An exotic herbivorous insect drives the evolution of resistance in the exotic perennial herb *Solidago altissima*

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Abstract. Invasive plants often experience rapid changes in biological interactions by escaping from their original herbivores at their new habitats, and sometimes re-associating with those herbivores afterwards. However, little is known about whether the temporal changes in herbivorous impact work as a selective agent for defensive traits of invaded plants. *Solidago altissima* (goldenrod) is a North American perennial that has widely invaded abandoned fields in Japan. Recently, an herbivorous insect *Corythucha marmorata* (lace bug), an exotic insect also from North America, which was first recorded in 2000 in Japan, has been expanding its habitat on *S. altissima* populations in Japan. In this study, we investigated whether the invasion of *C. marmorata* had a selective impact on the defensive traits of *S. altissima*, by conducting a field survey, a common garden experiment and microsatellite analysis. We compared quantitative genetic differentiation of traits (resistance, growth, and reproduction) and neutral molecular differentiation among 16 *S. altissima* populations with different establishment years of *C. marmorata*.

The common garden experiment, in which plants were grown in a greenhouse and treated to either *C. marmorata* herbivory or no herbivory, revealed the presence of higher resistance, sexual reproduction, and asexual (rhizome) reproduction in populations subjected to a longer history of *C. marmorata* pressure. Such phenotypic variability among establishment years of lace bugs was likely driven by natural selection rather than stochastic events such as genetic drift and founder effects. In addition, when plants were exposed to lace bug herbivory, resistance had a positive relationship with sexual and asexual reproduction, although no relationship was found when plants were free from herbivory. These findings suggest that defensive traits in *S. altissima* have evolved locally in the last decade in response to the selective pressure of *C. marmorata*.

Key words: biological invasion; Corythucha marmorata; *exotic insects; goldenrod; Japan; lace bug; perennial plant; plant defense; plant–insect interaction;* Q_{CT} *vs.* F_{CT}; *rapid evolution;* Solidago altissima.

Introduction

There is an increasing appreciation that ecological dynamics can be shaped over a very short time period by the rapid evolution of populations (Thompson 2013). In this context, biological invasion is being recognized as an ideal opportunity for studying adaptive evolution over contemporary timescales (Gurevitch et al. 2011). The genetic variation in introduced species often changes quickly because of selection and/or drift as these species expand into new ranges (Suarez and Tsutsui 2008). In spite of the fact that ecological and evolutionary processes that affect invading species can change over time, evidence for adaptive evolution in invasive species has been primarily obtained from short-term studies comparing traits in the native and invasive ranges. Thus, little is known about how ecological processes that influence trait evolution in invasive

species may change after its expansion (Lambrinos 2004, Hawkes 2007, Lankau et al. 2009).

Invasive plant species may evolve rapidly in response to changes in biological interactions (Mitchell et al. 2006); for instance, they can escape from their natural enemies, including their specialized herbivorous insects by moving to new areas (e.g., Elton 1958, Crawley 1987). The reduction in herbivory in a new range is hypothesized to cause a shift in the investment of resources from defense towards growth and/or reproduction and drive the evolution of increased competitive ability (EICA) in exotic populations (Blossey and Notzold 1995, Siemann and Rogers 2003, Colautti et al. 2004), although tests on this hypothesis have not been conclusive. Over a long timescale, herbivory in an exotic range may increase again due to interactions with generalist enemies in the exotic range (Parker et al. 2006) or re-association with enemies from the native range. For example, the chemical defense in parsnip, *Pastinaca sativa*, which initially declined in its new range upon release from a specialist herbivore, the webworm,

rapidly increased when the webworm was subsequently introduced into the new range (Zangerl and Berenbaum 2005). A recent study on the common ragweed, *Ambrosia artemisiifolia*, and its recently re-associated specialist herbivore, the leaf beetle, *Ophraella communa*, in the invasive range also suggested that *A. artemisiifolia* in the invasive range evolved its defense against the recently introduced specialist over the course of 10–12 years (Fukano and Yahara 2012). However, no studies have explicitly tested whether the re-association with herbivorous insects work as a selective agent for defensive traits of an invaded plant.

Identifying natural enemies as selective agents driving changes in plant defensive traits is difficult because other evolutionary forces, such as stochastic processes that include genetic drift and founder effect, can generate similar outcomes (Müller-Schärer et al. 2004). Thus, it is essential to separate out other factors to determine the selective agents of focal herbivores with regard to plant defensive traits. In addition, there is limited knowledge regarding the impacts of herbivores on the defensive traits of long-lived plants (Crawley 1985, Maschinski and Whitham 1989), since very few studies have attempted to assess the effects of herbivory on the lifetime reproductive success and growth of such plants (but see Doak 1992, Uriarte et al. 2002). Since many of the invasive plants are perennial, understanding how natural enemies alter traits in long-lived plants will provide new insights into evolution in a wide range of invasive plants.

