

Do vigour of introduced populations and escape from specialist herbivores contribute to invasiveness?

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Summary

1 Plant species may become invasive due to a lack of natural enemies (e.g. herbivores) in their introduced range. Absence of herbivores may result in selection for the loss of costly herbivore-resistance traits, which are expected to show a trade-off with vigour or competitive ability (the evolution of increased competitive ability, or EICA, hypothesis).

2 We conducted a common garden experiment in Switzerland, in which we compared herbivore resistance and vigour of *Senecio jacobaea* plants exposed to the specialist flea beetle *Longitarsus jacobaeae*, for four populations originating within the native range (Europe), and four from regions where it had been introduced (New Zealand, USA) and was unaffected by *L. jacobaeae*. Our predictions were that, compared with plants from the native populations, plants from introduced populations would experience greater herbivory (due to loss of resistance traits), and exhibit more vigorous growth.

3 As expected, we found that introduced *S. jacobaea* grew larger, and had greater reproductive output, than plants from the native range. Larger plants experienced more feeding damage, and introduced plants were consumed more even when size differences were controlled. Introduced plants also exhibited a greater relative ability to reproduce after damage was sustained, i.e. higher tolerance to herbivory.

4 Contrary to predictions, however, plants from introduced populations had higher total pyrrolizidine alkaloid production (chemical defence against herbivores).

5 Although plants from introduced ranges exhibited life-history traits consistent with EICA predictions, similar phenotypes were common in one of the populations from the native range, suggesting that EICA may not fully explain the invasion success of *S. jacobaea*.

6 Our results imply that increased competitive ability (vigour) of invasive plants may be associated with changes in resistance as well as tolerance to herbivory, and both types of anti-herbivore defence may need to be examined simultaneously to advance our understanding of invasiveness.

Key-words: enemy release hypothesis (ERH), evolution of increased competitive ability (EICA), herbivore resistance, herbivore tolerance, invasive species, *Longitarsus jacobaeae*, *Senecio jacobaea*, vigour

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Introduction

Invasive species present a serious problem in direct economic costs and in their impact on biodiversity (Pimentel *et al.* 2000). One explanation of the success

of the estimated 1% of naturalized species that become invasive (Williamson & Fitter 1996) highlights the lack of associated natural enemies in the new habitats (Maron & Vilá 2001; Mitchell & Power 2003; Torchin *et al.* 2003). In plant invasions, the enemy release hypothesis (ERH; for review see Keane & Crawley 2002; Hierro *et al.* 2005) predicts that a plant species introduced to areas outside its native range should increase in abundance as a result of the reduced impact of natural enemies (especially herbivores) in the introduced range.

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The argument is based on the assumption that, if specialist enemies present in its native range are absent from the introduced range, the invader will encounter few (or only generalist) enemies and these will have a relatively low impact, allowing population growth. The predictions of ERH have found support in some studies (Schierenbeck *et al.* 1994; Wolfe 2002; but see Agrawal & Kotanen 2003; Vilá *et al.* 2003), but this theory has traditionally been assumed to be true in order to justify introductions of biocontrol agents.

An extension of the ERH hypothesis applies the assumption of a trade-off between growth and defence (Simms 1992; Stamp 2003) to provide an alternative mechanism for plant invasion. The evolution of increased competitive ability hypothesis (EICA; Blossey & Nötzold 1995; Adler 1999) proposes that, under conditions of reduced herbivory in the introduced range, selection will favour genotypes with higher allocation towards competitive abilities and growth, and lower allocation towards resistance to herbivores. Over time, plants from populations introduced to areas outside the native range (hereafter 'introduced') are predicted to show increased vigour, and (if the host–specialist association is re-established) suffer greater damage by specialist herbivores, compared with plants from populations within the native range (hereafter 'native'). A test of EICA thus requires a comparison of the performance of native and introduced populations in a common environment. Several studies have found evidence for increased vigour or competitiveness of introduced plant species in new habitats (Willis & Blossey 1999; Siemann & Rogers 2001; Leger & Rice 2003; Siemann & Rogers 2003; Vilá & Weiner 2004; Wolfe *et al.* 2004; but see also Willis *et al.* 1999; Willis *et al.* 2000; Thebaud & Simberloff 2001; Simons 2003; van Kleunen & Schmid 2003; Hierro *et al.* 2005), but few of these have compared insect herbivory or plant resistance traits on introduced and native populations (Daehler & Strong 1997; Willis & Blossey 1999; Garcia-Rossi *et al.* 2003; Siemann & Rogers 2003).

The EICA hypothesis directly addresses the possibility that populations in the introduced range may be characterized by genotypes with reduced resistance, yet in order to defend against herbivores, plants can utilize either resistance traits (which reduce damage by herbivory) or tolerance traits (which reduce the impact of herbivory on plant fitness; Tiffin 2000). The resistance–tolerance trade-off (see review by Strauss & Agrawal 1999) predicts that reduced resistance may translate into increased tolerance to herbivory; for example, if resources reallocated from resistance traits to growth allow individuals to repair damage, or regrow after damage, such individuals will have greater reproductive capability than less tolerant individuals in which fitness will be more negatively affected. Therefore, we predicted that populations in the introduced range that have escaped from herbivory may evolve increased tolerance as well as reduced resistance.

