Hybridization in the invasive *Fallopia* complex

and its influence on sexual reproduction and herbivore resistance

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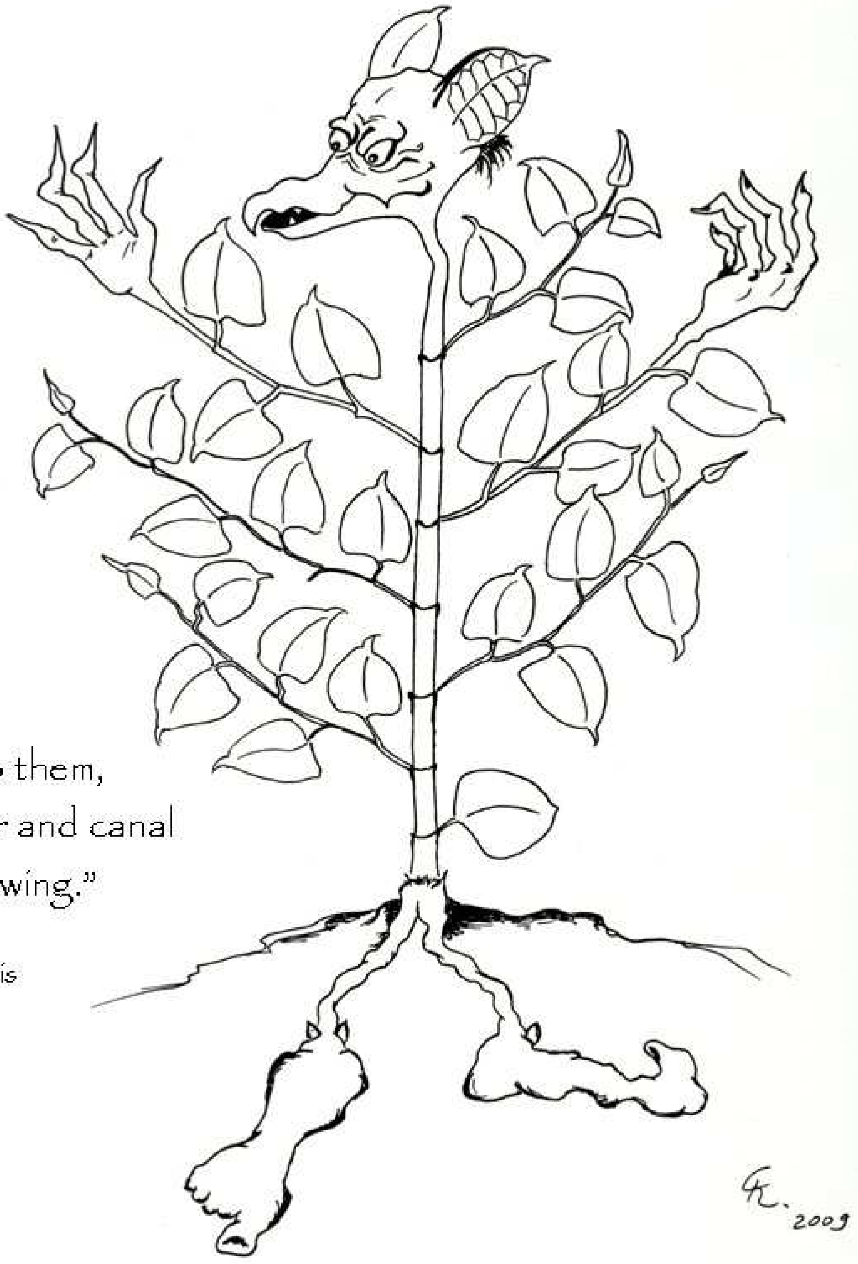

> “ Turn and run.
> Nothing can stop them,
> around every river and canal
> their power is growing.”
>
> Peter Gabriel, Genesis

CONTENTS

ABSTRACT

Biological invasions are considered to be an important cause of current biodiversity loss and can cause significant ecological problems. Several hypotheses have been proposed to explain why exotic plants become invasive.

An important determinant of the success of invasive plants in their adventive range is the efficiency of reproduction. High fecundity and the potential for sexual reproduction, which generates genetic variability but also vigorous clonal growth, are attributes frequently observed among the most successful invasive plants. However, external factors also can influence plant invasions. One commonly accepted mechanism for the invasion success of plants is that plants introduced into a new range experience less herbivory due to the release from natural enemies resulting in higher plant fitness. Furthermore, biological invasions may be promoted by climate change. Altered environmental conditions due to climate change could be more favourable for many invasive species and could increase the reproduction, establishment and spread of exotic plants in the introduced range.

Recently, it has been claimed that the ability of exotic plants to establish and rapidly expand in a novel range may also be shaped by evolutionary processes. Interspecific hybridization has been identified as a stimulus for the evolution of increased invasiveness in exotic plant species. Hybridization can increase genetic variation and provide the genetic material on which natural selection can act. Novel gene combinations may result in phenotypes that are stronger competitors, have higher reproduction and survival rates and are more resistant against herbivory and may therefore be favoured by natural selection. Moreover, hybridization may facilitate adaptation to different or altered environmental conditions, for example, due to climate change. Successful hybrid genotypes might be fixed and retained by vegetative propagation and increase the invasion success of exotic plants.

