
**The danger of re-introduction of invasive plants: A
novel experimental test with *Lythrum salicaria* from
introduced and native range**

Dissertation
der Mathematisch-Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr. rer. nat.)

vorgelegt von

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Tübingen, 2010

Citation

Joshi, S. 2010. The danger of re-introduction of invasive plants: A novel experimental test with *Lythrum salicaria* from introduced and native range. Ph.D.Thesis. University of Tübingen, Germany.

Datum der mündlichen Prüfung: 18. 1. 2011

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ACKNOWLEDGEMENTS

First and foremost, I would like to offer most sincere gratitude to my supervisor **Prof. Dr. Katja Tielbörger** for providing me this precious opportunity to work with her, for her supervision as well as for great advice throughout my PhD term. I thank her for understanding and ignoring serious mistakes committed by me. Thank you very much for continuous motivation, excellent guidance, constructive feedbacks and for providing me an opportunity to attend international conferences, this really helped me during this work and will be an asset in future too.

I would express my deepest gratitude and feel extremely honored to work with **Dr. Merav Seifan**. I extend my sincere thanks to her for her guidance and statistical help all throughout my PhD tenure. I owe my special thanks to **Dr. Mark Bilton** for his helpful discussions, proof reading and contributing comments on my dissertation. I thank **Dr. Pierre Liancourt** for his helpful suggestion. I thank **Prof. Dr. Kirk A Moloney** for helpful suggestion and instruction during the starting of my work.

Completion of this project would not be easy without dedicated logistical help. **Ms Ortrun Ebinger** provided excellent assistance whether collecting seeds in the field or in green house for over three years. I have to thank gardener **Mr. Golkary** and **Mr. Ernst** for their help in green house. I also thank **Petra Finkenbein** for her help during establishment of my common garden in first year.

Special thanks goes to **Anne, Christian, Clara, Johannes, Michael, Michal, Raúl, Sabine, Sara, Sarah, Sven, Tal, Udi, Wolfgang** and rest of the group member for their wonderful company. I also thank all my student helpers for their help. Very special thanks go to **Christiane Adler** and **Andrea Sebastian** for their help, especially translating almost all of my official letters.

I would like to express my sincere thanks to my parents especially my dad, who gave me consistent encouragement all through my academic career and my mum and sisters for their affection and encouragement. I appreciate the deep faith to my parents-in law for their support. I thank all my family members and friends for their love and encouragement. Words are insufficient to convey my gratefulness to my husband, **Dr. Moti Rijal**. He accompanied me through these PhD years. Thanks for being a source of constant support and encouragement.

I owe the last word of thanks to generous financial support from The Deutsche Forschungsgemeinschaft (DFG) for two years.

ABSTRACT

ABSTRACT

Biological invasions have been considered as an important component of global environmental change, causing serious threat to native communities and ecological processes. It is necessary to understand the mechanisms behind their success in order to prevent future invasion and to control the spread of existing ones. Biological invasions provide unique opportunities to study evolutionary processes that are involved in invasion success. The evolution of increased competitive ability (EICA) hypothesis have important evolutionary implications, which presumes that, when introduced plants are released from specialist enemies in the new range they not only experience a direct fitness advantage but also reallocate resources away from herbivore defence mechanisms to traits providing a competitive advantage.

To test the EICA hypothesis, a common garden experiment was established in the native range of *Lythrum salicaria* using seeds from population in introduced range (North America) and in the native range (European). Controlled crosses were performed between introduced and native populations to produced F1 generation and F1 hybrids seeds. In one of the study we expose plants to entire herbivore spectrum in the native range to compare herbivore damage and tolerance. Competitive effect and response (interspecific competition) was compared between native and introduced populations with naturally occurring neighbour *Urtica dioica*. Similarly, competitive ability of native, introduced and cross origin hybrid (German maternal hybrid, US maternal hybrid) was compared through intraspecific competition.

Leaf damage was found higher for introduced populations of *L. salicaria* than the native populations in both years suggesting that this difference is genetic rather than the maternal effects because plants were controlled for the maternal effects for F1 generation seeds for second year. Tolerance to herbivory was large and did not differ among origin. Invasive plants maintained a much larger size than natives

irrespective of damage. These results provide evidence for evolutionary changes in invasive plant.

In interspecific competition experiment introduced populations were found to be larger than the native populations of *L. salicaria*. North American *L. salicaria* demonstrated much stronger competitive effects and low responses than European *L. salicaria* against co occurring natural neighbours. Our results show strong support for the EICA hypothesis, suggesting a rapid evolutionary change in the invasive populations of *L. salicaria* which express a superior competitive ability of invasive populations than individuals from its native range.

In intraspecific competition experiment we found that introduced populations performed significantly better than the natives only in terms of height. We only found some pattern to support for the EICA hypothesis that introduced population of *L. salicaria* had more damage by herbivory than the other origin. The competition intensity for biomass and seed production was consistent for native, introduced and US hybrids, however German hybrids perform relatively bad under competition. These differences in hybrids performances imply that the alleles may follow the maternal line.

Overall, our study shows support to the EICA hypothesis and reintroduction of invasive plant in the native range may have large effect on native plant communities. Therefore, important consideration should be taken early for effective management. We highlight the importance of future studies to consider genetic studies to identify different pathway of introduction and reintroduction to prevent establishment of such a problematic invader.

ZUSAMMENFASSUNG

Biologische Invasionen werden als wichtiger Bestandteil des globalen Wandels der Ökosysteme angesehen und stellen als solche eine ernsthafte Bedrohung für heimische Gemeinschaften und ökologische Prozesse dar. Um zukünftige Invasionen zu verhindern und die Ausdehnung bereits existierender zu kontrollieren müssen die zu Grunde liegenden Mechanismen verstanden werden. Biologische Invasionen bieten einzigartige Gelegenheiten um die am Erfolg der Invasionen beteiligten evolutiven Prozesse zu untersuchen. Die “evolution of increased competitive ability hypothesis” (EICA, Hypothese der Evolution erhöhter Konkurrenzfähigkeit) beinhaltet wichtige evolutive Auswirkungen, da sie annimmt, dass Pflanzen durch die Abwesenheit spezialisierter Gegenspieler im neuen Habitat nicht nur einen direkten Fitness Vorteil erfahren, sondern durch Umverteilung der zuvor zur Abwehr von Fressfeinden benötigten Ressourcen auch konkurrenzstärker werden.

Die EICA Hypothese wurde mittels eines Gartenexperimentes im ursprünglichen Verbreitungsgebiet von *Lythrum salicaria* getestet, in welchem sowohl Samen aus dem heimischen Gebiet (Europa), als auch solche aus invadierten Gebieten (Nord Amerika) verwendet wurden. Durch kontrollierte Kreuzungen zwischen eingeschleppten und heimischen Populationen wurden F1 Samen und F1 Hybrid Samen gezüchtet. In einer der Studien setzen wir die Pflanzen dem gesamten Spektrum an Herbivoren des Ursprungsgebiets aus um so Beeinträchtigung durch und Toleranz gegenüber Herbivorie zu vergleichen. Der Konkurrenzeffekt und die Konkurrenzreaktion (Interspezifische Konkurrenz) von heimischen sowie eingeschleppten Populationen wurde gegenüber dem natürlich vorkommenden Nachbarn *Urtica dioica* verglichen. In ähnlicher Art und Weise wurde die Konkurrenzfähigkeit von heimischen, eingeschleppten sowie den Hybriden (Hybrid mit deutscher Mutterpflanze, Hybrid mit US Mutterpflanze) in einem intraspezifischen Experiment verglichen.

Der an Blättern der eingeschleppten Populationen von *L. salicaria* festgestellte Schaden war höher als derjenige bei ursprünglichen Populationen in beiden Jahren. Da die Pflanzen im zweiten Jahr im Hinblick auf maternale Effekte in der F1 Samengeneration kontrolliert wurden ist dies ein Hinweis auf genetische Unterschiede. Die Toleranz gegenüber Herbivorie war hoch und unterschied sich nicht zwischen den Ursprungsgebieten. Unabhängig vom Ausmaß der Herbivorieschäden wurden die invasiven Pflanzen viel größer als die einheimischen. Diese Ergebnisse dienen als Beleg für evolutive Veränderungen bei invasiven Pflanzen dar.

Im Experiment zur interspezifischen Konkurrenz wuchsen eingeschleppte Populationen von *L. salicaria* höher als einheimische Populationen. *L. Salicaria* aus Nordamerika wies gegenüber dem natürlichen Nachbarn einen viel stärkeren Konkurrenzeffekt und schwächere Konkurrenzreaktion als europäischer *L. salicaria* auf. Unsere Resultate unterstützen die EICA Hypothese in hohem Maß und weisen auf schnelle evolutive Veränderung der invasiven Populationen hin, die in höherer Konkurrenzfähigkeit invasiver Populationen gegenüber einheimischen Populationen zum Ausdruck kommen.

Im intraspezifischen Konkurrenz Experiment zeigten die eingeschleppten Populationen gegenüber den ursprünglichen allein bei der Wuchshöhe eine bessere Leistung. Bezüglich der Herbivorie konnten wir lediglich ein, die EICA Hypothese unterstützendes, Muster feststellen – eingeschleppte *L. salicaria* Populationen waren stärker beschädigt. Die Konkurrenzstärke bezüglich der Parameter Biomasse und Samenproduktion war für einheimische, eingeschleppte und Hybriden mit Mutter aus den USA einheitlich; Hybriden mit Deutscher Mutter hingegen wurden von Konkurrenz verhältnismäßig stark negativ beeinflusst. Diese Unterschiede in den Leistungen der Hybriden implizieren das die Allele der mütterlichen Linie folgen.

Insgesamt unterstützt unsere Studie die EICA Hypothese und daher könnte die Wiedereinführung von invasiven Pflanzen in das ursprüngliche Verbreitungsgebiet

einen großen Einfluss auf heimische Pflanzengemeinschaften haben. Daher sollten im Sinne eines wirkungsvollen Managements wichtige Erwägungen frühzeitig getätigt werden. Wir betonen die Wichtigkeit zukünftiger Studien welche genetische Studien in Betracht ziehen sollten um verschiedene Wege der Einführung und Wiedereinführung zu identifizieren um wiederum die Etablierung eines derart problematischen Eindringlings zu verhindern.

SYNOPSIS

Introduction

Biological invasion has become one of the major causes of economic and environmental damage (Pimental *et al.*, 2000). Indeed, it is believed that invasive plants are the second major threat to biodiversity after habitat destruction (Wilcove *et al.*, 1998). As a result of increasing global trade and transport, plants are either deliberately or accidentally introduced into new range (Sakai *et al.*, 2001). However, only small fraction of introduced plant becomes successful in the invaded range. For example, Williamson and Fitter (1996) proposed the “Tens Rule,” which suggests that only about one in 10 non-native species that establish in natural areas become successful. After successful establishment, invasive plant spread rapidly, displace native habitat and bring changes in structure and functioning of native communities and ecosystem processes (Gordon 1998; Lodge 1993).

Many studies and hypotheses have been proposed to explain the success of invasive plant but still the mechanism behind success of invasive plant is not yet resolved (Mack *et al.*, 2000). Many invasive plants possess trait that makes them invasive such as short life cycle, high growth rates, large number of seeds with good dispersal ability and colonizing capacities (Pysek & Richardson, 2007). Moreover, the invasion success of invasive plants also depends on both biotic and abiotic factors. Abiotic factors such as climate and soil conditions might be more favourable in the introduced range, while biotic component such as competition and herbivory might be an important determinants of invasiveness (Blossey and Nötzold, 1995). For example, one of the most influential hypothesis explaining the success of invasive plant is enemy release hypothesis (ERH), which presumes that an increase abundance of exotic plants in the introduced range is due to decrease in regulation by natural enemies because introduced plants leave behind their coevolved natural enemies in the native range (Keane & Crawley, 2002; Mitchell & Power, 2003).

Recently, it has been recognised that evolutionary process plays a major role in the success of invasive plant. The Evolution of Increased Competitive Ability (EICA)

hypothesis explicitly predicts that as introduced plants are released from specialist enemies in the new range they not only experience a direct fitness advantage but they may also reallocate resources away from herbivore defence mechanisms to traits providing a competitive advantage (Blossey & Nötzold, 1995). These traits could be a larger plant height, larger seed production or faster growth rate. Many studies shows evidence that invasive genotypes grow larger or produce more biomass than the native genotype (Blossey and Nötzold, 1995; Wolfe, 2002; Bossdorf *et al.* 2005), while few studies in invasive plant did not found evidence for the EICA hypothesis (van Kleunen & Schmidt, 2003; Bossdorf *et al.*, 2004).

Previous cross-continental studies have not addressed all key aspects of the EICA hypothesis simultaneously (Bossdorf *et al.*, 2005). This may be due to the fact that none of the previous studies investigated all aspects of the hypothesis in a comprehensive manner i.e. enemy release, competitive ability and evolutionary change. For example, previous tests of the EICA mainly focused on effects of only single enemies (e.g. van Kleunen & Schmidt 2003; Siemann & Rogers, 2003; Stastny *et al.*, 2005). In nature, plants are rarely attacked by single enemy, but have to deal with a diverse community of herbivores and pathogens. Several studies have also highlighted that specialist and generalist herbivores have differential effects on plant defence (Joshi & Vrieling, 2005; Hull-Sanders *et al.*, 2007; Abhilasha & Joshi, 2009; Cano *et al.*, 2009).

Despite the importance of competition in the EICA hypothesis only few studies have addressed the EICA hypothesis through competition (Vila *et al.* 2003; Leger *et al.* 2003; Bossdorf *et al.*, 2004 a; Blumenthal *et al.*, 2007; Zou *et al.*, 2008a; Ridenour *et al.*, 2008). Few of these studies measure interspecific competition and found that introduced population grow larger than the native population but only in competition free environment (Leger *et al.*, 2003; Blumenthal *et al.*, 2007). Some studies measured competition between invasive and native genotypes of *Alliaria petiolata* (Bossdorf *et al.*, 2004 a) and *Sapium sebiferum* (Zou *et al.*, 2008a), but did not measure competition with other neighbouring species. Only one study so far have

reported direct measurements of the competitive effects and competitive responses of an invasive species in its native and invaded range (Ridenour *et al.*, 2008). However, in their study they use same pot to measure both effects and response. To the best of our knowledge, no study has ever distinctly separated both effect and response. Competitive effect traits is important in the establishing phase of the invasion process; whereas competitive response is likely to be important once individuals get established in order to resist the impacts of the other locally residing neighbouring species.

For example, a number of other factors may affect the ability of native and invasive species to evolve, which include gene flow, genetic drift and genetic diversity. One of the important stimulus for the evolution of invasiveness is hybridization (Ellstrand and Schierenbeck, 2000). As there is evidence from genetic marker studies that repeated multiple introductions from different source populations are common in many introduced plants (Dlugosh & Parker, 2008; Rosenthal *et al.*, 2008) that provide the opportunity of gene flow among population and establish hybrid populations that exhibit new genetic variability in the introduced range (Dlugosh & Parker, 2008).

Greater genetic variation may allow introduced plant to adapt to novel conditions (Lavergne & Molofsky, 2007). Hybridization between native and invasive species may create novel genotype with stronger competitors, more aggressive with high biomass allocation and reproductive ability, more resistant to herbivory and better adaptation to certain environment than either of their parental species (Reiseberg *et al.*, 2007). In new environment, selection pressure may differ between native and invasive ranges which may result in evolutionary change and cause displacement of native species (Mooney & Cleland 2001; Vellend *et al.*, 2007). Therefore, main concern is that if invasive plants represents genetically distinct ecotype compare to native plants and has evolved special features of invasibility, they may exhibit their invasive nature everywhere, i.e. they may bear a potential risk of re-invasion in the native range.

The reinvasion of an exotic species back into its native range, and the incursion of it are poorly understood. Since most hypotheses assume that it is important to compare the two ecotypes one with aggressive characteristics and one with well- behaving ecotype in a common environment to identify which novel traits have evolved in the new range (Bossdorf *et al.*, 2005). Therefore, cross-continental studies where plants of native and introduced origins are transplanted reciprocally in the field and their performance measured with and without neighbours and/or herbivores is an ideal approach to measure competitive ability (Leger & Rice 2003; Genton *et al.*, 2005). However, conducting such experiments in native range may have a danger of reintroducing an aggressive ecotype into the native range.

Exotic species may become harmful as back invaders if they have been genetically and morphologically modified in their introduced range. In addition, under certain circumstances, if these reintroduced plants are able to hybridize with their native progenitors, the spread of aggressive allele may take rapidly in the native populations which may have detrimental effects on the native plant community. Therefore, present study presents a comprehensive test of the EICA hypothesis to evaluate the danger of back-invasions through cross-continental studies.

Study species

General history and impact of *Lythrum salicaria* on native communities:

Purple loosestrife (*Lythrum salicaria* L. Lythraceae) is native to Europe, occurring commonly in wetland habitat. It's a perennial plant first introduced during 1800's intentionally for medicinal and ornamental purpose and unintentionally through ship ballast. Presently, *L. salicaria* has been spreading in almost all states of the USA and nine adjacent provinces of Canada (Welk, 2004). *L. salicaria* usually occurs in low lying coastal area, floodplains of freshwater streams, rivers, lakes, and ponds (Mal *et al.*, 1992). It has invaded many habitats because of its prolific seed production and ability to tolerate wide range of environmental condition (Shamsi and Whitehead,

1974a, 1977). It can negatively affect native plant communities (Mal *et al.*, 1997) often leading to the decrease in floral and faunal diversity of invaded area (Thompson *et al.*, 1987). Its large size, rapid growth rate, high seed production (approx 2 million seeds per mature plant), high seed germination efficiency and fast germination allow it to easily out-compete native plant species (Mal *et al.*, 1997; Hager, 2004). Keddy *et al.*, (1994), found that invasive *L. salicaria* caused an average reduction of 60% in the yield of neighbour species.