To test the predictions of EICA, we performed an experiment using the well-studied system of an invasive

plant *Senecio jacobaea* (tansy ragwort) and its specialist herbivore *Longitarsus jacobaeae* (tansy ragwort flea beetle). A native in much of temperate Europe, *S. jacobaea* was introduced without its specialists into new regions in the late 19th and early 20th centuries (Coombs *et al.* 1999). It subsequently became invasive in grazed areas of western North America, southern Australia and Tasmania, and New Zealand. No direct comparison of native and introduced populations had been available until recently, when Willis *et al.* (2000) compared vegetative size of European and New Zealand/Australian populations in a common garden experiment and did not find significant differences. However, in order to fully test the EICA predictions, a comparison in the context of herbivory is required.

We performed a common garden experiment in the native range, in Switzerland, to compare vegetative growth and reproduction, as well as resistance and tolerance to herbivores between and within populations of different geographical origin and specialist enemy history (native and introduced populations), under herbivory by *Longitarsus jacobaeae*. On the basis of EICA, we predicted that compared with native populations, introduced populations would in general show (i) increased vigour and reproduction, and (ii) higher feeding damage by *L. jacobaeae* as a consequence of their putatively reduced defence (i.e. lower concentration of anti-herbivory compounds). In addition, we predicted that (iii) the introduced populations would show higher levels of tolerance to herbivory by *L. jacobaeae* than native populations.

Methods

STUDY SYSTEM

Senecio jacobaea (Asteraceae) and its specialist herbivores are among the most extensively studied systems in plant–insect interactions. A biennial, *S. jacobaea* spends the first year as a rosette and bolts the following spring before senescing, producing inflorescences with hundreds to thousands of capitula, each containing several dozen long-lived seeds (McEvoy *et al.* 1993; Coombs *et al.* 1999). It has a marked capacity for regrowth after damage (van der Meijden *et al.* 2000), and produces a complex and variable profile of pyrrolizidine alkaloids (PAs), which have been strongly implicated in defence against herbivores (Witte *et al.* 1992; Macel *et al.* 2002). Vrieling *et al.* (1993) have shown that phenotypic variation in the total PA concentration has a genetic basis. A cost of this constitutive, non-inducible (van Dam *et al.* 1993) defence has been demonstrated under light-limiting conditions in the laboratory (Vrieling & van Wijk 1994a); in contrast, no costs were detected under natural conditions (Vrieling & van Wijk 1994b), even when additional defence traits (leaf pubescence, toughness, regrowth capacity) were considered (Vrieling *et al.* 1996).

The biology and impact of one of the main specialist herbivores of *S. jacobaea*, *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae), has been the focus of a number of studies, especially for the purpose of bio-control. This univoltine, monophagous insect has a significant impact on plant fitness and population dynamics of *S. jacobaea* (McEvoy *et al.* 1991; Windig 1993) through larval feeding on the root crowns. Adult beetles feed on the foliage throughout the summer and fall, leaving characteristic feeding punctures (hereafter 'shotholes'), but seem to have minimal direct effects on plant growth (Windig 1993). Shothole density estimates adult population size, which in turn predicts density of larvae; thus, number of shotholes is a straightforward way to estimate herbivory levels in this system.

PLANT MATERIAL

Seeds from eight populations from the native and introduced ranges of *Senecio jacobaea* (hereafter, 'origin types' or 'origins', see Table 1) were collected in 2000, and stored at 5 °C until the spring of 2001. Biocontrol agents, including *Longitarsus jacobaeae* or the sibling species *L. flavicornis*, have been released in many areas of the introduced range, but we specifically chose only introduced populations which, up to that point, had been unaffected by these and other (e.g. *Tyria jacobaeae*) specialist herbivores. In contrast, all native populations support populations of *L. jacobaeae* and/or *L. flavicornis* (in the case of Chereng).

A hierarchical design was used in order to partition variance in the plant traits of interest between families within populations, between populations within origin, and between origins. Eight families (maternal lines) with a sufficient number of viable seeds were randomly chosen from each of the eight populations (total of 64 families). Seeds were sown in a glasshouse on 29 and 30 May 2001, using trays of peat pots containing a mix of potting soil, sand, vermiculite and a time-release fertilizer, and covered with a thin layer of sand. Up to three seeds (when available) were sown per pot to compensate for expected differences in germination. Trays were rotated periodically to minimize position effects. In each pot the neighbours of the first seedling to germinate were removed before any direct competition could occur. The Mettembert (CH) population, which showed poor germination, was mass-germinated in Petri dishes, and then transplanted into pots. In order to reduce damage by fungus gnats, we applied a spraying and then soaking application of *Bacillus thuringiensis* var. *israelensis* (Skeetal® FC, Pluss-Staufer AG/SA, Oftringen, Switzerland) to all the trays.

