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LETTER

The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores

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Abstract

The success of invasive plants has been attributed to their escape from natural enemies and subsequent evolutionary change in allocation from defence to growth and reproduction. In common garden experiments with *Senecio jacobaea*, a noxious invasive weed almost worldwide, the invasive populations from North America, Australia, and New Zealand did indeed allocate more resources to vegetative and reproductive biomass. However, invasive plants did not show a complete change in allocation from defence to growth and reproduction. Protection against generalist herbivores increased in invasive populations and pyrrolizidine alkaloids, their main anti-herbivore compounds, did not decline in invasive populations but were higher overall compared with native populations. In contrast, invasive plants lost additional protection against specialist herbivores adapted to pyrrolizidine alkaloids. Hence, the absence of specialist herbivores in invasive populations resulted in the evolution of lower protection against specialists and increased growth and reproduction, but also allowed a shift towards higher protection against generalist herbivores.

Keywords

Alkaloids, biodiversity, biological invasion, defence, enemy release hypothesis, generalist/specialist trade-off, herbivory, *Senecio jacobaea*, Tansy ragwort.

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INTRODUCTION

A small percentage of exotic plant species becomes invasive weeds although they are not pest species in their native range (Mack *et al.* 2000). The reasons why some species are capable of invasions following introduction, and which traits allow them to disrupt the systems into which they are introduced, remain intensively debated (e.g. Elton 1958; Blossey & Nötzold 1995; Callaway & Aschehoug 2000; Keane & Crawley 2002; Agrawal & Kotanen 2003; Mitchell & Power 2003; Torchin *et al.* 2003; Colautti *et al.* 2004; Lambrinos 2004). It has been postulated that invasive plants profit from a direct release from natural enemies (enemy release hypothesis, ERH; Keane & Crawley 2002). Since resistance can be costly (Koricheva 2002) and many herbivore resistance traits are genetically controlled (Kennedy & Barbour 1992), the low abundance or absence of herbivores in new environments could cause rapid evolutionary change through selection for reduced anti-herbivore defences. Improved competitive ability through a shift in allocation from defence to growth has been predicted by the Evolution of Improved Competitive Ability or EICA hypothesis (Blossey & Nötzold 1995), but evidence collected so far is contradictory (Colautti *et al.* 2004). Higher performance has been observed in several invasive species (Blossey & Nötzold 1995; Willis & Blossey 1999; Siemann & Rogers 2001; Leger & Rice 2003; Wolfe *et al.* 2004), but not in others (Willis *et al.* 2000; Vila *et al.* 2003; Maron *et al.* 2004) and in some cases invasive populations even showed decreased competitive ability (van Kleunen & Schmid 2003; Bossdorf *et al.* 2004). Similarly, the higher susceptibility to herbivory proposed by the EICA hypothesis (Blossey & Nötzold 1995) has only occasionally been demonstrated in invasive populations (Blossey & Nötzold 1995; Daehler & Strong 1997; Siemann & Rogers 2003; Wolfe *et al.* 2004; Maron *et al.* 2004) and decreased levels of defence because of the loss of natural enemies potentially leading to a compensatory release (Colautti *et al.* 2004) have only been directly measured in few studies (Willis *et al.* 1999; Siemann & Rogers 2001; Blair & Wolfe 2004; Maron *et al.* 2004).

When testing the ERH and EICA hypothesis, it is necessary to incorporate the fundamental difference between specialist (feeding on one or a few closely related plant species) and generalist (feeding on several non-related plant species) herbivores (Müller-Schärer *et al.* 2004). The specialist-generalist dilemma (van der Meijden 1996) argues that in native areas, intermediate levels of chemical defences are maintained by opposing selective forces of adapted specialists that use plant defence chemicals as host-plant recognition cues and feeding- and oviposition stimulants, and of non-adapted generalists that are deterred by the same chemicals. We therefore predict, combining the ERH/EICA and specialist-generalist dilemma, that plants introduced into areas where their specialist herbivores are absent but generalist herbivores are present, may evolve increased (qualitative, cheap) defences against generalist herbivores and decreased (quantitative, expensive) defences against specialist herbivores. If the defence chemicals against generalists are less 'expensive' than the defence chemicals against the specialists, such an evolutionary shift in defence strategy in invasive populations may result in a net gain of resources for the plant, which then can be invested into increased growth and reproduction.

We tested the above predictions using the monocarpic perennial *Senecio jacobaea* L. (Tansy Ragwort) as a model species. *S. jacobaea* is native to Eurasia (Bain 1991), and has become a noxious weed in New Zealand, Australia and North America, where it was first recorded from the second half to end of the 19th century, respectively (Wardle 1987; Bain 1991; Rice 2003). In the native area, specialist herbivores and defence mechanisms in *S. jacobaea*, especially the production of pyrrolizidine alkaloids, secondary compounds toxic to non-adapted (generalist) herbivores and livestock, have been intensively studied and related to the occurrence of specialist insect herbivores (e.g. Vrieling *et al.* 1991; Hartmann 1999; Macel & Vrieling 2003). Here, we compare herbivory by generalist and specialist insects, levels of chemical defence, growth, reproduction and regrowth between native and invasive populations of *S. jacobaea* grown in common experimental environments. It has been argued that regrowth capacity in *S. jacobaea* has evolved as a strategy to cope with predictable episodes of herbivory by univoltine specialist herbivores like *Tyria jacobaeae* (Cinnabar moth; van der Meijden *et al.* 2000). A decreased regrowth capacity is therefore expected to evolve in invasive areas as predicted by the EICA hypothesis.

