Parallel evolution in an invasive plant species : evolutionary changes in allocation to growth, defense, competitive ability and regrowth of invasive Jacobaea vulgaris

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Chapter 6

Summary

For the last hundreds of years, with an increase in human travel intensity, a large amount of plant species have been introduced into new environments around the world by human activities (Cassey et al. 2005). These non-native plant species that successfully establish and spread when introduced beyond their native range and become extraordinarily prominent in their new habitats and are defined as invasive plant species (Williamson 1996; Pysek et al. 2004). The introductions of organisms beyond the natural range of potential dispersal (aided by human transport) are commonly referred as biological invasions. Invasive plant species often received a pest status in their introduced areas since their invasiveness could lead to a loss of biodiversity, cause habitat degradation and disruption and pose a threat to livestock and human health (Mack et al. 2000). Therefore invasive plant species are regarded as one of the greatest threats to global biodiversity and cause enormous economic losses (Hobbs and Mooney 1998; Kark and Antonio 2002; Pimentel et al. 2005; Pejchar and Mooney 2009; Pyšek and Richardson 2010). For example common ragwort causes more than four million dollar of annual costs in Australia due to livestock losses, decreased pasture yields and increased management costs (McLaren et al. 2000).

In spite of their negative effects, invasive species provide an ideal opportunity to ecologists for studying the evolutionary changes over time scales that are not feasible in common laboratory and field studies. Biological invasions can be used as large scale experiments where selective forces have been changed for dozens of generations. Since many biotic and abiotic factors differ between the invasive and native range the evolutionary changes that promote invasiveness remain debated (Turner et al. 2014). After plant invasions, one of the most prominent changes in selective forces is the herbivore composition in the introduced ranges. After invasion, plants are freed from their native specialist herbivores (Enemy Release Hypothesis (ERH), (Elton 1958; Keane and Crawley 2002), though they are still under herbivore pressure by the local generalist herbivores and occasional local specialist herbivores of congeneric plant species (Frick 1972; Castells et al. 2013). Such a shift in the herbivore composition towards a guild that is dominated by generalist herbivores in the new range, was expected to exert altered selection in favor of invasive genotypes with reduced allocation to anti-herbivore strategies and increased allocation to growth. Therefore the invasive plants were hypothesized to have lower defense levels, grow faster and produce more seeds and thus show an increased competitive ability over the local plant species as suggested by the Evolution of Increased Competitive Ability hypothesis (EICA), (Blossey and Nötzold 1995).

However, it is worth to point out that while invasive species are freed from their specialist herbivores they still are under attack by generalist herbivores in the invasive range (Agrawal and Kotanen 2003; Siemann and Rogers 2003; Parker et al. 2006). Therefore as an extension to the Evolution of Increased Competitive Ability hypothesis, the Shifting Defense Hypothesis (SDH) takes the different selective pressures of specialist and generalist herbivores into account (Müller-Schärer et al. 2004; Joshi and Vrieling 2005; Doorduin and Vrieling 2011). The SDH states that the herbivore guild shifts from a specialist dominated community towards a generalist dominated community in the introduced range and hence invasive plants are selected for lower investment in their total defense by reducing the costly quantitative defenses against specialist herbivores and increasing the cheap qualitative defenses targeted at local generalist herbivores without having the side effect of attracting the specialist herbivores (Feeny 1976; Rhoades and Cates 1976; van der Meijden 1996). As a result a net gain can be saved for additional growth and resulting in an increased competitive ability.

Quantitative defenses are digestibility reducers and/or structural defenses such as leaf thickness and toughness that act against both specialist and generalist herbivores. They occur in high concentrations which make them expensive for plants to produce. Qualitative defenses are toxins or deterrents (e.g. alkaloids and glucosinolates) that are effective at in low concentrations and act against generalist herbivores. Because they occur at relatively low concentrations they are cheaper to produce than quantitative defenses. The drawback of qualitative defenses is that specialist herbivores are often adapted to the them and can even use them as feeding and oviposition stimulant or even sequester them for their own defense against predators (Feeny 1976; Rhoades and Cates 1976).