Plant morphology and growth:

L. salicaria is a widespread perennial herb growing in wetland habitat. It can germinate successfully on substrates with a wide range of pH of neutral to slightly acidic pH (Shamsi & Whitehead, 1974a). Seed germination starts late spring or early summer and the optimum temperature required for seed germination is in between 15-20°C (Shamsi & Whitehead, 1974a; Young & Clements, 2001). The maximum height was reported upto 2.7 m (Mal *et al.*, 1992). Leaves are 3-10 cm long, lower leaves are opposite or in a whorl of three leaves but the upper leaves are alternate. A 13-h photoperiod is threshold temperature for growth and flowering (Shamsi & Whitehead, 1974b). Flowering usually starts 8-10 weeks after germination; from late June to early October. Seeds are usually dispersed by water, wind, wild life and human activities.

Reproduction and pollination biology:

L. salicaria is a self incompatible and heterostylous plants with tristylly i.e. with three different flower morphs (short, mid and long style morph) that differ reciprocally in the relative heights of stigmas and anthers within flowers. Each flower of a given morph produces two anther whorls at levels corresponding to the levels of stigmas in flowers of the other two morphs. The short-styled flowers have mid and long staminal whorls; the intermediate-styled flower has short and long staminal whorls; and the long-styled flowers feature mid- and short-staminal whorls (Darwin, 1877; fig1). Pollen polymorphism was also observed in *L. salicaria*. Pollen

grains from the long stamens are significantly larger than those from the mid stamens, followed by those from the short stamens (Mal & Hermann, 2000).

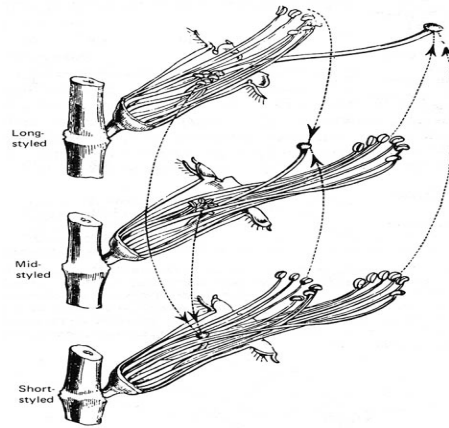


Fig 1: Diagrammatic view of three forms of flowers morph of *Lythrum salicaria* showing the reciprocal arrangement of stigma and anther heights among the three floral morphs (long-styled, mid-styled and short-styled). The dotted lines with the arrows show the direction in which pollen must be carried to each stigma to ensure full fertility. Darwin (1877).

Control measures:

In North America numerous insects were found to feed on *L. salicaria* (Maddox & Wiedenmaan, 2003). Diehl *et al.*, (1997) found 14 members of insects feeding on *L. salicaria* out of 41 genera collected from six sites of Canadian wetlands. In Europe, 120 species of phytophagous insects and 64 species of floral visitors were found associated with purple loosestrife (Batra *et al.*, 1986). Conventional methods such as physical, mechanical or chemical, have continuously failed to curb the spread of *Lythrum salicaria* or to provide satisfactory control. A biological control program has been considered the most effective methods for control of *L. salicaria*. Four species, *Hylobius transversovittatus*, a root-mining weevil, *Galerucella calmariensis* and *Galerucella pusilla*, two leaf-eating beetles, *Nanophyes marmoratus* and a flower-feeding weevil was introduced as biological control agent. Among them two Chrysomelid species (*Galerucella calmariensis* and *G. pusilla*) have been identified as an effective means of biological control for *L. salicaria* (Malecki *et al.*, 1993;

Blossey *et al.*, 1994; Dech & Nosko, 2002). The effect of feeding by these adult beetles on *L. salicaria* is minimum compare to the larvae stage, larvae are considered to be the most significant destructive stage for defoliation (Blossey, 1995).

Outline of thesis

In this thesis, I explain the several possible mechanism contributing to the success of invasive *L. salicaria* by mainly focusing on the evolution of increased competitive ability (EICA) hypothesis. I performed a common garden experiment in the native range with *Lythrum salicaria* as model species which is also a classical example of the EICA hypothesis. The thesis is mainly structured into three chapters. Each chapter is organized in manuscript form with abstract, introduction, methods, results, discussion, acknowledgements and references.

Chapter 1

A novel test of the evolution of increased competitive ability hypothesis with native and invasive *Lythrum salicaria* through full exposure to native herbivores.

We tested the EICA an extended part of the Enemy Release Hypothesis (ERH) by exposing *L. salicaria* from its native (Europe) and invasive range (North America) to the entire herbivore spectrum of natural populations in the native range. We performed this experiment both with first generation plants as well as with offspring of these where maternal effects were removed. We examined difference in herbivore damage and tolerance between native and invasive populations of *L. salicaria*. The main idea of this research was to observe whether release from natural enemies in invasive plants was accompanied by microevolutionary changes.

Key results: Leaf damage by herbivory was higher for introduced than for native populations and the response to herbivory was quiet strong in the second generation. Tolerance to herbivory was large and did not differ among origins. Despite more

damage from herbivores, introduced plants maintain higher fitness than the native plants.

Chapter 2

Separating competitive effect and response of native and introduced *Lythrum salicaria*.

One of the hypotheses explaining the success of invasive plant is shift in allocation from defence mechanism to growth. A common garden pot experiment was conducted by using F1 generation individual from both the native and invasive ranges of *L. salicaria* to assess competitive ability. *Urtica dioica* that naturally co-occur in both ranges was used as a target and neighbour to test for either ‘competitive effect’ or ‘competitive response’ of our study species. Several traits were compared between native and invasive populations to investigate whether invasive populations of *L. salicaria* have evolved into superior competitive ability than individuals from its native range.

Key results: The competitive effect of *L. salicaria* upon *U. dioica* was significantly stronger for the introduced populations than for the native plants. In addition, the introduced populations showed very little competitive response to the presence of neighbours in comparison to the native populations.

Chapter 3

Reinvasion a potential threat to native plant communities? A test using *Lythrum salicaria*

Evolutionary process such as hybridization among native and introduced populations may be one of possible outcome that can occur during reinvasion. Here we explain the possible consequences of reintroducing invasive plants into their native range. The EICA hypothesis was tested in different framework through direct interaction (dialed competition) between native, introduced and hybrid populations of *Lythrum salicaria*. The main objective of this study was to determine the different

outcome of reinvasion and to determine the potential threat that it may cause to the native plant communities.

Key results: We found that introduced populations performed significantly better in competition than native populations in terms of plant height but not for biomass and seed number. The performance for hybrid populations follows the maternal line, with US hybrid populations producing more biomass and more seeds than the German hybrids in competition.

Research significance

The EICA hypothesis is mainly based on the assumption that introduced plants are released from specialist enemies in the new range which may allow them to experience a direct fitness advantage by reallocating resources away from herbivore defence mechanisms to traits providing a competitive advantage. However, previous cross-continental studies are limited because they measured competitive ability through defense and growth and more importantly most of these studies have not addressed all key aspects of the EICA hypothesis such as microevolutionary changes, enemy release and competition among plants simultaneously. In order to address all key aspects of the invasion success, we tested reinvasion hypothesis to broaden the gap missing in the invasive plant. Herbivory experiment was performed to investigate that release from natural enemies favor invasive plant to evolve trait associated to reduced herbivore resistance through full exposure of plant to native herbivores. Competition experiment was performed to measure competitive effect and response separately to provide possibility of much stronger evolutionary changes in the introduced population. Hybridization among native and introduced population could occur during reinvasion, through direct competition interaction we explain whether hybrids are more vigorous (heterosis effect) than their parent or do they show intermediate pattern relative to their parents. Consequently, testing all these experiments we can draw a major conclusion about whether there is a danger of reintroducing invasive plants into the native range and such reintroduction has potential impact on native plant communities or not.

Discussion

During the last few decades biological invasion has become one of the major threats to biodiversity after habitat destruction. Recently, the study of invasions has received much more attention and much work in invasion ecology has focused on identifying underlying mechanisms that explain invasion success. However, still the mechanism explaining species to become a successful invader is a major unresolved question so far. Evolutionary processes are believed to play a major role in the success of invasive plants. When species are introduced into a new range they may experience new selective pressures due to novel biotic and abiotic environments (Mack *et al.*, 2000; Mooney & Cleland, 2001), which makes conditions favorable for rapid evolution of the invaders in the new range. Hence, the larger differences in the performances between native and introduced populations might be a result of rapid evolution of increased competitive ability (Blossey & Nötzold, 1995). Our main aim of the study is to test the different assumptions of the EICA hypothesis and we chose *L. salicaria* as a model species which is a well-known wetland invader and a classical example of the EICA hypothesis. Three different studies were performed in order to address the assumptions of the EICA hypothesis.

The results of our first study (chapter 1) demonstrate the role of herbivory in plant invasion. One of the key findings of the common garden studies show the indication of enemy release in *L. salicaria* and explain that the successful establishment and spread of invasive species is associated to evolutionary change. We found that leaf damage by herbivory was higher for introduced than for native populations and the response to herbivory was quite strong in the second generation. Moreover, our findings confirm the results from previous studies which showed lower resistance in invasive populations than the native populations when plants were exposed either to one or two specialist herbivores or more subsets of herbivory (Bossdorf *et al.*, 2004b; Meyer *et al.*, 2005; Siemann & Rogers, 2003; Zou *et al.*, 2008 a, b). In our study plants were controlled for maternal effects for F1 generation seeds for the second year, the higher leaf damage in invasive plants in both years clearly suggests that this

difference is genetic rather than the maternal effects supporting the EICA hypothesis.

Another important finding in first study was that we found that tolerance to herbivory was large and did not differ among origins and herbivore damage did not translate into reduced fitness. Tolerance mechanism is used to sustain tissue loss and maintain growth and high fitness after herbivore damage (Strauss & Agrawal, 1999). In this study invasive plants maintained a much larger size than natives irrespective of higher damage. This finding indicates that invasive plants have evolved into more vigorous phenotypes in the invaded range, and in addition, they are largely tolerant to herbivory. One limitation of our research is that we made tolerance estimate with direct measurement of plant fitness compare with damage plant, however better estimates of tolerance can be made by comparing damage vs undamaged plants (Strauss & Agrawal, 1999).

Overall, we believe that release from attack from natural herbivores has played an important role in the evolution of *L. salicaria*. Similar finding was observed in invasive genotypes of *Sapium sebiferum*. Invasive genotypes were less well defended, more tolerant and outperformed native genotypes even under higher levels of attack (Rogers & Siemann 2004; Zou *et al.*, 2008a, b). According to this study, it is more likely that invasive plants will be more heavily damaged when specialist enemies are reintroduced as biocontrol agents, but if invasive plants increased tolerance in response to herbivore damage it is more likely that introduction of such biological control program may not be very effective to control invasive plants (Müller-Schärer *et al.*, 2004). The high herbivore tolerance and increased vigour of *L. salicaria* are very likely the major reasons for the high invasibility observed for this plant. The study emphasizes the importance of genetically controlled experiment through full exposure to herbivore and studying defense mechanism for effective biological control of the invasive plant.

However, competition is another important process determining the possibility of plant invasion. To address the EICA hypothesis, competition experiment (chapter 2) was conducted between native and introduced populations of *L. salicaria* with naturally occurring neighbour *Urtica dioica*. Many studies have shown that larger plants are superior in their ability to suppress the growth of competitors (Goldberg, 1996) and therefore play an important role in affecting plant community structure. However, when measuring competitive ability it is important to incorporate which plant trait determines invasiveness. Gaudet & Keddy, (1988) found a strong relationship between competitive ability and plant biomass for *L. salicaria* and reproductive traits in general appeared important determinants of invasiveness (Pysek & Richardson, 2007).

The most prominent findings in this present work is that the plant biomass represents an important trait to determine invasiveness, introduced populations exerts significantly more effects to residing neighbour species than the native populations which show additional support to the previous study in *L. salicaria* (Gaudet & Keddy, 1988). Higher biomass allows species to become more successful as above ground biomass has been also used as a proxy for fitness (Tilman & Wedin, 1991) and this may consequently result in formation of dense monospecific stands. We found above ground biomass differed between native and invasive population of *L. salicaria* and introduced populations have larger effect on residing neighbour species signifying that selection might have taken place during the process of invasion (Vila *et al.*, 2003).

The important outcome of less response of introduced populations in presence of neighbour indicates that *L. salicaria* can be a good colonizer and persist once they are established. Enhanced colonization success of invading species may suppress native competitor species. The finding of larger effect and less response to neighbour, however, agree with finding of Ridenour *et al.* (2008) and suggest that a combination of both a strong competitive effect and a less response to neighbour

competition could be an evolved strategy that has enabled the species to successfully establish and spread throughout their invaded range.

To address the possible consequences of reinvasion, we conducted a common garden competition experiment in the native range with native, introduced and hybrid populations (Chapter 3). With increasing global trade, plants are introduced intentionally and unintentionally from one region to another region. Recent molecular study by Chun *et al.*, 2009 in the same subset of population that we were using have shown evidence for multiple introductions in *L. salicaria* and found that introduced populations genetically differ than the native populations. There is quiet high possibility that many of these genetically distinct invaders can be reintroduced into the native range.

Two different scenarios can be expected from reinvasion. Reinvasion of non-native populations could likely pose threat to the native populations and communities or it may not create potential harm to the native plant communities. Potential risk associated with reintroducing invasive plant in the native range is hybridization among reinvading species and native species. Hybridization is considered as one of the important evolutionary forces; as it can increase genetic variability and provide novel genetic material (Ellstrand & Schierenbeck, 2000). Such novel gene combination may result into stronger phenotypes with higher reproductive potential (Rieseberg *et al.*, 2007). In this study we didn't find evidence for increased competitive ability between introduced plants and native plants. This could mean that direct test using intraspecific competition is not the best way to determine competitive ability between two or more populations of this perennial species over a short time. For perennial species competitive ability may increase over multiple growing seasons (Pfeifer-Meister *et al.*, 2008).

One of the key and interesting patterns found between the two different hybrids type's performances is that the alleles appear to follow the maternal line with US hybrids found to be significantly different than the German hybrids. This shows that

such difference in hybrids performance may be due to inherited maternal cytoplasmic genome (Burgess and Husband, 2004). As we lack genetic analysis for this response further study should be carried out to test if the transmissions of alleles do truly pass down the maternal line. Hybrid vigor in the F1 generation might be due to heterozygosity and this may decline in subsequent generation hybrid (see Hufford & Mazer, 2003). We therefore advocate future studies to assess multigeneration hybrids to test if the fitness trait transmission continues in the same maternal pattern.

Conclusions and outlook

A major conclusion that can be drawn from all the experimental studies of this thesis (chapters 1-3) is that *L. salicaria* represent a highly invasive species with superior competitive abilities causing serious problems in the invaded range. Overall, two of our studies show full support to the EICA hypothesis, and one of the hybrids type (US hybrids) showed increased vigor, as a result, reintroduction of this highly invasive species to native range can cause serious problem to native plant communities. However, the mixed response for hybrids performances of *L. salicaria* in our study emphasizes the importance of hybridization mechanism to be watched and considered properly in the future studies.

Rapid evolutionary changes are important in invasive plant, therefore to better understand the evolutionary and ecological aspects of biological invasion future research should more specifically address the following points.

(i) genetically controlled experiment through full exposure to herbivore and to track whether the effect is more pronounced by specialist or generalist herbivores (ii) below ground interaction such as impact of herbivory (root feeding weevil) and below ground competition should be observed when grown with inter and intraspecific competitor (iii) competition experiment should include comprehensive analysis of fitness over multiple growing seasons (iv) to understand the function and dynamics of gene flow, comparison of more generation of hybrids and backcross should be performed (v) Molecular marker studies should be done to find different routes of invasion and re-invasion to prevent future introduction.

Overall, bio safety rules must be strictly kept while conducting common garden experiments in the native range.

DECLARATION OF MY OWN WORKING CONTRIBUTION

Srijana Joshi: Development of ideas together with Prof. Katja Tielbörger, planning of all experiments and studies, data collection, statistical analysis of all data, writing all first drafts of manuscripts with some initial comments and suggestions from Katja Tielbörger (**chapters 1-3**).

Katja Tielbörger: Initial ideas, help in manuscript preparations (**chapter 1**), supervision of all studies throughout the course of this research (**chapters 1-3**).

Mark Bilton: final proofreading .

Manuscript in preparation

Joshi, S & Tielbörger, K. A novel test of the evolution of increased competitive ability hypothesis with native and invasive *Lythrum salicaria* through full exposure to native herbivores (in preparation to submit to Biological Invasion)

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CHAPTER 1

A novel comprehensive test of the evolution of increased competitive ability hypothesis with native and invasive *Lythrum salicaria* through full exposure to native herbivores.