We ask (1) whether invasive populations show decreased defences against specialist herbivores, but increased defence against generalist herbivores; (2) whether levels of pyrrolizidine alkaloids, the main anti-herbivory compound in *S. jacobaea* (Hartmann 2004) are higher in invasive populations; and (3) whether invasive plants show higher growth and reproduction compared with native populations.

MATERIAL AND METHODS

Study species

Senecio jacobaea L. (Tansy Ragwort) is a self-incompatible, monocarpic perennial plant species that forms rosettes in its first growing season (Harper & Wood 1957; Wardle 1987). Its flowering is triggered by a cold period provided the plants have reached a certain critical threshold size (Wesselingh & Klinkhamer 1996). *Senecio jacobaea* is native in Eurasia where it is attacked by more than 70 herbivores (Harper & Wood 1957). In Europe, most herbivory is by the univoltine Cinnabar moth (*T. jacobaeae* L.; Lepidoptera: Arctiidae) and the specialized fleabeetle *Longitarsus jacobaeae* L. (Coleoptera: Chrysomelidae). Both have been introduced as biocontrol agents within the invasive range (McEvoy *et al.* 1993). In the USA, *c.* 42 species of generalist arthropods are recorded feeding on *S. jacobaea* (Frick 1972). *Senecio jacobaea* is recorded to contain more than 14 different pyrrolizidine alkaloids (PAs) (Witte *et al.* 1992). Variation in concentration and composition in PAs is to a large extent (50–100%) under genetic control (Vrieling *et al.* 1993). In the native range, populations have been found to belong to two major (PA) chemotypes, the jacobine type and the erucifoline type (Witte *et al.* 1992; Macel *et al.* 2004).

Plant growth

Climate-room experiment: seeds collected between 1998 and 2000 from 13 native and 16 invasive populations of *S. jacobaea* (see Table 1) were sown in a 1 : 1 v/v mixture of compost and dune sand. Two weeks after germination, equally sized seedlings were potted in 0.8–l pots in the same compost/dune sand mixture with 3 g per pot of 'Osmocote' added, a slow release fertilizer. Twelve plants per population (representing four maternal genotypes, except in populations where only bulk samples were available; see Table 1) were placed in a randomized block design with four blocks in growth chambers (12 h light/12 h dark with 18/14 °C temperature, relative humidity 80%). After 5 months, the length of the longest leaf and the number of leaves and rosettes of 12 plants per population were recorded. Chlorophyll content in two expanded leaves per individual was measured using a chlorophyll meter (Minolta SPAD-502). After 8 months, all leaves of three genotypes per population were removed and regrown biomass after 42 days in the climate room was compared with control plants. The leaf area of harvested leaves was measured with a portable area meter (LI-3000; LI-COR, Lincoln, NE, USA

Table 1 Sites of origin of populations used in the experiments (1) in climate rooms to measure vegetative growth, SLA, regrowth capacity, PA content of leaves, chlorophyll, and N-content of leaves, feeding preference of generalist and specialist herbivores (*Mamestra brassicae* and *Tyria jacobaeae*, respectively); and (2) in an experimental garden to assess flowering probability, reproductive biomass, PA content of flowers, and attack rates of the specialist herbivore *Platyptilia isodactylus*; (3) populations used to assess oviposition preference in *Spodoptera exigua*