Instead of deterring herbivores, plants can also reduce the negative fitness effects of herbivores by being tolerant to damage (van der Meijden et al. 1988). Tolerance is the ability of a plant to vegetatively or reproductively overcome the damage caused by herbivores. Tolerance is usually seen as a last resort against specialist herbivores which have broken through other defences (Agrawal et al. 1999; Strauss and Agrawal 1999; Fornoni 2011). It is considered to be costly because leaf tissue is lost through herbivory and reserves for tolerance cannot be used for growth (Bossdorf et al. 2004b). Regrowth ability is the most common tolerance strategy of plants and it is a compensatory response to replace damaged tissue after herbivory by using the stored nutrients and energy in plant roots that are relatively free from herbivory (McNaughton 1983; Rosenthal and Kotanen 1994; de Jong and van der Meijden 2000; Anten and Pierik 2010).

However, roots have multiple functions and they can be used by plants for maintaining structure, retrieving nutrients and water for growth and for storing resources. A large root to shoot ratio may be the result of high storage levels but can also be the consequence of low nutrient availability (van der Meijden et al. 2000). To understand regrowth capacity and the role of the size of the roots for regrowth it is necessary to study both the root storage and root size.

So far a lot of studies compared invasive plants with their conspecifics and only a few of them showed strong evidence that evolutionary changes have been occurred in invasive plant genotypes (Daehler 2003; Vila and Weiner 2004; van Kleunen et al. 2010; Palacio-Lopez and Gianoli 2011). However, even among the latter studies evidence showing that changes in the herbivore guild are the selective force for changes in allocation patterns is largely circumstantial as other biotic and/or abiotic factors cannot be ruled out (Willis and Blossey 1999; Colautti et al. 2004; Liu and Stiling 2006; Bradley et al. 2009; Colomer-Ventura et al. 2015). Therefore we set out to study a system where multiple invasive regions are compared that differ in climatological conditions. If a change in the herbivore guild is the main selective force acting on growth, competitive ability and anti-herbivore defenses, parallel evolutionary changes are expected in each of the geographically and climatologically differing invasive ranges.

In this thesis I used *J. vulgaris* as s study plant species. With several experimental setups I compared the differences in growth and growth related traits, qualitative defense (pyrrolizidine alkaloids), quantitative defense (structural defenses and tolerance) and competitive ability between native and invasive *J. vulgaris* genotypes. In addition, I specifically selected the invasive genotypes from ranges that are geographically isolated and that do differ in climatological conditions. The main goal of this thesis is to exam whether there are evolutionary changes in invasive *J. vulgaris* plants and if so, whether these changes are due to the release of the selection pressure from specialist herbivores in the introduced area?

In my thesis the following four specific research questions are proposed:

1. Do invasive *Jacobaea vulgaris* genotypes have decreased quantitative defenses (structural defenses and regrowth)?
2. Do invasive *Jacobaea vulgaris* genotypes have increased qualitative defense (chemical defense)?
3. Do invasive *Jacobaea vulgaris* genotypes have increased growth and competitive ability in the absence of herbivores?
4. Is the competitive ability of invasive *Jacobaea vulgaris* genotypes more affected by a specialist herbivore and less affected by a generalist herbivore than that of native genotypes?
5. Did evolution select growth, competitive ability, quantitative and qualitative defenses of invasive *Jacobaea vulgaris* genotypes from geographically and climatological distinct regions all towards the predicted directions?

Corresponding to these research questions I will divide my discussion into five parts.

1. Evolutionary changes in growth, structural defenses and potential regrowth ability in invasive *Jacobaea vulgaris*

In chapter 2 I studied the differences in growth, structural defense and potential regrowth ability (root-shoot ratio) between native and invasive *J. vulgaris* genotypes in which plants grew in individual pots in a climate room for 17 weeks. I examined structural defense related

traits such as leaf microscopically structural traits (e.g. leaf thickness, cell wall thickness), toughness of leaves, amount of cell wall proteins in leaves, and root-shoot ratio. In addition, plant biomass at the end of the experiment was measured. Following the EICA and SDH I hypothesized that due to the absence of specialist herbivores but the presence of generalist herbivores, invasive *J. vulgaris* genotypes have been selected for decreased structural defenses and regrowth but increased growth performance.

