosenberg 2007) and Distruct ver. 1.1 (Rosenberg 2004).

Since Structure may have limited explanatory power when comparing different cytotypes within one data set (Blanchet *et al.* 2014), we additionally investigated similarity in microsatellite composition with a principal component analysis (PCA) in R ver. 3.1.2 (R Development Core Team 2014), using POLYSAT ver. 1.4 (Clark and Jasieniuk 2011). We used Bruvo distances, which permits the comparison of individuals with different ploidy levels and takes into consideration that alleles, similar in size, could be closely related by mutation (Bruvo *et al.* 2004).

Genetic differentiation among populations

To test genetic differentiation between and within geo-cytotypes, F_{ST} (Nei 1978) pair-wise ρ_{ST} (Ronfort *et al.* 1998) and between geo-cytotypes, pair-wise F_{ST} (Weir and Cockerham 1984) were estimated in SpaGeDi. Meirmans and Van Tienderen (2013) demonstrated convenient properties of ρ_{ST} when comparing populations with different ploidy levels. These are identical expectations under identical gene flow conditions.

Ecological differences among populations

One seedling from each seed family was grown in 120 ml rocket pots filled with a 50:50 mixture of potting soil and sand. The location of the greenhouse was the Directorate of Plant Protection and Soil Conservation in Baranya County (Pécs-Hungary). Plants were grown at 18–25°C temperature and under 10 h light periods, 70 µEm-2s-1 illumination and 60% relative humidity. We watered them once every day over the course of 4 months. Height and leaf numbers for each seedling were obtained before outplanting.

For the common garden experiment, a regularly mown meadow was chosen where *Solidago* was already abundant nearby. The environmental conditions of the experimental sites were similar to the seed sample collection sites. The study site was close to Pécs, Hungary (46.06°N; 18.26°E, elevation: 142 m above sea level). The soil characteristics were the following: meadow soil, pHKCl: 7.23; pHH_2O: 7.6; salt: 0.18%; $CaCO_3$: 0.1%; organic matter: 5.08%; NO_3/NO_2:163 mg/kg; SO_4: 207 mg/kg Mg: 356 mg/kg; P_20_5: 1269 mg/kg; K_2O: 377 mg/kg; Na: 86.4 mg/kg; Zn: 45.6 mg/kg; Cu: 9.63 mg/kg; Mn: 53.5 mg/kg; nitrogen: 0.343 mg/kg. The moisture status of the soil was about 20–44% for the site. Soil characteristics were measured, and ecological data collected, in the flowering phenophase of the plants (around August). Both moisture parameters and soil samples were measured in 10–20 cm depths, in the most abundant zone of *S. gigantea* roots and rhizomes. Soil samples were taken in the second year of the experiment from three points in the common garden and soil moisture was measured at 15 points in the garden in the first and second years. In Hungarian grasslands, peak productivity ranges from around 395 to 535 g/m^2 (Molnár 2006; Nagy and Tuba 2008). We cleared existing vegetation from the sites using a weed whacker and demarcated them before planting. The seedlings were planted out in a random array in May 2010 at 50 × 40 cm apart, individually labeled to aid with identification, and then allowed to compete with the native vegetation of the sites without interference. In the first year, a few plants started to flower and those inflorescences were removed before they opened. In both the first and second year, performance attributes of the individuals (stem number, height, leaf number) and their interaction with pests (infection and herbivory rate) were recorded before the flowering phenophase (around August, according to Weber and Jakobs 2005). To avoid the problems of clonality, each ramet's connection to a genet was determined using a hand shovel. Aboveground harvest took place after the second year and the plants were weighed for dry biomass after 48 hours of desiccation in a 60°C heat chamber. We calculated the infection by fungi or bacteria and herbivory rates by counting all leaves that were affected. The number of leaves per each individual and the percentage of damaged leaves were calculated. Three observation points were established for statistical analyses according to the individual's phenological state. The first point was the seedling state before outplanting (referred to as greenhouse experiment) and the two other observation points were the individuals in their flowering phenophase in the first and the second year.