Country	Location	Longitude	Latitude	Jacobine/ erucifoline type	Climate room experiment	Common garden experiment	*Spodoptera* oviposition experiment	Bulk sample/ maternal genotypes
Native populations								
A Poland	Warsaw	E 21°	N 52°	E	x	x		B
B Sweden	Gotland	E 18°	N 57°	J	x	x	x	M
C Czech Republic	Zlin	E 18°	N 49°	E	x	x		B
D Germany	Buggingen	E 8°	N 48°	E	x	x		M
E Switzerland	Rothenturm	E 8°	N 47°	E	x	x		M
F Brochterbeck	E 7°	N 52°	E	x	x		B	
G Switzerland	L'Himelette	E 7°	N 47°	E	x	x		M
H France	Dijon	E 4°	N 47°	E	x	x		B
I The Netherlands	Leiden	E 4°	N 52°	J	x	x	x	M
J The Netherlands	Meijendel	E 4°	N 52°	J	x	x	x	M
K France	Chéreng	E 2°	N 50°	J	x	x	x	M
L UK	Wales	W 5°	N 51°	J	x	x		M
M Ireland	Baldoyle	W 6°	N 53°	J	x	x	x	B
Germany	Darmstadt	E 8°	N 49°	E		x		B
The Netherlands	Westervoort	E 5°	N 51°	J		x	x	M
Invasive populations								
N Canada	Abbotsford (British Columbia)	W 122°	N 49°	J	x	x	x	B
O Canada	Yarrow (British Columbia)	W 122°	N 49°	J	x	x	x	B
P USA	Island Lake Road (Montana)	W 114°	N 48°	J	x	x	x	M
Q USA	Suprise Hill-Sylvia Lake (Montana)	W 114°	N 48°	J	x	x	x	M
R USA	Silvertown (Oregon)	W 122°	N 45°	J	x	x	x	M
S USA	Larch Slope (Oregon)	W 121°	N 45°	J	x	x	x	M
T USA	South Cooper (Oregon)	W 121°	N 45°	J	x	x	x	M
U USA	Indian Creek Road (Oregon)	W 122°	N 44°	J	x	x	x	M
V New Zealand	Waikato, Hamilton (North Island) Island)	E 173°	S 48°	J	x	x	x	M
W New Zealand	Marlborough Sounds (South Island)	E 170°	S 41°	J	x	x	x	M
X New Zealand	Marble Hill (South Island)	E 172°	S 42°	J	x	x	x	M
Y New Zealand	Inchbonnie (South Island)	E 171°	S 42°	J	x	x	x	M
Z Australia	Southern Tasmania	E 147°	S 43°	J	x	x	x	M
AA Australia	Northern Tasmania	E 146°	S 41°	J	x	x	x	M
AB Australia	Mornigton Peninsula (Victoria)	E 144°	S 38°	J	x	x	x	M
AC Australia	Snake island (Victoria)	E 145°	S 38°	J	x	x	x	B

Populations were assigned to the jacobine chemotype if plants of these populations in the vegetative and/or in the reproductive stage produced jacobine pyrrolizidine alkaloids. At the Canadian sites, *Tyria jacobaeae* and *Longitarsus jacobaeae* have been successfully introduced as biological control agents since the 1960s. At the US sites in Oregon, *T. jacobaeae*, *L. jacobaeae* and *Botanophila jacobaeae* Waterhouse have been released. At the Tasmanian sites, *Longitarsus flavicornis* (Stephens) was released in 1979. The type of seed collection is indicated by M where seeds of a population have been separately bagged for each maternal genotype and with B for batch collections.

and leaves were subsequently weighed to calculate the specific leaf area (SLA, m^2 g^{-1}). At the same time, the fresh weight of washed roots was measured (g fresh weight).

Common garden experiment: seedlings of 15 native and 16 invasive populations (see Table 1) were placed in 2-L pots in a 1:1 v/v mixture of compost and dune sand and fertilized initially with 6 g per pot of 'Osmocote'. Five cohorts of each five genotypes per population were placed in a randomized block design with five blocks per cohort in an experimental garden in Leiden, The Netherlands (N 52°, E 4°) in July. The plants remained outside during winter. Afterwards, they were placed in a glasshouse to trigger flowering from March to July 2002. All inflorescences were harvested continuously from 3 to 25 June 2002 to obtain an estimate of cumulative reproductive biomass per individual.

Chemical analyses

One leaf of four 5-months old plants per population (climate-room experiment) representing four different maternal genotypes (except in populations where only bulk samples were available; see Table 1) and one flowerhead of two plants per population (common garden experiment) were dried (50° C, 3d), ground and the PA composition and concentration was determined (see Macel & Vrieling 2003 for methodological details). Percent nitrogen content of the same leaves from the climate room experiment was measured by dry combustion using an automated CHN analyzer (LECO, CHN-1000).

Herbivore assays

Susceptibility to herbivory by generalist herbivores was tested with the native European generalist herbivore *Mamestra brassicae* L. (cabbage armyworm; Lepidoptera: Noctuidae; 13 days old, kept at 17 °C on a diet consisting of 60% cabbage, 30% Nasturtium, 10% *S. jacobaea* leaves) and *Spodoptera exigua* Hübner (beet armyworm; Lepidoptera: Noctuidae; reared on artificial diet). In individual enclosures in a climate room, one early 3d-instar larva of *M. brassicae* was placed on each of four 5-months old plants per population. Caterpillars that had died during the first 2 days were replaced by substitute caterpillars that had been reared on spare plants of the same populations. After 2 weeks, caterpillar mortality, number of attacked leaves, and minimum and maximum percent damage per leaf were assessed. Mean herbivore damage was calculated as number of attacked leaves × median of per cent damage. Oviposition preference was assessed in individual cages where one female moth of *S. exigua* was given a choice for oviposition between equally sized leaves from randomly paired jacobine-type native and invasive plants (see Table 1). Each leaf was placed in a 10 mL vial filled with water and after 2 days (experiment 1) and 4 days (experiment 2) the number of eggs and egg batches on the leaves were counted. Experiment 1 and 2 were each repeated 25 times, i.e. 50 different female moths were used in total. In the second experiment, one male moth was added to each individual cage. There was no significant difference between the two experiments, hence the results of both experiments were pooled and analysed as a split-plot design with a repeated measures analysis.