EXPERIMENTAL DESIGN

The study was originally designed as a split-plot experiment divided into plots with herbivory by *L. jacobaeae* and control plots with a bi-weekly spray application of the broad-spectrum systemic insecticide dimethoate

Table 1 Location, climate and specialist herbivores for eight populations of *Senecio jacobaea* used as seed sources in the experiment

Population	Site of seed collection	Historical presence	Specialist herbivores	Mean January/July temperature, annual precipitation	Geographical coordinates/elevation
Meijendel (NL)	Meijendel, Leiden, W coast, the Netherlands	Native	<i>Longitarsus jacobaeae</i> , <i>Tyria jacobaeae</i>	3.4 °C/17.2 °C 878 mm	52°05' N, 04°16' E 10 m
Chereng (F)	Chereng, N Normandy, France	Native	<i>Longitarsus flavicornis</i> , <i>Longitarsus jacobaeae</i>	2.0 °C/17.5 °C 646 mm	49°30' N, 01°00' E 100 m
Mettembert (CH)	Mettembert, NE Jura, Switzerland	Native	<i>Longitarsus jacobaeae</i>	0.5 °C/16.0 °C 943 mm	47°24' N, 07°18' E 650 m
L'Himelette (CH)	L'Himelette, SE Jura, Switzerland	Native	<i>Longitarsus jacobaeae</i>	-2.0 °C/11.0 °C 1473 mm	47°12' N, 07°03' E 1200 m
Inchbonnie (NZ)	Inchbonnie, W coast South Island, New Zealand	1876 (for NZ)	<i>L. jacobaeae</i> introduced but failed to persist	16.5 °C/9.2 °C 2072 mm	42°43' S, 171°28' E 120 m
Linkwater (NZ)	Linkwater, N coast South Island, New Zealand	1876 (for NZ)	None	16.5 °C/5.7 °C 647 mm	41°18' S, 170°52' E 25 m
Larch Slope (USA)	Larch Slope, N Oregon, United States	1922 (for OR)	None	-1.2 °C/14.2 °C 2253 mm	45°07' N, 21°48' W 1000 m
Island Lake (USA)	Island Lake Road, NW Montana, United States	1970 (for MT)	None	-4.8 °C/19.4 °C 640 mm	48°15' N, 114°57' W 1100 m

(Perfekthion®, Dr R. Maag SA, Dielsdorf, Switzerland). Although no side-effects on plant growth had been reported for dimethoate, our measurements in 2001 and 2002 (see Data collection) suggested that the insecticide inhibited plant growth (data not shown). In addition, comparisons of above-ground, below-ground and total biomass in a glasshouse study using 80 randomly chosen potted seedlings revealed negative effects of dimethoate (data not shown). Thus, rather than making assumptions about similar (proportional) effects between the populations, we excluded our insecticide treatment altogether and present only data from the herbivory (insecticide-free) plots. It should be noted, however, that the relative trends observed in the insecticide treatment plots are in full agreement with the results discussed here, for all the growth parameters measured.

The plots were laid out in a uniform meadow with a southern aspect, adjacent to the CABI Bioscience Centre, Delémont, Switzerland. Both *S. jacobaea* and *L. jacobaeae* occur here naturally. Two 9 × 12 m plots were established with plants from eight populations, each represented by eight different families (total of 64 families). Each family consisted of four replicates per plot, regularized within plots in order to reduce position effects, except for a few families with poor germination. Therefore, a total of approximately 500 individuals were planted. Plants were spaced 0.5 m apart, and a row of plants from a random mix of families was added around the periphery of each plot to minimize edge effects. The site was mowed prior to transplanting, inducing a progressive increase in forb species. Seven populations were transplanted into the experimental plots on 11 July 2001, while the Mettembert (CH) population remained in the glasshouse for another week to compensate for late germination. Molluscicide granules (Blaukorn®, Pluess-Staufner AG, Oftringen, Switzerland) were scattered around each plant after transplanting. During summer 2001, vegetation surrounding our plants was clipped repeatedly, simulating grazing which facilitates the growth of *S. jacobaea* (McEvoy *et al.* 1993). The plots were irrigated in the summer.

As the local population of *L. jacobaeae* did not provide sufficient densities for the experiment, we increased herbivore density to the levels approaching those found in native ragwort infestations at nearby sites (M. Stastny, personal observation). Twice during the 2001 season, *L. jacobaeae* adults were collected from the surrounding region using a reverse leaf blower, and released at uniform densities throughout the plots. The sex ratios in populations collected by this method reflect the observed natural ratios, which are close to 1 : 1 (K.P. Puliafico, personal communication).

