ABSTRACT

Invasive species often evolve rapidly in response to the novel biotic and abiotic conditions in their introduced range. Such adaptive evolutionary changes might play an important role in the success of some invasive species. Here, we investigated whether introduced European populations of the South African ragwort Senecio inaequidens (Asteraceae) have genetically diverged from native populations. We carried out a greenhouse experiment where 12 South African and 11 European populations were grown for several months at two levels of nutrient availability, as well as in the presence or absence of a generalist insect herbivore. We found that, in contrast to a current hypothesis, plants from introduced populations had a significantly lower reproductive output, but higher allocation to root biomass, and they were more tolerant to insect herbivory. Moreover, introduced populations were less genetically variable, but displayed greater plasticity in response to fertilization. Finally, introduced populations were phenotypically most similar to a subset of native populations from mountainous regions in southern Africa. Taking into account the species’ likely history of introduction, our data support the idea that the invasion success of Senecio inaequidens in Central Europe is based on selective introduction of specific preadapted and plastic genotypes rather than on adaptive evolution in the introduced range.

Keywords
Aphids, biological invasions, EICA, genetic variation, microevolution, phenotypic plasticity.

INTRODUCTION

Evolutionary change can occur rapidly, and it is therefore relevant to the study of ecological questions (Thompson, 1998; Hairston et al., 2005). This may be particularly true in situations where organisms are exposed to dramatic environmental changes, because these will impose strong and possibly novel selection pressures and may therefore cause rapid adaptive evolution (Bone & Farres, 2001). A particularly good, and increasingly well-studied, series of examples of evolution in response to environmental change are biological invasions (Mooney & Cleland, 2001; Sakai et al., 2001; Bossdorf et al., 2005; Strauss et al., 2006).

Plant and animal species that are – intentionally or unintentionally – moved across biogeographical barriers by humans are often introduced to ecological communities that contain different competitors, antagonists, and mutualists than the communities in their native range. These species may impose rather different ecological interactions, and therefore selection pressures, particularly because they lack a coevolutionary history with the introduced species (Callaway & Ridenour, 2004; Hallett, 2006). As a consequence, introduced species may undergo rapid evolutionary divergence in their novel range (e.g. Blossey & Nötzold, 1995; Maron et al., 2004; Siemann et al., 2006), and so may the resident species in response to the novel selection pressures imposed by the exotics (Callaway et al., 2005; Lau, 2006; Strauss et al., 2006).

Adaptive evolutionary adjustments in important ecological traits may be one of the reasons why some introduced species start spreading and become extremely successful invaders only after a time lag of several decades or centuries (Williamson, 1996; Mack et al., 2000). A particularly intriguing hypothesis about evolutionary change in invasive plants is the Evolution of Increased Competitive Ability (EICA) hypothesis by Blossey & Nötzold (1995) which proposes that release from specialized insect herbivores may have caused adaptive evolution of less defended but more vigorous and therefore more competitive genotypes in successful plant invaders.

There are, however, several other hypotheses about rapid adaptation in invasive species one can conceive of. In principle,
any ecological trait that confers a fitness advantage to an invasive species in its novel environment will be under selection and may therefore evolve. A potentially very important but still under-studied class of traits in this context is phenotypic plasticity, the ability of a genotype to express different phenotypes in different biotic or abiotic environments (Pigliucci, 2001). Since phenotypic plasticity is related to both ecological niche breadth and opportunistic success, it may be expected to be beneficial, and thus under positive selection, in novel environments (Richards et al., 2006). Two types of plasticity that are generally considered to be important for the success of plants are (1) the re-allocation of resources in response to changes in nutrient availability, and (2) herbivore tolerance, the ability of plants to re-grow after damage (Bloom et al., 1985; Strauss & Agrawal, 1999).

In practice, demonstrating that adaptive evolutionary change in ecologically relevant traits has taken place in invasive plant species requires carefully designed common garden or reciprocal transplant experiments that combine ecological and genetic factors to test for genetic differentiation and local adaptation in native vs. introduced populations (Hinz & Schwarzlaender, 2004; Bossdorf et al., 2005; Hierro et al., 2005; Callaway & Maron, 2006).