Tall goldenrod, *Solidago altissima* (Asteraceae), is a rhizomatous, herbaceous perennial native to a variety of successional habitats such as prairies, woodland edges, and old agricultural fields throughout eastern North America. It was introduced to Japan in the early 20th century as an ornamental plant and has now spread throughout Japan (Shimizu 2003). Since more than 100 species of generalist and specialist herbivores feed on *S. altissima* in its native range, *S. altissima* has been well studied as a model plant for insect–plant interactions (e.g., Maddox and Root 1987, Abrahamson and Weis 1997). A number of studies have revealed large genetic variability in goldenrod's resistance to insect herbivory (Maddox and Root 1987, Craig et al. 2007, Utsumi et al. 2011), and herbivory was explained as a major agent of natural selection in *S. altissima* (Bode and Kessler 2012). Recently, two exotic insects have been introduced to Japan from North America: the aphid, *Uroleucon nigrotuberculatum* (Aphididae; Hemiptera), in the early 1990s and the lace bug, *Corythucha marmorata* (Tingidae; Hemiptera; Appendix E: Fig. E1) in 2000. While the aphid has been widespread and abundant in Japan (Ando et al. 2010, Sakata et al. 2013b), the lace bug is still expanding its habitat concentrically, and the number of years since its establishment exhibits geographic variability (Kato and Ohbayashi 2009). The lace bug is one of the most abundant herbivores of *Solidago* spp. in its native North America (Cappuccino and Root

1992, Fontes et al. 1994), and was noted to cause major herbivore damage during the summer in an experimental garden (Root 1996). The recent expansion of the lace bug in Japan provides a promising opportunity to examine the selective impacts of this herbivorous insect on defensive traits in *S. altissima*.

In this study, we hypothesized that defensive genotypes are selectively favored under lace bug damage in *S. altissima* populations, resulting in elevated resistance (i.e., less damaged) and tolerance (i.e., compensatory regrowth) in populations with a longer establishment of lace bugs. To test this hypothesis, we investigated geographic variation in defense levels in *S. altissima* populations across Japan and compared them in terms of the establishment years of lace bugs. Specifically, we addressed the following questions: (1) Do the abundances and damage levels of lace bugs differ among natural *S. altissima* populations with different establishment years of lace bugs? (2) Are there phenotypic differences in the defense, growth, and reproductive traits of *S. altissima* among populations with different establishment years? (3) If so, does natural selection imposed by the lace bugs play an important role in observed phenotypic differences in *S. altissima*? To accomplish these objectives, we first conducted a two-year field survey of lace bug abundance and leaf damage in populations with different establishment years of lace bugs to investigate current interactions between the exotic plant and its re-associated exotic enemy. Second, we used a joint approach, linking population genetics with molecular techniques and a common garden experiment, to elucidate whether populations of *S. altissima* have differentiated and evolved adaptively through diversification or stabilized selection in response to the selective agent of lace bugs. We compared the estimates of genetic differentiation at neutral loci (F_{CT}) and phenotypic differentiation (Q_{CT}) in 16 *S. altissima* populations among different establishment years of lace bugs. Additionally, because the impact of herbivores on plant fitness determines selection strength for defenses, we explored the relationship between resistance to the lace bugs, and other traits related to growth and reproduction to understand how resistance affects the fitness of *S. altissima*.

Methods

Field survey

Population surveys for *S. altissima* and *C. marmorata* were conducted in 50 *S. altissima* populations in June in 2011 and June 2012 (Appendix B: Table B1; Appendix F: Fig. F1), and six populations (KG, KA, KYM, TT, SF, and KR; see Appendix B: Table B1 for abbreviations) were surveyed again in July and August 2012. Populations were identified as aggregations of plants in abandoned areas such as riversides and old agricultural fields. All the surveyed populations were in an early successional stage with the patch sizes being larger than 25 m^2. The distance between two adjacent populations

was at least 1 km. We surveyed three ramets per genotype for 5–10 genotypes that were randomly selected, distinguished by clumps in each population, and 2–11 populations per year since lace bug establishment. For each ramet, the number of lace bug individuals and damage levels of lace bug herbivory were recorded. The damage level was calculated by classifying the damaged leaves exhibiting yellow scars induced by the lace bug feeding into four levels: (1) no damage, (2) <33% damage of total leaf area, (3) 33%–66% damage, and (4) >66% damage. Then, we counted the number of leaves for each damage level, added the values for all of the four levels, and finally divided this figure by the total number of leaves.