Statistical analyses

Statistics were carried out in R 3.1.2. Genetic differences in A_R and H_e between geo-cytotypes were analyzed with

two-sample *t*-tests. While statistics of the common garden experiment were carried out with linear mixed-effect models using the lm4-package (Bates *et al.* 2015) with populations treated as random factor. Height and number of leaves were analyzed using fixed effects for the interaction of geo-cytotype and observation point (three levels: after the greenhouse, 1st and 2nd year of the common garden experiment). In the case of stem number, interaction of geo-cytotype and observation point was analyzed too, but observation point had only two levels (1st and 2nd year of the common garden experiment). For these models, observation point was treated as a repeated measurement. In contrast, biomass, herbivory rate and infection rate only contained geo-cytotype as a fixed effect, because their collection was obtained in the second year. Herbivory and infection rates were measured in the second year to let the individuals acclimatize to the new environmental conditions. For biomass, we tried to avoid the effect of first year harvesting on the second year results. The height and number of stems were $\log_e$ transformed. Function dropterm with chi-squared test was used to estimate test statistics (Venables and Ripley 2002). Transformation was based on graphical evaluation according to Crawley (2014). For pair-wise comparisons, Tukey post-hoc tests were conducted in both cases with multcomp-package (Hothorn *et al.* 2008). For the completion of models and post-hoc tests, maximum likelihood estimation was used.

RESULTS

Ploidy analyses

All of our individuals from the native range were hexaploids and all individuals from the non-native range were tetraploids.

Genetic diversity and genetic structure

In total, we found 55 alleles across the six loci with the number of alleles per locus ranging from 6 to 17, and 3 to 10 within a population (see online supplementary Table 1). Most of the alleles were shared between both geo-cytotypes. Ten private alleles were found in the natives, while four were found in the non-natives (see online supplementary Table 2). A_R in the non-native tetraploids was 4.83, it was 6.04 in the native hexaploids and 8.21 overall. H_e in the non-native tetraploids was 0.69, it was 0.78 in the native hexaploids, and 0.75 overall (see online supplementary Table 2). Both A_R (t-value = −5.009; $P < 0.001$) and H_e (t-value = −7.412; $P < 0.001$) were significantly higher in the native hexaploids.

In the structure analysis of the full dataset, including both geo-cytotypes (Fig. 1), dividing plants into two clusters gave the maximum likelihood values. This revealed the different genetic structure of native and non-native geo-cytotypes. While the first non-native tetraploid population (Pop1) was distinct from the rest of non-natives, native populations seemed rather similar to one another. Non-native individuals were divided into four clusters, according to their ΔK results. This strengthened the differentiation of Pop1 from the others, while showing a similarity among the other populations. To investigate the structure of native populations, they were divided into two clusters which indicated Pop7 and Pop8 differ in genetic structure from the rest of the native groups.

The PCA plot (Fig. 2) shows the position of populations estimated by microsatellite loci. The differentiation of continents is visible. Pop7 and Pop8 showed a slight segregation from the rest of the native populations supporting the results of structure analysis. Within the non-native range there was no clear differentiation.

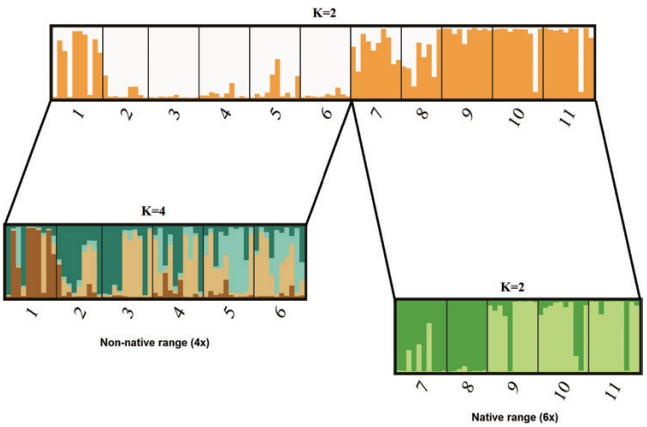

Figure 1: representative plots of the examined geo-cytotypes from the structure analyses based on the microsatellite loci results. K is the number of clusters into which the plants were divided, according to ΔK values. Bar plots represent individuals' posterior assignment probability to one of the genetic cluster.