PLANT VIGOUR AND HERBIVORY DAMAGE

In 2001, measurements were taken to estimate vegetative size and feeding damage (so that we could assess resistance which equals 1 – damage, Pilson 2000). We measured

the length of the longest leaf and the total number of green leaves in the rosette, and multiplied these two variables to obtain a surrogate of rosette size which is highly correlated with above-ground biomass (Wesselingh 1995). Rosette size was first measured at the time of transplanting, and then 3, 6 and 15 weeks later. Feeding damage by *L. jacobaeae* was estimated by counting the total number of shotholes on all green leaves in the rosette 4 and 7 weeks after transplanting and, finally, at week 18, when feeding activity started to decline sharply after the first frost.

Estimation of larval densities involves extraction or dissection and requires destructive sampling. Consequently, we were not able to measure larval attack rates on experimental plants in spring 2002, but obtained data by examining the root crowns of randomly selected 20 edge plants under the microscope.

In 2002, all plants producing flowers were harvested individually at their peak of flowering, defined as the point at which the most mature flowers in the inflorescence began to senesce. The harvested above-ground parts were oven-dried at 80 °C for 24 hours, and weighed. All capitula within an inflorescence (open and unopened) were then counted for each individual to serve as a surrogate measure for seed number, because obtaining mature seeds would have posed the risk of spreading the non-native genotypes. We estimated relative investment into reproduction for each flowering individual by dividing the total number of capitula by the above-ground biomass.

PYRROLIZIDINE ALKALOID ANALYSIS

One bolting plant per family was selected randomly from each population, and several leaves were sampled for the analysis of PAs as described in Macel *et al.* (2002). The leaves were dried and then frozen until further handling. For PA extraction, ground plant material was added to 0.1 M H₂SO₄ and put on a shaker. After 1 hour, excess Zn powder was added to reduce N-oxides, and the solution was agitated for 2 hours. The samples were then made basic (pH 9–10), centrifuged, and the aqueous phase was purified over Extrelut. The column was washed with CH₂Cl₂ and the combined extracts evaporated to dryness. The residue was dissolved in MeOH. GC-FID was used to determine PA composition for each plant (Vrieling & de Boer 1999). Total PA concentration was determined spectrophotometrically, and individual PA concentrations were then calculated by multiplying total PA concentration by the fractions of the respective PA determined from GC analysis.

STATISTICAL ANALYSIS

All statistical analyses were conducted using the SAS statistical package, version 8.2 (SAS Institute, Cary, NC, USA). Origin was treated as a fixed factor. Because we used specific criteria for the selection of the populations

and were interested in between-population comparisons, we treated population nested within origin as a fixed factor. Family nested within population was treated as a random factor, and was included in the model to control for expected genetic differences between families within populations. Plot was likewise included as a random factor. We performed the analyses detailed below in the PROC MIXED procedure in SAS.

Both above-ground biomass and number of capitula were fourth-root transformed to conform to ANOVA assumptions, and analysed using the mixed-model ANOVA described above. Differences between origins and between populations in relative investment into reproduction (number of capitula divided by above-ground biomass) were assessed after a square-root transformation.

Rosette size differences between populations and between origin types were analysed as repeated measures over time. The data were fourth-root transformed to improve normality and reduce heteroscedasticity. To compare differences in adult feeding damage, we first corrected for the effect of size by regressing rosette size (fourth-root transformed) on damage (cube-root transformed). We then analysed the residuals of this model as repeated measures over time.

For comparison of total PA concentration between populations, non-transformed data were analysed using ANOVA, with one replicate per family in each population. Several observations had below-detection levels, and were assigned equal arbitrary values below the minimum detected value to meet ANOVA assumptions. In this model, origin and population effects were included as above, but (due to lack of replication) family effects were not.

The confounding insecticide effect (see above) precluded testing differences in tolerance between populations and between origins by comparing the fitness of plants in the herbivory and herbivore-free treatments (Stowe *et al.* 2000). Instead, we performed an ANCOVA on family means of relative investment into reproduction (defined above), with origin as the main effect and adult feeding damage residuals (maximum damage corrected for final rosette size) as the covariate. We interpreted a significant interaction (difference in regression slopes) as a difference in relative tolerance for the

two origin types. Thus, we removed the effects of vegetative size both with respect to herbivory and reproduction, and tested whether native and introduced families differed in the effect of herbivory on their relative fitness.

Results

PLANT VIGOUR

Differences in growth rate between the different populations became more apparent as the season progressed (Fig. 1a, Table 2). The growth trajectories for all eight populations show considerable differences between populations of the same origin type; notably, the plants from Chereng (F) grew, on average, much larger than the other native populations, producing greater variation in final rosette size between the native populations than between the introduced populations. Nevertheless, introduced populations were, overall, significantly larger than native populations.