In this study I did not find strong evidence from microscopic analysis and leaf toughness analysis supporting the hypothesis that invasive genotypes have evolved lower structural defenses against herbivory. But I did find that invasive *J. vulgaris* had 7% thinner leaves (results are summarized in Table 1). Leaf thickness plays an important role in plant anti-herbivore defense. Peeters (2002) found it was negatively associated with densities of chewing herbivores. In addition, I found that the invasive *J. vulgaris* contained a 10.8% lower amount of cell wall proteins per leaf area than the native ones (Table 1). Leaf cell walls can constitute a substantial amount of nitrogen and account for 30-50% of leaf dry mass. Therefore having a smaller amount of cell walls can decrease leaf structural defense which, in turn, would render plants to be more susceptible to herbivores (Onoda et al. 2004). The results further showed that invasive *J. vulgaris* genotypes invested more in the aboveground parts than in underground parts resulting in a 13.7% larger shoot mass and a 8% smaller leaf mass area. Having larger shoots and thinner leaves may enable invasive genotypes to grow faster (Lake and Leishman 2004; Leishman and Thomson 2005; Grotkopp and Rejmánek 2007), and, independent of this growth potential, also enable them to compete more effectively for light (Schieving and Poorter 1999). Therefore plants from the invasive *J. vulgaris* genotypes were expected to have higher potential growth than native plants. At the final harvest we found no differences in total dry mass because invasive plants produced smaller roots. As a result, the invasive plants had a 18.7% lower root-shoot ratio. A low root-shoot ratio in the invasive plants can represent poorer potential regrowth ability. This is supported by the findings of Joshi and Vrieling (2005) who found that native *J. vulgaris* genotypes had a 12% higher regrowth ability after complete defoliation. In general, this study is consistent with the EICA which hypothesize that invasive *J. vulgaris* have evolved poorer structural defenses and lower tolerance ability to herbivory but a higher potential growth compared to native genotypes.

2. Evolutionary changes in growth, chemical defense and potential regrowth ability in invasive and native *Jacobaea vulgaris*

In chapter 3 I studied the differences in growth, chemical defense, and regrowth ability between native and invasive *J. vulgaris* genotypes. I grew plants individually in pots in a climate room for 9 weeks before harvest. After that I examined plant growth and underlying traits such as plant biomass, photosynthesis, specific leaf area and leaf mass fraction. In addition, the quantity and composition of leaf pyrrolizidine alkaloids (PAs) in each plant was measured by LC-MS as a measure of qualitative defense. Root carbohydrate storage (inulin) was measured as the energy source for regrowth. Following the EICA and SDH I hypothesized that due to the absence of specialist herbivores but the presence of generalist herbivores, invasive *J. vulgaris* genotypes have been selected for decreased regrowth ability but increased growth and qualitative chemical defense PA concentration.

In this study I found that invasive *J. vulgaris* had 5% higher specific leaf area, 12% higher leaf mass fraction, 7.7% higher Pmax and a 10.8% higher PNUE than native genotypes (Table 1). All these underlying growth traits are assumed to be positively correlated with relative growth rate (Poorter and Remkes 1990; Shipley 2006). Indeed at the end of this experiment invasive *J. vulgaris* produced a 12% larger total dry mass. This is in line with a previous study (Joshi and Vrieling 2005) who found that invasive *J. vulgaris* plants had higher vegetative growth and had a 37% higher reproductive output compared to native plants in a common garden experiment after eight months of growth. However, this result is not in line with Willis et al. (2000). The latter found no difference in size between invasive and native *J. vulgaris* which is may be due to the small sample size that has been used in this study (6 populations for each range).