Taxa of the genus *Fallopia* (Polygonaceae), native to eastern Asia, are among the most troublesome invasive species worldwide and are particularly aggressive in Europe and North America. The plants cause significant damage to native ecosystems and are especially a problem along rivers. In Europe, exotic *Fallopia* plants spread mainly by vegetative propagation. Of the widespread *F. japonica* var. *japonica* in Europe only a single genotype with male-sterile flowers has been found, and sexual reproduction in the exotic *Fallopia* complex is thus restricted mainly to hybridization among the taxa. The naturalized but less frequent species *F. sachalinensis* can act as pollen donor, resulting in the hybrid *F.* x *bohemica*. The hybrid is frequent and considered to have a higher invasion potential and a faster spread than its parental species. A third introduced species is the climbing *F.*

baldschuanica. Hybridization between this species and *F. japonica* var. *japonica* results in the hybrid *F.* x *conollyana,* which so far has only rarely been reported from Europe.

In my dissertation I present four studies on hybridization in the invasive *Fallopia* complex in Western Europe and its consequences. Specifically, I studied the genetic diversity and hybridization patterns (chapter 2), extent and variation in sexual reproduction (chapter 3), regional variation in seedling establishment and the potential effects of climate change (chapter 4) and the influence of hybridization on herbivore resistance (chapter 5).

The distribution of *F. japonica* var. *japonica*, *F. sachalinenis* and *F.* x *bohemica*, their genetic diversity and regional genetic structure was assessed in seven regions in Germany and Switzerland using RAPD analysis and flow cytometry. *Fallopia japonica* var. *japonica* was frequent in all regions, while distribution of the hybrid *F.* x *bohemica* decreased from south-east to north-west. *Fallopia sachalinensis* was present in all regions at a low abundance, except in Ticino where no populations were found, suggesting the absence of this species. All individuals identified as *F. japonica* var. *japonica* exhibited the same RAPD phenotype, confirming results from other studies that a single male-sterile plant has been introduced and has spread only vegetatively throughout Europe. In contrast, *F. sachalinensis* and *F.* x *bohemica* showed high genotypic diversity. The genetic diversity of *F. sachalinensis* may result from multiple introductions or from occasional sexual reproduction. Bayesian cluster analysis revealed three distinct genetic clusters. The majority of *F.* x *bohemica* individuals were assigned to a unique genetic cluster different from those of the parental species. Some *F.* x *bohemica* individuals, however, had different degrees of admixture to the three genetic clusters. These results might indicate different origins of the hybrids: by hybridization between the parental species, sexual reproduction among hybrids or multiple introductions of hybrids. I also found regional differentiation in the gene pool of *F.* x *bohemica,* i.e. individuals within the same region were more similar to each other than individuals from different regions. This result suggests that the high genetic variation in *F.* x *bohemica* results from hybridization in the introduced range. Such regionalisation of the gene pool of the hybrid may provide the raw material for adaptive evolution in different regions with different environmental conditions and may increase the invasiveness of the hybrid *F.* x *bohemica*.

We assessed seed production, seed set, seed germination, flower morphology and pollen viability of the invasive clonal species *F. japonica* var. *japonica, F. sachalinensis* and of their hybrid *F.* x *bohemica* in five European regions and determined the ploidy level of their offspring to potentially infer the pollen donor under natural conditions. In addition, crossing experiments were carried out to assess pollen suitability for intra- and interspecific sexual reproduction. Our study revealed a high level of variation in seed production, flower morphology and pollen viability of the invasive *Fallopia* taxa among and within regions in Europe. Sex of flowers partially explained variation in seed production of *F. sachalinensis* and *F. x bohemica*, but not in the single male-sterile clone of *F. japonica* var. *japonica* detected in Europe. A gradual variation in seed production and pollen viability was found among *F. sachalinensis and F. x bohemica* stands, with some stands with male flower morphology producing seeds and having low pollen viability. At the regional level, high seed production of *F. japonica* var. *japonica* was correlated with high ratios length of anthers/length of ovary of *F. x bohemica*, indicating predominantly male flowers in hybrid stands, which can serve as pollen donor. Seed set in the experimental crosses depended on the pollen donor and was comparable to the highest seed set observed in the field. These results indicate that, beside flower morphology, the local availability of compatible pollen is a key factor contributing to the variation in the seed production of invasive *Fallopia* taxa in Europe. The predicted increase in hybrid abundance is therefore likely to contribute to an increased seed production of invasive *Fallopia* taxa in Europe.

Although the high genetic diversity of the hybrid *F.* x *bohemcia* and the production of large amounts of viable seed in the introduced range with high germination rates under containment indicate spread by sexual reproduction, low seedling survival and establishment of *Fallopia* has been observed in the field. It has been stated that seedlings of *Fallopia* are sensitive to frost and a cold humid winter and do therefore not survive European winters. To test this assumption, I assessed the likelihood of successful seedling establishment under natural conditions across six different sites from Northern Germany to Southern Switzerland. By including sites with different climatic conditions, I further investigated if the risk of seedling establishment might increase under the predicted climate change. We found that a large amount of seeds of all three *Fallopia* taxa remained viable throughout the winter, and between 25 and 75% germinated in the following spring at the different field sites. Most of the seedlings established successfully and survived the first winter, but performance during the first season varied significantly among regions and among mother taxa, with offspring of *F. japonica* var. *japonica* showing the highest performance. The lowest seedling emergence and performance were recorded at the coldest site and highest values at the most southern site under climatic conditions that are similar to those predicted for large parts of northern Europe. Although vegetative spread might remain the main mode of reproduction in the invasive *Fallopia* complex in Europe, our results suggest that under the predicted climate change, sexual reproduction and successful establishment of *Fallopia* seedlings might increase in

Europe in the future. Increased levels of sexual reproduction could further promote the spread, increase the genetic variability and stimulate the invasiveness of the *Fallopia* complex.