Herbivory by specialist herbivores was tested with the univoltine *T. jacobaeae*, a specialist on *S. jacobaea* and known to sequester PAs. In *T. jacobaeae*, PA's stimulate oviposition (Macel & Vrieling 2003) and act as feeding stimulants (Bernays *et al.* 2004). Larvae of *T. jacobaea* have taste sensilla specific for PAs and behavioural studies showed that feeding bouts are longer when PAs are present in a diet (Bernays *et al.* 2004). In climate rooms, six first instar larvae of *T. jacobaeae* of mixed egg batches were each put on four individually caged plants per population of 13 native and 16 invasive *S. jacobaea* populations. After 21 days, larval mortality and weight were assessed, and two larvae of intermediate weight were put back on the same plant to obtain data on survival until pupation and pupal weight. Sex of the pupae was included as a covariate in the analysis of pupal weight.

Statistical analyses

All variables were analysed with hierarchical analyses of variance or deviance (sequential sum of squares or sum of deviance changes) using generalized linear models as implemented in the statistical software Genstat 5. The following terms were fitted in the regressions: block, cohort (planting dates of plants grown in the experimental garden), native vs. invasive populations (fixed factor), geographical regions within the invasive range (North America, New Zealand, Australia), presence of biocontrol in invasive populations (see Table 1) and population identity. The term 'region' was tested against the pooled residual variance among native and among invasive populations (term 'population'). To check whether potential differences in variability among native and invasive populations had an influence on the significance of the term region, the residual variance between populations was also split into variation among native vs. variation among invasive populations (see e.g. van Kleunen *et al.* 2005) and the term region was then tested against the variation among invasive populations. In both type of analyses, however, the term region was not significant in any of the herbivory, regrowth or PA analyses indicating that there were no significant differences in these traits among regions within the invasive range. Only if the number of rosettes was tested against the variation among invasive populations instead of the pooled variation among both invasive and native populations, regional differences within the invasive range became significant (see Results). To account for the possibility that all invasive populations descended from European populations of the jacobine chemotype (see Table 1), additional comparisons between native and invasive jacobine-type populations were performed. All populations that either in the vegetative stage and/or in the generative stage produced jacobine are referred to as jacobine-type populations (see Table 1). Significance tests were based on *F*-tests (analysis of variance) or quasi-*F*-tests (analysis of deviance, mortality data, see McCullagh & Nelder 1989). The effect of native vs. invasive populations was tested for significance with *F*-ratios using the pooled variation among both invasive and native populations (term 'population') as denominator. The interaction term 'regrowth × native vs. invasive' in the analysis of the regrowth experiment was tested against 'regrowth × population'.

RESULTS

Production of main defence chemicals

No reduction in the production of PAs (the main defence chemicals) was detected in invasive populations in our experiments. In the vegetative stage, plants from invasive populations even produced on average 90% more PAs than plants from native populations (3.84 ± 0.273 mg g^{-1} dry mass in invasives vs. 2.02 ± 0.279 mg g^{-1} dry mass in natives; $F_{1,25} = 14.88$, $P < 0.001$). There was no significant difference in PA concentrations between regions within the group of invasive populations (New Zealand, Australia and North America; $P > 0.6$). In the native range, populations have been found to belong to two major chemotypes (Fig. 1). One chemotype is predominantly distributed along the coasts of Western Europe, and produces jacobine as its main PA and sometimes also small amounts of erucifoline, whereas the second chemotype produces no jacobine-type PAs and relatively small amounts of erucifoline and occurs more in inland Europe (Fig. 1, Table 1). In the invasive range, however, all populations were found to belong to the jacobine chemotype producing nearly identical spectra of PAs (Fig. 1). Only in the one population from the northern island of New Zealand, small amounts of erucifoline were consistently found in the vegetative stage as well as in flower heads (Fig. 1V). Therefore, the erucifoline chemotype has either not been introduced to most of the invasive areas or has been selected against in all new environments. In contrast to the invasive populations, native jacobine-type populations had more variable spectras of PAs and, with the exception of the Irish population (Fig. 1M), always contained plants that did produce both, jacobine-type PAs and some amount of erucifoline.

When comparing native and invasive jacobine-type populations, no difference was found in the PA content of leaves ($P = 0.4$). No significant difference between PA contents was also detected in the flower heads of plants from native and invasive populations (2.71 mg g^{-1} dry mass in native vs. 2.61 mg g^{-1} dry mass in invasive plants; $P = 0.8$). This similarity in PA contents of flower heads was mainly because of an increase in erucifoline in the native populations during flowering, as compared with the vegetative stage, indicating enhanced investment into defence of the most vulnerable organs in monocarpic species (van Dam *et al.* 1996).

Generalist herbivory

The generalist herbivore *M. brassicae* had significantly lower survival on invasive than on native plants (3% vs. 38%, $F_{1,25} = 27.97$, $P < 0.0001$). If only European jacobine-type plants were compared with invasive populations, this difference remained highly significant (3% vs. 21%, $F_{1,18} = 16.24$, $P < 0.0001$). Invasive plants also had fewer leaves attacked ($F_{1,25} = 7.57$, $P = 0.01$), and overall suffered lower feeding damage (number of leaves attacked × per cent damage, Fig. 2a). No significant difference was observed between regions in the invaded range (all $P > 0.2$). Feeding damage was negatively correlated with total PA concentration ($r = -0.32$, $P < 0.001$). However, if only jacobine-type populations were compared, the difference in feeding damage remained significant ($F_{1,20} = 4.17$, $P = 0.05$) with invasive plants still having 33% less damage. If total PA concentration was fitted as a covariate, *M. brassicae* still had a lower survival on invasive populations ($F_{1,26} = 13.32$, $P = 0.001$), showing that next to PAs other defence factors play a role. The lower performance of *M. brassicae* was not an effect of N-content in leaves either, because the average nitrogen concentration in dry leaves (4.16%) did not vary between native and invasive populations ($P > 0.5$). If fitted as a covariate, foliar nitrogen had no influence on the number of leaves attacked by *Mamestra* ($P > 0.68$).