Here, we have tested for evolutionary change in invasive populations of the South African plant Senecio inaequidens, currently one of the most rapidly spreading plant invaders in Europe (Heger & Boehmer, 2005). S. inaequidens was introduced to Europe over a century ago, but started to spread only in the 1970s, i.e. after a time lag of some 80 years. In a recent field study, we found that plants in invasive populations of S. inaequidens in Europe were significantly larger and less parasitized than plants in native South African populations (Prati & Bossdorf, 2004). Here, we tested whether these differences have a genetic basis. We grew offspring from several native and introduced populations in a common environment for several months, subjected them to a factorial combination of insect herbivory and fertilization treatments, and asked the following questions: (1) On average, do plants from invasive populations grow more vigorously than plants from native populations? (2) Are plants from invasive populations generally more tolerant to herbivore damage? (3) Do plants from invasive populations show a greater average degree of plasticity in response to different levels of nutrient availability?

**METHODS**

**Study species**

Senecio inaequidens DC. (South African ragwort, narrow-leafed ragwort) is a perennial member of the Asteraceae that is native to the mountainous regions of southern Africa (Hilliard, 1977). It is a small shrub of 60–100 cm in height that naturally grows on outcrops, rocky slopes, and gravelly riverbeds. In the last century, S. inaequidens has also become a frequent colonizer of disturbed habitats in several South African provinces (Hilliard, 1977). The species occurs in diploid (2n = 20) and tetraploid (2n = 40) forms.

At the end of the 19th century, S. inaequidens was repeatedly introduced to Europe, most likely as a wool contaminant (Ernst, 1998). The species started to spread rapidly only in the last decades, and it is currently considered as one of the most rapidly spreading invaders in Europe (Heger & Boehmer, 2005). In the Mediterranean, S. inaequidens has become a noxious weed in vineyards and pastures (López-Garcia & Maillet, 2005). Like many other Senecio species, it contains pyrrolizidine alkaloids, which are toxic to many animals, in particular livestock. As a consequence, S. inaequidens is often avoided by grazing animals, which contributes to the species' success and competitive advantage on Mediterranean pastures. In Central Europe, S. inaequidens was first recorded in the Ruhr Basin and around Bremen, Germany. Starting in the 1970s, the species has spread rapidly along motorways and railroad tracks, and it is currently expanding particularly eastwards (Heger & Böhmer, 2005). In Germany, S. inaequidens grows predominantly in ruderal habitats, but it occasionally also invades old fields in early successional stages. A recent survey of the insect fauna on S. inaequidens in Germany found 62 different insect species feeding on S. inaequidens, most of them generalist herbivores (Schmitz & Werner, 2000).

**Seed collection and experimental set-up**

In February and March 2002, seeds of S. inaequidens were collected from 12 native populations in South Africa and Lesotho that covered a broad range of natural and ruderal habitats (Table 1). In each population, seeds were collected separately from up to 20 maternal plants. In the summer of 2002, similar collections were made in 11 European populations (Table 1). For each population, we created a mixture of seeds from 10 maternal families. In a few South African populations, however, fewer maternal plants had been sampled, so the seed mixtures were based on six to eight seed families.

In September 2002, 100 seeds from each population (= seed mixture) were placed into Petri dishes filled with moist potting soil and germinated at 20 °C. A few weeks later, 20 randomly chosen seedlings per population were transferred to 1.5-L plastic pots filled with washed silica sand. There were only 17 seedlings available for the Montpellier population, so the total number of plants in our experiment was 457. The pots were placed on four benches in a greenhouse and watered regularly with an automatic watering system. The temperature in the greenhouse ranged from 15 to 25 °C during the day and it never dropped below 5 °C at night. Additional light was provided by high-pressure potassium lamps (SON-T AGRO 400 W, Philips GmbH, Hamburg, Germany) with an initial 12/12 h day/night cycle that was gradually increased to a 16-h day period until the end of the experiment.

The plants were subjected to a factorial combination of two experimental treatments: (1) a nutrients treatment in which half of the plants received slow-release fertilizer (Osmocote, 15-9-9 NPK, Scotts International, Geldermalsen, The Netherlands) at a rate of 2 g per litre sand, while the other half were fertilized only once with 20 mL liquid fertilizer (7–3–6 NPK, Tip Blumendünger, Düsseldorf, Germany) to initiate flowering. (2) A herbivory
treatment in which half of the plants were fed on by a generalist insect herbivore, the aphid *Aulacorthum solani* (Homoptera, Aphididae), while the other half were treated with the insecticide dimethoate (Perfekthion, BASF, Ludwigshafen, Germany) at a concentration of 0.15% every 3 weeks.