We explored factors influencing the numbers and the damage levels of lace bugs using generalized linear mixed models (GLMMs). First, the number of lace bugs per ramet was selected as response variable, while the latitude and number of years since establishment were set as explanatory variables. We included latitude as an explanatory variable since there are prominent hypotheses that the amount of herbivory and investment in plant defense should increase with decreasing latitude (Dobzhansky 1950, Pennings and Silliman 2005), although these hypotheses have not been closely scrutinized (Moles et al. 2011). The year since lace bug establishment for each population was determined by the recorded date on the Special Notice of the Plant Protection Office in each prefecture. The natural logarithm of the leaf number was entered into the model as an offset term to control for differing numbers of leaves in each ramet. An assumed regression coefficient for this offset term is 1. We included the following three variables to the model as random effects: (1) genotypes nested within a population and populations nested within a prefecture, (2) date of the survey, and (3) year of the survey. Next, we considered the damage level per ramet to be a response variable and considered the same set of candidate explanatory variables, and offset and random effects as in the model for the lace bug numbers. We used a Poisson distribution to map the error structure and logarithms as link functions in the GLMM. We then selected the best model that had the lowest AIC value. The analyses described here were conducted using the lme4 package (Bates et al. 2011) of R 2.13.1 (R Development Core Team 2011).

Microsatellite analysis

We conducted a microsatellite analysis to examine the genetic differentiation at neutral loci in *S. altissima* populations among different establishment years of lace bugs. In June 2011, leaf samples of 349 plants from 16 populations (Appendix B: Table B1; Appendix F: Fig. F1) of *S. altissima* were collected, and we analyzed these leaves using previously developed 16 microsatellite markers (Sakata et al. 2013a) for analysis (Appendix A: section A-1). Since it was not possible to determine genotypes and allele frequencies from peak heights because of the high ploidy level (only hexaploid plants have been found in Japan [Sakata et al. 2013b]), multilocus phenotypes, recording the presence and identity of alleles at each locus, were produced for each individual. We measured genetic diversity, genetic relationship among individuals, and F_{CT} (Appendix A: section A-2). F_{ST} and Q_{ST} are the estimation among populations (where F_{ST} is the genetic differentiation at neutral loci among populations, and Q_{ST} is the phenotypic differentiation among populations), while F_{CT} and Q_{CT} are the estimation among groups or regions (i.e., invasion stages of lace bugs in our analyses).

Trait measurements

We conducted a common garden experiment to examine the phenotypic traits of *S. altissima* from populations with different establishment years of lace bugs. In June 2011, we collected rhizome segments of 10 genotypes from a widely separated area in each population during a field survey of 16 populations (Appendix B: Table B1). The segments were then planted in a greenhouse at the Center for Ecological Research, Kyoto University, Japan (34.97136° N, 135.957892° E) and cultivated for one year to remove maternal effects. Populations were classified into four categories of invasion stages of the lace bugs (A, no lace bug invasion; B, 3–5 years of lace bug establishment; C, 6–8 years of lace bug establishment; and D, 9–11 years of lace bug establishment; each invasion stage containing 3–5 populations) to calculate phenotypic differentiation (Q_{CT}). We could not estimate Q_{CT} among establishment years because more than two populations are required in each establishment year for this analysis, which would have been impractical for the common garden experiment. Hence, we estimated Q_{CT} among different invasion stages. All collected plants were checked with microsatellite analysis and confirmed to be different clones. On 20 May 2012, rhizomes were cut into 6 cm long segments with a diameter of 5 mm; four ramets of identical genotype (640 plants in total) were grown in a greenhouse for two months. In mid-July, all but one shoot were eliminated to adjust plants in the same size for each ramet. Two ramets per genotype were assigned to lace bug treatment (320 plants in total) and the other two to undamaged control plants (320 plants in total). We measured traits including resistance, vegetative growth and reproductive traits (Appendix A: section A-3).

Analysis of quantitative traits

We conducted four different analyses of quantitative traits. First, we explored factors influencing phenotypic differences using linear mixed effects models (LMMs). We set the establishment years of the lace bugs and the latitude of origin of these populations as candidate explanatory variables for resistance. Additionally, we measured the effect of treatment (damage or control)

and the interaction term of treatment and establishment year for other traits. We assumed that phenotypic differences were dependent on the effect of establishment year of the lace bugs. To meet the assumption of a normal distribution and homoscedasticity, the data were square-root-transformed. For lace bug population growth, the number of lace bugs (total, adults, and nymphs) divided by the initial population size (i.e., four individuals) was set as the response variable. For damage levels, damage scores divided by the number of green leaves were set as the response variable. For the growth rate of plant height, leaf number, and stem width, the last recorded measurement divided by the initial size (the first measurement) for each trait was set as a response variable. For flower and underground biomass, number of estimated flowers, and the number of rhizomes and rhizome length were set as response variables. For all models, genotypes nested within a population and populations nested within a prefecture were included in the model as random effects. In order to determine whether tolerance was associated with lace bug establishment year, we analyzed the relationship among the establishment year of lace bugs and tolerance related traits using linear models (LMs). Tolerance in each population was estimated as the slope of linear regression of the plant growth and reproductive traits against the lace bug damage level in sixth week per ramet. The analyses described here were conducted using nlme package (Pinheiro et al. 2013) of R 2.13.1 (R Development Core Team 2011).