Spodoptera exigua laid eggs preferably on native jacobine-type plants rather than on invasive (jacobine-type) populations (number of egg batches: 1.8 vs. 0.92, $F_{1,18} = 5.69$, $P < 0.05$; Fig. 2b). Both experiments show that generalist herbivores performed better or preferred plants from native populations even if only native jacobine-type populations were compared with invasive populations.

Specialist herbivory

In contrast to the generalists, native specialist herbivores adapted and attracted to PAs, preferred invasive plants. *T. jacobaeae* showed a higher survival on plants from invasive

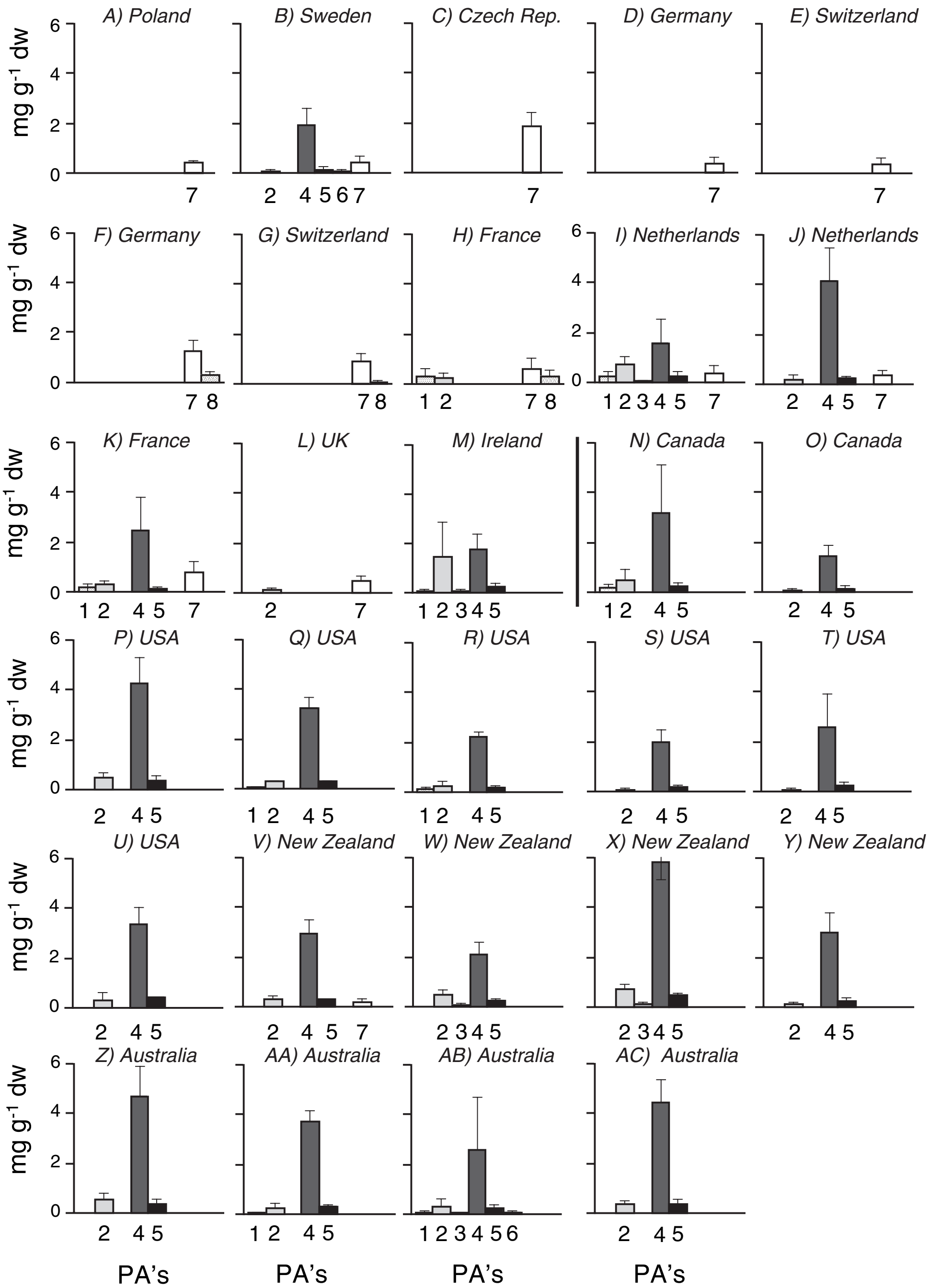

Figure 1 Pyrrolizidine alkaloid (PA) concentration in leaves of four plants per population of native and invasive *S. jacobaea* plants in the vegetative stage. Error bars denote ±1 SE. Individual pyrrolizidine alkaloids: 1, senecionine; 2, seneciphyline; 3, integerrimine; 4, jacobine; 5, jacozine; 6, jacoline; 7, erucifoline; 8, cf. acetylerucifoline. European sites are listed according to an East-West gradient mainly separating European inland vs. coastal populations. Coastal European populations and invasive populations were of the jacobine chemotype (see Table 1 for further details on the sites of origin).

