Native jewelweed, but not other native species, displays post-invasion trait divergence

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Invasive exotic plants reduce the diversity of native communities by displacing native species. According to the coexistence theory, native plants are able to coexist with invaders only when their fitness is not significantly smaller than that of the exotics or when they occupy a different niche. It has therefore been hypothesized that the survival of some native species at invaded sites is due to post-invasion evolutionary changes in fitness and/or niche traits.

In common garden experiments, we tested whether plants from invaded sites of two native species, *Impatiens noli-tangere* and *Galeopsis speciosa*, outperform conspecifics from non-invaded sites when grown in competition with the invader (*Impatiens parviflora*). We further examined whether the expected superior performance of the plants from the invaded sites is due to changes in the plant size (fitness proxy) and/or changes in the germination phenology and phenotypic plasticity (niche proxies).

Invasion history did not influence the performance of any native species when grown with the exotic competitor. In *I. noli-tangere*, however, we found significant trait divergence with regard to plant size, germination phenology and phenotypic plasticity. In the absence of a competitor, plants of *I. noli-tangere* from invaded sites were larger than plants from non-invaded sites. The former plants germinated earlier than inexperienced conspecifics or an exotic congener. Invasion experience was also associated with increased phenotypic plasticity and an improved shade-avoidance syndrome. Although these changes indicate fitness and niche differentiation of *I. noli-tangere* at invaded sites, future research should examine more closely the adaptive value of these changes and their genetic basis.

Biological invasions have profound ecological impacts on invaded communities (Lodge 1993, Levine et al. 2003). Exotic species reduce the abundance of native species through direct interactions, such as predation (Rodda et al. 1977) or competitive exclusion (Porter and Savignano 1990), or indirectly, e.g. by disruptions of mutualism among resident organisms (Stinson et al. 2006). Exotics may also negatively influence the populations of native species by changing environmental conditions, e.g. nutrient cycling (Vitousek et al. 1987), or by altering hydrologic (Zavaleta 2000) or fire regimes (D’Antonio 2000). As a consequence, invasive exotics may drive native species to local or even global extinction (Carlton et al. 1999).

Exotic species that dominate and thus replace native species have understandably received much attention. Multiple examples show, however, that resident species may coexist alongside invaders due to pre-adaptation or phenotypic plasticity (see examples in a review by Strauss et al. 2006). As an example of the former, exotic plants attract native herbivores and may be integrated into their diets (Solarz and Newman 2001). A study by Hejda et al. (2009) showed that loss of diversity is reduced if both the plant invaders and residents are similar in height. Phenotypic plasticity helps natives to cope with exotics by maintaining populations, even in invasion-altered environments (MacNeil et al. 2001, Peacor et al. 2006).

In a recent study, MacDougall et al. (2009) proposed a unifying framework based on a coexistence theory (Chesson 2000) to determine invasion outcomes. The authors assume that the success of exotics in native communities depends on either a fitness advantage or a difference in the niche of the resident species. The position of the invader along the two axes describing these two distinct sources of differences can be then used to determine whether coexistence is possible or whether natives are likely to be displaced. The third option is that residents repel the invaders.

Although the framework proposed by MacDougall et al. (2009) may greatly advance our understanding of invasions and their impact on native communities, it does not take into account that the relative differences in fitness and niche between invaders and residents can change during the invasion process. Evolutionary mechanisms, such as the rise of superior genotypes of invaders through hybridization (Ellstrand and Schierenbeck 2000, Lavergne and
Molofsky 2007) or through the emergence of clines adapted to local environments (Maron et al. 2004, Godoy et al. 2011), are well-documented. These processes may then lead to a larger fitness advantage or increased similarity between the niches of invaders and residents, making coexistence less likely. What has largely been ignored is the response of native species to invaders and the evolutionary consequences of this interaction. Given the profound impacts of invaders on the abundance and fitness of natives, exotics may exert strong and potentially novel selection pressures that can facilitate fast evolutionary adjustments in native species (Strauss et al. 2006, Carroll et al. 2007). Selection of the most adapted genotypes of residents can, contrary to the evolutionary differentiation between invaders and residents or reduce the fitness advantage of invaders and thus increase the probability of coexistence.

Evolutionary adjustments of native organisms to exotics are documented for a variety of ecological contexts, including invasive species as competitors and predators of native species or as hosts of native herbivores or pathogens (Mooney and Cleland 2001, Strauss et al. 2006). Studies documenting the adaptation of native plants to exotic plant invasions are limited (Callaway et al. 2005, Lau 2006, Mealor and Hild 2007, Leger 2008).