ABSTRACT

Introduced plants become successful because they lose their co-evolved natural enemies during introduction into their new range. We tested the Evolution of Increased Competitive Ability hypothesis (EICA) as an extended part of the Enemy Release Hypothesis (ERH) which assumes that escape from such natural enemies may thus have a fitness advantage over native plants. Surprisingly, despite myriads of empirical tests, most straightforward predictions of this hypothesis have not been investigated. First, if invasives have lost their enemies and their defence, they should be more negatively affected by their full pre-invasion herbivore spectrum than their native conspecifics. Secondly, it assumes an evolutionary change in the invasive range, which has not yet been taken sufficiently into account. We exposed *Lythrum salicaria* (purple loosestrife) from several populations in its native (Europe) and invasive range (North America) to the entire herbivore spectrum in two natural populations in the native range. We performed this experiment both with plants raised from field-collected seeds as well as with offspring of these where maternal effects were removed. Absolute and relative leaf damage was higher for introduced than for native plants. Tolerance to herbivory was large and did not differ among provenances. Invasive plants maintained a much larger size than natives irrespective of damage. Origin effects on response to herbivory were particularly strong in the second generation, indicating that invasion and enemy release was accompanied by genetic changes. Our overall findings support the EICA Hypothesis. We further advocate the importance of genetically-controlled experiments with the entire spectrum of enemies in the native range.

Keywords: Biological invasion, EICA hypothesis, Enemy Release Hypothesis, field experiments, herbivore tolerance

INTRODUCTION

Biological invasions have tremendous ecological impacts and economical costs, causing extinction of many native species and shifts in ecosystem function (Pimental *et al.*, 2000). Despite the large numbers of studies in invasion biology, the ultimate causes that lead to the success of invasive plants in novel environments are still unresolved (Kolar & Lodge, 2001). The Enemy Release Hypothesis (ERH) is among the most popular hypotheses that attempts to explain plant invasion success. It assumes that invaders are dislocated from their co-evolved natural enemies during introduction into their new range and thus have a fitness advantage over native plants (Keane & Crawley, 2002; Colautti *et al.*, 2004). However, invaders cannot completely escape all herbivores during introduction. For example, they could either suffer from new generalist herbivores or they can acquire a competitive advantage when such herbivores have a greater impact on native competitors than on the invader (Keane & Crawley, 2002).

Some indirect studies provide experimental evidence for higher herbivore loads on native species than on introduced species (Wolfe, 2002; Mitchell & Power, 2003; Cincotta *et al.*, 2008), while opposite pattern was found for exotic species being more susceptible to attack by native herbivores (Agrawal & Kotanen, 2003). While a number of studies focused on a single herbivore species, mostly with the aim of discovering means of biological control (Garcia-Rossi *et al.*, 2003; Goolsby *et al.*, 2004). It has also been argued that in nature, plants are rarely attacked by single enemy, but have to deal with a diverse community of herbivores and pathogens. Several studies have also highlighted that specialist and generalist herbivores have differential effects on plant defence (Joshi & Vrieling, 2005; Hull-Sanders *et al.*, 2007; Abhilasha & Joshi, 2009; Cano *et al.*, 2009). This illustrates the need to study the response of invaders to a whole suite of herbivores.

The Evolution of Increased Competitive Ability (EICA) hypothesis is an expansion of the ERH which assumes that release from natural enemies drives an evolutionary change in introduced plants, in which resources are reallocated away from defence mechanisms to growth and reproduction (Blossey & Nötzold, 1995). An apparently obvious prediction stemming from the EICA is that if we expose plants from the invasive to full natural habitats in their native range they should suffer more from herbivory than their native conspecifics in a common environment because they are less well defended. Comparisons of invasive and native plant population in common garden studies have showed mixed support for prediction of the EICA (Bossdorf et al 2005). Unfortunately, most tests of theories of invasion, including the EICA, were not sufficiently genetically controlled to distinguish genetic responses to enemy release in the novel range. In particular, potential maternal effects on plant performance have not been removed (van Kleunen & Schmid, 2003) and genetically controlled tests of the EICA are virtually lacking.

For example, response to enemies was only investigated in the invasive range (Siemann & Rogers, 2003), and only for a small subset of enemies (e.g. Joshi & Vrieling, 2005; Stastny *et al.*, 2005) or in a garden and not in natural populations (van Kleunen & Schmidt, 2003; Stastny *et al.*, 2005), i.e. the herbivore spectrum may not have been relevant to the study goal. The most remarkable experiments into the direction we suggest have been conducted with plants from both origins in the native range (Wolfe *et al.*, 2004; Meyer *et al.*, 2005; Zou *et al.*, 2008a, b). Albeit doing their experiments in gardens and not in natural populations, these authors were fully aware of this problem and mentioned that their natural populations were within several kilometres or even metres of the gardens. However, none of the above studies has attempted to test whether patterns of enemy release are maintained when conducted within natural populations in the native range, and when done with plants where maternal effects are removed.

Another understudied aspect of the EICA is the differentiation between the susceptibility to herbivores (e.g. the amount of tissue loss) and the fitness

consequences of herbivory damage. Most research has focused on resistance, while tolerance to consumer damage has only recently received more attention (Stowe *et al.*, 2000) and work on invaders is limited to few species. Tolerance is the ability of a plant to maintain its fitness through regrowth and reproduction and thus to compensate for tissue loss due to herbivores (Strauss & Agrawal, 1999). It has been argued that there is a trade-off between resistance, i.e. the ability for defence against herbivory, and tolerance mechanism (Fineblum & Rausher, 1995). This assumption is based on the EICA hypothesis which predicts increased allocation to growth in invasive plants due to enemy-release (Blossey and Nötzold, 1995), i.e. invasive plants may have a larger ability for compensatory growth (Rogers & Siemann, 2004). Yet, it has also been argued that increased competitive ability is realized at the cost of tolerance, i.e. invasive plants should exhibit lower tolerance to herbivores (van Kleunen & Schmid, 2003). Not only the theoretical predictions but also experimental evidence is equivocal, even though more studies have suggested increased tolerance in invasive plants coupled with faster compensatory growth (e.g. Rogers & Siemann, 2004; Stastny, *et al.*, 2005; Zou *et al.*, 2008 a, b). This highlights that tolerance and resistance need to be considered simultaneously in tests of the EICA.

In this study, we attempted to fill the above knowledge gaps by exposing plants from the invaded range and their native conspecifics to a full natural spectrum of herbivores in native habitats. We did this with both plants raised from field collected seeds as well as with their offspring where maternal effects were removed, and we quantified herbivory effects both as direct damage as well as tolerance. Our model species was purple loosestrife (*Lythrum salicaria*) because this species has been fundamental for developing the EICA hypothesis, which postulates microevolutionary changes in response to enemy release. Furthermore, biological control has also been applied with some success, indicating that enemy release is likely to have occurred (Blossey *et al.*, 2001; Landis *et al.*, 2003). In order to examine the role of enemy release and the evolutionary change in invasive plant, we tested the following hypotheses:

- 1) Introduced plants exhibit greater leaf damage than native plants when exposed to the natural herbivore spectrum in populations in the native range.
- 2) Introduced plants show a larger tolerance to herbivory than native plants.
- 3) The response to enemies is genetically fixed and thus more pronounced in plants grown from seeds where maternal environment effects are removed.

METHODS

Study species:

L. salicaria L. (Purple loosestrife, Lythraceae) is native to Europe and it is a well-known aggressive perennial invader in North America. The species was introduced to eastern North America accidentally by ship ballast and purposely for horticulture, as food source, and for ornamental and medicinal use in the early 19th century (Thompson *et al.*, 1987). Since then it has expanded into a large variety of wetland ecosystems and produced large monospecific stands, often at the expense of native plants (Thompson *et al.*, 1987). The observation of high genetic diversity in invasive *L. salicaria* populations has led to speculation that species are successful because populations were established by multiple introduction (Houghton-Thompson *et al.* 2005, Chun *et al.* 2009). Biological control programs exist for this species. For example, two Chrysomelid species (*Galerucella californiensis* and *G. pusilla*) have been identified as an effective means of biological control for *L. salicaria* (Malecki *et al.*, 1993; Blossey *et al.*, 1994; Dech & Nosko, 2002). The effect of feeding on *L. salicaria* by these adult beetles is believed to be minimal, with the larvae considered to be the most significant destructive stage for defoliation (Blossey 1995). In North America numerous insects were found to feed on *L. salicaria* (Maddox & Wiedenmaan 2003). Diehl *et al.* (1997) found 14 members of insects feeding on *L. salicaria* out of 41 genera collected from six sites of Canadian wetlands. In Europe, 120 species of phytophagous insects and 64 species of floral visitors were found associated with purple loosestrife (Batra *et al.* 1986)

Study sites:

The experiment was carried out in 2007 and 2008. Plant and seed material was raised in a common garden of the Botanical Gardens of the Tübingen University, Germany (48°32'N, 9°02'E). Two natural populations of *L. salicaria* were used for exposing the plants to native herbivores. The two populations are approx. 9 km and 14 km away from the common garden and are located near the villages Reusten (48° 55' N 8° 91' E) and Unterjesingen (48° 52' N 8° 98' E). *L. salicaria* occurs in natural densities (approx. 1 plant per square meter) at these sites which are characterized by naturally wet conditions, i.e. the root zone is saturated or even flooded throughout the year. Climatic conditions in the garden and field sites were identical and other natural *L. salicaria* populations grew within 2km of the garden. This ensured that plants were exposed to near-natural conditions even during the phases of cultivation in the garden.

Cultivation of plant material:

Ripe seeds of *L. salicaria* were collected in late summer 2006 from four native (German) and four invasive (North American) populations (Table 1). The seeds were collected from 20 randomly selected individuals (i.e. maternal sibships) in each population and bagged individually. Handling of seeds, germination, raising of plants and hand pollination followed a protocol that has been used successfully in our own previous studies (e.g. Moloney *et al.* 2009). Seeds were air dried and stored in paper bags during winter and stratified at 4°C for four weeks in March to maximise germination success. Two experiments were performed. In 2007 (Generation 1), we used the maternal sibships from the field collected seeds. In the year after (2008; Generation 2), we used seeds from eight plants per population that were raised from field collected seeds in our common garden in 2007. During the growing season in 2007, at least three flowering stalks per plant were covered prior to flowering with a light fabric organza to prevent access by insect pollinators. Plants were then moved to a greenhouse to perform controlled hand pollination. Flowers were carefully observed and fresh flowers were selected for pollination when their petals were fully expanded and the stigma had a bright colour.

Approximately fifty flowers per plant were then successively hand-pollinated. For the within-population crosses used in this experiment, each plant was pollinated with pollen from one other plant from the same native or invasive population that had the appropriate flower morph (three partly incompatible flower morphs, see Barrett 1993). The resulting seeds from hand pollination were collected for each seed family (i.e. mother plant) individually and treated and stored as explained above until the next growing season.

Seeds of both native and introduced *L. salicaria* were germinated in a greenhouse next to our common garden in May (2007, 2008) in standard potting soil that shares common properties with the natural soil conditions of the native range. Pots were placed among plastic trays filled with water and their position was randomised twice. After four weeks, plants with similar size were selected and transplanted into larger pots (30 cm diameter \times 26 cm depth). 20 g of slow release fertilizer Osmocote (18 + 10 + 11) NPK was applied to each pot 14 days after transplanting to ensure that growth was not limited by nutrients. Seedlings that died during the first two weeks of transplantation were replaced. The experimental design was similar in both generations, but differed in the number of replicates and seed material. In 2007 (field collected seeds), we used three replicates per population in each of the two sites resulting in a total of 48 pots (three plants \times eight populations \times two sites). In 2008 (second generation seeds), we used four replicates per population (i.e. seed families from within-population crosses) and two sites resulting in a total of 64 pots (four plants \times eight populations \times two sites).

Field experiments:

On 5 July, 2007 and on 7 July 2008, respectively, plants were transferred to the two field sites (Reusten and Unterjesingen) for exposure to the natural range of herbivores. To this end, potted plants were placed randomly among the plants of the natural populations. In order to avoid contamination of the natural populations with invasive plant material, we returned the plants to the common gardens prior to flowering. Therefore, the exposure in the natural sites was limited to four weeks.

Plants in the common garden were placed in flooded pools with six plants per pool. Due to drier conditions in the second year, two plants from the native range died prior to seed set, leading to a slightly unbalanced design with respect to populations.

Response variables:

We measured final above-ground biomass as a variable more closely related to fitness. Parallel studies have indicated that plant biomass is a good proxy for seed production in both origins ($r^2 = 0.346$; $P < 0.001$ for natives; $r^2 = 0.515$; $P < 0.001$ for introduced plants). Biomass was measured during October by harvesting all above-ground parts, drying them at 60°C for 24 hours and weighing them. We assessed both the total leaf area consumed and the percent leaf damage for each plant by randomly selecting ten leaves per plant prior to measuring biomass. The leaves were scanned with a STD 1600+ scanner (Regent Instruments) and the absolute area consumed per leaf and percent leaf damaged was estimated with the software WinFolia. The percent leaf damage was calculated by reconstructing the leaf area before damage and dividing the leaf area consumed by the original area.

Statistical Analysis:

All statistical analyses were done with **SPSS version 15.0** (SPSS Inc., 2007). Differences between origins in biomass, absolute leaf area per plant consumed, percent leaf damage were tested with hierarchical ANOVA models with origin and generation as a fixed factors and region (nested within origin) and population (nested within region) as random factor and location as a random factor. All data were log transformed to meet the assumptions of ANOVA. To compare herbivore tolerance between native and introduced individuals, linear regressions were performed with percent leaf damage as independent variable and above ground biomass as dependent variable. Biomass data fulfilled the assumptions of the analyses without transformation.

RESULTS

In both generations, above ground biomass was significantly larger for introduced than for native populations (Table 2; fig 1a). Differences between the origins were larger in the second generation as indicated by a significant origin x generation interaction (Table 2). Even though the sample size was comparatively low in both generation but we found similar pattern of damage in both generation. North American plants experienced larger absolute and relative leaf damage than the European plants in both generations. Similar to growth, differences between origins in damage were more pronounced in the second generation despite much smaller overall damage in the second year (Table 2; fig 2).

Regressions with percent leaf damage and above ground biomass showed that native and introduced populations did not differ in tolerance in either of the two generations (Fig 3,). Tolerance was very high - biomass was not affected by herbivore damage (first generation native $r^2 = 0.001$, $P = 0.868$; introduced $r^2 = 0.001$ $P = 0.864$; second generation native $r^2 = 0.015$, $P = 0.521$; introduced; $r^2 = 0.002$ $P = 0.821$) .

DISCUSSION

Our results show evidence for the EICA hypothesis for *L. salicaria* and indicate that the observed release from natural enemies in invasive plants was accompanied by micro evolutionary changes. In the following, we discuss our findings with respect to our initial hypotheses.

Introduced plants exhibit larger damage by native herbivores than native plants:

In both generations, absolute and relative leaf damage was markedly larger for introduced plants than for native ones when exposed to the natural herbivore spectrum in the native range. This indicates that release from enemies happened

during introduction of *L. salicaria*, and this was accompanied by evolutionary changes in resistance to herbivory. Empirical studies attempting to test the EICA hypothesis are plentiful, but they have yielded equivocal results. One possible reason may be that the approaches to test the EICA are rather diverse. For example, previous tests of the EICA mainly focused on effects of only single enemies (e.g. van Kleunen & Schmidt 2003; Siemann & Rogers, 2003; Stastny *et al.*, 2005; Zou *et al.* 2008b) and have produced equivocal results. Invasive provenances of *Sapium sebiferum* experience a higher amount of leaf area damage by generalist insects than native seedlings (Siemann & Rogers, 2003). Invasive populations of *Senecio jacobaea* were larger, and were more consumed by a specialist herbivore than native plants (Stastny *et al.*, 2005). van Kleunen & Schmidt, 2003 found no difference in response of native and introduced plant to simulated herbivory. Seedlings of invasive populations of *Sapium sebiferum* were more frequently attacked by specialist beetles relative to native populations (Zou *et al.*, 2008b). This various result provides inconclusive prediction about the response of invasive plants to single enemies.

Many studies have found that specialist and generalist herbivores may have different effects on invasive plant defence (Keane and Crawley, 2002; Bossdorf *et al.*, 2004b, Joshi and Vrieling, 2005). Therefore, exposing invasive plants to the entire herbivore spectrum in the native range automatically include specialist and generalist herbivores and it simply increases the probability of detecting reduced resistance compared to studies with single enemies. Therefore, there is an urgent need for studies that expose both native and invasive conspecifics to the entire enemy spectrum in the native range (Bossdorf *et al.*, 2005). There is some evidence from studies that shared some key aspects with our design (Wolfe *et al.*, 2004; Meyer *et al.*, 2005; Zou *et al.*, 2008a, b) and the results of these and our study were consistent and yielded strong support for reduced resistance in invasive plant against native enemies. While these studies primarily report only a subset of insects like leaf chewers and the sap-feeders, gap-makers and leaf miners found from the native range (Meyer *et al.*, 2005), plants were not fully expose to natural herbivory

condition because access to some predators was limited by greenhouse (Zou *et al.*, 2008 a).

Other key difference between our study and those above is that we used plants where maternal effects were removed. Furthermore, our 'common gardens' (i.e. field sites) were replicated, outcrossing of invasive genotypes with native plants was avoided outside in the field experiment, and plants were exposed to natural conditions in field populations. We believe that all these features are desirable for studies of the EICA in the native range. The lack of -and need for- control of maternal environment effects has also been highlighted before (Bossdorf *et al.*, 2005), but only few studies have included this consideration in their design (van Kleunen & Schmid, 2003; Meyer *et al.*, 2005). Furthermore, it has been shown that experiments conducted in a single garden in either or both ranges may yield spurious results (Maron *et al.*, 2004, Williams *et al.*, 2008; Moloney *et al.*, 2009). Maybe most important, bio safety rules must be strictly kept in field and common garden experiments in the native range. If they are done within a short distance to natural populations (Genton *et al.*, 2005; Meyer *et al.*, 2005; Zou *et al.*, 2008a) and plants are allowed to flower or set seed, this may bear the risk of introducing invasive genotypes into the native range, especially because these common garden studies indicated that this risk may be quite high. Namely, they found that the invasive plants maintain their superior morphology (higher reproduction and/or biomass) in the native range despite being more attacked by enemies. Therefore the length of exposures to herbivores in field was limited for only four weeks in our experiment. In summary, we would like to advocate our protocol as a comprehensive approach for future studies of EICA hypothesis. This could be further complemented by a systematic appraisal of several possible enemy groups (e.g. Wolfe *et al.*, 2004; Meyer *et al.*, 2005; Zou *et al.*, 2008 a, b), including below-ground enemies (Rogers & Siemann, 2004).