Hybridization can alter traits important for the invasion success of exotic plants. One of the most important determinants of invasiveness is herbivore resistance. Interactions between *Fallopia* taxa and their antagonists and the influence of hybridization on herbivore resistance were examined. I experimentally assessed resistance of parental taxa (*F. japonica* var. *japonica*, *F. sachalinensis*, *F. baldschuanica*), and hybrids (*F. x bohemica*, *F. x conollyana*) of the invasive *Fallopia* complex to four native European herbivores (the slug *Arion lusitanicus,* the moth *Noctua pronuba*, the grasshopper *Metrioptera roeselii* and the chrysomelid beetle *Gastrophysa viridula).* The native European plants *Rumex obtusifolius* and *Taraxacum officinale* were tested for comparison. To elucidate the potential for the evolution of increased herbivore resistance, we included different genotypes of the particularly invasive hybrid *F. x bohemica*. The leaf area consumed by herbivores and their relative growth rate on the hybrids in comparison to that on the parental species differed depending on the herbivore tested. In most of the cases, the hybrids resembled the more susceptible parent, followed by hybrids with intermediate levels of resistance. Genotypes of the hybrid *F. x bohemica* varied significantly in herbivore resistance, but never exceeded that of the parental species. In general, exotic *Fallopia* taxa were better defended against European herbivores than native plant species and herbivore growth rates on them were lower which might contribute to their invasion success in Europe. Differences in leaf traits known to influence palatability, i.e. specific leaf area, leaf dry matter content and C/N ratio only partly explained the differences in resistance against European herbivores between the exotic *Fallopia* and the native plants, suggesting that other factors like novel secondary compounds may also contribute to the high resistance of *Fallopia* plants to European generalist herbivores.

ZUSAMMENFASSUNG

Invasionen nicht-einheimischer Pflanzenarten stellen einen der wichtigsten Gründe für die Abnahme der Biodiversität dar und können bedeutende ökologische Probleme verursachen. Mehrere Faktoren werden dafür verantwortlich gemacht, dass eingeführte Arten invasiv werden. Für eine erfolgreiche Invasion spielt eine starke Vermehrung eine große Rolle. Hohe Fekundität und die Möglichkeit zur sexuellen Reproduktion, die eine hohe genetische Variabilität gewährt, aber auch klonales Wachstum, sind Eigenschaften, die häufig unter den erfolgreichsten invasiven Pflanzen beobachtet wurden. Externe Faktoren können ebenfalls den Invasionenserfolg beeinflussen. Eine erhöhte Konkurrenzfähigkeit exotischer Pflanzen durch das Fehlen von einheimischen Feinden ist einer der häufigst zitierten Gründe für den Erfolg invasiver Pflanzen. Darüber hinaus können globale Umweltveränderungen, wie Klimaveränderungen, ebenfalls zum Erfolg invasiver Arten beitragen. Veränderte Umweltbedingungen, die durch den Klimawandel hervorgerufen werden, können für viele invasive Arten geeignetere Bedingungen darstellen und somit auch Reproduktion, Etablierung und Verbreitung von exotischen Pflanzen im eingeführten Gebiet steigern.

Aktuelle Studien lassen darauf schließen, dass die Fähigkeit von exotischen Arten sich zu etablieren und sich in einem neuen Gebiet schnell auszubreiten, auch durch evolutionäre Prozesse geformt wird. Hybridisierung kann eine treibende Kraft in evolutionären Prozessen während Invasionen von Pflanzen sein. Die genetische Variabilität kann erhöht werden, wodurch das genetische Material zur Verfügung gestellt wird, auf das natürliche Selektion einwirken kann. Neue Genkombinationen können in Phänotypen resultieren, die stärkere Konkurrenten sind, höhere Reproduktion und Überlebensraten aufweisen und die resistenter gegenüber Herbivorenbefall sein können. Darüber hinaus kann Hybridisierung eine Anpassung an unterschiedliche oder veränderte Umweltbedingungen, beispielsweise durch Klimawandel, ermöglichen. Erfolgreiche Hybrid-Genotypen können durch vegetative Vermehrung erhalten werden und den Invasionserfolg exotischer Pflanzen steigern.

Taxa der Gattung *Fallopia* (Polygonaceae), ursprünglich aus Ostasien, gehören in Europa zu den besonders aggressiven invasiven Pflanzen, die den Charakter von Ökosystemen verändern. Heute zählen diese Arten vor allem entlang von Fließgewässern zu den problematischsten invasiven Pflanzenarten in Mitteleuropa. In Europa verbreiten sich exotische *Fallopia*-Pflanzen hauptsächlich vegetativ. Die weitverbreitete Art *F. japonica* var. *japonica* kommt in Europe mit nur einem Genotypen mit männlich-sterilen Blüten vor. Aus diesem Grund ist sexuelle Reproduktion im exotischen *Fallopia*-Komplex hauptsächlich auf Hybridisierung zwischen den Taxa beschränkt. Die etablierte aber etwas seltenere Art *F.*

sachalinensis kann als Pollendonor fungieren, wodurch der Hybrid *F.* x *bohemica* entsteht. Der Hybrid ist häufig und es wird behauptet, dass er ein höheres Regenerationspotential und eine schnellere Ausbreitung als seine Elternarten besitzt. Eine dritte Art, die Kletterpflanze *F. baldschuanica,* wurde ebenfalls eingeführt. Hybridisierung zwischen dieser Art und *F. japonica* var. *japonica* resultiert in den Hybrid *F.* x *conollyana*, der bisher nur in Großbritannien mit einem Individuum beobachtet wurde.

