Geographic variations in phenotypic traits of the exotic herb *Solidago altissima* and abundance of recent established exotic herbivorous insects

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Many invasive plants increase aggressiveness after introduction. Since evolutionary forces such as herbivore pressure may change over different time scales, understanding the changes in biotic interactions in invasive plants through time can clarify the mechanism of their evolution in aggressiveness. In this study we examined the geographic variation in phenotypic traits of *Solidago altissima* and the abundance of two exotic herbivorous insect species (the aphid, *Uroleucon nigrotuberculatum* and the lacebug, *Corythucha marmorata*), which are recently expanding their habitat on *S. altissima* populations over Japan. The two exotic insects were present at high density on *S. altissima* throughout their range. No differences in growth traits (plant height and number of leaves) were found among populations, and all plants examined appear to be exclusively hexaploid. Future studies on population genetics and common garden experiments are necessary to evaluate the potential evolutionary dynamics of the *S. altissima* after introduction.

**Keywords**
Corythucha marmorata, exotic insects, hexaploid, species invasion, Solidago altissima,

Uroleucon nigrotuberculatum

Introduction

Rapid evolution has been found in many successful invasive plants, including changes in traits such as biomass, reproductive output, competitive and dispersal abilities (e.g. Blossey & Notzold 1995, Maron et al. 2004, Brown & Eckert 2005). The enemy release hypothesis (ERH), one of the influential hypotheses considered as fundamental in explaining plant invasion success, is important by transporting the plant away from its natural enemies allowing the plant to grow vigorously. Many studies examining traits of invasive plants have focused on a snapshot in time, however, evolutionary forces such as herbivore pressure may change over different time scales (Hawkes 2007). Understanding the pattern of changes in potential drivers of invasive plants through time can clarify how invaders continue to be successful.

A perennial herb, Solidago altissima, was introduced to Japan in the early 19th century from North America, and has expanded its distribution rapidly over Japan after the 1960s (Shimizu 2003). Solidago altissima in North America consists of diploid,
tetraploid, and hexaploid (2n=18, 36, 54) (Halverson et al. 2008a). While the taxonomic
treatment of the species has been complex, recent treatments (Semple and Cook 2006)
have recognized two subspecific taxa. These subspecific taxa is associated with
cytotypic variation, with subsp. *gilvocanescens* reported as diploid and tetraploid across
its range and subsp. *altissima* primarily hexaploid (a few tetraploids have been reported
at the western edge of the distribution and across the southeastern US; Semple and
Cook, 2006). However, the ploidy level in Japan is unknown. Although it is considered
as one of the most invasive, of introduced plants in Japan, ecological traits and natural
enemies throughout its distributional range have been poorly explored. Recently, two
exotic insects have been introduced to Japan from North America: the aphid *Uroleucon
nigrotuberculatum* in 1990’s and the lacebug *Corythucha marmorata* in 2000. The aphid
has a large impact on the native insect community via changing *S. altissima* traits (Ando
et al. 2011). On the other hand, the lacebug has been rapidly expanding its habitat, and
has become a serious pest of crops such as chrysanthemum and sweet potato in Japan.
The aim of this study was to elucidate the geographic variation in the abundance of the
two exotic herbivorous insect species among *S. altissima* populations over Japan.
Because the abundance of the herbivorous insects is dependent on plant traits such as plant size (Lawton 1983) and ploidy levels (Halverson 2008b), we also examined plant traits related to the abundance of the two insects. This is fundamentally important for understanding the changes of the herbivore pressure on *S. altissima* after expansion in the invasive range.

**Methods**

In June in 2011 and 2012, we surveyed the abundance of the two exotic insects at 15 sites (1-5 *S. altissima* populations per site) in Japan (Table 1). Populations at each site occur within radius 10 km, and the distance of two adjacent populations was 1 km. We surveyed 5-10 individual plants (three ramets per individual) distinguished by clumps in each population. For each ramet, number of the two insects was counted.

We recorded plant height and number of leaves for all ramets which the insect survey was conducted. Then, we collected rhizomes from five individuals of one population at each site for determination of the ploidy level. Ploidy levels were determined by flow cytometry and chromosome numbers (chromosome counts in root
tip squashes of the cultivated plants from rhizomes). The root tips were treated with a 0.05% hydroxynole solution at 16-18°C for 5 h before they were fixed with an ethanol : glacial acetic acid solution (3 : 1) at 4°C for 24 h. They were macerated by 1N HCL at 60°C for 1 min before being stained with aceto-carmine solution for 24 h and were mounted on a microscope slide. In the flow cytometry analysis, for each sample intact nuclei were extracted from approximately 0.5 cm² of leaf tissue in a Petri dish. The sample was chopped for 30 sec using a sharp steel razor blade in 400 µl of extraction buffer (Partec, Görlitz, Germany) and filtered using a 30-µm CellTrics disposable filter (Partec). For each sample, the filtrate was mixed with 1.6 ml of staining solution (Partec) and the mix was incubated for 60 s. We analyzed these samples, using CyStain UV precise P (Partec). We converted fluorescence to chromosome number, using standard samples (hexaploid samples determined by root tip squashes). Only samples producing a histogram peak with a low coefficient of variation (< 5%) were retained. The standard sample was checked after every five samples.