Interestingly, decreased resistance did not translate into detectable fitness loss for the introduced plants, i.e. US plants grew consistently larger than European plants.

Because biomass is positively correlated with seed number in *L. salicaria*, our findings suggest that release from enemies may have resulted in reallocation of resources from defence to growth and reproduction. This corroborates the observation that was made for *L. salicaria* by Blossey & Nötzold (1995) which had initially inspired the EICA hypothesis. Other studies detecting increased 'vigour' coupled with reduced defence in invasive plants have interpreted this as strong evidence for the EICA hypothesis (Rogers & Siemann, 2004; Stastny *et al.*, 2005; Meyer *et al.*, 2005; Zou *et al.*, 2008 a, b). Though ideally, competitive ability should be tested directly rather than inferred from plant size (e.g. Bossdorf *et al.*, 2004 a), our results provide support for the EICA hypothesis. Namely, enemy release did happen in *L. salicaria* during transport and there were evolutionary changes in resistance and plant size associated with the invasion. Whether the large plant size of the US plants is a direct consequence of enemy release may be debated, but our consistent findings of plant size differences for both generations indicates that selection has occurred in the novel range. This is further supported by the fact that our experiment was controlled for maternal effects.

Herbivore tolerance in native and introduced populations:

Native and invasive populations of *L. salicaria* did not differ in tolerance to herbivory and tolerance was high for both origins. Therefore, invasive plants maintained a much larger size even when experiencing a larger absolute and proportional loss of tissue by herbivores than natives. This indicates that invasive plants have evolved into more vigorous phenotypes in the invaded range, and in addition, they are largely tolerant to herbivory. In combination, these two traits - herbivore tolerance and increased vigour- are very likely a main reason for the observed high invasibility of this plant. So far, relatively few studies have explicitly compared tolerance and resistance between native and introduced genotypes (Bossdorf *et al.*, 2005) and their findings were equivocal. High tolerance was found for invasive vines (Ashton & Lerdau, 2008), in *Senecio jacobaea* (Stastny *et al.*, 2005) and in *Sapium sebiferum* (e.g. Roger & Siemann, 2004; Zou *et al.*, 2008a, b). No difference in tolerance between native and invasive populations was detected in

Solidago species (van Kleunen & Schmid, 2003; Meyer & Hull-Sanders, 2008) and *Alliaria petiolata* (Bossdorf *et al.*, 2004 b), while smaller tolerance was found in invasive *Solidago gigantea* (Meyer *et al.*, 2005). One explanation may be that large plant vigour that has evolved as a consequence of enemy release is realized at the cost of reduced tolerance (van Kleunen & Schmid, 2003). However, tolerance was similar and very high for both provenances. Therefore, we believe that the most parsimonious explanation for our findings is that tolerance is a trait which is characteristic for the species and has not newly evolved. A similar conclusion was drawn in a recent study where high tolerance was found both in native and introduced populations of *Alliaria petiolata* (Bossdorf *et al.*, 2004b). This is interesting because it illustrates that traits that enable a species to become invasive have not necessarily evolved during the invasion process but may be characteristic to the pre-invasion stage. Because a main aim of invasion ecology is to predict the invasibility of species prior to invasion (van Kleunen *et al.*, 2010), tolerance could be an important trait to look at in the future.

Variation between generations:

Though our findings were qualitatively similar in both generations, there were large differences in susceptibility to herbivory. All plants exhibited a larger average damage by herbivores in the first than in the second generation but at the same time, relative differences between origins were more pronounced in the second year (fig 2).

Because absolute differences in performance between generations were similar across origins, they were probably affected by different growing conditions in the two years. Our second study year was markedly drier than the first year. Few studies have been done for more than a single season, but all detected large differences among years. For example, Funk & Throop, (2010) detected differences in herbivore damage between years, but these were in the opposite direction with larger effects in the drier than in the wetter year. Changes in herbivore fauna composition across years was observed, too, and could be another reason for annual variations in leaf

damage (Agrawal *et al.*, 2005). This also emphasizes the importance of multi-year studies.

The more interesting pattern in our study is the relative difference between origins in the two seed generations. Differences among origins were much larger in the second generation where maternal effects were removed. We deem it unlikely that this 'interaction' between generation and origin may be affected by year quality and more likely that it was in fact caused by the genetic differences of the two setups. This has important consequences for future studies of the EICA because maternal effects apparently influence the outcome of tests of enemy release and competitive ability. Bossdorf *et al.*, (2005) have highlighted this problem and suggested that inaccurate estimates of population differentiation are obtained if maternal effects are not removed. To the best of our knowledge, there are only two studies so far that have raised plants a second generation (van Kleunen & Schmid, 2003; Meyer *et al.*, 2005), but plants were raised from rhizomes, not from seeds which may have caused unwanted effects. For example, Meyer *et al.* (2005) used a similar setup as ours for testing the EICA hypothesis in *Solidago gigantea* and obtained a smaller difference between origins in their second year. They attributed this finding to possible induced defence in the first generation, which may have caused a larger resistance to herbivory in the second generation. Our finding highlights the importance of conducting studies with appropriate seed material. This is even more important for tests of hypotheses which assume evolutionary changes during and after introduction.

Conclusions:

The lack of comprehensive tests of the EICA not only limits our understanding of invasion success but may also deprive us from the possibility to discover effective means of biological control. For example, our findings of high tolerance indicate that though biological control with leaf-eating beetles is applied for *L. salicaria*, it may not be the most effective way of managing this species in the invaded range. Also, genetically-controlled experiments are needed for determining whether

biological control may be successful or not. Our results also support conclusions by previous authors (Bossdorf *et al.*, 2005; Williams *et al.*, 2008; Moloney *et al.*, 2009) that replicated common garden experiments with many populations from the native and invasive range are needed for testing theories of invasion success. We therefore advocate the basic protocol of our study as a useful template for future studies of invasive plants.

ACKNOWLEDGMENTS

We thank Merav Seifan for her great support with the statistics and K. Moloney for generously sharing his ideas about the design and for useful comments on an earlier draft of the manuscript. F. Schurr, K. Moloney and C. Holzapfel provided seed material. This study was funded by the German Research Foundation (DFG; TI-338/8-1).

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Table 1: Sources of native and invasive populations of *L. salicaria* used in the experiment.

Origin	Country	Population (region)	Latitude	Longitude
Native	Germany	Unterjesingen (Tübingen)	48° 52' N	8° 98' E
	Germany	Hagelloch (Tübingen)	48° 32' N	9° 01' E
	Germany	Grube (Potsdam)	52° 43' N	12°97' E
	Germany	Golm (Potsdam)	52° 40' N	12°97' E
Introduced	USA	Beaver Run (New Jersey)	40° 51' N	79°55' W
	USA	Hainesville county (New Jersey)	41° 25' N	74°80' W
	USA	Boone Forks (Iowa)	42° 17' N	93°56' W
	USA	Manly (Iowa)	43°16' N	93°07' W

Table 2: Summary of nested ANOVA constructed to test for the fixed effects of origin (invaded *vs.* native range) and generation (field collected seeds *vs.* seeds raised in a common garden) and the random effect location (two locations in the native range), above ground biomass, leaf area consumed and percent leaf damage. All data were log transformed prior to analysis.

Source of variation	df	<u>Above ground biomass</u>			<u>Leaf area consumed</u>			<u>Percent leaf damage</u>		
		MS	F	P	MS	F	P	MS	F	P
Origin	1	1.64	394.5	0.003	0.31	19.97	0.047	1.35	33.05	0.029
Generation	1	0.27	13.71	<0.001	3.87	210.58	<0.001	12.66	151.97	<0.001
Origin * Generation	1	0.16	7.94	0.006	0.02	0.88	0.351	0.24	2.83	0.096
Location	1	0.02	0.96	0.329	0.00	0.00	1.00	0.18	2.14	0.146
Region (O)	2	0.00	0.12	0.889	0.02	2.31	0.215	0.04	0.97	0.454
Population (R(O))	4	0.03	1.73	0.15	0.01	0.37	0.831	0.04	0.51	0.731
Error	99	0.02			0.02			0.08		

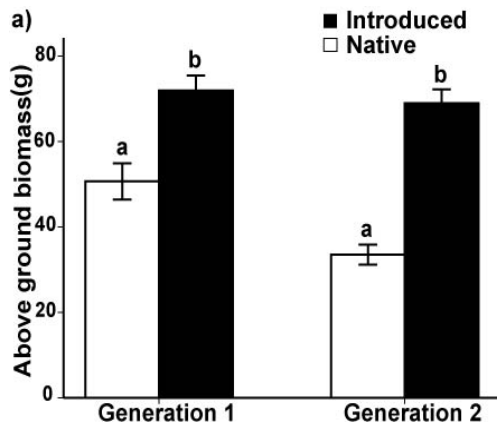


Figure 1. Mean (± 1 SE) (a) above-ground biomass for native and introduced populations of *Lythrum salicaria*. First generation (2007) plants were grown from field collected seeds, and second generation (2008) plants from seeds produced in common conditions.

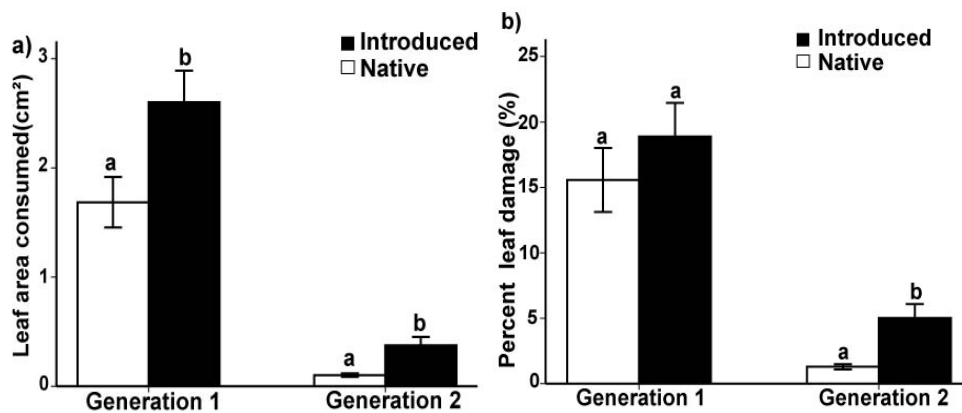


Figure 2. Mean (± 1 SE) (a) leaf area consumed and (b) percent leaf damage for native and introduced populations of *Lythrum salicaria*. First generation (2007) plants grown from field collected seeds and second generation (2008) plants grown from seeds produced in common conditions.

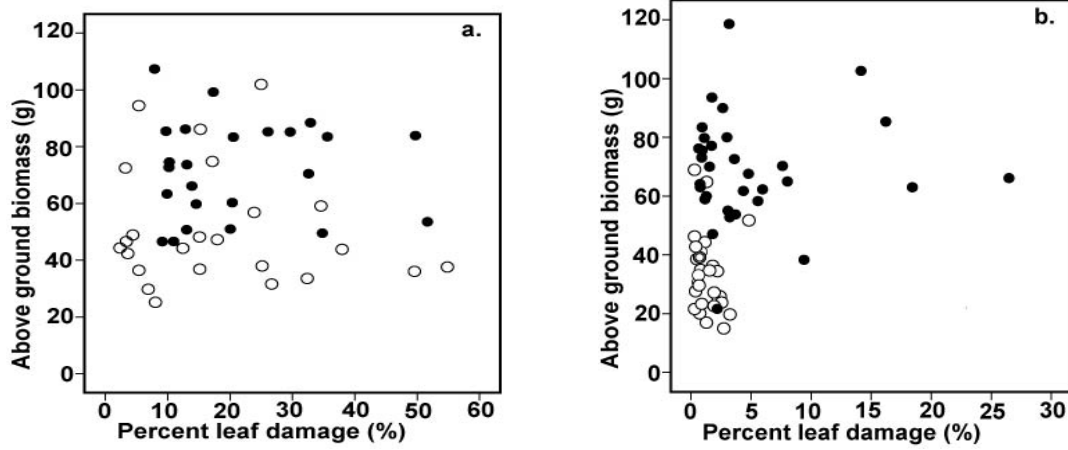


Figure 3. Relationship between the percent leaf damage and above ground biomass for two generations of plants. Tolerance is defined as the slope of the regression with fitness (i.e. biomass) and percent leaf damage. None of the regressions was significant ($p > 0.5$, $r^2 < 0.015$) and thus no fit lines are shown. (a) first generation 2007 (field collected seeds) and (b) second generation 2008 (seeds from plants grown in common conditions). The solid circles represent introduced populations; open circles represent native populations.

CHAPTER 2

Separating competitive effect and response of native and introduced
Lythrum salicaria

ABSTRACT

The evolution of increased competitive ability (EICA) hypothesis predicts that plants successfully introduced into a new range will evolve into relatively superior competitors to that of their native range, since a release from specialized herbivores allows for greater fitness to be achieved from a reallocation of resources to growth related traits. However, the few studies that have investigated the hypothesis are incomplete. In order to test the hypothesis we conducted a common garden competition experiment using F1 generation individual from population of both the native (European) and invasive (North American) ranges of a model species for EICA, *Lythrum salicaria*. The species, *Urtica dioica* commonly occurring in both ranges was used as both a target and neighbour to test for either ‘competitive effect’ or ‘competitive response’ of our study species. When grown without competition, plants from the introduced populations attained a larger size and produced more biomass than the native populations. The competitive effect of *L. salicaria* upon *U. dioica* was significantly stronger for the introduced populations than for the native plants. In addition, the introduced populations showed very little competitive response to the presence of neighbours in comparison to the native populations. Our results show strong support for the EICA hypothesis, suggesting a rapid evolutionary change in the invasive populations of *L. salicaria* which express a far superior competitive ability than individuals from its native range. This approach and findings may be a key to understanding why this species, and potentially others, may be successful establishing invaders into natural plant communities.

Keywords: Competitive effect, competitive response, EICA hypothesis, *L. salicaria*

INTRODUCTION

Introduction of non-native species into new areas is one of the major human induced impacts strongly affecting biodiversity on both a local and a global level (Vitousek *et al.*, 1997). There is growing evidence that after introduction to a new habitat, invasive plants encounter selection pressure that can lead to rapid evolutionary changes in invasive plants (Maron *et al.*, 2004). These adaptive evolutionary changes in the new environments might provide a key to understanding the major processes enabling an invader to be successful (Müller-Schärer *et al.*, 2004; Maron *et al.*, 2004, Bossdorf *et al.*, 2005). However, until recently, evolutionary processes have received very little attention in the framework of invasion ecology (Müller-Schärer *et al.*, 2004). The most intuitive hypothesis for evolutionary change in the invasive plant is the “evolution of increased competitive ability hypothesis (EICA)” which is based on the fact that invaders are released from their specialist natural enemies from its native range which allows them to reallocate resources away from herbivore defence mechanisms to traits providing a greater competitive advantage (Blossey & Notzold, 1995).

Results from previous tests of EICA are inconclusive, rarely showing evidence that either strongly supports or disproves the theory (Bossdorf *et al.*, 2005). This may be due to the fact that none of the previous studies investigated all aspects of the hypothesis in a comprehensive manner i.e. enemy release, competitive ability and evolutionary change. In particular, competitive ability is a key component of the hypothesis and has rarely been addressed in a manner consistent with current approaches in community ecology. When species are introduced into a new range, in many environments competition is likely to be the main interaction that the plants will face in the novel community. Therefore it is the competitive ability of the invading plants over the locally residing species, which is likely to determine its successful establishment (Callaway & Aschehoug, 2000). In extreme cases, where local biodiversity may be threatened, selection for even more competitive traits may

enable the invasive species to become dominant and displace many neighbouring species (Fynn *et al.*, 2009).

Two main approaches have been used in previous studies to measure the competitive ability of invasive species. Firstly, individuals from invasive plant populations have been grown in competition experiments with other neighbouring species from the invaded range (Callaway & Aschehoug, 2000; Maron & Marler, 2008). Secondly, individuals from populations in both the native and the invaded ranges of the study species have been grown together in direct competition with each other (Bossdorf *et al.*, 2004, Zou *et al.*, 2008). For example, the first approach lacks a cross continental view and does not compare the invaded populations with the individuals from the study species' native range, and therefore does not allow for testing any competitive differences that may have evolved. Although the second approach does test whether the plants from the invasive range can out-compete their native relatives, in general the native and introduced plants do not naturally co-occur. Therefore, this approach does not confirm if the plants are likely to be successful in either community, as it does not test if they are more or less competitive than any neighbouring species. An appropriate investigation of the EICA hypothesis would therefore be to compare the competitive ability of native and introduced genotypes when grown with a common neighbouring competitor from both range (Bossdorf *et al.*, 2005).