In meiner Dissertation präsentiere ich vier Studien zur Hybridisierung im invasiven *Fallopia*-Komplex in West-Europa und ihre Konsequenzen. Ich habe die genetische Diversität und Hybridisierungsmuster (Kapitel 2), Ausmaß und Variation in der sexuellen Reproduktion (Kapitel 3), regionale Variation in der Keimlingsetablierung und den möglichen Effekt von Klimawandel (Kapitel 4) und den Einfluss von Hybridisierung auf Herbivorenresistenz (Kapitel 5) untersucht.

Die Verteilung von *F. japonica* var. *japonica, F. sachalinenis* und *F.* x *bohemica,* ihre genetische Diversität und regionale genetische Struktur wurde in sieben Regionen in Deutschland und der Schweiz mithilfe von RAPD-Analysen und „flow cytometry“ untersucht. *Fallopia japonica* var. *japonica* war in allen Regionen häufig, während der Hybrid *F.* x *bohemica* von Süd-Ost nach Nord-West in seiner Häufigkeit abnahm. *Fallopia sachalinensis* war in allen Regionen in einer niedrigen Dichte präsent, ausser im Tessin, wo keine Bestände gefunden wurden. Alle Individuen, die als *F. japonica* var. *japonica* identifiziert wurden, besaßen denselben RAPD-Phänotyp. Dies bestätigt die Ergebnisse anderer Studien und deutet daraufhin, dass eine einzige männlich-sterile Pflanze eingeführt wurde, die sich nur vegetativ über Europa verbreitet hat. Im Gegensatz zeigt *F. sachalinensis* und *F.* x *bohemica* eine hohe genetische Diversität. Die genetische Variabilität von *F. sachalinensis* mag von mehrmaligen Einführungen oder gelegentlicher sexueller Reproduktion herführen. Bayesische Cluster Analyse deckte drei verschiedene genetische Gruppen auf. Die Mehrheit der *F.* x *bohemica* Individuen wurde einer eigenen genetischen Gruppe, unterschiedlich zu der Gruppe der Elternarten, zugeordnet, während die anderen *F.* x *bohemica* Individuen einen unterschiedlichen Grad an Beimischung zu den drei genetischen Gruppen zeigten. Diese Ergebnisse deuten auf verschiedene Ursprünge der Hybriden hin: durch Hybridisierung zwischen den Elternarten, sexuelle Reproduktion zwischen Hybriden oder mehrmalige Einführung von Hybriden in die verschiedenen Regionen. Ich fand ausserdem regionale Differenzierung im Genpool von *F.* x *bohemica.* Individuen innerhalb derselben Region waren sich ähnlicher als Individuen von verschiedenen Regionen. Hieraus kann man schliessen, dass die hohe genetische Variabilität von *F.* x *bohemica* aus Hybridisierung im eingeführten Gebiet resultiert. Regionalisierung des Genpools des Hybriden kann das genetische Rohmaterial für adaptive Evolution in den verschiedenen Regionen mit ihren unterschiedlichen Umweltbedingungen zur Verfügung stellen und dadurch die Invasivität des Hybriden *F.* x *bohemica* erhöhen.

Ich habe Samenproduktion, Samenansatz, Keimung, Blütenmorphologie und Pollenfruchtbarkeit der invasiven, klonalen Arten *F. japonica* var. *japonica, F. sachalinensis* und ihrem Hybriden *F.* x *bohemica* in fünf europäischen Regionen untersucht. Die Bestimmung des Ploidylevels der Keimlinge wurde benutzt, um auf den möglichen Pollendonor in natürlichen Kreuzungen schliessen zu können. Kreuzungsexperimente wurden durchgeführt, um die Eignung von Pollen für intra- und interspezifische sexuelle Reproduktion abschätzen zu können. Die Studie brachte eine hohe Variabilität in Samenproduktion, Blütenmorphologie und Pollenfruchtbarkeit der invasiven *Fallopia*-Taxa zwischen und innerhalb Regionen in Europa zum Vorschein. Geschlecht der Blüten erklärte teilweise die Variation in der Samenproduktion von *F. sachalinensis* und *F. x bohemica,* allerdings nicht in der Art *F. japonica* var. *japonica,* welche mit einem einzigen männlich-sterilen Klon in Europa vorkommt. Zwischen *F. sachalinensis* und *F. x bohemica* Beständen wurde eine Variation in der Samenproduktion und Pollenfruchtbarkeit gefunden, darunter einige Bestände mit männlicher Blütenmorphologie, die allerdings Samen produzierten und eine niedrige Pollenfruchtbarkeit aufwiesen. Eine hohe Samenproduktion von *F. japonica* var. *japonica* war auf regionaler Ebene mit einem hohen Verhältnis Länge der Antheren/Länge des Ovariums von *F.* x *bohemica* korreliert. Dies deutet auf männliche Hybride hin, die als Pollendonor für *F. japonica* var. *japonica* dienen. Der Samenansatz in den Kreuzungsexperimenten war vom Pollendonor abhängig und war vergleichbar mit dem höchsten Samenansatz, der im Feld beobachtet wurde. Diese Ergebnisse lassen darauf schliessen, dass neben Blütenmorphologie, die Möglichkeit Pollen von einem kompatiblen Partner zu bekommen ein wichtiger Faktor ist, der zur Variation in der Samenproduktion der invasiven *Fallopia*-Taxa in Europa beiträgt. Der vorhergesagte Anstieg in der Häufigkeit der Hybriden kann zu einer erhöhten Samenproduktion der invasiven *Fallopia*-Taxa in Europa beitragen.