The data further showed that invasive *J. vulgaris* produced on average a 43% higher concentration of total PAs and 123 % higher concentration of tertiary amine PAs than native genotypes while the concentration of N-oxide PAs was similar (Table 1). When we further compared the invasive *J. vulgaris* genotypes with the potential source populations from the western coast of Europe (Doorduin et al. 2010), they did not differ from native genotypes in total PA concentration but their PA composition had shifted to the more toxic jacobine-like PAs and only trace amount of Erucifoline-like PAs. Jacobine-like PAs are present in larger quantities as tertiary amines. These findings are in line with Joshi & Vrieling (2005). Since tertiary amines were observed to be more deterrent to insects than the PA N-oxides (Macel et al. 2005; Nuringtyas et al. 2014), this indicates that invasive genotypes have shifted to more toxic forms of PAs than native genotypes. Since generalist herbivores play a role in the evolution and maintenance of the diversity of PAs (Macel et al. 2005), I argue that the changes in the herbivore guild towards one dominant by generalists in the introduced areas has led to selection for increased qualitative defense in invasive *J. vulgaris*.

Invasive *J. vulgaris* stored 34% less carbohydrate (inulin) in the roots compared to native genotypes (Table 1). As one of the underlying traits of regrowth, root carbohydrate storage was found to be positively correlated with regrowth ability in several plant species (Donaghyē and Fulkersonǵ 1998; Sosnová and Klimešová 2009; Chen et al. 2013; McCormick et al. 2013; Aranjuelo et al. 2015). Also Joshi and Vrieling (2005) showed, by clipping in a in a common garden experiment, that native *J. vulgaris* genotypes had better regrowth after complete defoliation. This study clearly showed that evolutionary changes in the allocation patterns of invasive *J. vulgaris* populations have occurred and they have evolved less root storage for potential regrowth but increased growth and qualitative chemical defense (PAs) after invasion as predicted by the SDH hypothesis.

3. Evolutionary changes in growth and regrowth ability of invasive *Jacobaea vulgaris*

In the chapter 2 and 3 we found the invasive *J. vulgaris* have evolved poorer underlying regrowth traits such as smaller root-shoot ratio and less root carbohydrate storage. In order to test the actual regrowth ability, I conducted an experiment (chapter 4) with artificial defoliation to measure the effects on growth and regrowth in invasive and native *J. vulgaris*. In this experiment, I examined plant growth and root carbohydrate storage (inulin content) before defoliation and after four weeks of regrowth.

The results showed that invasive *J. vulgaris* genotypes had better growth by producing 17% higher total mass and a 44% larger total leaf area a than native genotypes in the control treatment without defoliation after 12 weeks of growth (Table 1). In contrast, the invasive *J. vulgaris* from the clipping treatment showed a poorer regrowth resulting in a 20% lower total leaf area and a 20% lower total dry mass than their native congeners after four weeks of regrowth. Plants from invasive *J. vulgaris* genotypes contain a 38% lower of root inulin content than that of native genotypes at the moment of clipping and I found that the amount of root inulin content is positively correlated with the net gain in total biomass after four weeks of regrowth. Similarly, root carbohydrate storage has been reported to play an important role in regrowth after defoliation in several plant species (Ta et al. 1990; Corre et al. 1996; Avice et al. 1997; McCormick et al. 2013; Janeček and Klimešová 2014). The results strongly suggest that invasive *J. vulgaris* have been selected to compromise their regrowth ability to increase plant growth through a lower investment in root carbohydrate storage. The data further suggest that a large root is not necessarily translated into better regrowth ability. In fact the size of structural root was negatively correlated with plant regrowth after damage. Therefore it is crucial to study both the root storage and root size in order to understand plant regrowth ability and the role of the size of the roots for regrowth.

4. Evolutionary changes in competitive ability and regrowth in invasive and native *Jacobaea vulgaris* with and without herbivory

Generally a higher growth rate is considered as a proxy for competitive ability (Grime 2006). Since plant performance in the absence of competition might not necessarily translate into competitive ability, it is essential to examine plant competitive ability in a competitive environment (Bossdorf et al. 2004a). The outcome of competition experiments between native and invasive plants is also likely to depend strongly on whether or not herbivory is included and which herbivores are present since the invasive and native genotypes have been selected to evolve different anti-herbivore strategies. Therefore, in chapter 5, I compared the competitive ability of invasive and native *J. vulgaris* genotypes with an intraspecific competition setup where native and invasive genotypes compete with each other. The mixed cultures were exposed to three treatments: no herbivores, a generalist herbivore (*Mamestra brassicae*) or a specialist herbivore (*Tyria jacobaeae*). I harvested half of the pots after 12 days of herbivory and from the remaining pots the herbivores were removed and the plants were allowed to (re)grow for another four weeks. Shoot biomass of individual plants was measured at both harvests.