Also to demonstrate whether individuals of a species from its invasive range have evolved a superior competitive ability than individuals from its native range, both the effect and response components of competition should be estimated separately (Vila & Weiner, 2004). The competitive effect (CE) relates to the ability of an individual to suppress growth and reproduction of its neighbours, whereas competitive response (CR) is the ability of an individual to tolerate the inhibitory effect of its neighbour or withstand competition (Goldberg & Werner, 1983; Goldberg & Fleetwood, 1987). It has been shown that competitive effect and response are not necessarily correlated (Goldberg & Landa, 1991). This probably

indicates that some different traits may confer either CE ability or CR ability, which in turn may determine how, why or if the potential invader is successful in different environments. These two aspects of competition could characterise two distinct stages in an invasion history: Traits related to competitive effect ability may be important in the establishing phase of the invasion process; whereas competitive response is likely to be important once some individuals are established in order to resist the impacts of the other locally residing neighbouring species. Separating these two distinct components of competitive ability is important as it may provide further understanding into the ways in which plants interact. Moreover, if combined with the simultaneous study of multiple traits related to fitness and competitive ability, it could provide some insights into traits related to invasiveness. For example, vegetative traits such as plant height are known to be indicators of plant competitive effect since they have an advantage in space occupation and interception of light (Grime, 1977), while biomass predicts the traits of dominant species and have large impacts on ecosystem properties (Lavorel & Garnier, 2002), and seed production is commonly thought to be particularly important for the rapid colonization of invasive species (Levin *et al.*, 2003). Moreover, evolution is a particularly important aspect of the EICA hypothesis. Therefore in order to distinguish the important evolutionary development of these traits within distinct population ranges, any seed compared in competition may be influenced by the environmental maternal effects of their respective populations. Therefore pre-cultivation of plants is required to control for maternal effects for any genetic variation investigation (van Kleunen and Schmidt, 2003).

A number of studies have comparatively tested the competitive effect and response of invasive species, but in most cases they investigated the introduced individuals of the study species with coexisting species from their new range (Hager, 2004; Suding *et al.*, 2004; Domenech & Vila, 2008; Schmidt *et al.*, 2008; Gomez-Gonzalez *et al.*, 2009; Stevens & Fehmi, 2009). However, to our knowledge only one experiment (Ridenour *et al.*, 2008) has compared the competitive effect and response within a suitable EICA framework, where they used *Centaurea maculosa* individuals from

both ranges competed with a neighbour *Pseudoroegneria spicata* or *Festuca idahoensis* from invaded range. Maternal effects were controlled for in this experiment, and although CE and CR were compared simultaneously in the same pots, as opposed to the often advised separate pots, Ridenour *et al.*, (2008) did find that plants from the invasive range had increased size, greater competitive effects and also responded less to competition.

Therefore, with the aim of creating a most intuitive test of the EICA hypothesis, we devised a common garden experiment to fully test the evolutionary emergence of more competitive traits within introduced populations. Here, we used populations from the native (European) and introduced (North. American) ranges of the wetland species *Lythrum salicaria* (purple loosestrife), the species for which the EICA hypothesis was developed (Blossey & Nötzold 1995). From our own previous studies (Joshi and Tielbörger unpublished manuscript chapter -1) we know that the invasive plants of *L. salicaria* are more susceptible to herbivore attack than individuals from the native range, therefore, any observations of an increased competitive ability strongly suggest an evolutionary shift in resource allocation in support of the EICA hypothesis. We addressed any impact that environmental maternal effects may play in the role of competitive ability by using seed material from plants that were grown in a common garden. We chose *Urtica dioica* (stinging nettle) as a suitable phytometer plant as it is a common neighbour with *L. salicaria* in both the native and invaded range. In addition, we investigated separately the two important components of competitive ability, namely competitive effect and competitive response.

Within this framework, we tested the competitive aspects of the EICA hypothesis. Therefore more precisely we predict (i) that due to the release from specialized herbivore pressure, the plants from the introduced populations of *L. salicaria* have evolved an increased performance (larger size, more seed production) to that of the native populations. In addition (ii), since competitive displacement by the invader is the key assumption of the EICA, we predict that the introduced populations should

exhibit a larger competitive effect than the natives on the naturally co-occurring *U. dioica*. However (iii), since tolerance to competition from neighbouring species is not a prerequisite of success, we predict the competitive response of *L. salicaria* may not differ among range origins.

METHODS

Study species:

Lythrum salicaria is an herbaceous, tristylous perennial plant in the Lythraceae family. It is native to Europe and has become a highly invasive plant of North American wetland habitats, roadsides and ditches (Thompson *et al.*, 1987). Mature plants of *L. salicaria* can grow to about 2-3m in height. It is also a prolific seed producer, producing about 120 seeds per capsule and up to 900 capsules per plant (Shamsi and Whitehead, 1974). Consistent with the EICA hypothesis, *L. salicaria* is grazed in its native range by a large number of specialist and generalist herbivores (Blossey and Nötzold, 1995). Therefore, absence of its natural enemies in North America could be one cause for its rapid spread here. Following multiple introductions, *L. salicaria* has become an aggressive invader in North America (Houghton-Thompson *et al.*, 2005; Chun *et al.*, 2009), often forming large monospecific stands, which prevents the growth and establishment of the locally residing species (Thompson *et al.*, 1987). In controlled pot experiments, introduced populations of *L. salicaria* have shown the ability to out-compete North American species, due to its high growth rate, large size and high biomass allocation pattern (Gaudet & Keddy, 1988; Weihs & Neely, 1997; Mal *et al.*, 1997; Hager *et al.*, 2004).

Urtica dioica L. is a tall perennial herb, which is dioecious, rhizomatous, and possesses numerous stinging hairs. It grows well in high nitrate soil and shows vigorous and luxuriant growth even at relatively low light levels (Olsen, 1921). *U. dioica* naturally co-occurs with *L. salicaria* in both ranges (Lambert, 1951; Wolin, 2005).

Plant material:

The F1 seed propagation (for *L. salicaria*), germination, and plant raising were all conducted in a green house in the Botanical Garden of Tübingen University (48°32'N, 9°02'E). During summer 2006, seeds were collected from four native (German) and four introduced (North American) populations of *L. salicaria* (see Table 1 Chapter 1). In 2007 plants, from the random sample of field collected seeds, were raised from eight individuals of each of the eight populations. Prior to the onset of flowering, at least three flowering stalks per plant were covered with a light fabric organza to prevent insect cross-pollination. Only previously covered, fresh flowers, with expanded petals and bright stigma were considered for hand pollination. We performed hand pollinations between plants originating from the same population, moving pollen for approximately fifty flowers per plant from an anther to a stigma of the same length with consideration to the pollination system of the species (three partly incompatible flower morphs). The resulting F1 generation seeds were collected for each seed family individually, were stored outside during winter to maximize germination, and were used throughout the competition experiment the following growing season in 2008. For *U. dioica*, seeds were randomly collected from two different populations near Tübingen, Germany (Reusten and Heuberger Tor Weg,) in 2007. Seeds were cold stratified during winter (January-March 2008) in order to maximize germination fraction.

In May 2008, we germinated seeds of both *L. salicaria* and *U. dioica* in small (13 x 13 cm) pots filled with a standard potting soil which had been successfully used in previous *L. salicaria* experiments (Moloney *et al.* 2009). Pots were randomly placed within plastic trays filled with water and were re-randomized after two weeks to minimize position effects. After approximately one month, individuals were transplanted into the experimental pots measuring 30 cm in diameter and 26 cm deep, filled with the same soil substrate used for germination. Pots were moved outside into a common garden field site, and placed into plastic pools containing water. The pots were arranged in a completely randomized design with 5 pots in each pool. The experimental pots were then continually watered every other day for

the remainder of the experiment. 14 days after transplantation, 20 g of slow release fertilizers Osmocote 18-10-11 (N-P-K) was applied to each pot. Any seedlings which died during the first two weeks of transplantation were replaced.

Competitive effect and competitive response measurement:

To test for the competitive effect (CE) of *L. salicaria* upon *U. dioica*, a single individual of *U. dioica* (target individual) was planted in the centre of each pot. The individuals were grown under two treatments: with and without neighbours. For the neighbour treatment, *U. dioica* was surrounded by three *L. salicaria* plants. In each pot the neighbouring *L. salicaria* individuals were all from the same seed family, and plants grown alone were randomly assigned one neighbour pot from each of the eight different populations (partner pots).

Similarly to test for the competitive response (CR) of *L. salicaria* to *U. dioica*, the single target individual planted in the centre of the pot was that of *L. salicaria*. Again, the individuals were grown under two treatments: with and without neighbours. This time the neighbours in the competition treatment were five individuals of *U. dioica*, which was reduced to three individuals after one week. Here, each plant grown alone was paired with a neighbour treatment pot, both of which contained *L. salicaria* individuals from the same seed family (partner pots).

Each target-neighbor combination, for each population of *L. salicaria*, as well as each plant grown alone was replicated five times. We used families as a sample of the plant population as a whole, without replication of families. In total, we had 125 pots: 4 populations of *L. salicaria* per origin; x 2 origins (European native and North American introduced); x 3 competition treatments (no neighbour, CE, CR); + 5 plant grown alone containing *U. dioica*; x 5 replicates. Four individuals (two *U. dioica* grown alone, one *U. dioica* target individual, and one European *L. salicaria* grown alone) died later than two weeks after transplantation and data from these pots were therefore omitted from the analyses.

Intensity of competition was estimated by using the Relative Interaction Index (RII) proposed by Armas *et al.*, (2004). RII values ranges from -1 to +1, where negative values indicate competition, and positive values indicate facilitation. When RII = 0 this would indicate that there was no effect caused by the presence of a neighbour.

For both competitive effect, and competitive response RII can be calculated for each of the assigned partner pots using the following equation:

$$\text{RII} = (T_{w/n} - T_{wo/n}) / (T_{w/n} + T_{wo/n}).$$

(T= Target individual; w/n = with neighbours; wo/n = without neighbours).

Therefore, a greater competitive ability of *L. salicaria* would be indicated for: competitive effect by a more negative value of RII (greater impact of *L. salicaria* upon *U. dioica*); and for competitive response by a larger value of RII (smaller impact of *U. dioica* upon *L. salicaria*).

Plant trait measurements:

Different plant traits were recorded on the target individual in each pot to assess the performance of both species, with and without competition. We measured plant height, after transplantation to give an initial measure and then again prior to harvesting to give a maximum height measure. After the completion of seed set, plants were harvested to yield biomass and seed production measures of plant performance. For above ground biomass measurements, target plants were dried at 60°C for 24 hours, and weighed. Seed output was only recorded for *L. salicaria*, since *U. dioica* are dioecious. The number of seeds per plant was estimated in the following way. Firstly, the number of capsules per plant was estimated from the number of flowering stalks per plant, multiplied by an average length of flowering stalk per plant (calculated from 6 randomly chosen stalks per plant), multiplied by the average number of capsules per unit length (calculated by counting the number of capsules and measuring the length of 6 differently chosen stalks). The final estimate of seed number could then be calculated from the estimate of capsules per plant, multiplied by the average number of seeds per capsule (derived from three capsules per plant from apical, middle and basal parts).

Statistical Analyses:

Firstly, we tested for differences in the raw performance data (height, biomass, seed number) for the target species in both the CE and CR experiments. We used a one-way ANCOVA for testing the CE experiments, where *U. dioica* was the target individual. The factor was defined by nine levels (plant grown alone, and the eight types of neighbourhood pots containing four populations of *L. salicaria* from either their native or introduced range), and initial plant height was used as a covariate. For the CR experiment, with *L. salicaria* as the target individual, we conducted a two-way-ANCOVA with origin (native or introduced range) and treatment (with or without neighbour) as fixed factors, and initial plant height as a covariate. For both CE and CR testing, Tukey tests were used in post-hoc analysis.

Secondly, we used the partnered pots of monoculture and neighbour treatment to calculate RII values for each trait recorded (height, biomass, and seed number). However, two of the *U. dioica* single grown pots died, so the mean value of the remaining plant grown alone samples was used as replacement values in these cases. We performed two-tailed t-tests to look for differences in RII values between populations from native and introduced ranges in both the CE and CR experiments. All statistical tests were performed using SPSS version 18.

RESULTS

In the CE experimental pots, *U. dioica* did not significantly differ in height whether plants were grown alone or in competition with *L. salicaria* neighbours ($F_{8, 32} = 0.168$, $P = 2.01$). The above ground biomass of *U. dioica* was strongly affected by the presence of neighbours ($F_{8, 32} = 6.506$, $P = 0.015$). Three introduced and one native population significantly differed from monocultures. *U. dioica* suffered 58% reduction in biomass when grown with native neighbours and 80% with introduced neighbours (Fig 2b).

In the CR experimental pots, there was an overall significant effect for origin for both *L. salicaria* plant height ($F_{1, 74}=5.405$, $P=0.023$) and biomass ($F_{1,74}=9.107$, $P=0.003$), with individuals from the introduced range producing higher values than those from the native range (Table 1). When grown alone and in competition with *U. dioica*, plant height of *L. salicaria* target individuals was similar. However, competition cause a significant reduction to both *L. salicaria* biomass ($F_{1,74} = 9.03$, $P = 0.004$) and seed production ($F_{1,74}=9.834$, $P=0.002$). In addition, there was a statistically significant interaction between origin and treatment (with or without neighbours) for both biomass ($F_{1,74}=6.034$, $P=0.016$) and seed production ($F_{1,74}=4.551$, $P=0.036$), indicating that native populations were more affected by competition than introduced populations (Table 1). For example, native plants suffered a 33% and introduced plants suffered a 7% loss of biomass (Fig 1b); likewise *U. dioica* caused 50% seed production loss for native and 27% loss for introduced populations (Fig 1c).

Using the Relative Interaction Index (RII), in terms of competitive effect (CE), the intensity of competition applied by *L. salicaria* upon *U. dioica* did not differ among native and introduced populations when calculated using height measurements ($t_{37} = 1.294$ $P=0.204$ fig 3a). However, introduced populations showed a significantly larger competitive effect (lower RII values) than native populations upon the biomass of *U. dioica* target plants ($t_{33.5} = -2.134$ $P= 0.04$ fig 3a).

In terms of competitive response (CR), RII values produced from *L. salicaria* plant height measures did not differ regardless of the origin of the populations ($t_{38} = -0.811$ $P= 0.423$ fig 3b). However, the RII values for biomass ($t_{38} = -2.370$ $P=0.023$ fig 3b) and to a lesser extent for seed production ($t_{38} = -1.842$ $P= 0.073$ fig 3b) were significantly greater (less impact of *U. dioica*) for introduced populations than for native populations.

DISCUSSION

A major prediction of the EICA hypothesis is that through a process of natural selection occurring over multiple generations introduced plants have the ability to reallocate their resources from traits related to herbivore defense mechanisms to those related to growth and reproduction (Blossey & Nötzold, 1995), and that this would infer a greater competitive ability in the invaded range. This hypothesis was initially formulated for the wetland species *L. salicaria* but observations were made in a competition-free environment. Here, we applied a new approach to studying competitive ability by carefully separating competitive effect (CE – the ability to out-compete neighbours) and competitive response (CR – the ability to withstand competition). We found that not only the plants from an invaded range (North America) were larger and produced more seeds than those raised from the native range (Europe) in monoculture, but that they also had a superior competitive ability when measured in terms of CE and, in contrast to our hypothesis, also for CR when competing with a naturally co-occurring species.

Plants raised from the native and introduced populations of *L. salicaria* were found to be phenotypically divergent when grown under common garden conditions. Introduced populations of *L. salicaria* grew higher and produced a higher biomass than the native populations both in the absence and presence of competition. However, it is when introduced populations perform relatively better than the native populations in the presence of neighbours, that a greater competitive ability can truly be inferred (Blair & Wolfe, 2004). Within the CR experiments, biomass of *L. salicaria* produced a significant interaction term between origin and treatment to indicate a greater competitive ability within the introduced populations. While there was no significant overall effect of origin range on seed production, the pattern for mean values was the same as for the other traits measured, and the interaction term between origin and treatment suggests an increased competitive ability response in this trait as well. Using Relative Index Index (RII) values, we found statistical evidence in both our CE (biomass) and CR (biomass and seed production)

experiments that the introduced populations showed a greater competitive ability than native plants. While we predicted such a response for CE, the additional results for CR indicate that we were apparently dealing with a "super-competitor" which is not only able to displace neighbours but also to hold the space once it is established.

The results of our study are in general support to the EICA hypothesis suggesting that *L. salicaria* has evolved into superior competitor in its invaded range. In combination with our previous findings (Joshi and Tielbörger, unpublished, chapter -1) showing that the introduced populations have less herbivory defence, this shows strong evidence for an evolutionary shift in resource allocation from traits related to herbivory defense to competitive ability. Similar results were observed by (Ridenour *et al.*, 2008) when investigating the EICA hypothesis for *Centaurea maculosa*, common in disturbed areas, and they were able to demonstrate that plants from the invaded range had increased size, greater competitive effects and responded less to competition.