Obwohl die hohe genetische Variabilität des Hybriden *F.* x *bohemcia* und die Produktion großer Mengen an keimfähigen Samen mit hohen Keimraten unter Laborbedingungen darauf hindeuten, dass Verbreitung durch sexuelle Vermehrung erfolgt, wird niedriges Keimlingsüberleben und niedrige Etablierung von *Fallopia* im Freiland beobachtet. Es wird vermutet, dass Keimlinge von *Fallopia* frostempfindlich sind und einen kalten, feuchten Winter in Europa nicht überleben. Ich untersuchte die Wahrscheinlichkeit erfolgreicher Keimlingsetablierung exotischer *Fallopia*-Taxa in Europa und ob das Risiko einer erfolgreichen Etablierung von Keimlingen und damit die Vermehrung durch sexuelle Reproduktion mit den zu erwartenden Klimaveränderungen ansteigen kann. Samen von *F. japonica* var. *japonica, F. sachalinensis* und ihrem Hybrid *F.* x *bohemica* wurden im Herbst an sechs verschieden Orten von Norddeutschland bis in die Südschweiz in Töpfen mit derselben Erde ausgebracht. Samen aller Taxa blieben über den Winter keimfähig und zwischen 25 and 75% der Samen keimten im folgenden Frühling an den verschiedenen Orten. Die meisten Keimlinge etablierten sich erfolgreich und überlebten den Winter. Das Wachstum variierte allerdings während der ersten Wachstumsperiode zwischen den Wuchsorten und der Taxa der Mutterpflanze. Keimlinge, die von *F. japonica* var. *japonica* stammen, zeigten das höchste Wachstum. Das niedrigste Keimlingsaufkommen und Wachstum wurde am kältesten Ort und hohe Werte wurden am südlichsten Standort aufgenommen, der in seinen klimatischen Bedingungen denen entspricht, die für große Teile in Nordeuropa vorhergesagt wurden. Die Ergebnisse deuten daraufhin, dass unter dem vorhergesagten Klimawandel, sexuelle Reproduktion und erfolgreiche Keimlingsetablierung von *Fallopia* in Europa in Zukunft ansteigen kann. Verstärkte sexuelle Vermehrung könnte zu einer erhöhten Verbreitung führen, die genetische Variabilität erhöhen und den Invasionserfolg im *Fallopia*-Komplex steigern.

Hybridisierung kann Eigenschaften ändern, die für den Invasionserfolg exotischer Pflanzen wichtig sind. Einer der wichtigsten bestimmenden Faktoren für Invasivität ist die Resistenz gegenüber Herbivoren. Interaktionen zwischen exotischen *Fallopia*-Taxa und ihren Antagonisten und der Einfluss von Hybridisierung auf Herbivorenresistenz wurden untersucht. Ich habe experimentell Resistenz von Elternarten (*F. japonica* var. *japonica*, *F. sachalinensis*, *F. baldschuanica*) und Hybriden (*F. x bohemica*, *F. x conollyana*) des *Fallopia*-Komplex gegenüber vier einheimischen europäischen Herbivoren, der Nacktschnecke *Arion lusitanicus,* dem Kleinschmetterling *Noctua pronuba*, der Laubheuschrecke *Metrioptera roeselii* und dem Blattkäfer *Gastrophysa viridula,* bestimmt. Zum Vergleich wurden ebenfalls die einheimischen europäischen Pflanzen *Rumex obtusifolius* und *Taraxacum officinale* getestet. Um das Potential der Evolution einer gesteigerten Herbivorenresistenz zu testen, wurden verschiedene Genotypen des besonders invasiven Hybriden *F. x bohemica* aufgenommen. Blattfrass durch die Herbivoren auf den Hybriden im Vergleich zu dem auf den Elternarten unterschied sich, abhängig vom getesteten Herbivoren. Ebenso unterschied sich die relative Wachstumsrate der Herbivoren zwischen

Hybriden und Elternarten. In den meisten Fällen glich der Hybrid dem anfälligeren Elternteil, gefolgt von Hybriden mit intermediärem Grad an Resistenz. Genotypen des Hybriden *F. x bohemica* variierten signifikant in ihrer Herbivorenresistenz, allerdings zeigten sie niemals eine höhere Resistenz als ihre Elternarten. Generell waren exotische *Fallopia*-Taxa gegenüber europäischen Herbivoren besser verteidigt als einheimische Pflanzenarten und die Wachstumsraten der Herbivoren auf *Fallopia*-Taxa waren niedriger. Dies könnte unter anderem zum Invasionserfolg von exotischen *Fallopia*-Pflanzen in Europa beitragen. Unterschiede in Blatteigenschaften, die bekannt sind die Geniessbarkeit von Blättern zu beeinflussen, wie das spezifische Blattgewicht, Gewicht der Blatttrockensubstanz und das C/N-Verhältnis, erklärten nur teilweise Unterschiede in der Resistenz gegenüber europäischen Herbivoren zwischen exotischen *Fallopia*-Taxa und einheimischen Pflanzen. Dies lässt darauf schliessen, dass andere Faktoren, wie neue sekundäre Inhaltsstoffe, ebenfalls zur hohen Resistenz von *Fallopia* in Europa gegenüber europäischen Generalisten beitragen.