In this study, we explored whether invasion experience leads to adaptations in two native plants to the presence of an invader. Our study system consisted of the exotic annual jewelweed Impatiens parviflora and two native annual species, congeneric species Impatiens noli-tangere and the more distantly related but ecologically similar species Galeopsis speciosa. In a series of common garden experiments, we examined the differences in performance of plants from invaded and invasion-free sites, assuming better performance of the former populations when interacting with the invader.

Prior to the common garden experiments, we first assessed the differences between the invaded and non-invaded sites with respect to 13 environmental and biotic parameters. We did this because the differences in performance between plants from invaded and non-invaded sites can arise due to not only invasion but also contrasting environmental or biotic conditions between both site types already present before the establishment of exotics.

In the second part, we assessed the competitive effects of I. parviflora in the field and common garden. In the field study, we were interested in whether the effect of the co-occurring biomass on the biomass of I. noli-tangere and G. speciosa differed between plots with and without the presence of I. parviflora. We hypothesized that divergence between the invaded and non-invaded native populations is more likely if the exotic invader has a more profound competitive effect on the two study native species than the co-occurring native plants. In the common garden experiment, we tested the competitive response and competitive effect of invaded and non-invaded populations of I. noli-tangere and G. speciosa. We predicted that invasion would select for larger plant size in natives (but see Rowe and Leger 2011 for the opposite pattern). Our assumption was based on studies demonstrating that larger plants have a stronger competitive effect and a weaker competitive response (Goldberg and Fleetwood 1987, Gaudet and Keddy 1988, Dostál 2011).

Because the coexistence probability of exotic and native species is a function not only of fitness differences but also of niche differences (MacDougall et al. 2009), in addition to changes in traits related to competitive ability we studied changes in traits linked to niche differentiation. Therefore, in the final two parts of our study, we studied post-invasion changes in germination and phenotypic plasticity. We expected that invaded populations would differ in germination phenology from not only non-invaded conspecifics but also the invader because asynchrony of species resource use is assumed to promote their coexistence (Grubb 1977, Rathcke and Lacey 1985). Finally, we analyzed differentiation in phenotypic plasticity. Although we did not directly measure how invasion of I. parviflora changes resource availability for the native species, we assumed that resources are less available at invaded sites (which was tested by the field assessment of competitive effects of the invader on both native species in the second part of this study). We thus hypothesized that native plants from invaded sites would be more tolerant to low levels of these resources but would not differ from plants from non-invaded sites when resources are abundant.

Methods

Study system

Impatiens parviflora (Balsaminaceae), which is native to central Asia, was first introduced to Europe in a botanical garden in Geneva in 1831 (Coombe 1956). In the Czech Republic, it was first cultivated in 1844 at a botanical garden in Prague and first recorded in the wild in 1870 (Slavík 1997). At present, I. parviflora is one of the most widespread invasive species in Europe and is reported in 34 European countries (Lambdon et al. 2008). It can form large, sometimes monospecific, dense stands (covering several hundred square meters, with densities up to 100 fruiting individuals per square meter; Dostál unpubl.) and presumably displaces native species (Skálová and Pyšek 2009).

Post-invasion trait divergence was investigated in two native species: Impatiens noli-tangere, a close native relative of I. parviflora, and Galeopsis speciosa (Lamiaceae). The habitats and annual life histories of the three species are similar. They occur on damp to wet soils, mainly in damp forests (Hatcher 2003). The study species germinate in spring, begin to flower in early summer and die by winter. They are tall plants with heights of up to ca 130–150 cm (Coombe 1956, Hatcher 2003, Dostál unpubl.). In the database of Thompson et al. (1997), most of the records indicate only transient soil seed banks for all three species (persistance < 1 year). Perglová et al. (2009) showed that seeds of I. noli-tangere remained viable in the soil for three years, indicating a short-term persistent soil seed bank (sensu Thompson et al. 1997).

For the purposes of this study, seeds of I. noli-tangere were collected from four invaded and five non-invaded sites. At the invaded sites, we also collected seeds of I. parviflora. The sampling sites for G. speciosa seeds were generally identical to those for I. noli-tangere (n = 8 in total; Table 1). In the study area, G. speciosa is a common species typical to plant communities with both Impatiens species, and therefore it
was included in our study. Another reason for including this species was its life history, which is similar to that of *I. noli-tangere*.

In each population, we conducted a bulk seed collection in July 2008 from more than 100 fruiting individuals per species that grew no closer than 1 m to other conspecifics. Seeds were kept at room temperature, and in the autumn of 2008, they were processed as described below. All of the native populations that were sampled hosted more than 1000 conspecific individuals. In the case of invaded sites, the population size of the exotic species *I. parviflora* was estimated to exceed more than 10 000 individuals. The seed source localities are situated in central Bohemia in the Czech Republic, with a minimum distance of 3 km between the closest sites and a distance of 55 km between the farthest ones.