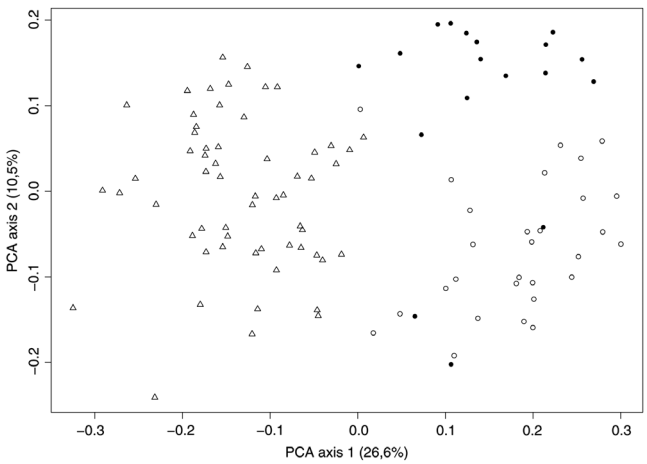

Figure 2: principal component analysis of the examined geo-cytotypes based on the microsatellite loci results. Distances were calculated using Bruvo's distance index. Δ: Non-native range (4x); ○: native range 1 (6×) (Pop9, Pop10, Pop11) and •: native range 2 (6×) (Pop7, Pop8). % of variance explained by each axis is provided within the figure.

Genetic differences among populations

The genetic separation among native, hexaploid populations (F_{ST} = 0.047; ρ_{ST} = 0.179) was higher than among non-native, tetraploids (F_{ST} = 0.043; ρ_{ST} = 0.137), but the differences were highest between tetra- and hexaploids populations (F_{ST} = 0.051; ρ_{ST} = 0.183).

Ecological differentiation among populations

The groups of native and non-native geo-cytotypes separated ecologically. Results of the statistical analyses are summarized in Table 1. Interaction of geo-cytotype and observation point did not influence stem development significantly. Stem numbers differed significantly only in the first year, when natives developed less stem than non-natives. Nevertheless, in the second year, the difference between ranges disappeared (first year non-native vs. first year native: *t*-value = −3.787; second year non-native vs. first year native: $P < 0.01$; *t*-value = −6.166; $P < 0.001$; second year native vs. first year native: *t*-value = −4.057; $P < 0.001$; Fig. 3). Height was significantly affected by the interaction of geo-cytotype and observation point (Table 1). There was no difference between the two geo-cytotypes in the greenhouse conditions and in the first year common garden. However, in the second year, a significant difference appeared between them (*t*-value = −5.225; $P < 0.001$; Fig. 4A), as the non-native individuals grew 20–25 cm taller on average. The number of leaves was significantly affected by the interaction of geo-cytotype and observation point also (Table 1). There was no significant differences between them in the first two phases, but in the second year, non-natives developed more leaves than the native ones (*t*-value = −5.852; $P < 0.001$; Fig. 4B). Also, in the second year, non-native individuals produced more biomass (average: non-native = 16.19 g; native = 7.31 g) than the native ones (*t*-value = −2.637; $P < 0.01$; Fig. 5A). Additionally, the second year non-native individuals suffered less herbivory attack on leaves than the natives (*t*-value = 2.271; $P < 0.05$; Fig. 5B), while in the rate of infection there was no significant difference (*t*-value = 1.768; $P > 0.05$; Fig. 5C).