Second, to determine how resistance affected plant fitness, we analyzed relationships among lace bug growth rate, damage level, and other traits related to growth and reproduction (growth rate of height, leaf number, stem width, flower number, rhizome number, and rhizome length) in both lace bug and control treatments using linear models (LMs). Each trait was set as response variable, while the lace bug growth rate and damage level were set as explanatory variables. For the control treatment, we measured the lace bug growth rate and damage level of the same genotypes as in the lace bug treatment. The analyses described here were conducted using R 2.13.1.

Finally, we estimated Q_{CT}, the estimation of phenotypic differentiation among invasion stages (Appendix A: section A-4). On the basis of the method used by Keller and Taylor (2008), combining the LMM analyses and the Q_{CT} vs. F_{CT} test can be interpreted as follows: (1) a significant effect of establishment year with $Q_{CT} > F_{CT}$ suggests that selection drives phenotypic divergence; (2) a significant effect of establishment year with $Q_{CT} = F_{CT}$ indicates phenotypic divergence driven by stochastic processes; (3) a nonsignificant effect of establishment year with $Q_{CT} < F_{CT}$ means that stabilizing selection results in the maintenance of similar traits; and (4) a nonsignificant effect of establishment year with $Q_{CT} = F_{CT}$ suggests that no statistically detectable phenotypic evolution is present.

Results

Field survey

Lace bugs were the most abundant herbivores, and native generalist herbivores were scarce during our survey. The only herbivores that were abundant other than lace bugs were the exotic aphid, *Uroleucon nigrotuberculatum*. However, these aphids were abundant only in June across the sites (Y. Sakata, *unpublished data*). No clear differences were observed in lace bug abundance and damage level in June among different establishment years (Appendix G: Fig. G1). The GLMM analyses revealed that neither the establishment year nor latitude significantly affected both lace bug abundance and damage level in June. The number of lace bugs did not change significantly in July and August, while the damage level gradually increased in most populations up to August (Appendix G: Fig. G2).

Genetic diversity and differentiation

The 16 microsatellite loci were highly polymorphic with a range of 11–50 (average 26.8) alleles per locus, and each individual exhibited a unique allele phenotype. The intra-population genetic diversity indices (N_a [number of alleles per locus, averaged over loci], N_p [number of private alleles per locus, averaged over loci], H_o [gametic heterozygosity], Shannon, and 1/Simpson) are indicated in Appendix C: Table C1.

Relationships between individuals, as visualized by principal coordinate analysis (PCoA) based on the two distance measure, illustrated that Hokkaido samples largely clustered separately from the other regions' samples (Appendix C: Fig. C1). This suggests that allele frequencies differ between the two regions, but the pattern is poorly related, or weakly unrelated, to allele size.

AMOVA results revealed that most genetic variation was distributed within populations, with only small variances attributing to differences between populations (7% and 9% for the Bruvo and Lynch distance matrices, respectively; see Appendix C: Table C2).

Genetically based differentiation in defense, growth, and reproduction

Lace bug population growth rate (except for the adult lace bug in sixth week) and damage level were negatively affected by the number of years of establishment of lace bugs (Fig. 1a–d; Appendix D: Table D1), but were not affected by latitude. The proportion of plants whose aboveground parts succumbed to lace bug damage was significantly higher in non-invaded populations than in other populations (A, non-invaded, 36% ± 8%; B, 3–5 years, 14% ± 3%; C, 6–8 years, 9% ± 5%; D, 9–11 years, 5% ± 2%; see Appendix H: Fig. H1a).