**Site characteristics of invaded and non-invaded sites**

We tested the differences between both site types with respect to 13 environmental and biotic parameters in 2009 (Supplementary material Appendix 1 Table A1a, b). The density of *I. parviflora* varied at the invaded sites, and this allowed us to collect vegetation and other measurements in plots free of the invader (or with invader cover <10%), presumably reflecting pre-invasion conditions.

**Competitive effects study: field and pot trials in a common garden**

In August 2010, we performed biomass sampling at three invaded and three non-invaded sites (Table 1). At each site, we randomly selected 30 plots (25 × 25 cm), and in each plot, we clipped the aboveground biomass at a height of ca 2 cm above ground. The biomass was divided into 1) *I. parviflora*, 2) *I. noli-tangere*, 3) *G. speciosa* and 4) the remaining natives. The samples were then dried to a constant mass at 70°C (n = 180 plots total).

In late May 2009, we initiated the competition experiment in the Inst. of Botany ASCR experimental garden in Průhonice (322 m a.s.l.; 49°59′N, 14°57′E). The seedlings for this experiment and for the phenotypic plasticity experiment were prepared as follows. In late October 2008, seeds of each population were planted in pots (each 20 × 25 × 5 cm) filled with a 1:1 mixture of commercially available peat and sand. The pots were randomly placed in a bed of an experimental garden and covered with mesh netting to exclude seed predators. In early May 2009, the seedlings were transplanted to trays (cell size 3 × 3 cm) filled with sterile soil and maintained for one month in a bed of an experimental garden covered with a shade cloth (shading = 30%). The positions of the trays were randomized twice during this cultivation phase. To control for initial seedling size, the height and length of the longest leaf of each seedling were measured. To calculate the aboveground biomass of the seedlings, a calibration set of randomly selected seedlings (three seedlings per population) was measured for the same parameters. The seedlings from the calibration set were then clipped immediately above ground, dried to a constant weight at 70°C and weighed.

The competition experiment consisted of planting one juvenile of *I. parviflora* at a distance of ca 5 cm from a target native individual in pots with low and high nutrient treatments (see the phenotypic plasticity experiment for details) placed under a 30% shade cloth. Seedlings of *I. parviflora* were selected at random from four populations of different seed origins without retaining information on population identity. As a control for this experiment, we used plants grown without an exotic competitor but in identical nutrient and light conditions.

**Seed and germination characteristics**

To determine the seed mass for each of the populations, we weighed five batches consisting of 10 seeds of each native population in October 2008 and conducted the same procedure for four exotic populations. Each seed batch was then planted in a separate pot (10 cm in diameter) filled with a 1:1 mixture of commercially available peat and sand in late October 2008. The pots were randomly placed in the bed of an experimental garden and covered with mesh netting to exclude seed predators. There was no sign of germination during the winter period. From early March 2009 onward, the pots were checked for the number of emerged seedlings on a weekly basis. The first seedlings were observed on 1 April 2009. On 6 May 2009, we terminated the experiment because no new seedlings had appeared for over two weeks. From the data collected, we determined the 1) germination onset (in weeks), 2) time when at least half of the germinated seeds had emerged ($T_{50}$ in weeks), and 3) the final proportion of the emerged seeds. We set 25 March 2009 (one week before the first seedlings emerged) as the beginning of the germination dynamics measurements.

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**Table 1. Invaded and non-invaded sites of *I. noli-tangere* and *G. speciosa* used as seed sources for this study (*G. speciosa* was missing from site ‘Jevany’). Additional details on abiotic and biotic site characteristics are provided in Supplementary material Table A1. Asterisks indicate sites where field competitive effects study was performed.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Site type</th>
<th>Geographical co-ordinates</th>
<th>Elevation (m a.s.l.)</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blatnice</td>
<td>invaded</td>
<td>49°31′086″N, 14°53′517″E</td>
<td>624</td>
<td>alder carr with spruce plantation</td>
</tr>
<tr>
<td>Okrouhlice*</td>
<td>invaded</td>
<td>49°46′943″N, 14°44′560″E</td>
<td>360</td>
<td>stream banks in spruce forest</td>
</tr>
<tr>
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<td>386</td>
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</tr>
<tr>
<td>Litíno*</td>
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<td>425</td>
<td>alder carr along stream</td>
</tr>
<tr>
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<td>600</td>
<td>alder carr along stream</td>
</tr>
<tr>
<td>Pojšuky</td>
<td>non-invaded</td>
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<td>555</td>
<td>alder carr along stream</td>
</tr>
<tr>
<td>Osředek*</td>
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<td>alder carr along stream</td>
</tr>
<tr>
<td>Moninec*</td>
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<td>591</td>
<td>spring area in spruce–beech forest</td>
</tr>
<tr>
<td>Jevany</td>
<td>non-invaded</td>
<td>49°57′290″N, 14°49′209″E</td>
<td>479</td>
<td>stream banks in spruce–beech forest</td>
</tr>
</tbody>
</table>
 Phenotypic plasticity experiment