The differences in vegetative growth observed in 2001 increased in 2002, during which the surviving plants bolted and flowered, with the exception of approximately 170 plants (excluded from the analyses) regrowing after heavy damage by voles (M. Stastny, personal observation). We found significant differences in above-ground biomass at the peak of flowering both between origin types and between populations nested within origins (Fig. 2a, Table 3). The native populations remained more heterogeneous than the introduced ones, as the Chereng (F) population maintained its high vigour (Fig. 2a), but introduced populations were still larger on average than the native populations (99.87 ± 15.10 g vs. 70.92 ± 22.96 g). Similar patterns were found for reproductive output (1831.00 ± 318.91 vs. 1038.75 ± 299.57 capitula; Fig. 2b, Table 3), because above-ground biomass and number of capitula were strongly correlated ($R = 0.91$, $P < 0.0001$). Again, both origin-level and population-level variation were highly significant (Table 3), with more variability within the native than within the introduced origin type.

The analysis of relative investment into reproduction also revealed significant differences at the level of origin

Table 2 Repeated-measures analysis of variance on the difference between native and invasive populations in vegetative rosette size and adult feeding damage corrected for rosette size. 'Origin' indicates native or invasive range. 'Time' represents the time of rosette size and feeding damage measurements. Size data were fourth-root transformed, damage data were cube-root transformed

Source	Rosette size			Adult feeding damage		
	d.f.	<i>F</i>	<i>P</i>	d.f.	<i>F</i>	<i>P</i>
Between subjects						
Origin	1	34.53	< 0.0001	1	7.50	0.0082
Population	6	7.60	< 0.0001	6	6.68	< 0.0001
Within subjects						
Time	3	2883.61	< 0.0001	2	0.00	0.9998
Time × Origin	3	6.74	0.0002	2	0.50	0.6042
Time × Population	18	5.94	< 0.0001	12	0.77	0.6849

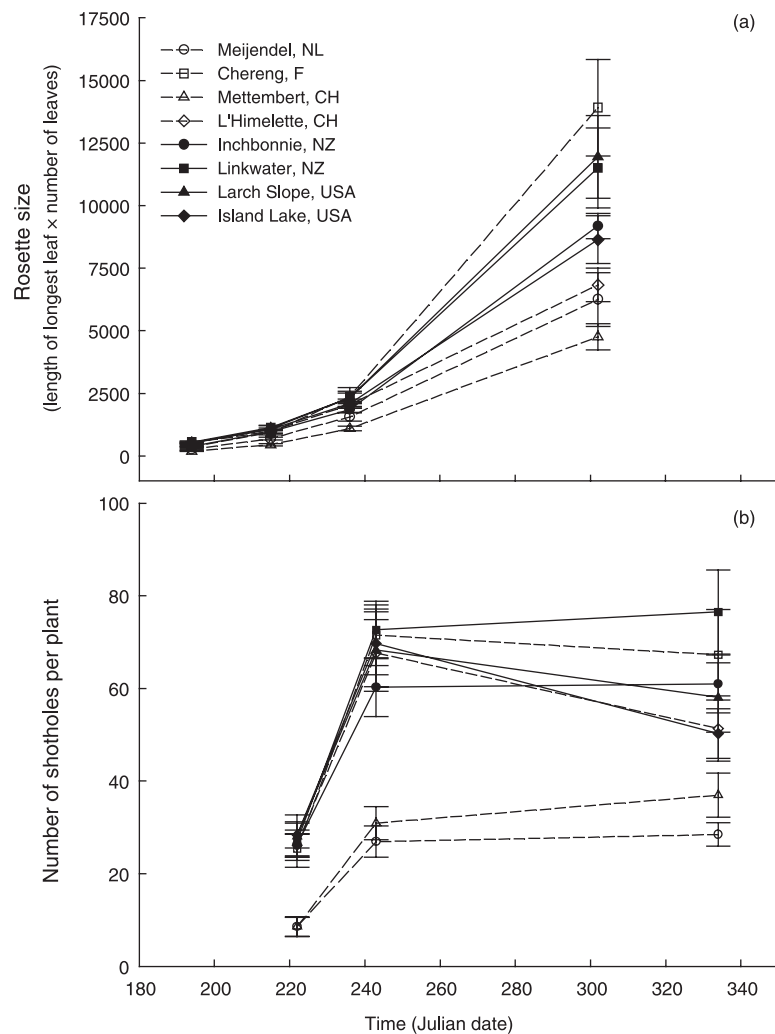


Fig. 1 Growth and herbivore damage over time in 2001 for populations of *Senecio jacobaea* from the native and introduced ranges. In all figures, error bars are ± 1 SE. Native populations: open symbols, dashed line. Introduced populations: filled symbols, solid line. (a) Vegetative size (length of the longest leaf \times total number of leaves in rosette); (b) Damage by *Longitarsus jacobaeae* (number of shotholes).