Many previous tests of the EICA hypothesis were done in competition free environments, where performances of native and introduced population cannot reliably predict competitive ability (Bossdorf *et al.*, 2005). Only a few studies show evidence of competitive ability of an invader from its native and invaded range through interspecific competition and the results are ambiguous (Leger & Rice, 2003; Blair & Wolfe, 2004; McKenney *et al.*, 2007; Blumenthal & Hufbauer, 2007; Barney *et al.*, 2009). In some studies, invasive populations were found to be superior to native populations but only in the absence of competition (Leger & Rice, 2003; Blumenthal & Hufbauer, 2007). McKenney *et al.*, (2007) found no difference in competitive ability between native and introduced populations of the agricultural weed *Lepidium draba* when grown with either strong or weak competitors. The diverse results from the testing of the EICA hypothesis could be due to the inappropriate selection of specific competitors in those studies because the neighbour competitors do not naturally co-occur in both ranges (Bossdorf *et al.*,

2005). This is likely to be extremely important in any competition experiment, and particularly when looking at genetic differences, because the strength of the competitive interaction and therefore the observed difference in response by native or introduced populations will be highly influenced by the identity of the competing neighbor (Weigelt *et al.*, 2002). Therefore, the most promising approach in interspecific experiments testing the EICA hypothesis would be to select neighbour species which co-occur with the study species in both ranges (Bossdorf *et al.*, 2005). One rare study so far to have taken this approach compared native and introduced populations of *Silene latifolia* competing with a few grass species that naturally co-occur in both their ranges (Blair & Wolfe, 2004). This study supports some prediction of the EICA hypothesis but no differences in competitive ability were found between origins; however it seems likely that choosing competing species that are similar in life-form to the invaders is also important for reliable differences in competitive ability to be observed (Vila & Weiner, 2004). However, these studies included native competitors that were functionally different in terms of their life-form from the invaders (Vila & Weiner, 2004). Equally, the diversity of results from tests of the EICA hypothesis could be related to study species themselves. Whilst we believe this hypothesis holds true for *L. salicaria*, it could be that evolution towards a more competitive strategy within other species is slower to occur or may not be occurring at all (Orians & Wards, 2010).

In general, most of the studies investigating the EICA hypothesis have merely compared the competitive effect of the native and introduced plants of a species whereas its competitive response ability has been largely ignored in the invasion literature (Bossdorf *et al.*, 2005). Vila and Weiner (2004) emphasized that to fully test if introduced populations have evolved a superior competitive ability in relation to their natives, it is important to incorporate both CE and CR experiments. We know only few studies that have adopted an effect and response approach in invasive plant ecology (Hager, 2004; Suding *et al.*, 2004; Domenech & Vila, 2008; Schmidt *et al.*, 2008; Ridenour *et al.*, 2008). Suding *et al.*, 2004 found that *Centaurea diffusa* was superior at tolerating neighbourhood competition than other

species from its invaded range but that it had no strong effect on its neighbours. Interestingly, the opposite trend was observed in another experiment investigating invasive populations of our study species *L. salicaria*, where plants showed stronger competitive effects on other species but were not superior tolerators in neighbourhood competition (Hager, 2004). However, from such a comparison we cannot generalize that invasive plants have further evolved into more superior competitors than their native relatives because these studies were limited to plants from the invaded range (Bossdorf *et al.*, 2005). However, the study by Ridenour *et al.*, (2008) made a proper distinction between effect and response, albeit not separating the pots for measuring CE and CR, and found both a difference in CE and CR for *Centaurea maculosa* across origin ranges. Our study certainly supports this finding. It is therefore possible for *L. salicaria*, and maybe for *C. maculosa*, that a combination of both a strong effect upon and a less response to neighbour competition could be an evolved strategy that has enabled these species to successfully establish, remain established, and spread throughout their invaded range. Our results support the contention that strong competitive effect may be a necessary but not a sufficient condition for strong invasive ability and other traits, such as competitive response, are also necessary for successful plant invasions (Fynn *et al.*, 2009).

The search for particular traits which define the threat of potential invasive species makes up a large part of the literature (see Kleunen & Fischer, 2010). However, by showing the evolution of increased performance of introduced species we may gain a greater insight into what traits may change, and to what degree they may change and possibly even an insight into how we can control established invasive plants. One trait for which we found a common response to both competitive effect and response is for above ground biomass. It is also possible, particularly for comparisons within species, that an increase in above ground biomass correlates with an increase in root biomass, indicating an increase in its competitive ability for the capture of below ground resources as well (Campbell *et al.*, 1991). Introduced populations of *L. salicaria* were taller and had higher biomass than the native

populations. Larger plants have the advantage in productive environments of being able to suppress the growth of smaller and slower growing species because of their ability to overtop them and intercept light while avoiding being overtopped by their neighbours (Miller and Werner, 1987). For example, many studies of the EICA hypothesis have emphasized plant height and fecundity as proxy of competitive ability (van Kluenen & Schmidt 2003; Bossdorf *et al.*, 2004). However, we found above ground biomass, but not height, as an important correlate of competitive ability in *L. salicaria* (see also Gaudet and Keddy, 1988). This corresponds with the result of Fynn *et al.*, (2009) who indicated that other fitness related traits such as total leaf mass, SLA, and tiller number, can play a more important role than plant height in determining invasiveness.

We also showed evidence that the impact of neighbours on the seed production of *L. salicaria* was less in the introduced populations than for the native populations. The low response of the invasive plants to neighbours indicates that even in resource limited conditions, the invasive plants may still be able to take advantage of their high seed production. In combination with their increased ability to outcompete neighbouring species, the introduced populations of *L. salicaria* also have the potential to increase the local abundance of seeds, seedlings, and established plants, leading to potential dominance of the natural communities (Lortie *et al.*, 2009). It seems highly likely, as shown in many other studies, that an increased ability to grow bigger, quickly and produce large amounts of seeds, equates to a good colonizer and a successful invader (Grotkopp *et al.*, 2002).

In conclusion, our results demonstrate that introduced populations of *L. salicaria* from North America are larger than from the native European range. More importantly, our findings strongly suggest that after introduction to North America, *L. salicaria* have evolved to superior competitors because of its strong negative effect on neighbours and strong ability to withstand the negative effect of neighbours. In combination with herbivory results (chapter -1), our results further strengthen the EICA hypothesis that release from enemies may influence invasion

by an evolutionary shift in resource allocation from traits related to herbivory defense to competitive ability. Moreover, there appears to be multiple events of introduction recorded throughout history in *L. salicaria* (Houghton-Thompson *et al.*, 2005, Chun *et al.*, 2009). Therefore, most likely evolutionary changes and the EICA, which makes individual of these species even more competitive than their native relatives, and is possibly a reason for the successful establishment and spread of this problematic invader.

ACKNOWLEDGEMENTS:

We would like to thank Merav Seifan for statistical help and comments, and Mark Bilton for careful proofreading and Pierre Liancourt for helpful suggestions. This work was supported by German Research Foundation (DFG; TI-338/8-1).

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Table 1: Results of two-way ANOVAs comparing the performance of native and introduced populations of *L. salicaria* in presence and absence of neighbours.

Source of variation	df	<u>Height</u>			<u>Above ground biomass</u>			<u>Seed output</u>		
		MS	F	P	MS	F	P	MS	F	P
Initial plant height	1	0.021	4.546	0.036	0.609	20.699	0.000	3.196	13.306	0.000
Origin	1	0.025	5.405	0.023	0.268	9.107	0.003	0.01	0.041	0.841
Treatment	1	0.000	0.05	0.823	0.266	9.03	0.004	2.362	9.834	0.002
Origin * Treatment	1	0.004	0.82	0.368	0.177	6.034	0.016	1.093	4.551	0.036
Error	74	0.005			0.029			0.24		

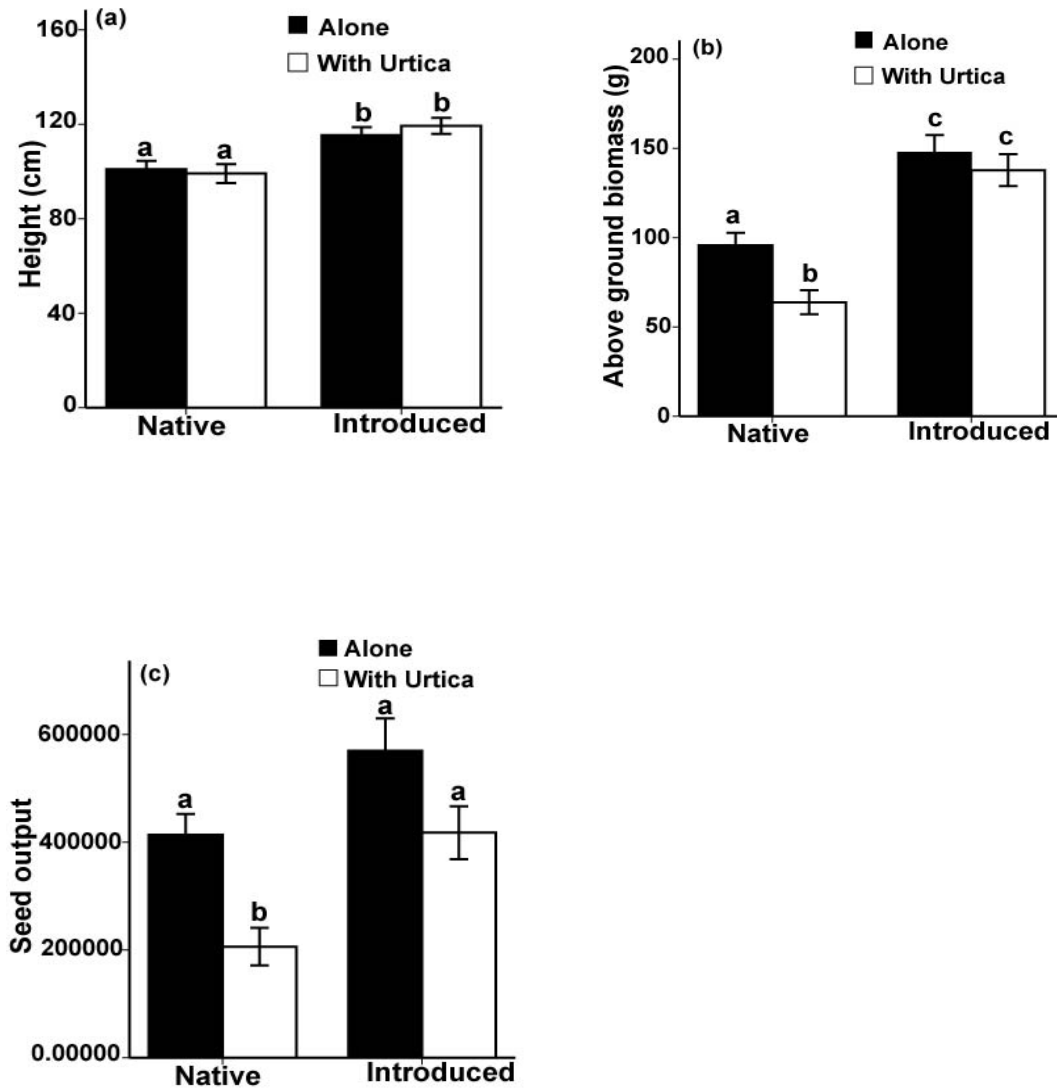


Fig 1: Effect of neighbour treatment of *L. salicaria* with *U. dioica* as neighbour on (a) Plant height, (b) Above ground biomass, (c) Seed production. Data represent means ± 1 SE. Different letters indicate significant difference in means (Tukey test).

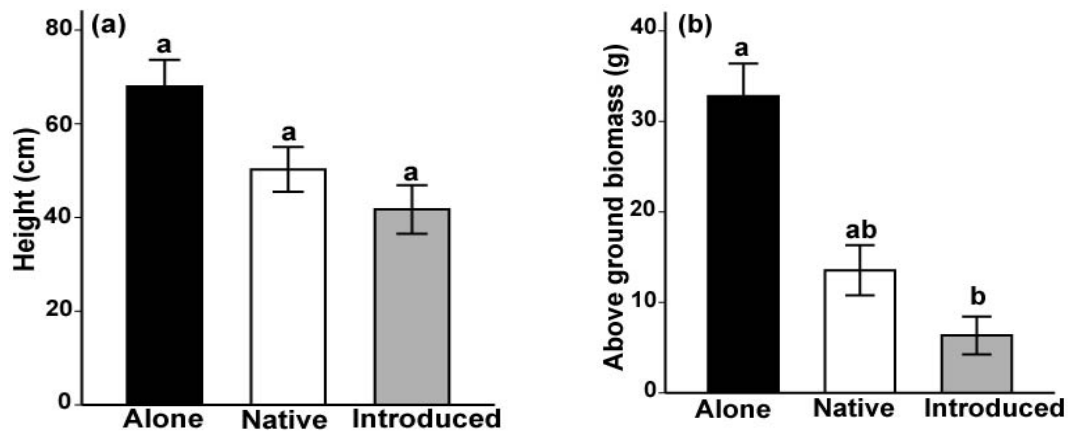


Fig 2: Plant height and biomass of *U. dioica* when grown alone and in competition with native and introduced *L. salicaria*. Data represent means \pm 1 SE. Different letters indicate significant difference in means (Tukey test).

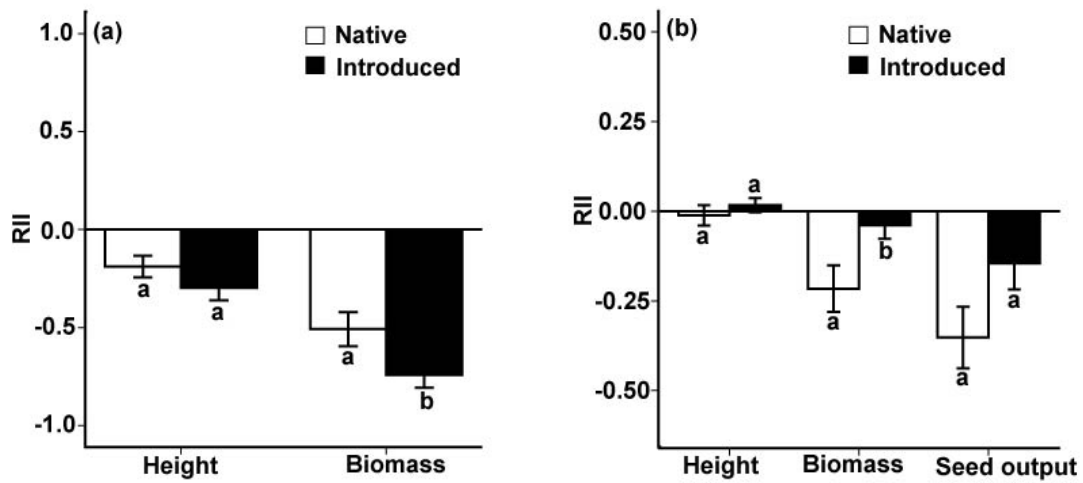


Fig 3: Competitive effect (a) and competitive response (b) among native and introduced plants measured in terms of relative interaction index (RII). A positive RII value indicates that neighbour facilitates growth and negative RII values indicate that neighbours inhibit growth. Data represent means ± 1 SE. Different letters indicate significant difference in means.

CHAPTER 3

Does reinvasion cause potential threat to native plant communities? A test using *Lythrum salicaria*

ABSTRACT

Several mechanisms explain that invasive plants evolve into superior competitors in their introduced range. Here we explore the possible consequences of reintroducing the genetically different individuals from the introduced range back into their native communities. Hybridization among native and introduced lineages is highly likely to occur during reinvasion, and the traits maintained in these initial generations are likely to provide valuable indications of reinvasion scenarios. We reexamined the evolution of increased competitive ability (EICA) hypothesis in a novel framework comparing native (European), introduced (North American) and cross-origin hybrid (German maternal hybrid, US maternal hybrid) individuals of *Lythrum salicaria* in a common competitive environment. The individuals were grown alone and in all possible intraspecific pair-wise mixtures, in the presence of natural enemies, in order to determine their fitness with respect to their competitive abilities, as well as their resistance to native herbivores. For both the raw data and Relative Interaction Index (RII), we show that introduced populations performed significantly better in competition than native populations in terms of plant height. Similar patterns for mean values were observed in biomass and seed production, no significant differences were found between origins. However for these traits it seems apparent that in raw measures the expression of performance follows the maternal line for hybrid populations, with US hybrid populations producing more biomass and more seeds than the German hybrids in competition. The RII for these traits were relatively consistent for native introduced and US hybrids whereas German hybrids perform significantly worse when under competition. In support of the EICA hypothesis, we found that introduced populations of *L. salicaria* had higher percentage of leaf loss than native populations, however all result of herbivory were non-significant. Although further confirmation is required, our preliminary results suggest that the alleles of the origin populations are withheld to a greater extent, or even exaggerated on the maternal line. During reinvasion this may result in the formation of monospecific stands in a local area rather than widespread invasion, therefore, essential consideration should be taken early for effective management.

Key words: EICA hypothesis, hybridization, intraspecific competition, *Lythrum salicaria*

INTRODUCTION

Non native plant invasions involve the intentional or accidental movement of a species from one region into a new region (Sakai *et al.*, 2001). However, only a small fraction of introduced plants get established and become invasive in a new range (Williamson & Fitter, 1996). A few theories based on ecological and evolutionary processes have been suggested to explain invasive success of exotic species in new environments. For example, successful invaders are frequently characterized by rapid growth, high fecundity, a persistent seed bank, a high dispersal ability and higher capacities for colonization (Pysek & Richardson, 2007). The introduced individuals are often regulated by different biotic (herbivory and competition) and abiotic (climate and soil) factors in their new range compared to those experienced in their native range (Hierro *et al.*, 2005). These potential forces of natural selection have been shown to cause rapid evolutionary changes in trait expression of the introduced plants (Muller-Schärer *et al.*, 2004; Bossdorf *et al.*, 2005), which may additionally facilitate the successful spread of the invasive plant (Sakai *et al.*, 2001). One well studied theory of evolutionary change in introduced plants is the Evolution of Increased Competitive Ability (EICA) hypothesis (Blossey & Nötzold, 1995). This states that under reduced exposure to specialized enemies (herbivores and diseases), over a number of generations, selection may cause a shift in the resource allocation of invasive species away from enemy defence towards traits associated with faster growth and reproduction.