CHAPTER 1

General introduction

Biological invasions

“. . . we are seeing one of the great
historical convulsions of the world’s
fauna and flora.”
Charles Elton

Invasions of exotic animals and plants are widespread and a significant component of human-caused global environmental change (Dukes and Mooney 1999; Mack et al. 2000; Mooney and Hobbs 2000). Biological invasions are considered to be the second most important cause of current biodiversity loss, after habitat destruction (Pimentel et al. 2000). Invasive plant species can outcompete native species (Vitousek 1990; Beerling et al. 1994; Pyšek and Prach 1995; Moore 2000; Gerber et al. 2008) and they have the potential to alter ecosystems and their processes (Vitousek 1990; Mack and D'Antonio 1998; Moore 2000). In addition, biological invasions can cause significant economic costs (Vitousek et al. 1996; Mack et al. 2000; Pimentel et al. 2000).

Why do exotic plants become invasive?

Many plant species are more vigorous, grow taller, have higher rates of survival and reproduction and spread more rapidly in their introduced than in their native range (Crawley 1987; Mack et al. 2000; Ward et al. 2008). Several hypotheses have been proposed to explain why exotic plants become invasive. The invasion success of exotic plants has been attributed to abiotic factors like climate and soil conditions, which might be more favourable in the introduced range (Crawley 1987). However, competition and herbivory may also be important biotic determinants of invasiveness (Blossey and Nötzold 1995). One commonly accepted mechanism for the invasion success of plants has been proposed by the enemy release hypothesis (ERH), which postulates that introduced plants experience a reduced top-down regulation by natural enemies when introduced into a new range, allowing them to increase in distribution and abundance (Maron and Vilà 2001; Keane and Crawley 2002). In the absence of herbivores, selection may favour genotypes with improved competitive abilities due to a shift in resource allocation from herbivore defence to enhancement of other traits, resulting in greater fitness of the plants in the introduced range (hypothesis of evolution of increased competitiveness (EICA); Blossey & Nötzold 1995). However, attributes of the plants itself like morphology, survival strategy, genetic variability and mode of reproduction also may be important determinants of the invasion success of exotic plants (Williamson and Fitter 1996).

The importance of reproduction for invasiveness

The mode and the efficiency of reproduction can directly influence the invasion process, as it determines the potential for range expansion (Daehler and Strong 1994; Daehler and Strong 1996; Rejmànek and Richardson 1996; Pyšek 1997). High fecundity and the potential of sexual reproduction, which generates genetic variability (Crawley 1997), but also vigorous clonal growth, are attributes frequently observed among the most successful invasive plants (Baker 1974; Rejmànek 1995; Pyšek 1997; Pyšek et al. 2003). The success of clonal plants can be related to high levels of phenotypic plasticity (van Groenendael and de Kroon 1990). Moreover, clonal propagation provides the potential for rapid spread of favourable genotypes (Silander 1985). In contrast, sexual reproduction permits plants to adapt to new environments by increasing their genotypic diversity (Levin 2000).

Biological invasion and climate change

Climate change can play a fundamental role in the invasion process and may both directly and indirectly promote biological invasions and their evolutionary consequences (Dukes and Mooney 1999; Mooney and Hobbs 2000; Willis and Hulme 2002). Altered environmental conditions due to climate change could be more favourable for many invasive species (Dukes and Mooney 1999; Menzel 2003) and could increase reproduction, establishment and spread of exotic plants in the introduced range (Dukes and Mooney 1999).

Evolution and hybridization in invasive plants

Recently it has been claimed that the ability of exotic plants to establish and rapidly expand in a novel range may be shaped by evolutionary processes (Ellstrand and Schierenbeck 2000; Maron et al. 2004; Ward et al. 2008). Exotic species should not be regarded as stable genetic entities as there is growing evidence that recently introduced populations often experience rapid evolutionary changes in morphology, behaviour, and life history (Sakai et al. 2001; Lee 2002; Callaway and Maron 2006; Friedmann et al. 2008). Evolution following the colonisation of new environments and leading to adaptation to novel environments can occur within 20 generations or less (Prentis et al. 2008).

Two important phenomena in plant species that might promote rapid evolution are hybridization (Anderson and Stebbins 1954; Levin 1966; Stebbins 1969; Arnold and Hodges 1995; Ellstrand et al. 1996) and polyploidy (Tiébré et al. 2007; Prentis et al. 2008). One of the first examples of the evolutionary potential created by introduction of exotic species was *Spartina anglica* in the UK (Thompson 1991). It is one of the most famous examples of hybridization and polyploidization resulting in a fertile, highly competitive and invasive plant. In the UK, the introduced species *S. alterniflora* hybridized with the native *S. maritima*, resulting in the sterile hybrid *S.* x *townsendii*, which as a result of subsequent chromosome doubling, produced the new species *S. anglica* and escaped from infertility (Thompson 1991). Polyploid plants often have a higher fitness and are better colonizers than diploids, which might contribute to the greater incidence of invasiveness among taxa that have higher ploidy levels (Prentis et al. 2008).