populations (survival of first-instar larvae to day 21: $F_{1,24} = 5.93$, $P < 0.05$, Fig. 2c; survival d. 21 to pupation: $F_{1,24} = 4.08$, $P = 0.05$) and larval and pupal mass were also higher when individuals were reared on plants from invasive than from native populations (difference in larval mass: + 3.84 mg; $F_{1,24} = 7.84$, $P = 0.01$, difference in pupal mass: + 11.1 mg; $F_{1,23} = 4.69$, $P = 0.04$). If only jacobine-type plants were compared, these differences became even

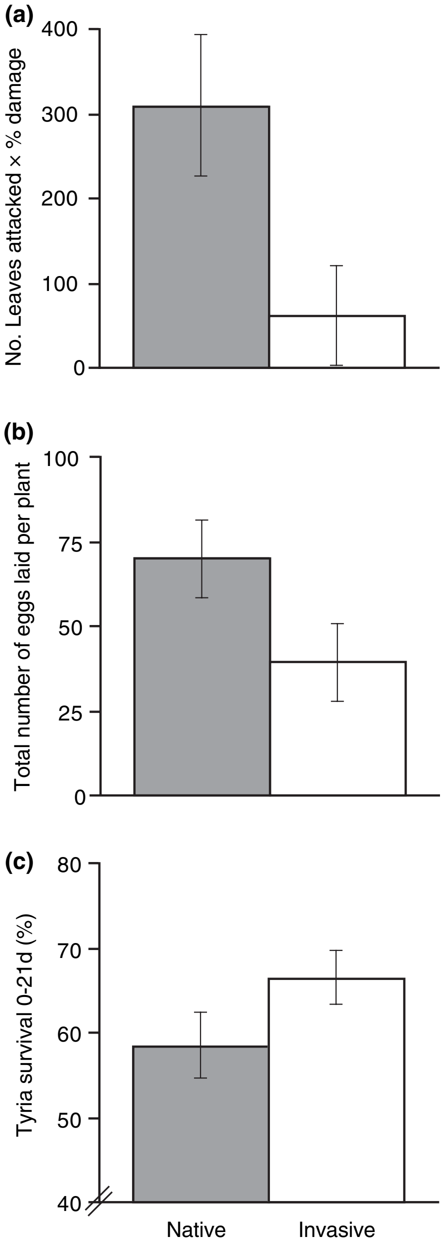

Figure 2 Herbivore assays on *S. jacobaea* plants from all native and invasive populations. (a) Herbivory by the generalist *Mamestra brassicae* calculated as the number of leaves attacked per plant × median of % damage per leaf ($F_{1,25} = 11.10$, $P < 0.01$); (b) total number of eggs laid on native and invasive jacobine-type plants by the generalist herbivore *Spodoptera exigua* ($F_{1,18} = 7.37$, $P = 0.01$); (c) larval survival of the specialist herbivore *Tyria jacobaea* after 21 days on native and invasive *S. jacobaea* plants ($F_{1,24} = 5.93$, $P < 0.05$). Error bars denote ±1 SE.

more pronounced (+ 6.76 mg, $F_{1,18} = 11.0$, $P = 0.004$, and + 16.6 mg, $F_{1,18} = 6.41$, $P = 0.02$, respectively) indicating that, in addition to lower levels of PAs and therefore lower attractiveness, native plants must have had other defences against *Tyria* that were lost in invasive plants. Again, no significant differences were found between regions within the group of invasive populations (all $P > 0.4$).

An accidental infestation by the plume moth *Platyptilia isodactylus* (Zeller), a specialist stem-borer on *Senecio*, at the end of the experiment in the experimental garden showed that this specialist herbivore also infested invasive plants 1.6 times more often than native plants ($F_{1,27} = 4.99$, $P = 0.03$). Here as well, this difference became more significant if only jacobine-type populations were compared ($F_{1,19} = 15.84$, $P < 0.001$).