A number of factors are likely to impact upon the continued evolution of successful invasion strategies in a new range; those include gene flow, genetic drift and genetic diversity within and between both the introduced and resident species (Leger & Espeland, 2010). One particular mechanism associated with the evolution of invasiveness is inter- and intraspecific hybridization (Ellstrand & Schierenbeck,

2000). There is evidence from genetic marker studies that repeated multiple introductions from different source populations are common in many introduced plants (Dlugosh & Parker, 2008; Rosenthal *et al.*, 2008, Lachmuth *et al.*, 2010). These introduction events provide the opportunity for gene flow among populations and the establishment of hybrid populations that exhibit new genetic variability in the introduced range (Dlugosh & Parker, 2008). Such admixture is predicted to contribute to invasion success by directly increasing fitness through hybrid vigor (Keller & Taylor, 2010; Verhoevan *et al.*, 2010). Such populations have the potential to create monospecific stands through rapid colonization of the habitat sites by outcompeting and preventing the establishment of the local species. The outcome of interspecific hybridization among introduced and native species has shown variable results (Ellstrand & Schierenback, 2000). In most studies hybrids were found to be more aggressive relative to their parents, and create a potential threat to the native plant communities (Campbell *et al.*, 2006; Daehler & Strong, 1997; Grosholz, 2010). However, so far, the consequences of intraspecific hybridization in invasive plant ecology have been explored to a far lesser extent, providing evidence of hybridization between native and introduced lineages (Culley & Hardiman, 2009; Meyerson *et al.*, 2010).

In particular, intraspecific hybridization could occur during the reinvasion of an exotic species back into its native range, and the impacts of this type of incursion are poorly understood. There are many possible opportunities by which a species could be unintentionally re-introduced through different pathways. Exotic species may become harmful as back invaders if they have been genetically and morphologically modified in their introduced range (Guo *et al.*, 2006). In addition, under certain circumstances, if these reintroduced plants are able to hybridize with their native progenitors, the aggressive alleles may also spread rapidly in the native populations which may have detrimental effects on the native plant community. Some recent studies have performed intraspecific hybridization among invasive plants (see Wolfe *et al.*, 2007, Meyerson *et al.*, 2010, Paul *et al.*, 2010), however their results are inconclusive. For example, Wolfe *et al.*, (2007) performed intraspecific hybridization

between plants from different populations within the same continent and found that intraspecific hybridization did not cause invasiveness, whereas Meyerson *et al.*, 2010 performed hybridization among native and invasive lineages and explain the potential for formation of aggressive hybrids. Exploring the possible consequences of reinvasion has the potential to fill some knowledge gaps in the EICA hypothesis specifically relating to the evolution and maintenance of traits in different environments.

Many studies on the EICA hypothesis have shown evidence that plants from the introduced populations are more competitive (Vila *et al.* 2003; Leger & Rice, 2003; Blumenthal & Hufbauer, 2007) but also more susceptible to specialists herbivores than plants from the native populations (Blair & Wolfe, 2004; Bossdorf *et al.*, 2004; Meyer *et al.*, 2005; Stastny *et al.*, 2005; Zou *et al.*, 2008a). Therefore, there are a number of scenarios for reinvasion. A harmless reintroduction may occur if invasive plants have evolved into new ecotypes without antiherbivore defence, and thus exhibit lower performance than the natives due to higher damage. In contrast the reinvasion could pose a potential risk to the native populations and communities if, whilst in their introduced range, invasive evolved a higher tolerance to herbivores or invest in compensatory growth. This would mean that on reinvasion the loss of defence mechanisms would be less important because individual fitness would be maintained or enhanced through a capacity for higher growth (Rogers & Siemann, 2005; Zou *et al.*, 2008 a, b). Critically, if a reinvasion is going to have any impact on the native populations or communities gene flow and alleles coding for aggressive traits would have to be maintained over several generations.

Our objective therefore was to reexamine the EICA hypothesis in a novel framework investigating the consequences and potential risks of reinvasion. To do this we used populations from the native (European) and introduced (North American) ranges of the wetland species *Lythrum salicaria* (purple loosestrife), the species for which the EICA hypothesis was developed (Blossey & Nötzold, 1995).

To the best of our knowledge no other study has taken this approach, despite invasive ecologists being aware of the potential problems of reinvasion, and therefore often preventing gene flow from invasive individuals back into the native communities by harvesting common garden experiments prior to flowering or seed set (Genton *et al.*, 2005; Franks *et al.*, 2008). One way to investigate the potential impact of reinvasion is to compare the performance of native, introduced and hybrid populations in direct competition with each other. Common competitive environments are ideally suited for unraveling how genetic and environmental factors contribute to the success of invasive species (Bossdorf *et al.*, 2005).

Testing performance under intraspecific competition, and crucially in the presence of natural enemies, may allow us to identify which fitness related traits have evolved during invasion, and which may be withheld if reinvasion occurs. Controlled-crosses were performed between native and introduced populations to produce intraspecific hybrids. By using hybrids we hoped to gain additional information of the trait maintenance we may expect in future generations after reinvasion. Therefore, using the species *L. salicaria* within this experimental framework, we specifically asked: (1) When grown individually (without competition), do individuals raised from the introduced range express a more vigorous growth response and less resistance to herbivores than the individuals raised from the native range, as predicted by the EICA hypothesis?; (2) as substantial support for EICA, when grown together in a common competitive environment (pair-wise intraspecific competition), do the introduced individuals consistently out-compete the plants raised from the native range?; (3) and finally to determine trait inheritance during reinvasion, do the crossed hybrid individuals show an intermediate response in plant performance between that of the native and introduced populations or do they show heterosis i.e. increased hybrid vigor in some traits expression.

METHODS

Study species:

Lythrum salicaria (Purple loosestrife) is a herbaceous, self incompatible, tristylous, wetland perennial plant first introduced into North America from Europe in the early 19th century (Thompson *et al.*, 1987). In its introduced range it is generally regarded as one of the most aggressive species mainly colonizing road sides, marshy places, coastal areas, stream banks and ditches (Thompson *et al.*, 1987). Once established, it can form monospecific stands that exclude establishment of other locally residing species (Mal *et al.*, 1992). Molecular marker studies show evidence for multiple introductions to North America with admixture among introduced populations of *L. salicaria* (Houghton-Thompson *et al.*, 2005; Chun *et al.*, 2009). Factors such as the evolution of increased competitive ability due to release from native herbivores (Blossey & Notzöld, 1995, chapter 2), hybridization with the North American native *Lythrum alatum* (Houghton-Thompson *et al.*, 2005), and an inherent strong dispersal capability, together with its high fecundity (Thompson *et al.*, 1987; Weihe & Neely, 1997) have been suggested as potential explanations for the success of *L. salicaria* in its invaded range.

Plant material:

Seeds of *L. salicaria* were collected from 20 randomly chosen individuals within each of four native (European) and four introduced (North American) populations during late summer 2006 (see Table 1 for seed source). The germination of the seeds commenced in early May 2007, in a green house at the Botanical Garden of Tübingen University (48°32'N, 9°02'E). The seeds were germinated in small (13 X 13 cm) pots filled with Flora plus Aussaaterde premium soil. The pots were randomly placed among green plastic trays filled with water and were re-randomized after two weeks to minimize position effects. After approximately one month plants were transplanted into larger pots and placed outside into a common garden field site.

Plants were grown outside in small plastic pools from beginning of June 2007. During the flowering season, flowering stalks were covered with a light fabric organza to prevent access by insect pollinators. As flowering commenced (mid July) plants were then transferred back into the green house for controlled pollination.

Cross-pollination was performed to produce F1 generation seeds within populations and F1 hybrids between origins (native and introduced populations). Therefore, three types of pollination crosses were performed: (I) within population crosses i.e. pollen transfer from fathers to mothers within the same population; (II) crossing for German hybrids i.e. pollen transfer from fathers of North American origin to mothers of European origin; (III) crossing for US hybrids i.e. pollen transfer from fathers of European origin to mothers of North American origin. Flowering was observed earlier for native populations (mid July) compared to introduced populations (end of July). For within population crosses, each origin was only represented by two populations throughout the competition experiment (see Table 1), so eight plants per population were randomly chosen from within the two populations of each of the two origins (European or North American) meaning pollen was transferred to a total of 32 plants. For between continents crosses there were 4 combinations of crosses for each hybrid type (4 populations within origin X 4 populations from different origin), with 2 hybrid types (German or US hybrid) and each combination was replicated two times, so involving a total of 64 plants. Only fresh flowers with expanded petals and bright stigma were considered for pollination. Pollen was collected in paper bags and using forceps hand pollinations between plants were performed moving pollen for approximately fifty flowers per plant from an anther to a stigma of the same length with consideration to the pollination system of the species (three partly incompatible flower morphs). Four days after pollination the plants were moved back outside into the flooded pools. The resulting seeds were collected for each plant individually, were cold stratified during winter (January-March 2008) in order to maximize germination fraction, and were utilized during the competition experiment the following year in 2008.

Experimental design:

In May 2008, we germinated the F1 generation seeds of *L. salicaria* in small (13 x 13 cm) pots filled with a standard potting soil which had been successfully used in previous *L. salicaria* experiments (Moloney *et al.*, 2009). Pots were randomly placed within plastic trays filled with water and were re-randomized after two weeks to minimize position effects. The first germination occurred 5 days after sowing, with germination occurring earlier in the US hybrids than in the other populations. After one month, individuals were transplanted into the experimental pots measuring 30cm in diameter and 26cm deep, filled with the same soil substrate used for germination. The pots were moved outside into a common garden field site and placed into plastic pools containing water. Pots were arranged in a completely randomized design with 5 pots in each pool, and were watered continually every other day for the remainder of the experiment. Two weeks after transplantation, 20 g of slow release fertilizers Osmocote 18-10-11 (N-P-K) was applied to each pot.

North American (two populations), European (two populations), German hybrids, and US hybrids were grown alone and in all possible pair wise mixtures with each other (two plants per pot). Therefore, throughout the experiment there were: 6 population types; 2 populations of *L. salicaria* per origin x 2 origins (European native and North American introduced), + 2 hybrid types (German hybrids and US hybrids); giving a total of 21 possible combinations of intraspecific interaction (with neighbour), and 6 types of plants grown alone (without neighbour). Each plant grown alone and neighbourhood combination was replicated 4 times giving a total 108 pots and 192 plants. However, one introduced plants grown alone died during the experiment period leaving 107 pots for analysis. For the native and introduced populations each replicate was represented by a different seed family; however each family was represented in both monoculture and mixture creating a pair for competition analyses. For the hybrid replicates we randomly chose plants produced from the different possible crosses between native and introduced populations, however the pairs for competition analyses were always comprised of hybrids created from the same populations within alternate origins.

Plant trait measurements:

At the end of the flowering period in October all the plants were harvested. We recorded numerous plant traits (height, total above ground biomass, seed output, and leaf damage) to assess the fitness of the populations both with and without competition. Firstly, we measured height of the plant. Secondly, we created a measure for the percentage of leaf damage for each plant by randomly selecting ten leaves. The leaves were scanned with a STD 1600+ scanner (Regent Instruments) and analysed using the software Winfolia. We estimated the percentage of leaf damage by reconstructing the leaf area before damage and dividing the leaf area consumed by the original area. Thirdly, for aboveground biomass (including both vegetative and reproductive parts) the plants were oven dried at 60° C for 24 hours and weighed. Finally, seed output was measured by estimating the number of seeds produced per plant. Initially, estimates of the number of capsules for each plant was made by taking the average length of six randomly selected flowering stalks, multiplying this by the average number of capsules per unit length, and multiplying again by the total number of flowering stalks each plant produced. Seed number was calculated by multiplying the average number of capsules by the average number of seeds per capsule (derived from three capsules per plant from apical, middle and basal parts).

Competition indices:

To quantify the competitive ability of each population type, we calculated their Relative Interaction Index (RII) as proposed by Armas *et al.*, (2004). RII has value ranges from -1 to +1, where negative values indicate competition (i.e. the growth of the target individual is reduced), and positive values indicate facilitation (i.e. growth of the target individual is promoted). When RII = 0 this would indicate that there was no effect caused by the presence of a neighbour. In order to calculate the competitive ability in this way, each individual within a mixture pot was paired with a plant grown alone during the experimental design (see above) and their performance was compared using the following equation: $RII = (T_{w/n} - T_{wo/n}) / (T_{w/n} + T_{wo/n})$. (T= Target individual; w/n = with neighbours; wo/n = without neighbours).

Using this method, two measures of competitive ability could be estimated: competitive effect (the ability of the neighbour to reduce the growth of target individual – calculated by averaging across the same neighbour types); and competitive response (the ability of the target individual to withstand the impact of competition – calculated by averaging across the same target types). Therefore, an individual with a good competitive ability is likely to show a lower RII value for competitive effect, and a higher RII value for competitive response. Since both individuals in each mixture pot were used as either the target or the neighbour individual, the different measures of competitive ability are unlikely to be completely independent

Statistical Analysis:

SPSS (version 18) was used throughout all the statistical analyses. We tested for differences in the separate raw performance data (height, biomass, seed number or herbivore damage) by using a two-way ANCOVA with origin (native, introduced, German hybrids and US hybrids) and treatment (with or without neighbours) as fixed factors, and using initial plant height as a covariate. Each ANCOVA analysis was followed by specific post hoc comparisons (Tukey tests) to compare differences in height, aboveground biomass and seed number among origin. We used the paired pots of plant grown alone and neighbour treatment to calculate RII values for each trait recorded (height, biomass and seed number). However, one of the introduced plants grown alone died, so the mean value of the remaining plants grown alone samples was used as replacement values in this case. We performed one way ANOVAs to test for the difference between RII values among origins. When calculating competitive effect ‘neighbour individual’ origin was used as the fixed factor and for the competitive response ‘target individual’ origin was used. For measuring difference among origins, Tukey tests were used in post-hoc analysis.

RESULTS

Comparing the raw performance measures of *L. salicaria* grown alone without neighbour and to that when grown in competition (fig 1, Table 2), we found competition did cause a significant reduction to both *L. salicaria* biomass ($F_{1, 182} = 60.4$ $P = 0.000$) and seed production ($F_{1, 182} = 2.9$ $P = 0.000$) but not plant height (fig 1a, Table 2). There was an overall significant effect for origin (native, introduced, German hybrid, US hybrid) of *L. salicaria* populations for both plant height ($F_{3, 182} = 3.5$ $P = 0.016$) and above ground biomass ($F_{3, 182} = 2.9$ $P = 0.038$). Although, when grown alone, there was no difference among origins in plant height, biomass and seed number (fig 1), many differences in performance measures were found when grown in competition. For example, in general, mean values for introduced populations were higher than those of native populations in all three measures of performance, but only significant for plant height (Tukey Test $P < 0.05$ Fig 1a). Also in the mixture pots, the hybrid populations produced an intermediate response (i.e. in between the values of the native and introduced populations) in terms of plant height (fig 1a), whereas for both biomass and seed production measures the hybrids were similar to their maternal line (i.e. German hybrids similar to natives; US hybrids similar to introduced) but were significantly different to each other ($p < 0.05$ Tukey Test, fig 1b & c). There was also a significant interaction between origin and treatment (with and without neighbours) for seed production ($F_{3, 182} = 2.9$ $P = 0.037$), where native populations suffered a 55.98%, introduced populations 56.21%, German hybrids 76.92% and US hybrids 33.42% seed production loss (Table fig 1c).

Using the Relative Interaction Index (RII) to determine competitive ability of the population types, we found overall differences occurred in terms of competitive response of plant height ($F_{3, 167} = 7.65$ $P < 0.001$), seed output ($F_{3, 167} = 3.39$ $P = 0.019$) with marginal significant differences for biomass ($F_{3, 167} = 2.25$ $P = 0.085$). For plant height, RII values were significantly higher which shows greater competitive ability for introduced populations than for natives or German hybrids, while US hybrids showed an intermediate response (Table 2 fig 2a). For biomass the RII values

showed that US hybrids exhibited significantly less response to competition (greater competitive ability) than German hybrids, while native and introduced populations showed intermediate pattern (Table 2 fig 2b). For seed production German hybrids showed more response to competition (less competitive ability) than all other population types (Table 2 fig 2c). However, when calculating competitive effect using the relative interaction index, we did not find any significant differences among origin across any of the trait measures (Table 2). Percentage leaf damage did not significantly differ among origin ($F_{3, 182} = 0.174$ $P = 0.914$). Although the origin effect was not significant, individuals from the introduced populations of *L. salicaria* had greater leaf damage caused by herbivores than the native populations (fig 3). However, plants growing under competition had significantly higher amounts of leaf damage than plants growing alone ($F_{1, 182} = 4.42$ $P = 0.037$).

DISCUSSIONS

The EICA hypothesis presumes that invasive species do not invest to defend themselves against their specialist natural enemies and can therefore re-allocate resources from defence to growth (Blossey & Nötzold, 1995). Therefore, the aim of this experiment was to find the potential risk of reintroducing such invasive plants back into their native range, using the EICA hypothesis as a framework. When grown alone, all the population types of *L. salicaria* studied performed relatively well and did not show any significant difference among origins in plant height, biomass and seed number, contradicting hypothesis (1) (fig 1). However, when grown in all combinations of intraspecific pair-wise competition, some patterns emerged in the performance of all fitness traits measured which was consistent with the EICA hypothesis. Plant height was found to be significantly higher in introduced populations than in native populations, and non-significant patterns in mean values indicated the possible presence of differences in biomass, seed output and percentage leaf loss (introduced > native). Although RII showed increased competitive ability expressed in plant height, the other traits were in contradiction to the precise interpretation of hypothesis (2). For biomass and seed output, the only difference

found in the actual competitive ability of the different population types was that German hybrids performed worse than all other origins in terms of competitive response. It was for the hybrids where the strongest and most interesting relationships were observed (hypothesis 3). In addition to finding a lower competitive ability of German hybrids, we also showed in the pair-wise mixtures that plant performance, for biomass and seed output, was similar to that of their maternal line, and significantly higher in the US hybrid (US mother x German father) populations compared to the German hybrid (German mother x US father) populations.