Attack by lace bugs decreased plant height and stem width, but it resulted in increased leaf number (Fig. 1e–g; Appendix D: Table D1). The number of years of lace bug establishment did not affect aboveground plant
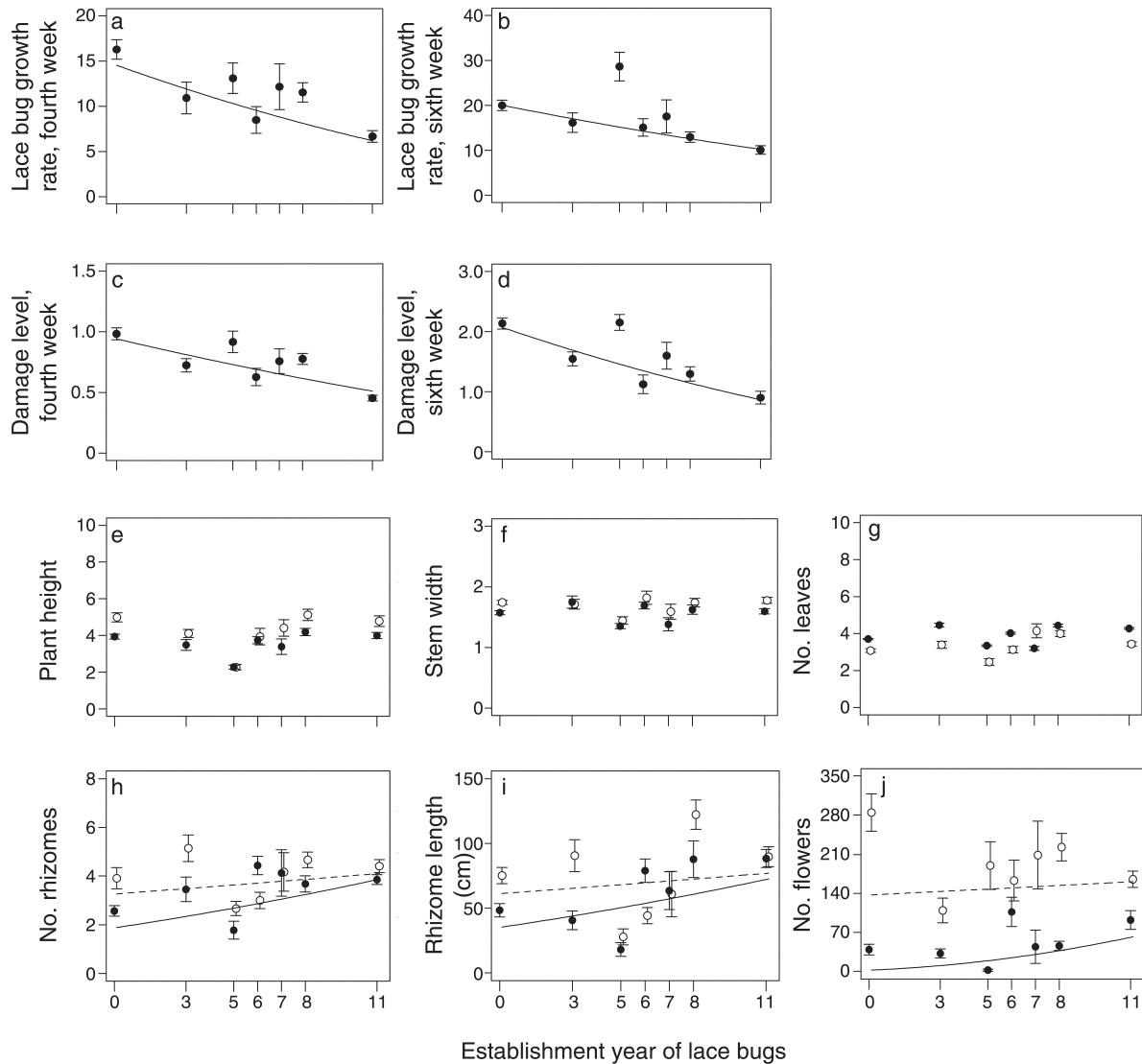
FIG. 1. (a–d) Resistance indices and (e–j) growth and reproductive traits (mean ± SE) among all ramets of goldenrod (*Solidago altissima*) for each establishment year of lace bugs (*Corythucha marmorata*) in the common garden experiment. Growth rate shows the lace bug population growth, and damage level show the damage scores of lace bugs divided by the number of green leaves. Solid and open circles represent the lace bug treatment and control treatment, respectively. Plant height, stem width, and number of leaves indicate the growth rate (×100%) of each trait in a two-month period from 10 July to 10 September. Lines represent a significant relationship with the establishment year in the lace bug treatment (solid line) and control treatment (dashed line). The number of populations in each establishment year (0, 3, 5, 6, 7, 8, and 11) was 5, 2, 2, 1, 1, 1, and 4, respectively.

growth rate in terms of height, stem width, or number of leaves (Fig. 1e–g; Table D1). For underground biomass, lace bug treatment had a negative effect and establishment year had a positive effect (Fig. 1h, i; Table D1). Because the interaction term of establishment year and treatment had a positive effect, and the P value of the establishment year effect was high for both rhizome number and length, the establishment year term did not significantly affect rhizome number or length in the control treatments (Appendix D: Table D1). For the number of flowers, which represents a sexual reproduc-

tion index, many plants from the lace bug non-invaded populations and earlier invasion stage-populations failed to flower in the presence of lace bug damage (Fig. 1j). The proportion of plants that did not flower was significantly higher in non-invaded and invasion stage B populations than in invasion stage C and D populations (A, 77.3% ± 9%; B, 53.4% ± 23%; C, 33.9% ± 11%; D, 42.2% ± 5%; see Appendix H: Fig. H1b). The lace bug treatment negatively affected the number of flowers (Fig. 1j), while latitude and establishment year had a positive effect (Appendix D: Table D1). Because

TABLE 1. Mean and 95% confidence limits (CL) of Q_{CT} (phenotypic differentiation) with that of F_{CT} (genetic differentiation at neutral loci), and the probability in the Lewontin and Krakauer (1973) test for each quantitative trait of goldenrod (*Solidago altissima*).