The phenotypic plasticity experiment was initiated in late May 2009. Juveniles of *I. noli-tangere* and *G. speciosa* were prepared as described in the competition experiment. Seedlings were transplanted to 2.75-l pots (one plant per pot) and subjected to a full-factorial experimental design with two factors: nutrient and light. Two different nutrient levels were achieved using two different mixtures of sand and garden soil at the beginning of the experiment. Plants in nutrient-poor conditions were grown in pots with a mixture of sterilized soil and sterilized sand at a 1:4 ratio; in nutrient-rich conditions, this ratio was reversed. The differences in nutrient levels were further maintained by fertilization every other week with 0.1- and 0.5-strength Hoagland nutrient solution (Hoagland and Arnon 1950) in nutrient-poor and nutrient-rich pots, respectively. The light treatment consisted of two different shading intensities under 30% and 70% shade cloths, hereafter referred to as high and low light treatments, respectively. Deficiency in either factor is well documented to reduce plant performance independently as well as in combination (Chapin 1980).

In the competition and phenotypic plasticity experiments, ten plants per population of the native species were subjected to each treatment combination. The exception was the population of *G. speciosa* from the Moninec site (Table 1), for which five plants per treatment combination were used. Additionally, we planted 80 exotic juveniles individually, with 20 plants for each nutrient and light treatment combination for the assessment of reciprocal competitive effects of native species. In total, there were 1070 pots, with 1400 juveniles planted at the beginning of the experiment. The pots were randomly distributed among six blocks covered with a 70% shade cloth. The blocks were alternated in beds, and 10 blocks were covered with a 30% shade cloth. Each block (approximate size 3 × 2 m) consisted of a wooden frame erected ca. 1.5 m above the bed and covered with a shade cloth from the top and the sides. To simulate realistic field conditions, the soil in all pots was inoculated with a soil suspension (100 ml l⁻¹ pot soil) prepared from soil collected from damp forest around the experimental garden. The plants were harvested after approximately 100 days in early September 2009 during the fruiting peak of all species. Individuals were removed from the pots, and soil remnants were washed from their roots. Plants were then divided into below- and aboveground biomass, dried to a constant mass at 70°C and weighed. Plant survival was monitored every other week. If any individual plant grown alone or in a competition pair died during the course of the experiment, data from that pot were no longer used.

The results are based on biomass measurements rather than on more direct fitness measures, such as the number of seeds, because the assessment of lifetime fitness of the study species would require prolonged measurements until the first frost. However, in such a case, we would lose information on vegetative structures such as roots. Additionally, the seed production of *I. noli-tangere* is correlated with its total biomass ($r = 0.47$, $p < 0.001$, $n = 48$) and root biomass ($r = 0.51$, $p < 0.001$, $n = 48$), as was found previously (Weiser unpubl.).

Statistical analyses

**Competitive effects study: pot trials in a common garden**

Linear mixed models were used with total biomass, root biomass, and plant height as response variables (analyzed separately) and site type, nutrient and competition treatments, and their interactions as fixed factors. Block, population identity, and their interactions with the treatments were included as random factors. The initial estimated seedling biomass was used as a covariable. Total and root biomass were ln-transformed to meet assumptions of homoscedasticity and normality of residuals.

In addition, for each plant grown in competition, we also calculated the log response ratio (lnRR; Goldberg et al. 1999) as follows:

\[ \text{lnRR} = \ln \left( \frac{B_{\text{neigh}}}{B_{\text{no neigh}}} \right) \]

where $B_{\text{neigh}}$ is the mean total biomass of individuals when grown alone ($n = 10$ if all plants survived), and $B_{\text{no neigh}}$ is the total biomass of target individual when grown with a neighbor. We then performed a linear mixed model with lnRR as the dependent variable and site type, nutrient treatment, and their interactions as fixed factors. Block, population identity, and their interactions with the nutrient treatment were included as random factors. The initial estimated seedling biomass of the target plant and the measured final biomass of the neighbor were used as covariables. Competition intensity was thus measured per unit biomass of the competitor. We performed two separate analyses with lnRR of the native species and *I. parviflora* as neighbors and vice versa.