Our results identify different scenarios to interpret for reinvasion. On one hand, reinvasion of non-native populations could likely pose threat to the native populations and communities. Previous studies clarify that the introduced populations of *L. salicaria* are better competitors (Gaudet & Keddy, 1988; 1995; Keddy *et al.*, 1994, Hager, 2004), our own previous study also show *L. salicaria* as a superior competitor than their native relatives when competing with a common interspecific neighbour (chapter 2) and that this has evolved from a reduction to specialized herbivore defense over many generations (Blossey & Nötzold, 1995; Joshi & Vrieling, 2005; Meyer *et al.*, 2005). Therefore, determining whether the reinvading individuals are likely to survive back in the native communities is dependent on the overall fitness of individuals, when both competition and herbivore re-exposure to their native enemies were taken into consideration (Bossdorf *et al.*, 2005). Within this study, we certainly produce support that the fitness under competition appears to be higher for the introduced populations, even with some exposure to native herbivores. However, interbreeding is most likely to occur between native and reintroduced individuals and the success and transmission of genes to the offspring will ultimately determine the reinvasion success. Our results show, that in the early generations at least, that the aggressive alleles follow the maternal line. Therefore, a risk is posed to the conservation of the native community and to the genes of the native species not only by the reinvading individuals themselves but from the seed set of the reinvading individuals as well.

On the other hand, evidence from this experiment does not support an increased competitive ability posed by the introduced populations over native individuals. In fact, we showed that the native maternal offspring (German hybrids) have less competitive ability than all the other population. If the competitive ability measured in this experiment is the only means for determining reinvasion success, our conclusion must be that there is no threat of reinvasion as no greater advantage is gained or posed by the reinvading populations. In fact, a reduction in competitive ability would suggest through natural selection that these traits would be lost again on reinvasion.

The discrepancy in whether the experiment predicts either reinvasion success or failure is closely linked to the finding that there is no difference in competitive ability between introduced or native populations. One reason for this could be related to the design of the competition experiments themselves. In previous studies we have shown that introduced and native populations do show differences in their competitive ability (chapter 2). However, in those experiments, *L. salicaria* was competing with an interspecific neighbour (*Urtica dioica*). It could be that direct test using intraspecific competition is not the best way to determine competitive ability between two or more populations of this perennial species over a short time. The competitive ability of perennial species may increase over multiple growing seasons (Pfeifer-Meister *et al.*, 2008). Therefore, a stronger competitive environment is required to identify the differences e.g. using a weaker competitor as a neighbour, using three neighbours to one target individual, or continuing for a sufficient length of time to highlight strong differences.

Only a few studies have tested the EICA hypothesis by intraspecific competition between individuals from native and invasive regions (e.g. Bossdorf *et al.*, 2004; Zou *et al.*, 2008). In one of those, Bossdorf *et al.*, (2004) found the counterintuitive EICA result that individuals of *Alliaria petiolata* from its invasive range were less competitive than native individuals. However, *L. scaliaria* is generally a very

competitive species (chapter 2, Gaudet & Keddy 1988, 1995; Keddy *et al.*, 1994; Hager, 2004), it could be that intraspecific competition may show different results to those from interspecific experiments. For example, few studies in *L. salicaria* have shown that intraspecific competition is stronger rather than interspecific competition (Gaudet & Keddy, 1988; Weihe & Neely, 1997). For the persistence of the aggressive invasive alleles and a risk created to the native populations and communities, our results suggest that the German hybrids would not persist in dominant monospecific stands, however with very little difference in the competitive abilities of the other population types, performance of fitness traits may be more important for future generations, as well as competition with other species. A recent study by Zou *et al.*, (2008) using *Sapium sebiferum* from native and invasive populations was competed against each other and the invasive populations grew to a larger size than natives despite having greater herbivore damage. This study did not measure competitive ability using RII, but assessed differences in performance finding very similar results to our stronger patterns which match the EICA hypothesis. By finding general differences in the fitness traits between native and introduced populations, and for hybrids of alternate maternal origin, we can therefore make some valid conclusions about the performance of these population types, even if they don't express a different competitive ability in the experimental framework used here.

The most interesting and the most crucial, results we found for suggesting potential reinvasion success were that the alleles appear to follow the maternal line, (German hybrids were more similar to native individuals; US hybrids similar to introduced individual), with significant differences between the two hybrid types. Interspecific, as opposed to intraspecific, hybridization has more commonly been investigated in the study of invasive species; however the results have been variable. Some plant hybrids were found be inferior to their parents, with reduced fertility or viability (see review Arnold & Hodges, 1995), while others have intermediate pattern to their parents (Blair & Hufbauer, 2010). The other studies showed hybrids to be superior (heterosis), with increased vigor than their parents (Vila & D'Antonio, 1998; Travis

et al., 2010). However, in an experiment with *Senecio jacobaea* and *Senecio aquaticus*, offspring from *S. jacobaea* mothers exhibited higher fitness than those from the *S. aquaticus* mothers (Kirk *et al.*, 2005) and they concluded that maternal effects may indeed play a substantial role in the fitness of interspecific hybrids. Moreover, Burgess & Husband, (2004) mentioned that in a common garden setting, fitness of hybrids was determined predominantly by their maternal background. Some evidence suggests that the variation observed in hybrid performance within our study may be due to inherited maternal cytoplasmic genome (Burgess & Husband, 2004; Levin, 2003).

A few investigations have compared intraspecific hybrids. For example, a recent study by Meyerson *et al.*, (2010) attempted to make crossings between native and introduced lineages of *Phragmites australis* in both directions, and found that only crosses with introduced *P. australis* pollen donors and native recipients exhibited seed set, suggesting gene flow was unidirectional. Wolfe *et al.*, (2007) made crossings between plants from different populations within the same continent from native and an introduced lineage and concluded that hybridization does not play a significant role in the success of *Silene latifolia*.

As there was no distinct patterns observed within other species and no clear reasoning for our findings, we therefore suggest that the hybrid performance results within our experiment are taken cautiously. Further study should be carried out to test if the transmissions of alleles do truly pass down the maternal line. Hybrid vigor in the F1 generation is due heterozygosity and this may decline in subsequent generation hybrid (see Hufford & Mazer, 2003). It would therefore be useful to test these further generations to investigate if the fitness trait transmission continues in the same maternal pattern; and perhaps a more specific experimental design to identify the differences in hybrids F1, F2 and their parents is required, possibly looking at phenotypic responses under community environments through exposure to native competition and native herbivores. However, at least for these early generations it seems that both introduced individuals and offspring produced from

their seed (F1 US hybrids) do express higher fitness than the native populations. Assuming hybridization could occur through natural pollination from native populations of *L. salicaria*; it will lead to declines of the native populations through genetic swamping and, potentially, through increased competition as hybrids may exhibit increased vigor. More importantly, backcrossing may provide a route for introgression of genetic material, if these hybrids back cross with their parental individuals and exhibit more aggressive behavior this could create a serious problem for native populations and communities. However, the observation that allele transfer is restricted to the maternal line, may suggest that any reinvasion would be limited in spatial expansion by the initial reinvasion and the resulting seed set, as opposed to the pollen dispersal. Most likely this would limit the spread of the invasion to a relatively more local area, increasing the chance of forming monospecific stands but decreasing the chance of rapid widespread invasion. If identified early, this type of gene invasion could be prevented by local eradication.

In conclusion, although further confirmation is required, these preliminary results suggest that the alleles of the origin populations are withheld to a greater extent, or even exaggerated, on the maternal line. During initial reinvasion this may result in formation of monospecific stands in a local area rather than widespread invasion, therefore, important consideration should be taken early for effective management. Further research should also be conducted with backcrosses of F1 generation to both parents and compare resultant vigor of successful crosses to F1 and original parent populations. However, in absence of molecular data the role of hybridization in plant invasion can be difficult to confirm. Therefore, future studies should also consider genetic studies and analysis of multigeneration hybrids to test if the fitness trait transmission continues in the same maternal pattern.

ACKNOWLEDGEMENTS:

This work was supported by German Research Foundation (DFG; TI-338/8-1) to Katja Tielbörger. We thank Merav Seifan for statistical help and Mark Bilton for helpful comments on early drafts of this manuscript.

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Table 1: Sources of native and invasive populations of *L. salicaria* used in the cross experiment. Population in bold letters were used for the competition experiment.

Origin	Country	Population (region)	Latitude	Longitude
Native	Germany	Unterjesingen (Tübingen)	48° 52' N	8° 98' E
	Germany	Hagelloch (Tübingen)	48° 32' N	9° 01' E
	Germany	Grube (Potsdam)	52° 43' N	12°97' E
	Germany	Golm (Potsdam)	52° 40' N	12°97' E
Introduced	USA	Beaver Run (New Jersey)	40° 51' N	79°55' W
	USA	Hainesville county (New Jersey)	41° 25' N	74°80' W
	USA	Boone Forks (Iowa)	42° 17' N	93°56' W
	USA	Manly (Iowa)	43°16' N	93°07' W

Table 2: Results of two-way ANOVAs comparing the raw data performance of native, introduced, German hybrids and US hybrids of *L. salicaria* in presence and absence of neighbours.

Source of variation	df	MS	<u>Height</u>		<u>Above ground biomass</u>			<u>Seed output</u>			<u>Percent leaves damage</u>		
			F	P	MS	F	P	MS	F	P	MS	F	P
Initial plant height	1	1376.5	6.7	0.01	34662	30.5	0.000	1.5 x 10 ⁴	28.1	0.000	12	0.25	0.615
Origin	3	722.1	3.5	0.016	3264	2.9	0.038	8.9 x 10 ¹⁰	1.7	0.178	8.2	0.17	0.914
Treatment	1	134.9	0.7	0.418	68722	60.4	0.000	2.4 x 10 ¹²	45.6	0.000	209.4	4.43	0.037
Origin * Treatment	3	209.1	1	0.385	1359.5	1.2	0.313	1.5 x 10 ¹¹	2.9	0.037	1.2	0.02	0.995
Error	182	204.9			1137.4			5.3 x 10 ¹⁰			47.3		

Table 3: Results of one way ANOVAs comparing the competitive effect (CE) and competitive response (CR) among native, introduced, German hybrids and US hybrids measured in terms of relative interaction index (RII).

Source of variation	df	MS	<u>RII Height</u>		<u>RII Above ground biomass</u>			<u>RII Seed output</u>		
			F		MS	F		MS	F	
Origin (competitive response)	3	0.07	7.65	***	0.22	2.25	(*)	0.44	3.39	*
Total	167									
Origin(competitive effect)	3	0.01	1.36	ns	2235	0.05	ns	0.086	0.629	ns
Total	167									

(*) p = 0.08; p<0.05; p<0.01;***p<0.001; ns= non sig.

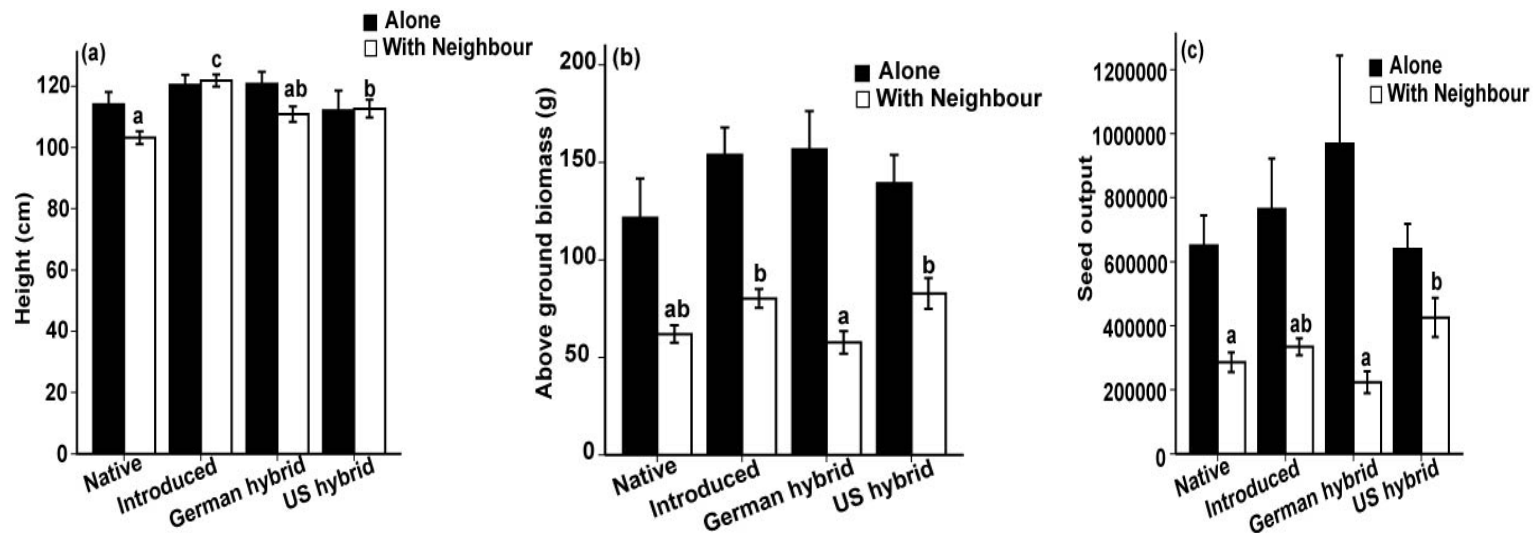


Fig 1: Effect of neighbour treatment of *L. salicaria* on (a) Plant height, (b) Above ground biomass, (c) Seed output. No significant difference was found among origins when grown alone. Data represent means \pm 1 SE. Different letters indicate significant difference in means in competition (Tukey test).

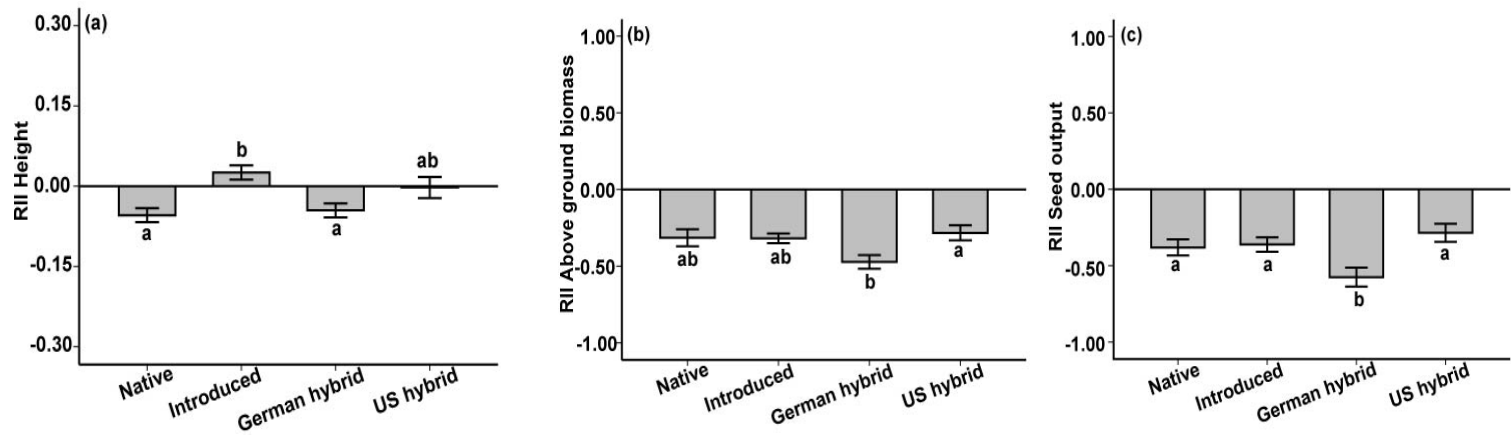


Fig 2: The response of competition of *L. salicaria* among native, introduced, German hybrid and US hybrid plants measured in terms of relative interaction index (RII). A positive RII value indicates that neighbour facilitates growth and negative RII values indicate that neighbours inhibit growth. Data represent means ± 1 SE. Different letters indicate significant difference in means (Tukey test).

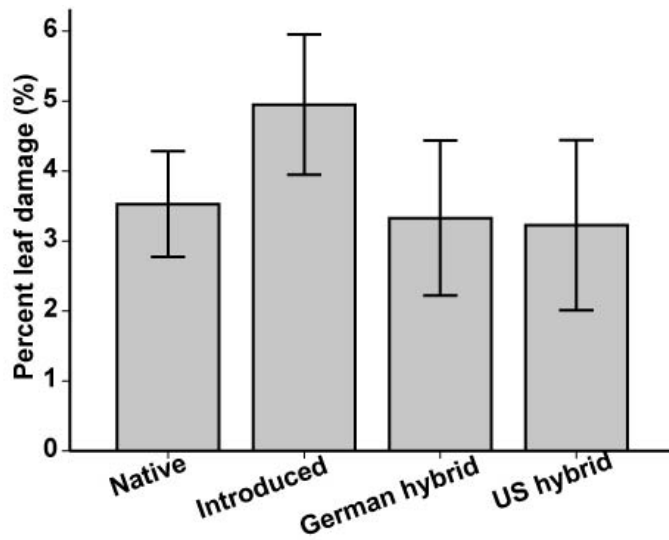


Fig 3: Percent leaf damage among native, introduced, German hybrid and US hybrid grown in common garden experiment. Data represent means \pm 1 SE.

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