The aphid *A. solani* is not host-alternating and feeds on a wide range of plant taxa. Aphids spontaneously occurred on *Senecio inaequidens* in the summer and were cultivated on additional plants in a separate greenhouse chamber. In December 2002, half of the plants received three to four aphids that were carefully placed on the leaves with a brush. The procedure was repeated 10 days later. Dimethoate is a wide-ranging, systemic insecticide that is allocated throughout the plant, but not re-allocated into newly produced tissues. The amount of insecticide applied ranged from 10 to 50 mL, depending on the size of the individual plant.

Plants receiving the same combination of treatments were grouped together into plots of 23 plants, each of which contained one plant per population. There were 20 plots overall, with five replicates per population in each treatment combination. The plots were assigned randomly to the four greenhouse benches, subject to the constraint that each treatment combination was present on each bench at least once.

**Measurements and statistical analyses**

All plants were harvested at the end of February 2003. At this point, the plants were approximately 5 months old, they had been subjected to the experimental treatments for about three months, and 80% of them had reached the flowering stage. On each plant, we estimated the abundance of aphids, using an ordinal scale from zero (none) to three (heavily infected). We counted the numbers of buds (unopened flower heads) and flower heads and used their combined total as an approximation of plant reproduction. Plants that did not flower were scored as zero and included in the analyses. As a measure of the degree to which plants showed a branched, bushy growth form, we counted the number of side branches of at least two-thirds of the length of the main stem. It is those major side branches, not the numerous small ones, that are responsible for the bushy appearance of *S. inaequidens*. On each plant, we also measured the length and width of three randomly chosen leaves, and the diameter of three flower heads. Finally, all plants were separated into shoot and root biomass, dried at 70 °C and weighed.

The data were analysed with nested analysis of variance using the JMP software package (SAS Institute Inc, Cary, NC, USA). Generally, the effects of nutrients and herbivory, as well as their interaction, were analysed at the plot level, i.e. using the plot effect as residual, while the effects of continent and population nested within continent, as well as their interactions with the experimental treatments, were analysed at the level of individual plants. Continent effects and their interactions were tested against the respective population effects or interactions. We used the continent main effect as a test for overall genetic differentiation between native and introduced populations. The continent

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**Table 1** The geographical locations of the southern African and European populations of *Senecio inaequidens* where seeds were collected for this experiment.