Table 3 Analysis of variance on the differences between native and invasive populations in above-ground biomass, reproductive output, relative investment into reproduction, and total PA concentration. Only fixed effects presented under MIXED model

Source	d.f.	Above-ground biomass		Total number of capitula		Relative investment into reproduction		Total pyrrolizidine alkaloid concentration	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Origin	1	17.18	0.0001	28.35	0.0047	16.05	0.0002	13.90	0.0004
Population	6	9.64	< 0.0001	6.52	< 0.0001	6.40	< 0.0001	3.55	< 0.0001

and of population nested within origin (Table 3). Overall, the introduced populations produced nearly 20% more flowers per unit above-ground biomass than the native populations (18.50 ± 0.74 vs. 15.89 ± 1.64 capitula g^{-1}).

LONGITARSUS JACOBAEAE HERBIVORY

Introduced populations had 40% more shotholes than the native populations (Fig. 1b; mean maximum damage = 86.40 ± 5.60 vs. 61.90 ± 13.11 shotholes), a significant difference even when corrected for rosette size differences. Overall levels of adult feeding damage

differed between plants from different origin types and populations nested within origins (Table 2). In all three periods, rosette size explained a significant amount of variation in feeding damage ($R^2 = 0.2164$ – 0.2621 ; $P < 0.0001$). Mean feeding damage was more variable between populations than between the origin types, with damage to plants from the native Chereng (F) population similar to that observed in the introduced populations (Fig. 1b).

A random survey of the edge plants along the periphery of the plots showed that 17 out of 20 dissected edge plants were infested with *L. jacobaeae* larvae, indicating

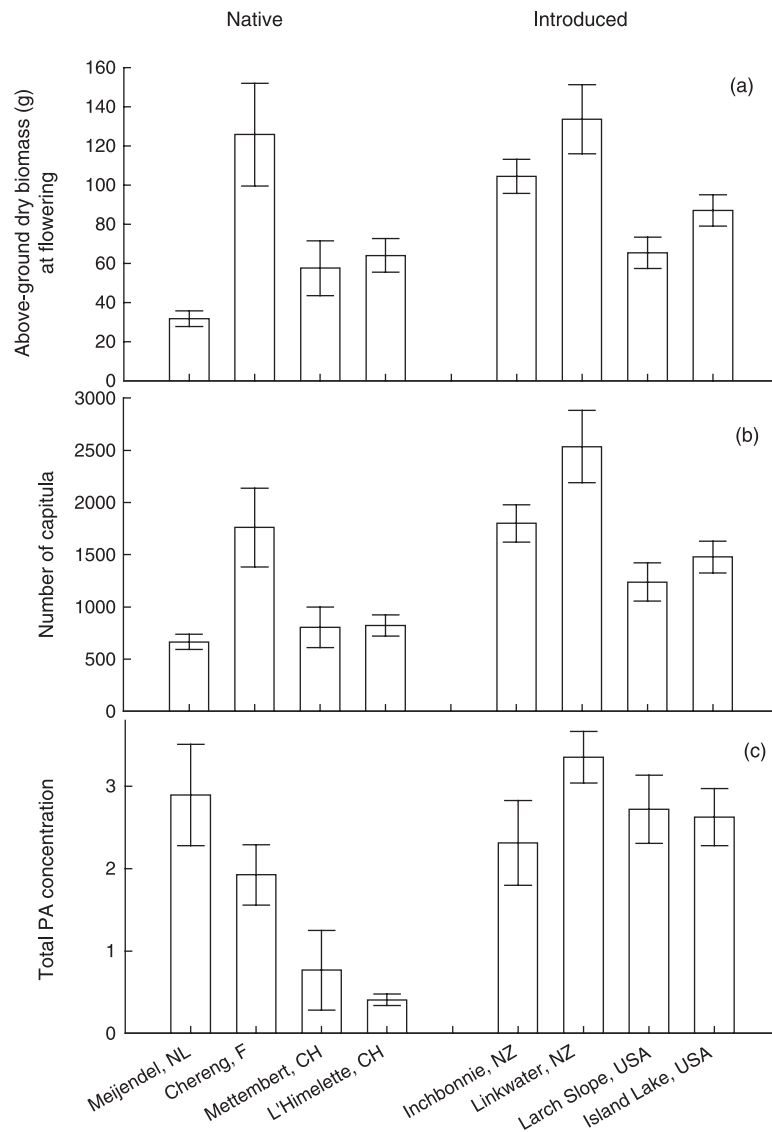


Fig. 2 Final plant mass, reproductive output and defence chemistry in 2002 for populations of *Senecio jacobaea* from the native and introduced ranges. (a) Dry above-ground biomass (g) at the peak of flowering. (b) Reproductive output (number of capitula). (c) Total concentration of pyrrolizidine alkaloids (PA, in $\mu\text{g mg}^{-1}$ leaf dry mass) at the time of bolting.

that oviposition and larval development was occurring throughout the experimental meadow.

Regression of relative damage on relative investment to reproduction (Fig. 3) showed that families from introduced and native populations differed in relative tolerance to feeding damage, when the slopes of the regression lines were compared in an ANCOVA (interaction term: $P = 0.0349$). While plants from the introduced populations did not appear to suffer reduced reproductive investment with increasing feeding damage (test of whether slope differs from zero: $P = 0.5204$), a reduction was observed in plants from the native populations (negative slope; $P = 0.0369$).