However, hybridization has a greater effect on genomic changes than chromosome doubling (Prentis et al. 2008). Interspecific hybridization is a widespread feature of plants and has been identified as a stimulus for the evolution of increased invasiveness in exotic plant species (Abbott 1992; Ellstrand and Schierenbeck 2000; Vila et al. 2000; Hänfling and Kollmann 2002; Lee 2002; Callaway and Maron 2006). When introduced into new regions, related plant species may be brought into contact with other exotic species or with species native to the introduced range, that previously had been evolving in isolation from each other, giving rise to new opportunities for hybridization with potentially important consequences for the invasion potential of the introduced species (Ellstrand and Schierenbeck 2000). Hybridization is an extremely rapid mechanism for increasing genetic variation and provides the genetic material on which natural selection can act (Anderson 1949; Anderson and Stebbins 1954; Stebbins 1969; Ainouche et al. 2003). Deleterious alleles can be unloaded by hybridization, heterotic genotypes with high vigour and phenotypic plasticity can be fixed, or taxa with transgressive phenotypes can be created (Lambrinos 2004). Novel gene combinations resulting from hybridization may result in phenotypes that are stronger competitors, more aggressive, have higher reproduction and survival rates and are more resistant to herbivory, and are better adapted to certain environments than either of their parental species and may therefore be favoured by natural selection (Stebbins 1969; Arnold and Hodges 1995; Rieseberg et al. 2007). Moreover, hybridization may facilitate adaptation to new and extreme habitats or altered environmental conditions, for example, due to climate change (Abbott 1992; Pyšek et al. 2003). Ecological adaptation can therefore be a significant factor for the range expansion of plant species, with hybrids occupying a wider range of habitat types (Ward et al. 2008). For example, colonization of extreme habitats like sand dunes, deserts and salt marshes, was made possible by interspecific hybridization and subsequent selection of extreme or “transgressive” hybrid phenotypes of the sunflower species *Helianthus annuus* and *Helianthus petiolaris* (Rieseberg and Linder 1999). Another aspect of hybridization is repeated backcrossing of hybrids with their parental species, which might transfer individual genes and associated adaptive and beneficial traits from one parent to the other (Barton 2001; Lee 2002; Whitney et al. 2006). By this introgression traits like herbivore resistance (Whitney et al. 2006) or increased clonal growth (Vilà and D`Antonio 1998) can be acquired (Ward et al. 2008). Successful hybrid genotypes resulting from hybridization might be fixed and retained by vegetative propagation and increase invasion success of exotic plants (Ellstrand and Schierenbeck 2000; Lee 2002; Moody and Les 2002).

Species and hybrids of the invasive *Fallopia* complex

Invasive status, origin and impact of exotic Fallopia plants

Japanese knotweed (*Fallopia japonica* var. *japonica* (Houtt.) Ronse Decraene, also known as *Reynoutria japonica* and *Polygonum cuspidatum*), Giant knotweed (*Fallopia sachalinensis* (F. Schmidt Petrop.) Ronse Decraene) as well as their hybrid *F.* x *bohemica* (Chrtek et Chrtková) J.P. Bailey form one of the most troublesome invasive species complexes worldwide and are particularly aggressive in Europe and North America (Weber 2003; GISD 2005). *Fallopia japonica* var. *japonica* and *F. sachalinensis* are native to eastern Asia and were introduced into Europe in the mid-nineteenth century as ornamental and fodder plants. Exotic *Fallopia* taxa have been claimed to cause significant damage to native ecosystems (Richardson et al. 2000) and are especially a problem along rivers (Child et al. 1992). The plants outcompete native plants and displace native fauna (Gerber et al. 2008). During the vegetation period the dense stands can exacerbate flooding through the inhibition of water flow (Child and Wade 2000). In winter the above ground biomass dies back and leaves bare soil, increasing the risk of soil erosion (Child and Wade 2000). *Fallopia* plants also can cause economic problems including damage to flood defence structures (Starfinger and Konold 2003). In Europe, the most frequent taxon is *F. japonica* var. *japonica*, often found along roads and railways, the hybrid *F.* x *bohemica* is also widespread and seems to be most common along water courses, while *F. sachalinensis* is naturalized but rare and more confined to gardens and parks (Mandàk et al. 2004).

Biology of Fallopia

Fallopia japonica var. *japonica*, *F. sachalinensis* and their hybrid *F.* x *bohemica* (Polygonaceae) are rhizomatous perennial geophytes with erect stems and an extensive woody rhizome system (Beerling et al. 1994). They form vast stands and produce a large amount of above-ground biomass, which dies back in winter (Beerling et al. 1994; Child and Wade 2000). The plants overwinter with buds below the surface. In Europe, exotic *Fallopia* plants spread mainly by vegetative propagation (Beerling et al. 1994; Child and Wade 2000). They have a high regeneration ability and can regenerate from small rhizome or shoot fragments (Brock and Wade 1992; Pyšek et al. 2003). Dispersal occurs, for example, by transportation of soil, horticultural waste or via river systems (Child and Wade 2000). However, there is growing evidence that sexual reproduction also occurs in Europe (Bailey et al. 2007; Tiébré et al. 2007). *Fallopia* plants can produce large amounts of viable seeds in the introduced range (Bailey 1994; Beerling et al. 1994; Bram and McNair 2004; Tiébré et al. 2007) with high germination rates under containment (Bailey 1994; Bailey et al. 1995; Alberternst 1998; Forman and Kesseli 2003) and seeds are able to overwinter and germinate under natural conditions (Forman and Kesseli 2003). However, in Europe, low seedling survival and establishment of *Fallopia* has been observed in the field (Bailey et al. 1995; Hollingsworth et al. 1998). It has been proposed that seedlings of *Fallopia* are sensitive to frost and a cold humid winter and do not survive the European winter (Beerling et al. 1994).