<table>
<thead>
<tr>
<th>Collection site</th>
<th>Habitat</th>
<th>Elevation</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Southern Africa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bultfontein (Free State)</td>
<td>Roadside</td>
<td>1340</td>
<td>28.16’S</td>
<td>26.08’E</td>
</tr>
<tr>
<td>Dealesville (Free State)</td>
<td>Roadside</td>
<td>1240</td>
<td>28.40’E</td>
<td>25.46’E</td>
</tr>
<tr>
<td>Dewetsdorp (Free State)</td>
<td>Roadside</td>
<td>1550</td>
<td>29.35’S</td>
<td>26.40’E</td>
</tr>
<tr>
<td>Giant’s Castle (KwaZulu-Natal)</td>
<td>River bed</td>
<td>1950</td>
<td>29.20’E</td>
<td>29.30’S</td>
</tr>
<tr>
<td>God’s Window (Mpumalanga)</td>
<td>Roadside</td>
<td>1550</td>
<td>24.50’S</td>
<td>30.50’E</td>
</tr>
<tr>
<td>Golden Gate I (Free State)</td>
<td>River bed</td>
<td>2150</td>
<td>28.31’S</td>
<td>28.25’E</td>
</tr>
<tr>
<td>Golden Gate II (Free State)</td>
<td>Rocks</td>
<td>2050</td>
<td>28.31’S</td>
<td>28.25’E</td>
</tr>
<tr>
<td>Joel’s Drift (Lesotho)</td>
<td>Roadside/Pasture</td>
<td>2000</td>
<td>28.46’S</td>
<td>28.25’E</td>
</tr>
<tr>
<td>Memel (Free State)</td>
<td>Roadside</td>
<td>1741</td>
<td>25.10’S</td>
<td>30.01’E</td>
</tr>
<tr>
<td>Mount Lebanon (KwaZulu-Natal)</td>
<td>Pasture</td>
<td>1800</td>
<td>29.35’S</td>
<td>29.40’E</td>
</tr>
<tr>
<td>Port St. Johns (Eastern Cape)</td>
<td>Roadside</td>
<td>10</td>
<td>31.37’S</td>
<td>29.32’E</td>
</tr>
<tr>
<td>Semonkong (Lesotho)</td>
<td>River bed</td>
<td>2200</td>
<td>29.49’S</td>
<td>28.03’E</td>
</tr>
<tr>
<td><strong>Europe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amersfoort (The Netherlands)</td>
<td>Ruderal vegetation</td>
<td>5</td>
<td>52.09’N</td>
<td>5.23’E</td>
</tr>
<tr>
<td>Amsterdam (The Netherlands)</td>
<td>Roadside</td>
<td>0</td>
<td>52.21’N</td>
<td>4.54’E</td>
</tr>
<tr>
<td>Basel (Switzerland)</td>
<td>Railroad tracks</td>
<td>254</td>
<td>47.33’N</td>
<td>7.36’E</td>
</tr>
<tr>
<td>Bremen (Germany)</td>
<td>Railroad tracks</td>
<td>15</td>
<td>53.05’N</td>
<td>8.48’E</td>
</tr>
<tr>
<td>Cologne (Germany)</td>
<td>Ruderal vegetation</td>
<td>46</td>
<td>50.56’N</td>
<td>6.57’E</td>
</tr>
<tr>
<td>Halle (Germany)</td>
<td>Car park</td>
<td>104</td>
<td>51.28’N</td>
<td>11.58’E</td>
</tr>
<tr>
<td>Hamburg (Germany)</td>
<td>Car park</td>
<td>11</td>
<td>53.33’N</td>
<td>10.00’E</td>
</tr>
<tr>
<td>Karlsruhe (Germany)</td>
<td>Railroad tracks</td>
<td>115</td>
<td>49.00’N</td>
<td>8.24’E</td>
</tr>
<tr>
<td>Kiel (Germany)</td>
<td>Railroad tracks</td>
<td>10</td>
<td>54.20’N</td>
<td>10.08’E</td>
</tr>
<tr>
<td>Montpellier (France)</td>
<td>Ruderal vegetation</td>
<td>23</td>
<td>43.36’N</td>
<td>3.53’E</td>
</tr>
<tr>
<td>Weissenfels (Germany)</td>
<td>Railroad tracks</td>
<td>159</td>
<td>51.12’N</td>
<td>11.58’E</td>
</tr>
</tbody>
</table>
by aphid interaction term was used as a test for genetic divergence of native and introduced populations in terms of their tolerance to insect herbivory. Likewise, the continent by nutrients interaction term was used as a test for genetic divergence in phenotypic plasticity. Prior to the analyses, all variables were log-transformed to meet the assumption of ANOVA. To validate the aphid treatments, we analysed the degree of aphid infestation with a generalized linear model for ordinal data.

We analysed overall phenotypic similarity among South African and European populations with a hierarchical cluster analysis of the population means data of all phenotypic traits. This analysis was also done in JMP.

To test for a general difference in genetic variability among native vs. introduced populations, we calculated separate coefficients of variation for South African and European populations based on the least square population means from the ANOVA of each trait, and used the asymptotic test statistic by Miller (1991) to test for differences between these CVs (a SAS macro is available from the authors upon request).

As a measure of phenotypic plasticity in response to fertilization, we calculated for each population the ratio of mean root biomass with and without fertilizer. Similarly, we calculated the ratio of mean shoot biomass with and without aphids as a measure of plant tolerance to insect herbivory. To test for genetic trade-offs between different components of plant growth and reproduction, plasticity, and tolerance, we calculated Pearson’s correlation coefficients for all pairwise combinations of these variables.