Trait	Q_{CT}			
	Mean	2.5% CL	97.5% CL	P
Growth rate, fourth week	0.140	0.105	0.184	**0.000**
Growth rate, sixth week	0.113	0.068	0.158	**0.000**
Damage level, fourth week	0.237	0.166	0.316	**0.000**
Damage level, sixth week	0.156	0.069	0.269	**0.000**
Plant height	0.081	2.15×10^{-8}	0.196	0.196
No. leaves	0.045	4.07×10^{-9}	0.123	0.489
Stem width	1.42×10^{-5}	0.000	0.000	**1.000**
No. rhizomes	0.086	0.068	0.101	**0.000**
Rhizome length	0.176	0.145	0.208	**0.000**

Notes: The mean F_{CT} value was 0.039, with 2.5% and 97.5% CL of 0.032 and 0.047, respectively. P values in boldface type indicate where significant differences between F_{CT} and Q_{CT} occurred. $P < 0.025$ indicates $Q_{CT} > F_{CT}$, while $P > 0.975$ indicates $Q_{CT} < F_{CT}$. The sample sizes for resistance indices (lace bug [*Corythucha marmorata*] growth rate and damage level in the sixth week) and other traits were $n = 320$ and $n = 640$, respectively. The number of populations for each invasion stage (Appendix A: section A4), A (no lace bug invasion), B (3–5 years of lace bug establishment), C (6–8 years of lace bug establishment), and D (9–11 years of lace bug establishment), was 5, 4, 3, and 4, respectively.

the interaction term of establishment year and treatment had a positive effect on the number of flowers, and the P value of the establishment year term was high, the establishment year did not significantly affect the number of flowers in the control treatments (Fig. 1j; Table D1).

Negative relationships were found between resistance indices and growth rate of leaf numbers (except for lace bug growth rate in the lace bug treatment). In the lace bug treatment, additional negative relationships were found between (1) resistance indices and flower numbers, and (2) damage level and rhizome production, while there was a positive relationship between the damage level and flower numbers in the control treatment (Appendix D: Table D2). On the other hand, we did not find any significant relationships between establishment year and tolerance related traits (Appendix D: Table D3).

Selection vs. stochastic events

In the Q_{CT} vs. F_{CT} analysis, both the lace bug growth rate and damage level in the fourth and sixth weeks, and traits tied to asexual reproduction (rhizome number and length) showed significant differences across all the four invasion stages with Q_{CT} being significantly larger than F_{CT}, indicating selection-driven divergence (Table 1). Because many plants did not flower, it was impossible to estimate Q_{CT} for the number of flowers. Plant height and the number of leaves displayed nonsignificant invasion stage effects and had similar Q_{CT} and F_{CT} values, indicating no statistically detectable phenotypic evolution. On the other hand, F_{CT} was significantly larger than Q_{CT} for stem width, indicating stabilized selection maintaining similar traits.

DISCUSSION

In this study, we found a strong possibility of rapid evolution of defense of *S. altissima* across natural populations with different years of establishment of lace bugs, suggesting that the lace bug invasion may have worked as an evolutionary force selecting for defensive traits in the invasive perennial plant.

Our LMMs analysis in the common garden experiment showed that *S. altissima* populations with longer establishment years of lace bugs had higher resistance, sexual reproduction, and underground asexual reproduction than populations that were non-invaded or had shorter establishment years of lace bugs (Appendix D: Table D1). The result that latitude did not affect resistance indicates that phenotypic difference in resistance could not be explained by latitudinal clines across introduced populations. Although the range of the latitude in this study might not be large enough to detect the latitudinal gradients in herbivory, this is consistent with the meta-analyses by Moles et al. (2011). They argued that it was unlikely that defense and herbivory covary with latitude, or at least empirical studies that apply consistent, rigorous methods across many sites along broad latitudinal gradients are lacking. In our field survey, neither effect of the establishment year nor latitude significantly affected both lace bug abundance and damage level. This discrepancy between the results of the field survey and the common garden experiment may be due to weather conditions on the survey days or differences in local predator communities and seasonal variations, making it difficult to detect variations in plant resistance among natural populations with different lace bug establishment years.

The analysis of Q_{CT} vs. F_{CT}, which was not affected by genetic admixture, showed that *S. altissima* populations with different invasion stages exhibited significantly higher phenotypic differentiation in resistance and underground asexual reproduction. On the other hand, the growth rate of aboveground plant parts showed no difference in stem height and leaf numbers, or significantly lower phenotypic differentiation in stem width

than neutral marker differentiation among invasion stages (Appendix D: Table D2). This indicates that directional selection on resistance and underground asexual reproductive traits resulted in the observed phenotypic divergence among invasion stages, although phenotypic divergence in other above-ground growth traits was not detected among invasion stages.