The results showed that invasive *J. vulgaris* genotypes were less preferred by the generalist *Mamestra brassicae* but more preferred by the specialist *Tyria jacobaeae*. Consequently the competitive ability of invasive genotypes was significantly increased by the first and reduced by the latter (Table 1). The increased competitive ability of the invasive genotypes was accompanied by a decrease in regrowth ability after attack by either type of herbivores. The latter result is in line with the results of the previous chapters. All together my results are in line with the EICA and SDH hypotheses and show that herbivore species can play a decisive role in shaping the competitive ability of invasive plants. The shift in the herbivore guild in the introduced range towards a community dominated by generalists has selected for invasive plants with better competitive ability in the presence of generalist herbivores. This

most likely contributed to their invasion success. More importantly, my results indicate that a competition experiment without generalist herbivores underestimates the competitive ability of plants from the invasive range.

Besides this thesis, I did some preliminary experiments about the preference of other generalist herbivores (slugs, leaf miners, and thrips) using *J. vulgaris* leaf discs. I found that slugs and leaf miners preferred invasive genotypes while thrips preferred native ones. Although at the first sight this is not in line with our hypothesis, it should be noted that damage by both slugs and leaf miners did not show a negative correlation with Jacobine concentration while damage by thrips did show such a negative correlation (Wei et al. 2015). An increase in Jacobine concentration was the most prominent evolutionary change in qualitative defense in invasive *Jacobaea vulgaris* genotypes. In addition, these results may be explained by differences in feeding behaviors among herbivore species. Accordingly, selection forces plants to co-evolve relevant strategies by distributing their defensive metabolites to different locations (Martin et al. 2001). Indeed, plant metabolites have been reported to be highly diversely distributed in different leaf tissues such as epidermis, cell walls, vacuoles, and cell nuclei (Hrazdina et al. 1982; Hutzler et al. 1998; Nuringtyas et al. 2012). Therefore the conclusion from chapter 5 should be treated with some cautions. More generalist herbivore species should be further tested with this study system and the differences in defensive metabolites between native and invasive *J. vulgaris* genotypes among different leaf tissues should be studied in more detail as well.

5. Parallel evolution in different invasive regions

In this study we found that of the traits measured (growth, competitive ability, anti-herbivore defenses and regrowth ability) which are significantly different between native and invasive genotypes of *J. vulgaris*, the invasive *J. vulgaris* populations from different geographically distinct regions all changed consistently towards the predicted direction. Combined with the fact that the local climate conditions differed significantly among the invasive regions, our result strongly suggests that the parallel evolution have occurred among the invasive *J. vulgaris* populations from geographically distinct regions in response to the shift in the herbivore pressure. Besides that, it is worth to point out that alternative explanations for parallel evolution e.g. bridgehead or anthropogenically induced adaptation to invasive regions are less likely to play a role (Lombaert et al. 2010; Hufbauer et al. 2012). Since genetic analyses of native and invasive *J. vulgaris* showed that multiple introductions have occurred in the invasive ranges (Doorduin et al. 2010), suggesting that the changes in the traits can be best explained by a parallel evolution in the invasive *J. vulgaris* populations from the different introduced regions.

Synthesis

This thesis shows that natural selection within less than 70 generations changed allocation patterns of invasive *J. vulgaris* genotypes to evolve better growth and competitive ability as well as higher qualitative defense while the investment in structural defenses and regrowth ability are reduced (Table 1). These results support the Evolution of Increased Competitive Ability hypothesis and the Shifting defense hypothesis. Several other invasive plant species