DISCUSSION

Genetic diversity and genetic structure

Our genetic analyses show differences among the investigated native, hexaploid and non-native, tetraploid populations. We found a higher number of unique alleles in the native hexaploids than in the non-native tetraploids. Also, allelic richness and expected heterozygosity showed higher diversity in favor of the native hexaploids, which can be caused by their higher ploidy level, as Mahy *et al.* (2000) suggested. However, genetic diversity does not only depend on ploidy, but gene flow among populations amplifies this effect (Sosa *et al.* 2014). As we discussed earlier, changes in sexual/asexual rate also can alter diversity. Alterations of sexual/asexual reproduction may depend on polyploidization (Liu *et al.* 2015), and non-native populations may exhibit stronger clonal growth than natives (Jakobs *et al.* 2004; but Schlaepfer *et al.* 2010). Both ploidy and sexual/asexual rate may suggest reasons for loss of genetic diversity. However, the results of our common garden experiment only showed differences in asexual reproduction during the early stage of the invasion process between the examined geo-cytotypes. Thus, it does not play an important role in the loss of genetic diversity in our investigated non-native, tetraploid populations. Our diversity results are consistent with the severe founder effect of an invader (Kliber and Eckert 2005) and parallel to earlier studies of *Solidago* which described higher diversity in the native range (Schlaepfer *et al.* 2008b). As our common garden experiment revealed, genetic diversity does not improve the success of *S. gigantea*, supporting the results of Rollins *et al.* (2013).

Genetic differences among populations

While F_{ST} showed little genetic differentiation between and within geo-cytotypes, ρ_{ST} showed clear differentiation at both cases. Whereas F_{ST} is more suitable for the analysis of identical ploidy level, ρ_{ST} is independent of the ploidy level, the degree of inbreeding and the amount of double reduction (Ronfort *et al.* 1998). Therefore, it may reveal more robust results for polyploid analyses and the comparison between cytotypes (Meirmans and Van Tienderen 2013). Moreover, ρ_{ST}, PCA and structure analyses showed strong separation between native hexaploids and non-native tetraploids, weak separation within the non-native and moderate separation within the native geo-cytotypes. Within the native geo-cytotype, two populations (Pop7 and Pop8) differed from the others. In contrast, within the non-native geo-cytotype, there was only one population (Pop1) which showed clear differentiation from

Table 1: results of the linear mixed-effect model analyses testing the interaction effect of geo-cytotypes and observation points (greenhouse experiment, 1st and 2nd year of common garden experiment) on the measured parameters of *S. gigantea*

	Number of stems			Height			Number of leaves			Biomass			Herbivory rate			Infection rate		
	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*
Range	1	9.418	<0.01	1	8.323	<0.01	1	9.418	<0.01	1	4.984	<0.05	1	5.131	<0.05*	1	3.140	>0.05
Observation point	2	81.342	<0.001	2	124.482	<0.001	2	81.342	<0.001			n.t.			n.t.			n.t.
Range: observation point	2	1.9457	>0.05	2	4.659	<0.05	2	5.871	<0.05			n.t.			n.t.			n.t.

In the models, observation point was treated as a repeated measurement. Biomass, herbivory and infection rate were measured only in the second year, therefore only geo-cytotype was included in their models. n.t. = not tested.

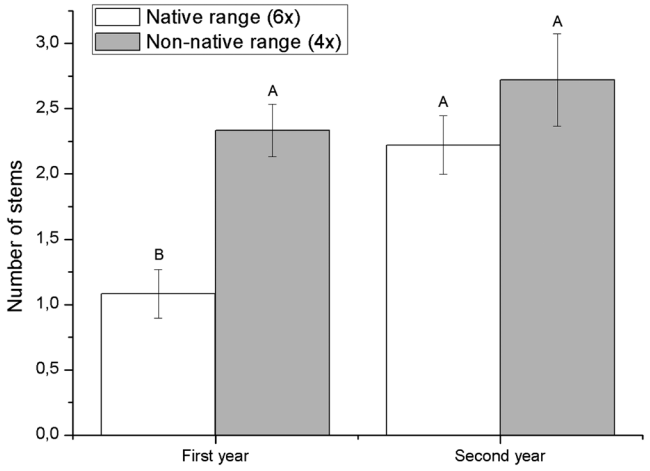

Figure 3: differences in number of stems between geo-cytotypes (non-native tetra- and native hexaploids) and their changes in time. Same individuals were investigated. Error bars represent S.E.M. Bold capital letters represents groupings which are based on Tukey post-hoc tests.

the remaining non-native ones. These populations were the most distinct in geographic distance and in original habitat. The isolated populations were from drier hillsides in both ranges, suggesting that distance and environment jointly influence genetic structure (Alberto *et al.* 2010).