Investment in growth

As predicted by the ERH and EICA hypothesis, invasive plants under identical experimental conditions grew larger than native plants indicating that this difference has a genetic basis and is not just because of phenotypic plasticity. Even though flowering probability was not significantly different between native and invasive populations ($P > 0.6$), reproductive biomass of invasive plants was significantly higher (+ 37%; $F_{1,21} = 22.97$, $P < 0.0001$; Fig. 3a), also if only native populations of the jacobine type were compared with invasive populations (+ 34%; $F_{1,13} = 4.91$, $P < 0.05$). Reproductive biomass did not differ among regions in the invasive range ($P > 0.13$). Similarly, vegetative growth was consistently higher in invasive plants if measured as 1) the number of leaves and rosettes per plant in a climate room ($F_{1,25} = 5.73$, $P < 0.05$, Fig. 3b; $F_{1,25} = 8.1$, $P < 0.01$); (2) the root biomass per plant in a climate room $F_{1,25} = 12.29$, $P < 0.001$); (3) or the diameter of the root crown in the experimental garden ($F_{1,27} = 18.79$, $P < 0.001$). There were no significant differences among regions within the invasive range in vegetative growth with the exception of rosette number: if the number of rosettes was tested against the variation among invasive populations, regional differences within the invasive range were significant ($F_{2,13} = 4.88$, $P < 0.05$) with populations from New Zealand slightly differing in average rosette number from European populations (3.48 ± 0.183 vs. 3.42 ± 0.155), but populations from North America and Australia having clearly a higher number of rosettes per plant (4.47 ± 0.146 and 4.13 ± 0.178, respectively). No differences in ploidy level were found in a subset of native and invasive populations.

Despite the higher investment in growth and reproduction, no trade-offs in foliar chlorophyll or nitrogen content were detected, whether all plants or only jacobine-type plants were compared (all $P > 0.3$). Increased size was also not correlated with higher SLA (i.e. greater leaf area per unit leaf mass suggesting higher palatability). In contrast, native populations invested less in leaves, i.e. had a higher SLA

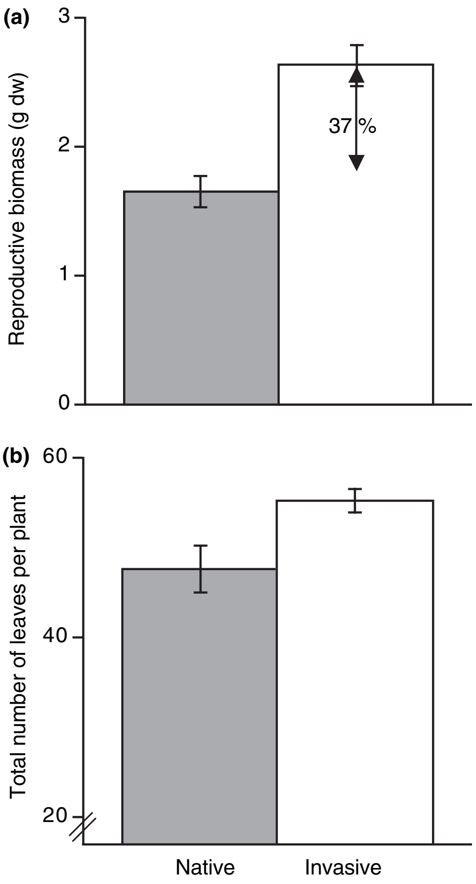

Figure 3 Plant performance in all native and invasive populations of *S. jacobaea*. (a) Reproductive biomass of native and invasive plants ($F_{1,21} = 22.97$, $P < 0.001$); (b) total number of leaves per plant of individuals grown for 5 months under identical climate-room conditions ($F_{1,25} = 5.73$, $P < 0.05$). Error bars denote ±1 SE.

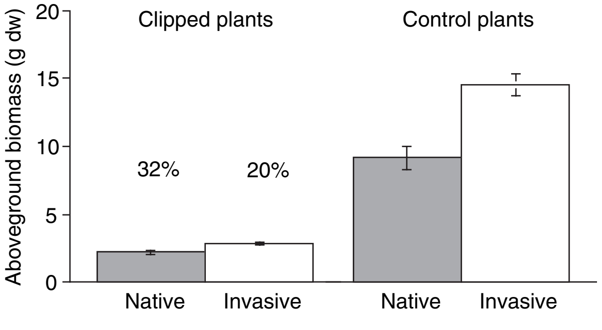

Figure 4 Regrowth potential in native and invasive *S. jacobaea* plants. Regrowth capacity in invasive populations was smaller in relative terms ($F_{1,23} = 9.90$, $P < 0.01$). Percentages denote the amount of regrown biomass compared with control plants. Error bars denote ±1 SE.

than invasive populations (0.016 $m^2\ g^{-1}$ vs. 0.013 $m^2\ g^{-1}$; $F_{1,23} = 9.03$, $P < 0.01$). The difference between native and invasive jacobine types remained significant, but was less pronounced (0.015 $m^2\ g^{-1}$ vs. 0.013 $m^2\ g^{-1}$; $F_{1,20} = 4.3$, $P = 0.05$).

In relative terms, regrowth capacity in invasive populations was smaller than in native populations ($F_{1,23} = 9.90$, $P < 0.01$; Fig. 4). In absolute terms, however, invasives still had 28% more aboveground biomass 42 days after defoliation than did native plants.