RESULTS

Both of the experimental treatments employed in this study strongly affected the growth and fecundity of S. inaequidens. On average, fertilization caused a more than fivefold increase in shoot biomass, and a more than threefold increase in root biomass (Fig. 1a,b); after 3 months of treatment, unfertilized plants had produced 2.7 flower heads and less than one side branch, whereas fertilized plants had produced almost 50 flower heads and over seven side branches (Fig. 1c,d). While there was significant variation among populations in the degree of this response to fertilization (Pop[C] × F interaction, Table 2), the direction of the response generally remained the same.

Infestation by aphids had a negative effect on plant performance. Compared with uninfested plants, plants that were infested with aphids had a significantly lower average shoot biomass (–18%; Fig. 2a) and root biomass (–35%; Fig. 2b), and they produced fewer flower heads (–42%; Fig. 2c) and side branches (–31%; Fig. 2d). The effect on side branches was inconsistent among populations (significant Pop[C] × A interaction, Table 2) with a few native and introduced populations showing even increased branch numbers in the presence of aphids (data not shown). Only one of the plants treated with aphids did not become infested. Some of the control plants treated with insecticide became infested with aphids, too, but this infestation was substantially lower ($\chi^2 = 371.84$, $P < 0.001$) and affected only the youngest leaves and flower heads. Mean infestation rates did not differ between South African and European plants (quasi-$F = 0.055$, $P > 0.8$).
Genetic differentiation among and between native and introduced populations

There were significant differences between South African and European populations of *S. inaequidens* (Table 2). On average, and across treatments, plants from invasive European populations produced significantly fewer flower heads (−56%) and side branches (−57%) than plants from native South African populations (Fig. 2c,d). In addition, European plants had a significantly higher average biomass (+41%; $F_{1,21} = 10.28$, $P < 0.001$), which

Table 2 Results of analyses of variance that test for the effects of fertilization, aphid herbivory, continent and population of origin, and their interactions, on the growth and reproductive output of *Senecio inaequidens*. The continent main effect tests for overall genetic differentiation between native and introduced populations. The continent by aphids interaction tests for genetic divergence of native and introduced populations in terms of their tolerance to herbivory. The continent by nutrients interaction tests for genetic divergence in phenotypic plasticity. Nesting is indicated by square brackets.

<table>
<thead>
<tr>
<th>Source of variation†</th>
<th>d.f.</th>
<th>SS Shoot biomass $(r^2 = 0.951)$</th>
<th>$F$ ratio</th>
<th>SS Root biomass $(r^2 = 0.853)$</th>
<th>$F$ ratio</th>
<th>SS No. of flower heads $(r^2 = 0.834)$</th>
<th>$F$ ratio</th>
<th>SS No. of side branches $(r^2 = 0.833)$</th>
<th>$F$ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fertilizer$^4$</td>
<td>1</td>
<td>357.64</td>
<td>1645.83***</td>
<td>156.51</td>
<td>108.25***</td>
<td>644.85</td>
<td>818.34***</td>
<td>317.74</td>
<td>938.21***</td>
</tr>
<tr>
<td>2. Aphids$^4$</td>
<td>1</td>
<td>5.99</td>
<td>27.59***</td>
<td>30.51</td>
<td>21.10***</td>
<td>28.93</td>
<td>36.71***</td>
<td>7.93</td>
<td>23.40***</td>
</tr>
<tr>
<td>3. F × A$^4$</td>
<td>1</td>
<td>0.23</td>
<td>1.05</td>
<td>4.14</td>
<td>2.86</td>
<td>3.12</td>
<td>3.86(*)</td>
<td>1.08</td>
<td>3.20(*)</td>
</tr>
<tr>
<td>4. Plot(F,A)$^{13}$</td>
<td>16</td>
<td>3.48</td>
<td>3.52***</td>
<td>23.13</td>
<td>6.02***</td>
<td>12.61</td>
<td>1.45</td>
<td>5.42</td>
<td>1.17</td>
</tr>
<tr>
<td>5. Continent$^6$</td>
<td>1</td>
<td>3.32</td>
<td>2.39</td>
<td>111.91</td>
<td>22.17***</td>
<td>63.00</td>
<td>10.71**</td>
<td>41.03</td>
<td>14.03**</td>
</tr>
<tr>
<td>6. Population[C]$^{13}$</td>
<td>21</td>
<td>29.15</td>
<td>22.52***</td>
<td>106.01</td>
<td>21.01***</td>
<td>123.48</td>
<td>10.80***</td>
<td>61.42</td>
<td>10.15***</td>
</tr>
<tr>
<td>7. C × F$^8$</td>
<td>1</td>
<td>0.01</td>
<td>0.00</td>
<td>5.02</td>
<td>4.40*</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>9. C × A$^{10}$</td>
<td>1</td>
<td>1.60</td>
<td>18.73***</td>
<td>1.96</td>
<td>4.03(*)</td>
<td>0.10</td>
<td>0.14</td>
<td>0.08</td>
<td>0.14</td>
</tr>
<tr>
<td>10. Pop[C] × A$^{13}$</td>
<td>21</td>
<td>1.80</td>
<td>1.39</td>
<td>10.21</td>
<td>2.02*</td>
<td>14.38</td>
<td>1.26</td>
<td>12.86</td>
<td>2.12**</td>
</tr>
<tr>
<td>11. C × F × A$^{15}$</td>
<td>1</td>
<td>0.03</td>
<td>0.40</td>
<td>1.27</td>
<td>4.10(*)</td>
<td>2.52</td>
<td>2.11</td>
<td>0.46</td>
<td>0.51</td>
</tr>
<tr>
<td>12. Pop[C] × F × A$^{15}$</td>
<td>21</td>
<td>1.62</td>
<td>1.25</td>
<td>6.50</td>
<td>1.29</td>
<td>25.09</td>
<td>2.19**</td>
<td>18.87</td>
<td>3.12***</td>
</tr>
<tr>
<td>13. Residual</td>
<td>349</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance levels: (*) $P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. †Superscripts behind a model factor indicate the line of the factor that serves as error term. d.f., degrees of freedom; SS, sum or squares.