Therefore, by combining the results of the two analyses using LMM and Q_{CT} vs. F_{CT}, we concluded that stochastic events and latitudinal clines are unlikely to explain the observed phenotypic divergence in resistance and underground asexual reproduction when comparing populations with different lace bug establishment years. Rather, it was more likely to be driven by stage-specific selective pressure, indicating a role of lace bugs as a selective force. In addition to the previous studies showing that herbivorous insects can exert selection pressure on defensive traits in invasive short-lived plants (e.g., Zangerl and Berenbaum 2005, Fukano and Yahara 2012), our study suggests that herbivorous insects may also impose selection on defensive traits in long-lived plants. Thus, the rapid evolution of defensive traits in invasive plants, regardless of the difference in their life histories, may be widespread and ubiquitous.

A large number of studies based on optimal defense theory (McKey 1979, Rhoades 1979) and the resource availability hypothesis (Coley et al. 1985) have attempted to explain observed patterns in investment to defense and growth in plants (Stamp 2003). On the other hand, the defense investment may not always have a trade-off with the investment to growth because a wide range of defensive chemicals have other functions involved in ecological interactions such as intra- and interspecific competition (Lankau and Kliebenstein 2009). Our LMMs analysis in the common garden experiment indicates that the length of the lace bug establishment period positively affected sexual and asexual (rhizome) reproduction in lace bug treatments, but had weak or insignificant effects in control treatments (Appendix D: Table D1). In addition, there was a positive relationship between resistance and rhizome production in lace bug treatments, but not in control treatments (Appendix D: Table D2). This implies that under lace bug herbivory, lace bug-resistant plants have greater underground asexually reproductive ability. Furthermore, the relationship between resistance and flower numbers, which was positive in the lace bug treatment, but negative in the control treatment (Appendix D: Table D2), implies that low resistant plants have larger sexual reproductive ability in the absence of lace bugs, and that there may be a trade-off between resistance and sexual reproduction. On the other hand, a positive relationship was found between resistance and leaf growth rate with respect to aboveground growth traits (Appendix D: Table D2). This suggests that the evolution of defensive traits may depend on not only multiple types of costs, but also multiple types of benefits, such as secondary compounds that act as both defenses and allelopathic agents

(Lankau and Kliebenstein 2009). Although we did not find any significant relationships between establishment year and the tolerance related traits in the common garden experiment (Appendix D: Table D3), we cannot deny a possibility that the measure of tolerance is confounded with resistance in our experiment. An additional experiment to separate the effects of the reduced herbivory and increased tolerance in resistant genotypes is necessary to examine the difference in tolerance.

The considerable genetic diversity observed within populations of *S. altissima* (Appendix C: Tables C1 and C2) is consistent with some clonal species that maintain sexual reproduction, resulting in surprisingly high levels of within-population genetic diversity even when combined with clonal spread, which might reduce genetic diversity within populations (e.g., Ward et al. 2008). In addition, the high ploidy level of *S. altissima* in Japan may contribute to its high genetic diversity within populations, since polyploids are expected to maintain high levels of heterozygosity and diversity of genome doubling and/or hybridization during formation (Soltis and Soltis 2000). This provides fundamental advantages for rapid adaptation toward selective pressure such as herbivory in new ranges (Fawcett and de Peer 2010).

Additional mechanisms may contribute to increasingly stronger resistance in *S. altissima* in populations with longer lace bug establishment periods. First, multiple introductions from genetically distinct demes in the native range may lead to divergence in quantitative traits of introduced species (Keller and Taylor 2008). The PCoA analysis clearly separated the populations of Hokkaido from other regions, suggesting that the lower resistance in lace bug absent populations (including Hokkaido region) may be simply reflected by the differences between the native demes of introduced populations. However, even when we eliminated the Hokkaido population data sets from the LMM and Q_{CT} vs. F_{CT} analyses, the establishment year significantly explained the resistant indices, and Q_{CT} was significantly larger than F_{CT} for the resistant indices (e.g., $F_{CT} = 0.038$; $Q_{CT} = 0.10$ for lace bug growth rate [$P < 0.025$], $Q_{CT} = 0.16$ for damage rate in the sixth week [$P < 0.025$]). Second, it cannot be ruled out that the lower resistance in populations with fewer years of lace bug establishment may be explained by the effect of plant stress; environmental stress such as drought increases plant susceptibility to insect herbivory by altering leaf chemistry and whole plant physiology (White 1969, Jones and Coleman 1991). Since the common garden is located in a mid-latitude location, plants from the high- and low-latitudinal populations that have shorter lace bug establishment periods, may have lowered performance in resistance because of environmental stressors, such as extremes in temperature and precipitation. In future research, it is necessary to conduct a reciprocal common garden experiment to separate the environmental factors from evolutionary effects. Third,