**Seed and germination characteristics**

Differences in mean seed mass and germination rates were analyzed using a linear mixed model with site type (invaded vs non-invaded) as a fixed factor and population identity (random factor) nested within type. In the germination analysis, the mean seed mass of each population was used as a covariable to control for possible maternal effects. The final proportions of germinated seedlings were arcsine square-root-transformed to meet assumptions of homoscedasticity and normality of residuals.

**Phenotypic plasticity experiment**

Linear mixed models were used with total biomass, root biomass and plant height as response variables (analyzed separately) with effects of site type, light and nutrient treatments, and their interactions as fixed factors. Block, population identity, and their interactions with the treatments were included as random factors. The initial estimated seedling biomass was used as a covariable. Total and root biomass were ln-transformed to meet assumptions of homoscedasticity and normality of residuals.

All linear mixed models were performed using the PROC GLIMMIX procedure in SAS 9.2. For F-tests of fixed effects, the denominator degrees of freedom were calculated using the Satterthwaite approximation. The level of significance of each random term was determined by $\chi^2$-tests for changes in deviance between the complete model and a model with the term in question omitted.
(Littell et al. 1996). All analyses were conducted separately for I. noli-tangere and G. speciosa.

Results

Site characteristics of invaded and non-invaded sites

The environmental and biotic parameters measured (13 variables in total; Supplementary material Appendix 1 Table A1a) did not vary significantly between the invaded and non-invaded sites. For example, both site types were at a similar mean altitude and did not differ in aboveground plant productivity. The presence of the invader Impatiens parviflora did not seem to alter the enemy diversity and herbivory of Impatiens noli-tangere plants (Supplementary material Appendix 1 Table A1b).

Competitive effects study: field and pot trials in a common garden

The presence of I. parviflora is likely to change a competitive environment at invaded sites. Based on field sampling from 2010, the biomass of I. noli-tangere decreased significantly with an increasing biomass of co-occurring vegetation (Fig. 1a), but the decline was steeper when I. parviflora was present in the plots (ANCOVA results; biomass of co-occurring vegetation: F1,61 = 8.80, p = 0.004; presence of I. parviflora: F1,61 = 7.64, p = 0.008). The biomass of Galeopsis speciosa also tended to decrease with increasing amounts of co-occurring biomass but the effect was not significant. Nor was significant the effect of I. parviflora presence in the plots (Fig. 1b).

In pot trials, the total biomasses of I. noli-tangere and G. speciosa decreased by 26.5% and 14.1%, respectively, due to the presence of the exotic competitor. The reciprocal competitive effects of I. noli-tangere on I. parviflora were similar to those of G. speciosa; the former species reduced the biomass of the invader by 47% and the latter by 45%, on average.

The total biomass of I. noli-tangere plants from invaded and non-invaded sites was similar in the presence of I. parviflora. When released from competition, however, the plants from invaded sites were larger in terms of root and total biomass (Table 2a, Fig. 2a, b). Consequently, the competitive response (lnRR) of plants from invaded sites was larger than that of plants from non-invaded sites (F1,13.9(invasion) = 5.83, p = 0.03; Fig. 2c). lnRR of I. parviflora did not depend on the site type from which I. noli-tangere originated (not shown). No differences in competitive response and competitive effect were found in the second native species, G. speciosa, with respect to the site type (not shown).

Seed and germination characteristics

The seed mass of I. noli-tangere differed significantly among populations ($\chi^2 = 19.52, p < 0.001$) but was not influenced by site type (i.e. invaded and non-invaded sites; F1,7 = 0.08, p = 0.78). The final germination rate of I. noli-tangere was also similar in plants from both site types. Seedlings from invaded sites, however, emerged significantly earlier (−0.4 week) than conspecific seedlings from non-invaded sites (Table 3, Fig. 3a) and also earlier than seedlings of the exotic congener (F1,6.1 = 4.30, p = 0.08; Fig. 3a). The T50 of I. noli-tangere seedlings from invaded sites was shorter by 0.5 weeks on average than that of conspecific plants from non-invaded sites (Table 3; Fig. 3b). It was also significantly shorter than the T50 of the exotic congener (F1,6.1 = 5.32, p = 0.05; Fig. 3b). Impatiens noli-tangere from non-invaded sites did not differ in germination onset or in T50 from I. parviflora (both parameters p > 0.05).

The seed mass of G. speciosa differed among populations ($\chi^2 = 4.16, p = 0.04$) but was not influenced by site type (F1,6 = 0.34, p = 0.58). Seedlings of G. speciosa germinated one week earlier than those of the exotic species, and T50 was shorter by 1.5 weeks. No germination parameter was influenced by invasion in this species (T50: F1,37 = 1.59, p = 0.22; final germination rate: F1,5 = 0.05, p = 0.83).