Environmental and life stage dependence of ecological differences

During the two-year study of the examined individuals, we found a significant interaction of geo-cytotype and observation point in the common garden experiment among examined parameters (except infection rate). These parameters suggest that the outcome of native hexaploid versus introduced tetraploid comparison is affected more by life stage than we earlier thought. In particular, genetic differences between geo-cytotypes did not manifest ecologically in the early stage of the invasion process or in controlled conditions. The differences between geo-cytotypes needed more time to appear. Greenhouse and field experiments create different environments for plants which can strongly alter or even reverse their response (Lankau *et al.* 2010; Zeller *et al.* 2010). These responses are due to the different composition of stressors. For example, competition is a stronger factor in common garden experiments, while abiotic stress more influential in greenhouses (Hartman *et al.* 2014). Thus, the controlled condition can mask differences, which would appear under natural conditions. Some evidence of this phenomenon arose when plants of the examined geo-cytotypes started to separate ecologically in the common garden experiment. As our data reflects, clonal growth of the non-native geo-cytotype is stronger than the native geo-cytotype, but only in the first year. This factor emerges in the non-native environment and leads to a rapid spread of the invader. It also leads to high reproductive investment in the early life stages,

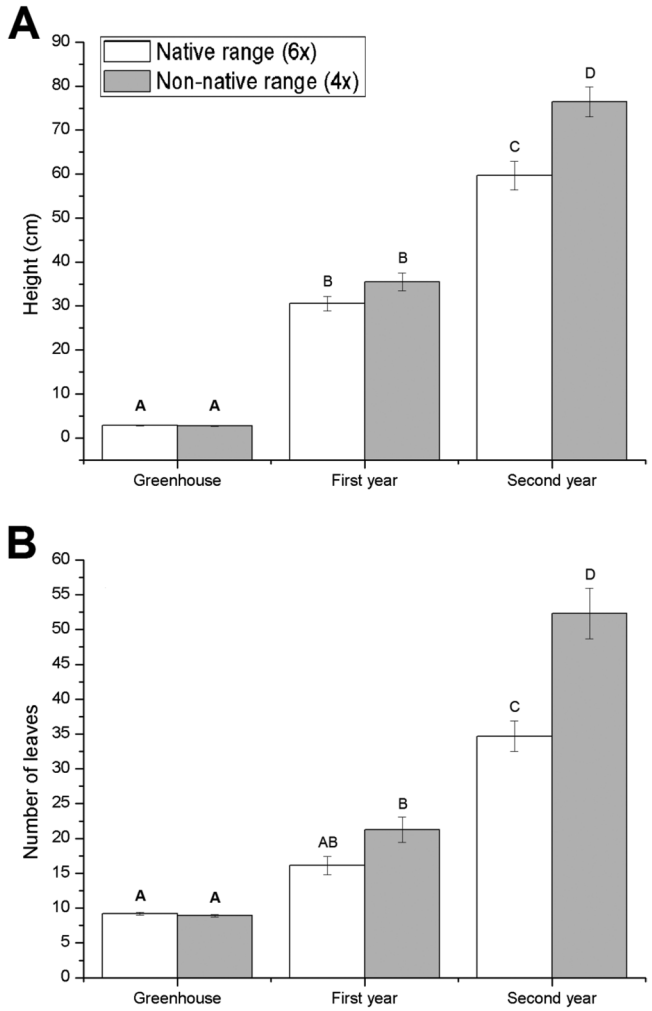

Figure 4: Differences in height (**A**) and number of leaves (**B**) between geo-cytotypes (non-native tetra- and native hexaploids) and their change under different conditions and in time. Same individuals were investigated. Error bars represent SEM. Bold capital letters represents groupings which are based on Tukey post-hoc tests. SEM = standard error of mean.

while the competitive conditions of native environment did not. Throughout the second year, native hexaploids reached the same stem number as non-native tetraploids, yet differences in other parameters strengthened. Producing photoassimilates and accumulating nutrients in the early period of spreading helps to translocate more energy into the development of each ramet (Aguilera *et al.* 2010). These results show how strongly the environmental conditions and the period of the research can influence the final conclusions.