Figure 2 The effect of aphid infestation on the growth and reproductive output of South African (light bars) and European (dark bars) populations of *Senecio inaequidens*. The values are backtransformed least square means (+95% confidence intervals) from analyses of variance. In those cases where continent main effects (C) or continent by aphid interactions (C × A) were statistically significant, their significance levels are indicated. All aphid main effects were significant at $P < 0.001$. ***$P < 0.001$, **$P < 0.01$. 

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was almost exclusively due to a large (+139%; Fig. 2b) increase of root biomass in European populations, as average shoot biomass did not increase significantly (Table 2, Fig. 2a). Consequently, plants from European populations had higher root:shoot ratios than plants from South African populations (0.66 vs. 0.34; $F_{1,21} = 31.31$, $P < 0.001$).

We found highly significant population effects in all of the phenotypic traits analysed (Table 2), indicating strong genetic differentiation among these populations. However, in most traits the coefficient of variation of European populations was significantly smaller than that of South African populations (Fig. 3), suggesting that overall genetic variation is significantly reduced in the invasive range.

Phenotypic plasticity and tolerance to herbivory

Native and introduced populations of *S. inaequidens* differed in their plastic responses to fertilization, as indicated by a significant continent by fertilizer interaction for root biomass (Table 2, Fig. 1b). Compared with their conspecifics from South Africa, plants from European populations showed a 64% greater increase of root biomass in response to fertilization (+289% vs. $+176\%$). Moreover, we found significant population variation for plasticity, as indicated by significant population by fertilizer interactions, in all phenotypic traits analysed (Table 2). There was significant negative population-level correlation between the plasticity of root biomass and average flower head production, suggesting that this type of plasticity may incur a fitness cost in *S. inaequidens* (Fig. 4a).

We found a significant continent by aphids interaction in the analysis of shoot biomass (Table 2), indicating that native and introduced populations of *S. inaequidens* also differed in their tolerance to insect herbivory. In the presence of aphids, the average shoot biomass of South African plants was reduced by 27%, whereas in European plants it was reduced by only 9% (Fig. 2a). The result was virtually identical when we used the aphid abundance categories as a categorical covariate. There was a negative genetic correlation between the tolerance of shoot biomass and the number of flower heads (Fig. 4b), suggesting that tolerance, too, may have fitness costs. Moreover, we found a positive genetic correlation between the average root biomass of a population and its degree of tolerance to aphid herbivory (Fig. 4c), which indicates that the ability of this species to compensate for damage may have to do with the amount of resources stored under ground. There was very little genetic variation for tolerance among populations (Table 2).