Figure 1. Biomass of I. noli-tangere and G. speciosa plotted against biomass of co-occurring vegetation in the plots (25 × 25 cm) sampled at three invaded and three non-invaded sites. From a total of 180 sampled plots, I. noli-tangere was present in 65 plots (21 also contained I. parviflora) and G. speciosa in 60 plots (21 also with I. parviflora). Plots containing I. parviflora are indicated by full symbols and a solid line, and those without invader are shown by empty symbols and a dotted line.
Phenotypic plasticity experiment

Based on plants from the calibration set, seedlings of *I. noli-tangere* from both site types had similar shoot biomass ($F_{1,69} = 0.81, p = 0.40$) and height ($F_{1,76} = 0.31, p = 0.59$). However, seedlings from invaded sites tended to be taller than juveniles of the same biomass originating from non-invaded sites (Supplementary material Appendix 1 Fig. A1; $F_{1,73} = 6.94, p = 0.03$). No invasion-related differences in the shoot biomass or height of *G. speciosa* seedlings were found (not shown).

Regarding the response of adult plants to experimental treatments, the total biomass of *I. noli-tangere* decreased by 26.5% in the low light treatment and by 64.4% due to fewer nutrients. The same factors reduced total biomass in *G. speciosa* by 14.2% and 54.0%, respectively.

Plants of *I. noli-tangere* from invaded sites differed from plants from non-invaded sites in the response to light and nutrient treatments; whereas the root biomass of plants from both site types was similar under low light or nutrient treatments, high resource treatments led to a significantly pronounced increase in the root biomass of plants from invaded sites (Table 2a, b, Fig. 4a, b). *Impatiens noli-tangere* from invaded sites also differed from the other plants in the response of height to the interaction of nutrient and light treatments. When grown in nutrient-rich conditions, plants from both site types were taller under low light treatment (Fig. 5a). At a decreased nutrient level, however, plants from non-invaded (as opposed to invaded) sites exhibited decreased heights in response to low light treatment (Table 2b, Fig. 5b). No invasion-related differences in phenotypic plasticity were found in *G. speciosa* (not shown).

We summarize all our findings in Table 4 and compare them with the pattern that we expected.

**Discussion**

In this study, we tested whether invasion may select for adaptation in two native species to the presence of an invader. When grown with the invader *Impatiens parviflora*, no differences were found in the performance of *Galeopsis speciosa* plants from invaded and non-invaded sites. Moreover, no differences between population types were found in any other experimental part. Regarding the second native

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**Table 2. Results of Competitive effects study and Phenotypic plasticity experiment: summary of linear mixed models of total and root biomass, and of plant height of invaded and non-invaded sites (factor ‘Invasion’) of *I. noli-tangere*. In (a), results are shown for plants grown with and without competition from *I. parviflora* under high light treatment and two nutrient levels. In (b), plants were grown in all combinations of light and nutrient treatments. Significant effects ($p \leq 0.05$) are in bold.**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Total biomass</th>
<th>Root biomass</th>
<th>Plant height</th>
</tr>
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<tr>
<td>Fixed terms</td>
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<td></td>
<td></td>
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<td>Initial mass</td>
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<tr>
<td>I × N × C</td>
<td>1,20.6</td>
<td>0.22</td>
<td>0.644</td>
</tr>
<tr>
<td>Random terms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
<td>1</td>
<td>6.31</td>
<td>0.012</td>
</tr>
<tr>
<td>Population (I)</td>
<td>1</td>
<td>3.08</td>
<td>0.079</td>
</tr>
<tr>
<td>P × N (I)</td>
<td>1</td>
<td>$&lt;0.01$</td>
<td>0.999</td>
</tr>
<tr>
<td>P × C (I)</td>
<td>1</td>
<td>$&lt;0.01$</td>
<td>0.999</td>
</tr>
<tr>
<td>P × N × C (I)</td>
<td>1</td>
<td>4.07</td>
<td>0.044</td>
</tr>
</tbody>
</table>