Ecological trade-offs in terms of EICA

We found significant differences between the investigated geo-cytotypes based on the percentage of plants that sustained herbivory. Our results prove that herbivores of the non-native range prefer native over non-native geo-cytotypes. These findings contradict the EICA hypothesis. As

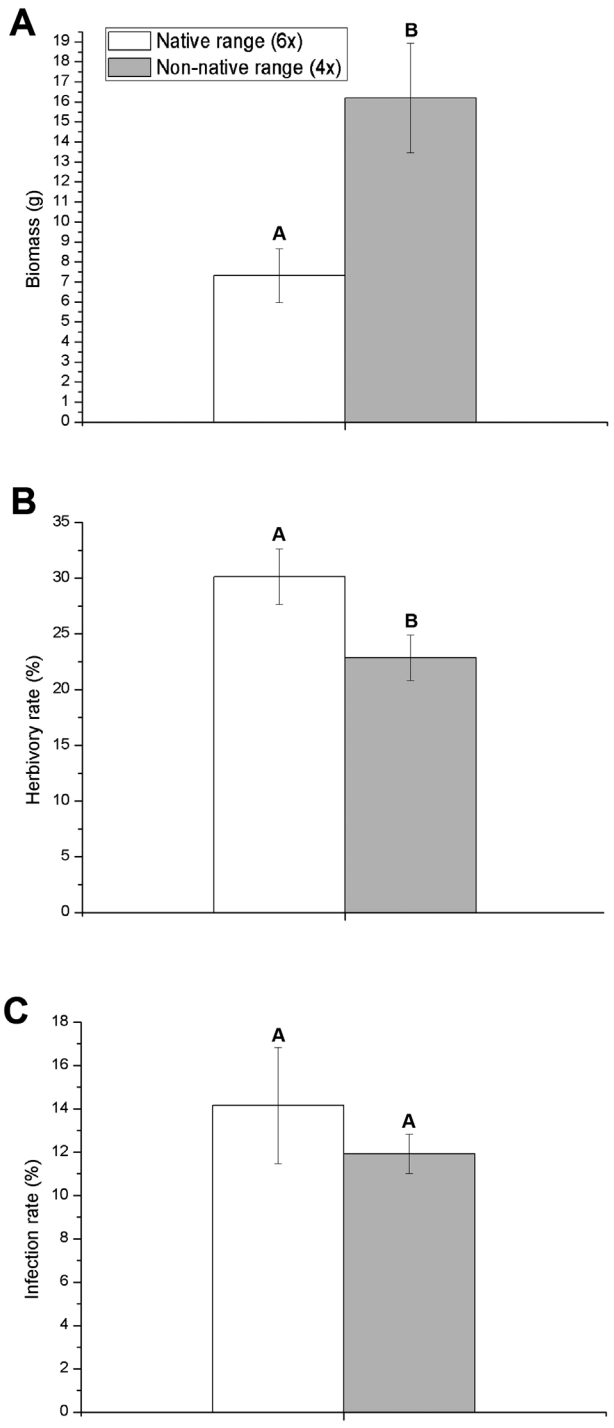

Figure 5: Differences in aboveground biomass (**A**), herbivory rate (**B**) and infection rate (**C**) between geo-cytotypes (non-native tetra- and native hexaploids) in the second year of the common garden experiment. Error bars represent SEM. Bold capital letters represents groupings which are based on Tukey post-hoc tests. SEM = standard error of mean.