**Genetic correlations and similarities among populations**

The analyses of population-level correlations among phenotypic traits showed that there were significant positive correlations between plant allocation to shoot and root biomass, and between the numbers of flower heads and side branches produced (Fig. 5). In contrast, there were significant negative relationships between root biomass on the one hand, and the numbers of flower heads and side branches on the other (Fig. 5). Since these correlations were calculated at the level of populations, they are likely to have a genetic component.

A closer examination of the genetic correlations showed that native and invasive populations often formed two distinct clusters in phenotypic space. In fact, much of the correlation pattern appeared to be due to the overall difference between native and invasive populations (Fig. 5). However, there were notable exceptions. Two South African populations (Golden Gate I and II; grey triangles in Fig. 5) consistently grouped together with the European populations, while one European population (Montpellier; black triangles in Fig. 5) was generally more similar to the South African populations than to other populations from Europe. This pattern is corroborated in a cluster analysis of the population means data of all phenotypic traits (including leaf form and flower head diameter), where populations from the two continents were generally well separated, but the two Golden Gate populations clustered together with the European populations, and Montpellier was placed among the South African populations (Fig. 6).
DISCUSSION

In this study, we have asked whether invasive European populations of *S. inaequidens* have diverged genetically from their native South African conspecifics. We found significant differentiation between native and invasive populations with regard to several important ecological traits such as growth, patterns of resource allocation, herbivore defence, and phenotypic plasticity. Thus, our study adds to the growing body of research showing that invasive species often undergo rapid adaptive evolutionary changes in their novel environments, and that ignoring these changes may lead to wrong predictions about their invasive potential and ecological impact (Hinz & Schwarzlaender, 2004; Bossdorf et al., 2005; Callaway & Maron, 2006). Below, we discuss the ecological implications of our results, and we suggest a possible scenario for the invasion history and ecological success of *S. inaequidens*.

Genetic differences in growth and reproduction

We found that plants from invasive populations of *S. inaequidens* were consistently larger but had a lower reproductive output, estimated by the number of flower heads, than plants from native populations. These findings are in contrast to recent field observations where individuals from invasive populations of *S. inaequidens* were larger and produced more flower heads than those from native populations (Prati & Bossdorf, 2004), and they highlight the importance of common garden studies for distinguishing genetic differences from plastic responses to the environment.

It is unlikely that these differences in mean performance between native and introduced populations are the result of maternal carry-over effects, at least of those mediated by differences in seed provisioning, since there were no overall differences in seed mass between the native and introduced populations used in this study (Prati & Bossdorf, 2004).

The observed population and continent differences in flower head production were to some extent accompanied by differences in phenology. Some populations already flowered after few weeks, while others just started doing so at the end of the experiment. While these phenological differences did not affect plant biomass or branch number, there was a tendency for later flowering populations to end up with fewer flower heads at the time of harvesting. We do not consider this phenological variation to be problematic (or an alternative explanation for differential reproductive success) but rather a part of the overall shift of life-history strategy in invasive populations. However, it is important to keep in mind that our estimates of plant fitness were incomplete, and therefore genetic correlations involving these fitness estimates should be interpreted rather cautiously, particularly with regard to their adaptive nature.

Genetic differences in herbivore defence

Compared to native populations, invasive populations of *S. inaequidens* appeared to be equally resistant (since they were equally infested with aphids), and they showed a higher tolerance to herbivory by aphids. At first sight therefore our results do not support the EICA hypothesis which predicts increased growth and reproduction but reduced herbivore defence in invasive populations (Blossey & Nötzel, 1995). However, the aphid *Aulacorthum solani* used in our study is a generalist herbivore, and invasive plants are commonly expected to be released from specialist but not from generalist insect herbivores. Therefore,
invaders are expected to reduce defences against the former but not the latter (Keane & Crawley, 2002; Müller-Schärer et al., 2004). We found that invasive populations were even better defended against a generalist insect herbivore, which suggests the possibility of a trade-off between generalist and specialist defence traits (Van der Meijden, 1996), and that the release from specialist insect herbivory (Prati & Bossdorf, 2004) may in fact have triggered increased allocation to generalist defence.