| (b) | | | |
| Fixed terms | | | |
| Initial mass | 1,243.6 | 27.19 | $<0.001$ | 1,204.6 | 10.65 | 0.001 |
| Invasion | 1 | 5.9 | 3.43 | 0.114 | 1,6.9 | 4.54 | 0.080 |
| Nutrients | 1,16.0 | 154.28 | $<0.001$ | 1,17.0 | 131.58 | $<0.001$ |
| Light | 1,17.0 | 132.62 | $<0.001$ | 1,17.4 | 171.36 | $<0.001$ |
| I × N | 1,16.0 | 0.16 | 0.703 | 1,7.08 | 0.68 | 0.437 |
| I × L | 1,15.8 | 0.61 | 0.467 | 1,13.9 | 7.08 | 0.019 |
| N × L | 1,16.4 | 15.35 | 0.007 | 1,14.11 | 5.41 | 0.035 |
| I × N × L | 1,16.1 | 4.92 | 0.070 | 1,13.8 | 4.00 | 0.065 |
| Random terms | | | |
| Block | 1 | 5.86 | 0.015 | 1 | 1.14 | 0.286 |
| Population (I) | 1 | 0.01 | 0.920 | 1 | $<0.01$ | 0.999 |
| P × N (I) | 1 | 4.48 | 0.039 | 1 | 3.31 | 0.069 |
| P × L (I) | 1 | 1.57 | 0.210 | 1 | $<0.01$ | 0.999 |
| P × N × L (I) | 1 | $<0.01$ | 0.999 | 1 | 0.70 | 0.403 |
species, *Impatiens noli-tangere*, the competitive response (log response ratio, *lnRR*) of plants from invaded sites was significantly larger (i.e. the performance decreased) than that of plants from non-invaded sites due to competition from the invader (Fig. 2c). This result contradicts the assumption that invasion experience may select for genotypes with increased resistance to the invader (Table 4), as shown in other study systems (Callaway et al. 2005, Mealor and Hild 2007, Rowe and Leger 2011), and questions the adaptive value of remnant populations that survived the invasion (cf. Leger 2008). However, this conclusion based on *lnRR* is determined by the plant performance in a competition-free environment; whereas plants from both site types were similar in total biomass in the presence of the invader, plants originating from invaded sites were significantly larger in a competition-free environment (Fig. 2b). This finding nevertheless indicates that larger size, which is often associated with stronger competitive ability, did not improve the performance of plants from invaded sites when in competition with the invader.

![Graph](image)

**Figure 2.** The effects of competition of *I. parviflora* on the root (a) and total biomass (b) of *I. noli-tangere* originating from invaded (full symbols and solid lines) and non-invaded sites (empty symbols and dotted lines) (means ± SE). In (c), *lnRR* of *I. noli-tangere* from invaded and non-invaded sites is shown. Shared letters indicate no significant differences among the means, as determined from post-single-factor ANOVA LSD tests.

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Table 3. Summary of linear mixed models testing the differences between invaded and non-invaded sites (factor ‘Invasion’) of *I. noli-tangere* for germination parameters. Significant effects (*p* ≤ 0.05) are in bold.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Germination onset</th>
<th>T&lt;sub&gt;50&lt;/sub&gt; (time needed for half of germ. seeds to emerge)</th>
<th>Final germination rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF, DDF</td>
<td>F or χ²</td>
<td>p</td>
</tr>
<tr>
<td>Fixed terms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed mass</td>
<td>1,40</td>
<td>1.07</td>
<td>0.307</td>
</tr>
<tr>
<td>Invasion</td>
<td>1,40</td>
<td>4.35</td>
<td><strong>0.044</strong></td>
</tr>
<tr>
<td>Random terms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population (l)</td>
<td>1</td>
<td>&lt;0.01</td>
<td>0.999</td>
</tr>
</tbody>
</table>
Ecological significance of trait divergence and implications for coexistence

A pronounced increase in total biomass and, in particular, root biomass in response to a more favorable environment (i.e. high light availability, more nutrients and no competition) was one of the most striking differences in *I. noli-tangere* plants from invaded sites. It contradicts our original assumption about the higher tolerance of plants from invaded sites to resource-poor conditions (Table 4). This finding indicates that invasion may have selected for genotypes of *I. noli-tangere* that are better able to exploit nutrient-rich patches that are locally released from competition from exotics. Increased phenotypic plasticity can then be similarly advantageous, as are plastic foraging responses of clonal plants in heterogeneous environments (van Kleunen and Fischer 2001).

Aside from pronounced plasticity, the plants from invaded sites seem to be better adapted for shade-avoidance, as demonstrated by morphological differences at the seedling and adult stages (Aphalo and Ballaré 1995, Donohue et al. 2000, Franklin 2008). Initially, juveniles from the invaded sites were significantly taller than similarly sized juveniles from the non-invaded sites (Supplementary material Appendix 1 Fig. A1). This corresponds with the results of McGoey and Stinchcombe (2009), who found that the hypocotyl length of *Impatiens capensis* is under positive selection when it interacts with heterospecifics, allowing *I. capensis* to grow faster than competitors. Secondly, we found that adult plants from the invaded but not the non-invaded populations were able to maintain a tall height under low light treatment and nutrient-poor conditions (Fig. 5b). These findings suggest altered evolution of the shade-avoidance syndrome (Franklin 2008) following invasion by exotics.