Results & Discussion

Both the lacebugs and the aphids were observed in high densities while native
generalist herbivores including grasshoppers and geometric moth larvae were rare.

Although there was a considerable variation in the aphid abundance among populations, aphids were found in all sites (Table 1). On the other hand, the lacebugs were absent in sites of Hokkaido and Sado, indicating that they have not invaded those sites yet (Table 1). The effect of the latitude was marginally significant on the abundance of the lacebugs and it was greater in the lower latitudinal populations [generalized linear mixed model (GLMM): random effects = individual nested with population, offset = leaf number (as plant size), $z = -1.82$, df = 343, $P = 0.07$], while greater aphid abundance was apparent in higher latitudinal populations [GLMM: random effects = individual nested with population, offset = plant height (as plant size), $z = 2.91$, df = 343, $P = 0.004$].

No latitudinal clinal patterns were found in both plant height [GLMM: random effects = individual nested with population, $z = -1.38$, df = 343, $P = 0.17$] and leaf number [GLMM: random effects = individual nested with population, $z = -0.032$, df = 343, $P = 0.975$]. Flow cytometry analyses mostly yielded high-resolution histograms, with average sample CV of 3.25% (range 1.99–4.86%). Flow cytometry data for 75
individuals of *S. altissima* from 15 sites showed that all individuals had the same value. This indicates that all individuals of *S. altissima* examined in the present study had the same ploidy level and they were hexaploid (2\(^n\) = 54) (Table 1). Therefore, they were all subsp. *altissima*.

Our field survey suggests that the two exotic insects were dominant herbivores on *S. altissima*. In particular, the lacebugs may have a selective impact to the traits of *S. altissima* because they continue causing severe damage to the plant by sucking the leaf tissue until the end of autumn. Moreover, because *C. marmorata* is expanding its range concentrically, the dates of population establishment differ geographically (including absent sites). This provides an excellent opportunity to test the potential of the selective impacts of this herbivore on traits of *S. altissima*. Although no differences in plant height and leaf number among populations indicate no difference in plant growth traits throughout the range, other traits such as reproduction and resistance may differ among populations with different abundance of the exotic insects.

There are two possible explanations for the result that only hexaploid plants were found. One is that only hexaploid plants had been introduced. Another is that other ploidies had
been also introduced, but they failed to establish or expand its ranges. Studies of the ploidy level of *S. gigantea* in the invasive and native range revealed that tetraploids were more invasive than diploids and it was the only cytotype found in the invasive range (Schlapfer et al. 2008; 2010). Future research on population genetics and common garden experiments is necessary to evaluate the potential evolutionary dynamics of *S. altissima* after introduction.

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**References**


Table 1. Geographical information of *S. altissima* populations and the means (±1 SE) of two traits (height and no. leaves), ploidy level and mean number (±1 SE) of two exotic insects.

<table>
<thead>
<tr>
<th>Site</th>
<th>No. populations</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Height</th>
<th>No. leaves</th>
<th>Ploidy level</th>
<th>No. lacebugs</th>
<th>No. aphids</th>
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<tbody>
<tr>
<td>Hokkaido</td>
<td>5</td>
<td>42.83</td>
<td>141.30</td>
<td>99.45±3.34</td>
<td>32.48±1.02</td>
<td>Hexaploid</td>
<td>0</td>
<td>34.76±9.00</td>
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<td>Tochigi</td>
<td>2</td>
<td>36.67</td>
<td>139.95</td>
<td>80.10±8.38</td>
<td>38.00±3.26</td>
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<td>13.10±3.79</td>
<td>14.80±8.09</td>
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<td>Sado</td>
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<td>37.80</td>
<td>138.24</td>
<td>85.32±3.25</td>
<td>35.34±1.59</td>
<td>Hexaploid</td>
<td>0</td>
<td>2.34±1.69</td>
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<td>Nigata</td>
<td>2</td>
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<td>139.04</td>
<td>92.86±4.54</td>
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<td>1.79±1.79</td>
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<td>Tokyo</td>
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<td>139.65</td>
<td>82.98±2.25</td>
<td>31.10±1.12</td>
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<td>92.33±5.56</td>
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<td>135.53</td>
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<td>Hyogo</td>
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<td>Kumamoto</td>
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<td>130.8</td>
<td>100.44±2.26</td>
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<td>Hexaploid</td>
<td>14.88±2.20</td>
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