evolved the similar changes in the allocation patters as *J. vulgaris* (Liu and Stiling 2006; Doorduin and Vrieling 2011; Felker-Quinn et al. 2013). However, evidence collected so far was still insufficient to prove that the changes in the herbivore guilds are the responsible selective force because other biotic and/or abiotic factors cannot be ruled out as being important (Willis and Blossey 1999; Colautti et al. 2004; Liu and Stiling 2006; Bradley et al. 2009; Colomer-Ventura et al. 2015). Therefore this thesis is one of the first studies that have compared native populations with invasive populations of the same plant species from multiple invasive regions which are geographically and climatologically distinct. Using such a setup, the other abiotic factors can be possibly ruled out since the climate conditions are considered as the most potential abiotic selective force for post-invasive evolution (Bradley et al. 2009; Colomer-Ventura et al. 2015). My results showed that all the studied traits changed consistently in the same predicted direction in all invasive *J. vulgaris* populations from multiple regions. This strongly suggests that the absence of specialist herbivores was the selective force leading to these parallel evolutionary changes in invasive *J. vulgaris*. It remains to be seen if the same holds for other invasive plant species. Clearly more species should be studied using a similar set-up to evaluate the role of herbivores in the invasiveness of plant species. Understanding the selective forces leading to evolutionary changes in invasive species can help to control invasive pests.

Table 1 Summary of the differences in the main traits between native and invasive *Jacobaea vulgaris* populations. Values are Mean ± SE, P values are from a nested ANOVA, with origin (native versus invasive) as fixed factor, invasive regions nested within origin and populations nested within invasive regions as random factors. P (origins): significance level of nested ANOVA between invasive and native origins. NS= not significant.

Chapter #	***Traits***	***Invasive***	***Native***	***P (Origins)***
Chapter 2	Leaf thickness (µm)	264.53±3.08	276.62±2.95	0.023
(17weeks of growth)	Leaf toughness (kJ•m^{-2})	0.227±0.009	0.210±0.007	NS
	Leaf mass area (g•m^{-2})	57.55±1.50	62.74±1.88	0.038
	Cell wall proteins (g•m^{-2})	9.48±0.31	10.63±0.25	0.037
	Total dry mass(g)	11.74±0.52	12.20±0.49	NS
	Root-shoot ratio (g•g^{-1})	0.750±0.037	0.922±0.046	0.030
Chapter 3	Total dry mass (g)	3.73±0.14	3.33±0.11	0.041
(9 weeks of growth)	Specific leaf area (cm^2•g^{-1})	238.09±2.14	228.3±2.48	0.044
	Leaf mass fraction (g•g^{-1})	0.728±0.005	0.649±0.006	<0.001
	Pmax (µmol CO_2•m^{-2}•s^{-1})	23.13±0.43	21.48±0.38	0.005
	PNUE(µmol CO_2•g^{-1}•s^{-1})	18.86±0.44	17.02±0.41	0.011
	Total PA (µg•g^{-1} DW)	4144.6±152.8	2893.3±162.6	0.001
	Total PA (Tertiary amines) (µg•g^{-1} DW)	1954.9±90.02	876.90±98.28	<0.001
	Total PA (N-oxide) (µg•g^{-1} DW)	2189.7±114.4	2016.4±104.8	NS
	Total inulin content in root (g)	0,397±0,025	0,597±0,028	<0.001
Chapter 4	Total dry mass at the moment of clipping (g)	2,14±0,09	1,90±0,08	NS (0,061)
(8 weeks of growth and 4 weeks of regrowth)	Total dry mass in the control treatment (g)	8,69±0,27	7,45±0,32	0,01
	Total dry mass after 4 weeks of regrowth (g)	1,58±0,06	1,97±0,09	0,003
	root inulin content at the moment of clipping (g)	0,209±19,84	0,339±22,64	<0,001
Chapter 5	Shoot dry mass (g)			
(8 weeks of growth, 12 days of herbivory and 4 weeks of regrowth)	After specialist herbivory	1.31±0.07	1.41±0.09	NS
	After generalist herbivory	2.18±0.10	1.23±0.07	<0.001
	Without herbivore	2.34±0.09	1.63±0.08	<0.001
	Shoot dry mass after regrowth (g)			
	After specialist herbivory	1.90±0.20	2.49±0.15	0.037
	After generalist herbivory	3.22±0.26	2.27±0.18	0.010
	Without herbivore	5.16±0.42	3.31±0.28	0.002

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