A recent study with another invasive Senecio species, *S. jacobaea*, found that invasive populations contained higher levels of pyrrolizidine alkaloids (PAs) and were therefore better defended against generalist herbivores, but at the same time they were less resistant to specialist herbivores adapted to PAs (Joshi & Vrieling, 2005). However, as a phloem sap-feeder the aphid species used in our study is unaffected by PA. Therefore, tolerance in our study system was likely the result of higher resource allocation to roots (Joshi & Vrieling, 2005). Fluctuating resources are typical for the ruderal habitats currently invaded by *S. inaequidens* in Central Europe.

Figure 5 Phenotypic correlations across 12 native South African (light symbols) and 11 invasive European populations (dark symbols) of *Senecio inaequidens* that were grown in a common environment. The South African populations Golden Gate I and II are indicated by light triangles, and the European population from Montpellier is indicated by black triangles. Regression lines are drawn only for significant correlations. ***P < 0.001.

Differences in resource allocation, and their ecological consequences

We found that, compared to native populations, invasive populations of *S. inaequidens* had significantly higher root:shoot ratios, i.e. they generally allocated a greater proportion of their resources into roots. Possibly as a result of this, invasive populations were not only more tolerant to insect herbivory, but they also showed greater phenotypic plasticity in response to nutrient addition. This ability of plants to quickly capitalize on increased resource availability is often considered a key characteristic of successful invaders (Davis et al., 2000). Fluctuating resources are typical for the ruderal habitats currently invaded by *S. inaequidens* in Central Europe.

A high root:shoot ratio is frequently also correlated with higher plant tolerance to drought, frost, or competition by neighbours.
and European (in bold) populations of Senecio inaequidens. The analysis is based on the population averages of eight phenotypic traits measuring plant growth, reproduction, plant architecture, and leaf shape. (Lloret et al., 1999; De Kroon & Visser, 2003). Indeed, a side experiment with plants from the same populations as in this study showed that the higher root allocation of European populations was correlated with greater frost resistance and winter survival (D. Prati, unpublished data). Thus, higher allocation to roots may confer a threefold advantage to invasive populations: increased frost resistance, nutrient uptake, and tolerance to herbivory. Interestingly, high root:shoot ratios are often found in species of later successional stages, presumably because of the increased competition intensity in these habitats (Gleeson & Tilman, 1994). If this is true, then invasive European populations of S. inaequidens, which are currently restricted to ruderal and largely competitor-free habitats (Scherber et al., 2003; Heger & Böhmer, 2005), may currently develop the potential to also invade established native communities in Central Europe, just as they already do in the Mediterranean (López-García & Maillet, 2005).

Pathways of introduction
In principle, adaptive genetic differentiation between native and invasive populations can be due to (1) natural selection and recombination creating novel genotypes in the invasive range, or (2) a differential success or sorting-out of different genotypes that already existed in the native range. Here, we found that invasive populations of S. inaequidens were less genetically variable than native populations, and they were ecologically most similar to a subset of native populations from mountainous regions (Golden Gate populations) in southern Africa. This strongly suggests that the genetic differences in ecological traits observed in this study are the result of a sorting-out process, where pre-adapted genotypes of S. inaequidens from the mountainous regions of southern Africa have become successful invaders in Central Europe. This may also explain the lag time between first introduction and spread of S. inaequidens in Central Europe; probably the species could start to spread only after these frost-resistant and competitive genotypes had been introduced by chance.

This scenario is corroborated by a recent cytological study showing that mountainous southern African populations of S. inaequidens are mainly tetraploid, including the two Golden Gate populations in our study, whereas lowland populations are generally diploid. In contrast, all European populations tested were tetraploid (Lafuma et al., 2003), which also suggests that invasive European populations originate from the mountainous regions of southern Africa. Unfortunately, we do not have cytological information for all of the populations in our study, so this line of evidence must remain anecdotal.

Our study also included one Southern European population from Montpellier, which in terms of its ecological traits consistently clustered among South African populations. However, whether this is due to another, independent introduction, or due to local adaptation to the warmer climatic conditions, remains to be clarified by future research.

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