Van Kleunen and Schmid (2003) previously revealed, EICA does not play a role in the invasion of the close relative *S. canadensis*. Meyer and Hull-Sanders (2008) tested the effect of the EICA hypothesis on *S. gigantea* and found no evidence that the range of origin would influence the compensation from herbivory. To get a more detailed picture, Hull-Sanders *et al.* (2009a) investigated herbivory from the viewpoint of two native insect species. They involved all *Solidago* geo-cytotypes and found that the preference of generalist herbivores is more sensitive to both range and cytotype. However, other studies suggest that the intensity of herbivory do not depend on the cytotype of a host plant (Boalt *et al.* 2010; Collins and Müller-Schärer 2012). Nevertheless, while previous studies only investigated some selected native specialist herbivores, our study focused on the effects of the non-native specialist and generalist herbivore fauna in a natural environment. As was mentioned earlier, in the non-native range, generalist herbivores consume *S. gigantea*, while specialist herbivores are the main consumers in the native range (Weber and Jakobs 2005). We found that herbivores of the non-native range preferred native hexaploids, which we hypothesize could be the result of the selecting forces of generalists on non-native tetraploids as it was proved by Maron and Vilà (2001) and Parker *et al.*(2006). Therefore, we suggest that the reaction to herbivory results from the adaptation of plants with different origins to the different composition of local insect fauna in each range as Sakata *et al.* (2014) also suggested. In contrast to herbivory, the percentage of the infected leaves was not significantly different between geo-cytotypes. This data suggests that the development of pathogen responses requires a longer period of time.

Effects of ploidy on invasion success

Our results contradict the assumption that an increasing number of chromosomes positively influences invasiveness in any time by increasing biomass and competitive ability (Pandit *et al.* 2014; te Beest *et al.* 2011). Native hexaploids performed worse in all body parameters than non-native tetraploids in our experiments. As we previously discussed, increased DNA content, which leads to higher cell volume, does not necessarily manifest in plant size (Hansen *et al.* 2007; Otto and Whitton 2000). Some studies have disputed the importance of polyploidy in the success of a plant species (Combes *et al.* 2012). In addition Suda *et al.* (2015) reported the existence of a genome size/ploidy trade-off that suggests that at a certain threshold, plants cannot increase the quantity of their genetic material with obligate benefits. A positive relationship between stem density, biomass and higher ploidy level of *S. gigantea* was described by Jakobs *et al.* (2004) and Schlaepfer *et al.* (2010) when they examined di- and tetraploid individuals, but hexaploids were left out from both experiments. Hull-Sanders *et al.* (2009b) went further when they took all geo-cytotypes into account and found differences in plant growth related to cytotype. However, these differences mainly affected the comparison between continents and not within them. The experiments also suggest that changes in the introduced range could be a result of stochastic events more than differences in ploidy level. Additionally, non-native cytotypes may not be independent lineages. The occurrence of genetic changes could drive the ecological differentiation within

the species (Hull-Sanders *et al.* 2009b). As we discussed earlier, average genetic diversity decreased in the non-native tetraploid populations due to bottlenecks during their introduction into Europe, and this may or may not inhibit its success in the non-native range. Williams and Fishman (2014) had the same results investigating *Cynoglossum officinale*. Our results suggest that both higher chromosome number and greater genome size does not increase the risk of invasion in every case, and that other genetic factors may stand behind the success of this species, including ploidy and gene diversity.

CONCLUSIONS AND PERSPECTIVES

Our results reveal that range of origin and ploidy level together have a strong impact on the microsatellite composition and that it can appear in ecological parameters. The native and non-native geo-cytotypes under investigation differed both in their genetic structure and ecologically. Our findings contradict previous literature, which emphasized the dominance of higher ploidy levels over lower ploidy levels in invasive species. We suggest that native hexaploids do not present a greater risk of invasion due to their increased number of chromosomes. In contrast, hybridization of the geo-cytotypes could give new dynamism to the success of the invader. However our current study did not investigate this aspect of hybridization. Therefore, it is possible that chromosome multiplication does not influence invasion success as much as we earlier thought. Perhaps, it only helps other genetic processes to increase adaptation skills, up to a point, where more chromosome sets do not equate more benefits. For further clarification and to examine other aspects of ploidy, region and their risk to natural communities, a simultaneous genetic and ecological investigation of several populations from all geo-cytotypes would need to be implemented.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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