microsatellite markers, which were highly variable, may underestimate F_{CT} (Hedrick 2005), because F_{CT} is largely dependent on within-population heterozygosity (Hedrick 2005, Jost 2008). Our estimates (the unbiased estimate of F_{CT}, $G'_{ST} = 0.13$ and $D_{EST} = 0.11$) that accounted for this problem were greater than F_{CT}. However, it has been recently reported that these measures are inappropriate to compare with Q_{CT} (Edelaar and Bjorklund 2011), and our estimates were still low enough to suggest gene exchange among populations. Furthermore, given that there were only two sub-clonal replicates, the within- and among-individual variation were likely to be overestimated; hence, our estimation of Q_{CT} tends to be conservative. Finally, carryover effects, which are known to affect plant phenotypic plasticity, may have inflated the additive genetic variation within populations. For instance, plants collected from lace bug invaded populations may have exhibited higher resistance due to an induced defense in response to herbivory. Hakes and Cronin (2012) experimentally demonstrated that defensive phenotypes in *S. altissima* may exhibit qualitative changes during succession, indicating phenotypic plasticity. Recently, epigenetic effects (i.e., DNA methylation and histone modification) were demonstrated to contribute to ecologically important phenotypic differences (Richards et al. 2010) and become especially active in response to stressful environments (Verhoeven et al. 2010) such as strong herbivory. Although the *S. altissima* plants used for the common garden experiment were raised for one year to alleviate carryover effects, using second or later generation plants may be necessary to completely rule out the inheritance of plastic responses.

Recent studies on short-lived plants using multi-generation selection experiments have demonstrated that herbivores play a major role in shaping plant defenses and that clear evidence of changes in genotype frequencies exists between herbivory and non-herbivory treatments (Agrawal et al. 2012, Züst et al. 2012). Because *S. altissima* is a perennial plant that can persist for decades, it is unlikely that genotypes susceptible to the lace bug completely disappeared in the populations with 12 years of lace bug establishment. We hypothesize that the susceptible genotypes may decrease their abundance in populations by failing to establish new seedlings because of their lower sexual reproductive rates, in addition to decreasing in coverage (i.e., number of ramets per genotype) due to the difference of rhizome production in following years. Our results are also consistent with an experimental study using *S. altissima* in the United States (Bode and Kessler 2012), which demonstrated that plants released from herbivory by the use of insecticides for 12 years displayed greater proportions of genotypes resistant to two different specialist herbivorous insects than plant populations that were exposed to natural herbivory. The results presented in our study clearly demonstrated the potential for the rapid evolution of enhanced resistance in a long-lived plant enforced by a re-associated enemy in nonindigenous environments.

Conclusions

Although there is an increasing body of evidence suggesting the occurrence of rapid evolution in defensive traits in invasive plant species (e.g., Blossey and Notzold 1995, Siemann and Rogers 2003, Colautti et al. 2004, Huang et al. 2010, Franks et al. 2012), most studies have mainly focused on defensive traits between invasive and native ranges in a snap shot of time. Our results demonstrated the longer temporal dynamics of plant defense revealed by a gradual increase in resistance over a decade. These findings will promote the understanding of how biological interactions after establishment are critical in shaping evolution in invasive plants. Finally, a growing number of studies are reporting interactions of exotic plants and exotic herbivorous insects in both natural (Ando et al. 2010, Chang et al. 2011, Stricker and Stiling 2012) and managed systems (Wheeler and Schaffner 2013). Although Garcia-Rossi et al. (2003) pointed out that the large genotypic variation in plant tolerance to the original specialist planthopper *Prokelisia marginata* is likely to decrease effectiveness of biological control using the planthopper in the invasive *Spartina* spp. cordgrass populations, evolution of defensive traits in weeds under the introduction of its natural enemy as a biocontrol agent is still largely overlooked. Our results make a caution that classical biocontrol programs for weeds, irrespective of short lived or long lived, may not be sustainable in even a short period of time. These results highlight the importance of considering the evolutionary dynamics of invaders for successful weed management.

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Supplemental Material

Appendix A

Supplemental information for material and methods of the microsatellite genotyping and analyses, trait measurements and analyses, and Q_{CT} vs. F_{CT} analyses (*Ecological Archives* E095-224-A1).

Appendix B

A table of population collections of *Solidago altissima* used in this study (*Ecological Archives* E095-224-A2).

Appendix C

Tables and a figure of the results of the microsatellite analysis of *S. altissima* (*Ecological Archives* E095-224-A3).

Appendix D

Tables of the results of traits of *S. altissima* in the common garden experiment (*Ecological Archives* E095-224-A4).

Appendix E

A photograph of lace bugs (*Corythucha marmorata*) and *S. altissima* with lace bug damage (*Ecological Archives* E095-224-A5).

Appendix F

A map of geographic distributions of field survey sites of *S. altissima* populations and geographic differences of lace bug establishment years (*Ecological Archives* E095-224-A6).

Appendix G

A figure of lace bug abundance and damage level in the field survey and common garden experiment (*Ecological Archives* E095-224-A7).

Appendix H

A figure of the effect of the invasion stage of lace bugs on the proportion of aboveground die down and on the proportion of plants without flowers (*Ecological Archives* E095-224-A8).