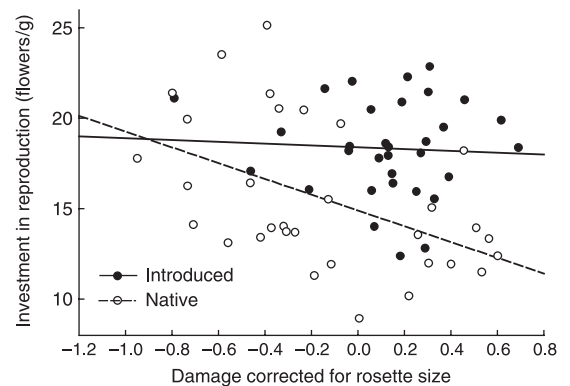


Fig. 3 Regression of adult feeding damage in 2001, corrected for rosette size, against relative investment into reproduction in 2002 (number of capitula per dry above-ground biomass). Slopes of the regression lines: native populations, -0.1579 ; introduced populations, 0.0356 .

PA ANALYSIS

The analysis of pyrrolizidine alkaloids (PAs) revealed significant differences between populations both in

total PA concentration (Fig. 2c, Table 3) and the specific alkaloid profiles (data not shown). Introduced populations had similar PA levels, which overall were nearly double those of native populations (2.75 ± 0.22 vs. $1.50 \pm 0.57 \mu\text{g mg}^{-1}$, respectively). The two native populations from Switzerland (Mettembert and L'Himelette) had much lower PA levels than any other native or introduced population (Fig. 2c).

In a regression of PA concentration on damage (corrected for rosette size), PAs explained a significant amount of variation in herbivory ($P = 0.0140$). When the effects of plant size were accounted for, *L. jacobaeae* fed relatively more on plants with lower PA concentration (negative regression slope). However, larger plants that, collectively, also had a higher PA concentration received more damage in absolute terms. Both origin types and populations still differed in the level of herbivory when PA concentration was used as a covariate ($P < 0.0001$ and $P = 0.0274$, respectively), indicating that differences in resistance were not entirely due to PA concentration.

Discussion

PLANT VIGOUR AND DEFENCE

We found that in a competitive environment, plants from introduced populations were generally larger and more vigorous, and had a greater reproductive output, than plants from native populations. These findings are in agreement with results from experiments comparing 13 native with 16 introduced *S. jacobaeae* populations (J. Joshi and K. Vrieling, unpublished observations), and support the evolution of increased competitive ability hypothesis (EICA), which predicts increased investment in life-history traits such as growth and reproduction in introduced ranges. On average, the more vigorous populations also had higher levels of pyrrolizidine alkaloids, suggesting the absence of a trade-off between vigour and allocation to PAs, and supporting the conclusion that PAs do not incur a detectable cost (Vrieling & van Wijk 1994a; Vrieling *et al.* 1996). An alternative explanation is then required to account for the increased vigour and increased PA levels in the introduced populations.

One hypothesis for the variation in life-history traits observed here is that populations may respond differently to the relative quality of the physical environment. These factors may produce a plastic response to a novel habitat (as suggested by Willis *et al.* 2000; but see Willis & Blossey 1999). Nevertheless, five out of the six non-Swiss populations in our study did not appear to be negatively affected by the novel conditions. These populations were able to outgrow and especially outflower the two potentially locally adapted populations from Switzerland, even though the Swiss plants were at least as vigorous as they would be under natural conditions (M. Stastny, personal observations). It appears that genetic constraints, possibly unrelated to *L. jacobaeae*

herbivory, may limit the potential for increased vigour and reproduction of the native populations. The ability to produce secondary meristems, and consequently multiple stems and inflorescences, appeared to be the most important determinant of increased vigour (M. Stastny, personal observation), but was not explicitly measured in this study. If such growth traits are common in introduced populations, but relatively rare in at least some native populations (e.g. Meijndel, L'Himelette, Mettembert), they may reflect the outcome of selection associated with the introduction of *S. jacobaeae* outside Europe.

Interestingly, our results are in contrast with the results of Willis *et al.* (2000), which did not show evidence of increased vigour in *S. jacobaeae*. The discrepancy could be attributed to the fact that in our study the plants were grown directly in a turf of established meadow vegetation, whereas Willis *et al.* used weed-suppressing matting. Intense interspecific competition could alter the relative levels of vigour, while reflecting more natural conditions under which the introduced populations of *S. jacobaeae* achieved their invasion success.