Finally, we showed that seedlings from invaded sites germinated significantly earlier than conspecifics from non-invaded sites and earlier than the exotic congener, which corresponds with our original hypothesis on the evolution of asynchronous phenology (Fig. 3, Table 4). In previous studies, earlier germinants have been documented to have a greater biomass, providing them a competitive advantage (Verdú and Traveset 2005, Orrock and Christopher 2010). Earlier phenology in perennial native grasses following the invasion of the exotic annual grass *Bromus tectorum* was also found to reduce resource availability for the invader (Leger 2008, Goergen et al. 2011).
however, phylogeny probably plays only a minor role in competitive interactions (Cahill et al. 2008, Dostál 2011; but see Cadotte et al. 2008). In this study, greater resource use overlap between both *Impatiens* species than between *Impatiens* and *G. speciosa* can be implied by the more similar germination phenology. Moreover, the field assessment demonstrated a pronounced competitive effect of *I. parviflora* on *I. noli-tangere* but not on *G. speciosa* (Fig. 1, Table 4). However, as we included only one closely related and one more distantly related native species in this study, we cannot draw a more robust conclusion about the role of relatedness in the observed trait divergence.

Limitations of the present study

One of the limitations of this study is that we cannot state whether the trait divergence in *I. noli-tangere* is genetically based or a result of maternal environmental effects. Although we conducted all of our experiments in a common environment, we cannot exclude the possibility that the trait divergence is due to reasons other than genetic differentiation. Several of the observed differences between the invaded and non-invaded populations were related to seed germination traits, which may be particularly influenced by maternal

<table>
<thead>
<tr>
<th>Field study</th>
<th>Expected</th>
<th>Observed <em>I. noli-tangere</em></th>
<th>Observed <em>G. speciosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Competitive effect of <em>I. parviflora</em> vs effect of native vegetation</td>
<td>↑</td>
<td>↑</td>
<td>0</td>
</tr>
<tr>
<td>Common garden study</td>
<td>Competitive response (lnRR) of invaded vs non-invaded populations to <em>I. parviflora</em></td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Competitive effect of invaded vs non-invaded populations on <em>I. parviflora</em> (lnRR)</td>
<td>↑</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Germination phenology of invaded vs non-invaded populations</td>
<td>← →</td>
<td>←</td>
</tr>
<tr>
<td></td>
<td>Tolerance of invaded vs non-invaded populations to low light and nutrient treatments</td>
<td>↑</td>
<td>0 in tolerance but ↑ in response to more light and nutrients</td>
</tr>
</tbody>
</table>
environmental effects (Donohue et al. 2007). Although we attempted to control for differences in seed provisioning by including seed mass in the germination analyses, Bischoff and Müller-Schärer (2010) showed that fitting of seed mass as a covariable may not be a successful procedure for reducing maternal environmental effects.

Another limitation is related to causality between trait changes and invasion. Although we found no differences between the invaded and non-invaded sites with respect to 13 abiotic and biotic factors, the number of sites was rather modest for observing significant differences. In addition, there are unmeasured factors that may differentiate both habitat types and may include characteristics driving both trait divergence in *I. noli-tangere* and the probability of invasion by *I. parviflora*. Consequently, the invasion by exotics need not be a cause of trait changes.

The detailed invasion history of *I. parviflora* in the study region is unknown, and thus we cannot explain why it is absent from some sites of *I. noli-tangere*. Different ecological requirements of the two *Impatiens* species are one of a number of possible explanations (Godefroid and Koedam 2010). Seed addition experiments, however, demonstrated that the lower abundance of *I. parviflora* in ancient rather than recent forests in Denmark is due to lower propagule pressure (Graae et al. 2004). Chmura and Sierka (2007) assume that the establishment and invasion of *I. parviflora* is driven predominantly by forest management practices, including seed transport, not by forest habitat differences. We assume that the same is true for our study sites (Dostál unpubl.).

**Conclusion**

To date, the greatest attention has been paid to the evolutionary changes of exotic species after transfer from old to new ranges and how these changes contribute to their invasiveness. No less important, however, is the investigation of how the natives respond both ecologically and evolutionarily to these novel interactions. In this study, we showed post-invasion divergence in populations of a native species, *I. noli-tangere*, with respect to germination phenology and phenotypic plasticity, but we have not yet been able to demonstrate the adaptive value of these changes.

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**References**


Supplementary material (available as Appendix O20333 at <www.oikosoffice.lu.se/appendix>). Appendix 1.


