

## RESEARCH ARTICLE

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

Yuzu Sakata<sup>a\*</sup>, Takayuki Ohgushi<sup>b</sup> and Yuji Isagi<sup>a</sup>

<sup>a</sup>Laboratory of Forest Biology, Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan; <sup>b</sup>Center for Ecological Research, Kyoto University, Otsu 520-2113, Japan

(Received 23 January 2013; accepted 19 February 2013)

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, 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 the 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 nineteenth century from North America and has expanded its distribution rapidly over Japan after the 1960s (Shimizu 2003). *S. 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 & Cook 2006) have recognized two subspecific taxa. These subspecific taxa are associated with cytotypic variation, with subsp. *gilvocanescens* reported as

diploid and tetraploid across its range and subsp. *altissima* primarily as hexaploid (a few tetraploids have been reported at the western edge of the distribution and across the southeastern USA; Semple & 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 1990s 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 et al. 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.

\*Corresponding author. Email: [sakata@ecology.kyoto-u.ac.jp](mailto:sakata@ecology.kyoto-u.ac.jp)

## Methods

In June 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 a radius of 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 the ramets for 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-carmin 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 cm<sup>2</sup> 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 sec. 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 and 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 the 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 ( $2n = 54$ ) (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

Table 1. Geographical information of *S. altissima* populations and the means ( $\pm 1$  SE) of two traits (height and number of leaves), ploidy level and mean number ( $\pm 1$  SE) of two exotic insects.

Site	No. populations	Latitude	Longitude	Height	No. leaves	Ploidy level	No. lacebugs	No. aphids
Hokkaido	5	42.83	141.30	99.45 $\pm$ 3.34	32.48 $\pm$ 1.02	Hexaploid	0	34.76 $\pm$ 9.00
Tochigi	2	36.67	139.95	80.10 $\pm$ 8.38	38.00 $\pm$ 3.26	Hexaploid	13.10 $\pm$ 3.79	14.80 $\pm$ 8.09
Sado	2	37.80	138.24	85.32 $\pm$ 3.25	35.34 $\pm$ 1.59	Hexaploid	0	2.34 $\pm$ 1.69
Nigata	2	37.88	139.04	92.86 $\pm$ 4.54	28.33 $\pm$ 1.15	Hexaploid	9.00 $\pm$ 2.68	1.79 $\pm$ 1.79
Tokyo	2	35.65	139.65	82.98 $\pm$ 2.25	31.10 $\pm$ 1.12	Hexaploid	8.67 $\pm$ 2.56	33.20 $\pm$ 15.23
Sizuoka	1	35.13	138.64	92.33 $\pm$ 5.56	33.38 $\pm$ 1.66	Hexaploid	3.85 $\pm$ 0.96	54.05 $\pm$ 19.52
Kyoto	3	34.84	135.53	95.44 $\pm$ 4.22	28.56 $\pm$ 1.70	Hexaploid	6.61 $\pm$ 1.39	4.55 $\pm$ 2.48
Shiga	2	34.80	135.66	96.87 $\pm$ 3.28	37.73 $\pm$ 1.49	Hexaploid	8.86 $\pm$ 2.08	32.10 $\pm$ 9.61
Osaka	3	34.90	135.45	88.29 $\pm$ 3.54	34.35 $\pm$ 1.31	Hexaploid	4.3 $\pm$ 0.77	17.22 $\pm$ 5.16
Hyogo	5	35.20	135.23	84.22 $\pm$ 2.24	34.17 $\pm$ 1.00	Hexaploid	7.29 $\pm$ 0.84	20.66 $\pm$ 4.76
Kochi	1	33.56	133.56	119.24 $\pm$ 4.07	21.84 $\pm$ 1.29	Hexaploid	1.32 $\pm$ 0.39	1.05 $\pm$ 3.04
Fukuoka	4	33.62	130.37	96.98 $\pm$ 2.43	37.33 $\pm$ 1.07	Hexaploid	4.78 $\pm$ 0.76	12.64 $\pm$ 2.04
Saga	3	33.32	130.27	83.89 $\pm$ 3.34	42.81 $\pm$ 2.25	Hexaploid	8.25 $\pm$ 2.18	41.17 $\pm$ 2.15
Kumamoto	4	32.92	130.8	100.44 $\pm$ 2.26	37.82 $\pm$ 1.18	Hexaploid	15.67 $\pm$ 2.25	11.50 $\pm$ 4.39
Kagoshima	3	31.65	130.47	110.54 $\pm$ 2.89	33.09 $\pm$ 1.16	Hexaploid	14.88 $\pm$ 2.20	3.86 $\pm$ 1.79

*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 (Schlaepfer 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.

#### Acknowledgments

We thank Dr Y. Ando for the helpful advices for the field survey. We thank Dr H. Choi and Dr W. Shinohara for helping with the chromosome counts. This work was supported by the JSPS Core-to-Core Program (No. 20004) from Japan Society for the Promotion of Science.

#### References

Ando Y, Utsumi S, Ohgushi T. 2011. Community-wide impact of an exotic aphid on introduced tall goldenrod. *Ecol Entomol.* 36:643–653.

- Blossey B, Notzold R. 1995. Evolution of increased competitive ability in invasive nonindigenous plant – a hypothesis. *J Ecol.* 83:887–889.
- Brown JS, Eckert CG. 2005. Evolutionary increase in sexual and clonal reproductive capacity during biological invasion in an aquatic plant *Butomus umbellatus* (Butomaceae). *Am J Bot.* 92:495–502.
- Halverson K, Heard SB, Nason JD, Stireman III JO. 2008a. Origins, distribution, and local co-occurrence of polyploid cytotypes in *Solidago altissima* (Asteraceae). *Am J Bot.* 95:50–58.
- Halverson K, Heard SB, Nason JD, Stireman III JO. 2008b. Differential attack on diploid, tetraploid, and hexaploid *Solidago altissima* L. by five insect gall-makers. *Oecologia.* 154:755–761.
- Hawkes CV. 2007. Are invaders moving targets? The generality and persistence of advantages in size, reproduction, and enemy release in invasive plant species with time since introduction. *Am Nat.* 170: 832–843.
- Lawton JH. 1983. Plant architecture and diversity of phytophagous insects. *Annu Rev Entomol.* 28:23–39.
- Maron JL, Vila M, Arnason J. 2004. Loss of enemy resistance among introduced populations of St. John's Wort (*Hypericum perforatum*). *Ecology.* 85:3243–3253.
- Schlaepfer DR, Edwards PJ, Billeter R. 2010. Why only tetraploid *Solidago gigantea* (Asteraceae) became invasive: a common garden comparison of ploidy levels. *Oecologia.* 163:661–673.
- Schlaepfer DR, Edwards PJ, Semple JC, Billeter R. 2008. Cytogeography of *Solidago gigantea* (Asteraceae) and its invasive ploidy level. *Journal of Biogeogr.* 35: 2119–2127.
- Semple JC, Cook RE. 2006. *Solidago*. In: Flora North America Editorial Committee, editor, *Flora of North America*, vol. 20. Asteraceae, Part 2. Astereae and Senecioneae. Oxford (UK): Oxford University Press; p. 107–166.
- Shimizu T. 2003. Naturalized plants of Japan (in Japanese). Tokyo, Japan: Heibonsha.