A common feature in all our results was the finding that variation between populations of the same origin was often equal to or greater than the difference between the origin types. While this effect was partly due to the limited number of populations in our experiment, it suggests important implications about the potential history of introduction. Particularly distinct was the contrast between the population from Chereng (F) and the other native populations. In terms of its growth, reproductive output, levels of herbivory, and even general morphology, this population resembled the introduced populations. It appears that the native Chereng population could display the same potential for invasiveness as any of the introduced populations. The observed vigour of the introduced populations may therefore not be solely due to post-introduction evolutionary change (as predicted by EICA), but rather related to which genotypes were introduced (which may have been random) and which were able to persist under novel conditions (which may be due to selection).

LONGITARSUS JACOBAEAE HERBIVORY

The introduced populations, which have been freed from *L. jacobaeae* herbivory for almost a century, generally experienced higher attack by the specialist herbivore, in agreement with EICA predictions. *L. jacobaeae* has been shown to have dramatic effects on individual fitness and on population dynamics of *S. jacobaea* (McEvoy *et al.* 1993), and is likely to have been a strong contributing factor in the evolution of resistance. *L. jacobaeae* can also be expected to tolerate or even prefer plants with high PA levels, because it is a specialist herbivore able to sequester PAs and to use them for its own defence (Dobler *et al.* 2000). Although absolute damage by *L. jacobaeae* increased with PA

concentration in our study, relative damage was lower on high PA plants. This seemingly contradictory result can be explained in two ways. First, plants with higher absolute damage were the larger, more vigorous plants from introduced populations (and therefore, as this group also had higher mean total PA concentration, damage appeared to increase with PA content). However, when we controlled for variation in plant size, relative damage was lower in introduced plants, consistent with higher PA levels. Similarly, Vrieling & van Wijk (1994b) found a negative correlation between PA concentration and both adult and larval herbivory by *L. jacobaeae*. On the other hand, neither their study nor ours was designed to test for the direct effect of PAs on feeding. The lack of direct negative correlation in our study may indicate that while total PA levels play a role in host selection by adult *L. jacobaeae*, other factors may be important. PAs represent only one measure of resistance, and herbivore damage is a more relevant reflection of overall resistance. The less vigorous populations, which had lower damage levels, all come from areas where *L. jacobaeae* acts as an important herbivore, and are likely to have been subject to selection to deter this specialist by increasing quantitative defence traits, such as tannins and lignin. Increased concentrations of quantitative defence traits may be negatively correlated with relative growth rate (Lambers *et al.* 1998).

We were unable to quantify larval feeding in 2002 directly, but larval densities can be expected to reflect the feeding preference of the adults in 2001, as found in other systems where females are likely to oviposit near host plants where they spend proportionally more time feeding (Scheirs *et al.* 2000). In a separate host-choice experiment in 2001/02, larval extraction and dissection of potted *S. jacobaeae* in a field adjacent to our own revealed that introduced populations had significantly higher larval densities than native populations (K.P. Puliafico, Master's thesis). If the introduced populations were less defended against specialized herbivores, as EICA predicts, we would expect a greater impact of larval feeding on these populations. After winter and spring herbivory, the magnitude of the relative difference in vigour between the introduced and native populations should then be reduced or even reversed compared with the difference observed before the initiation of larval feeding (final rosette size; introduced populations larger by 30% in 2001). We did not observe any such reduction and, on the contrary, the relative difference actually increased for both above-ground biomass (41%) and flower output (76%) in 2002, in favour of the introduced populations.

INVASIVE POPULATIONS AND TOLERANCE

Tolerance can be defined as the ability of a plant to experience damage without a reduction in fitness (see Mauricio 2000). We compared the relative tolerance of native and introduced populations by comparing the

slopes of the regression lines of adult feeding damage corrected for rosette size against flower output per unit above-ground biomass (relative investment into reproduction) for the two origin types (Fig. 3). The regressions indicate that the fitness of the native populations was negatively affected by herbivory, while there was no detectable impact on the introduced populations. Comparisons of herbivore tolerance of native vs. introduced populations have been largely neglected; our results are supported by a recent study of Rogers & Siemann (2004) in which invasive ecotypes of the Chinese tallow tree showed increased tolerance, but are in contrast with the generally opposite trend found in the cordgrass *Spartina* (Garcia-Rossi *et al.* 2003).

The possibility that increased vigour may allow higher tolerance to herbivory (Weis *et al.* 2000) has important implications for the topic of invasiveness. If a tolerant invasive genotype has high fitness both when undamaged and damaged, tolerance is unlikely to be a key factor explaining its high competitive ability and invasiveness. However, at the population level, tolerance will reduce the collective impact of generalist herbivores, thereby diminishing their role in slowing down the spread of the invader. Tolerance may be especially important in a novel environment because it is a non-specific type of defence; plants can compensate for damage inflicted by a wide variety of herbivores (Mauricio *et al.* 1997). Whether an exotic is more or less resistant to native generalists will be less important if it happens to be more tolerant to herbivory in general. Investigations of resistance may not directly detect this possibility. The potential for an association between invasiveness and increased competitive ability on the one hand, and higher tolerance rather than reduced resistance on the other, is an intriguing question that deserves more attention.

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