DISCUSSION

We expected qualitative defences to increase in the invasive areas where no specialist herbivores are present and plants are only attacked by generalist species (Frick 1972). Indeed, PA levels in the invasive area increased by 90%. A doubled PA concentration in leaves of invasive plants in the reproductive stage was also found in a field experiment comparing four European populations (two jacobine-type and two erucifoline-type populations) with two populations each from the USA and New Zealand (Stastny *et al.* 2005). However, in our study, only the jacobine chemotype was found in the invasive area and the total PA concentration of jacobine chemotypes did not differ between native and invasive areas. Therefore, the erucifoline chemotype has either not been introduced to most of the invasive areas or has been selected against in all new environments. Although the physiological reason is not known, it seems if jacobine is present, plants can obtain higher total PA concentrations (Macel *et al.* 2004). Hence, selection for jacobine is also selection for higher concentrations of PAs in general. We cannot exclude the possibility that all invasive populations were descendants of the coastal European populations of the jacobine chemotype (or of populations that were not sampled in this study). However, in the invasive populations where specialist biological control agents had been introduced 25–30 years ago (see Table 1), there was a trend of PA-levels to decrease again ($F_{1,24} = 3.46$, $P = 0.08$). Hence, specialist herbivores seem to impose a selective pressure on the production on PAs and the uniformity of invasive populations with regard to patterns of PAs (see Fig. 1) might not solely be a result of shared common ancestry, but rather or in addition a result of rapid evolution.

The results of our herbivory assays suggest that rapid evolution of decreased defence against specialist herbivores (absent in the invaded areas), and higher protection against generalist herbivores are factors contributing to the invasion success of *S. jacobaea*. In the native range, defence traits of this species are maintained at intermediate levels by opposing selection pressures of specialist and generalist insect herbivores (van der Meijden 1996). In introduced areas, however, invasive plants have often escaped specialist insect herbivores and are exposed to a natural enemy complex that is dominated by generalists only (e.g. Frick 1972; Keane & Crawley 2002; Wolfe 2002). Therefore, the observed shift towards higher defences against generalist herbivores and lower defences against specialist herbivores, shown by the lower performance of generalist herbivores and higher preference and performance of specialist herbivores in invasive populations, was the expected evolutionary response. Remarkably, this difference in performance of generalist vs. specialist herbivores was even significant if only the chemically more similar native European populations of the jacobine chemotype were used for the comparison with invasive populations (all of the jacobine chemotype, see Figure 1) and might therefore be related to other, not measured, chemical defences.

If the defence chemicals against generalists are less 'expensive' than the defence chemicals against the specialists, such an evolutionary shift in defence strategy in invasive populations may result in a net gain of resources for the plant, and may explain the increased growth and reproduction of invasive populations of *S. jacobaea*. Invasive populations produced more biomass in the climate room and the experimental garden and also reproductive biomass was 37% higher in plants from invasive areas (also if only native jacobine types were compared with invasive populations). Higher vegetative and reproductive biomass in invasive populations has also been found in a field experiment comparing some European with few populations from North America and New Zealand under grassland field conditions (Stastny *et al.* 2005). In contrast, Willis *et al.* (2000) did not find an increased vigour in invasive *S. jacobaea* plants comparing three invasive populations each from Australia and New Zealand with three native populations each from the UK and continental Europe.

In this study, the investigated invasive populations of *S. jacobaea* from three geographic regions (North America, New Zealand and Australia) showed the same herbivory and growth-related differences in comparison with native populations spread across the native European range (with the exception of the number of rosettes that differed between populations from New Zealand and populations either from Australia or North America). This suggests three possible scenarios: (1) subsequent introductions from one invasive area to the other have taken place or (2) separate introductions from the same native source populations with similar defences have taken place in the different invasive areas or (3) parallel evolution of these characters has occurred in response to the disappearance of selection pressures, likely from specialist herbivores, after invasion. In other traits, which are probably not under strong selection from herbivores, for example the minimal size for flower induction (larger in North America than in New Zealand and Australia, $x^2 = 21.12$, d.f. = 2, $P < 0.001$; J. Joshi, personal communication), invasive populations showed divergent differences in comparison with native populations. This indicates, that they had probably responded to geographically divergent selection pressures between the different areas of introduction (see e.g. Weber & Schmid 1998 for an example of latitudinal differentiation in flowering time among invasive populations of another invasive composite species).

There is growing evidence that ecological-evolutionary interactions may have a more pervasive influence on contemporary invasion dynamics than assumed previously (Lambrinos 2004). Given the lack of differences in PA levels between invasive and European jacobine-type populations, the significant differences in response to generalist vs. specialist herbivores even if only European jacobine-type populations were compared with invasive populations (see previous paragraph) must have been because of other defence mechanisms. Potential candidates for other defence chemicals are sesquiterpenes (Gonzalezcoloma *et al.* 1995) that have been detected in *Senecio* species, but have not been studied in *S. jacobaea*. In addition, chlorogenic acids (H. Kirk, unpublished data), and flavonoids (H. Kirk, unpublished data) were recently detected in *S. jacobaea* and are currently under research.

Invasion success based on release from the specialist-generalist trade-off (van der Meijden 1996) followed by increased production of toxic secondary compounds against generalist herbivores is a scenario that to our knowledge has not been considered so far by the EICA hypothesis. It should be included in future assessments of the invasion potential of introduced species that harbour chemically adapted specialist herbivores in their native range. Given that, e.g. of 5000 native British insect species 80% can be regarded as specialists (Schoonhoven *et al.* 1998), the potential number of invasive plant species that may have evolved similarly as *S. jacobaea* could be considerable.

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