Hybridization, genetic diversity and polyploidy in the Fallopia complex

The octoploid Japanese knotweed *F. japonica* var. *japonica* (2n = 88) has been identified in Europe with a single genotype (Bailey et al. 1995; Fritz et al. 1996; Hollingsworth et al. 1998; Hollingsworth 2000; Mandàk et al. 2005; Tiébré et al. 2007; Krebs et al. chapter 2). This suggests that there was a single introduction of a male-sterile plant which therefore spreads only vegetatively throughout central Europe (Bailey et al. 1995; Fritz et al. 1996; Hollingsworth et al. 1998; Hollingsworth 2000; Mandàk et al. 2005; Tiébré et al. 2007). In contrast, several genotypes with either male-sterile or male-fertile flowers of the tetraploid *F. sachalinensis* (2n = 44) occur in Europe resulting from multiple introductions or from occasional sexual reproduction (Mandàk et al. 2005; Tiébré et al. 2007; Krebs et al. chapter 2). This species can produce pure-bred seed and act as a pollen donor for *F. japonica* var. *japonica* (Bailey 1994; Tiébré et al. 2007), resulting in the hybrid *F.* x *bohemica*, which exhibits various levels of ploidy, but with the majority of individuals hexaploid in nature

(Bailey et al. 2007). The hybrid can backcross with its parental species and crosses between hybrids are also possible (Bailey et al. 2008). Genetic analyses indicated that the hybrid between *F. japonica* var. *japonica* and *F. sachalinensis*, *F.* x *bohemica* (2n = 66) has a higher genetic diversity in Europe than both parental species (Mandàk et al. 2005; Bailey et al. 2007; Tiébré et al. 2007; Krebs et al. chapter 2). It is frequently found in Europe (Hollingsworth et al. 1998; Hollingsworth 2000; Pyšek et al. 2003; Mandàk et al. 2005; Tiébré et al. 2007) and is considered to have a higher invasion potential and a faster spread than its parental species (Bimovà et al. 2003; Pyšek et al. 2003). In addition, to male *F. sachalinensis* and *F.* x *bohemica* plants, the climbing species *F. baldschuanica* (Regel) Holub (also referred to as *F. aubertii* L. Henry; 2n = 20), which occurs mainly as an ornamental plant, produces fertile pollen and can act as pollen donor within the *Fallopia* complex. This diploid species was apparently introduced into Europe with only a single genotype with hermaphrodite flowers (Bailey 1994; Tiébré et al. 2007). Hybridization between *F. japonica* var. *japonica* and *F. baldschuanica* (Bailey 1988; Tiébré et al. 2007) results in the pentaploid hybrid *F.* x *conollyana* J.P. Bailey (2n = 54; Bailey et al. 2007). Only one individual of this hybrid has ever been found established in nature (Bailey et al. 2007).

Outline of the dissertation

Interspecific hybridization plays an important role in the invasion process of the exotic *Fallopia* complex in its introduced range by providing the opportunity for sexual reproduction and increasing genetic diversity (Bailey and Stace 1992; Bailey 2003; Mandàk et al. 2003; Tiébré et al. 2007), thus giving the potential for evolutionary processes. By the origin of novel genotypes with higher fitness due to beneficial traits like herbivore resistance and the potential to adapt to new or changed environments, hybridization could increase the invasive success of exotic *Fallopia* plants. In this dissertation, I present four studies on hybridization in the invasive *Fallopia* complex in Western Europe and its consequences. Specifically, I study genetic diversity and hybridization patterns, the extent and variation in sexual reproduction, regional variation in seedling establishment and the potential effects of climate change and the influence of hybridization on herbivore resistance.

Chapter 2 deals with the distribution of the *Fallopia* taxa, their genetic diversity and the existence of regional patterns of hybridization in the invasive *Fallopia* complex in seven regions in Germany and Switzerland using RAPD analysis and flow cytometry. We examined whether genetic differentiation among regions is ongoing at the European scale and compared the hybridization and genetic patterns with those in other European regions. To study distribution, frequency and genetic diversity of *F. japonica* var. *japonica, F. sachalinensis,* and their hybrid *F.* x *bohemica* and the regional genetic structure of the complex could contribute to further understanding of patterns, extent and consequences of hybridization in Europe. Moreover, this knowledge could contribute to our further understanding of causes, mechanisms and consequences of invasion by plants in general.

In chapter 3 the extent and variation of sexual reproduction within the *Fallopia* complex was assessed on a large scale throughout Western Europe. Seed production, seed set, seed germination, flower morphology and pollen viability of the parental species *F. japonica* var. *japonica* and *F. sachalinensis* and their hybrid *F.* x *bohemica* were measured in five European regions using a hierarchical sampling design. Crossing experiments were carried out to assess pollen suitability for intra- and interspecific sexual reproduction and the ploidy level of offspring was determined to infer the paternal origin in natural crosses.

Chapter 4 studies the likelihood of successful seedling establishment and thus spread through sexual reproduction of exotic *Fallopia* species and their hybrids in Europe. So far only low seedling establishment has been observed in the field. We were especially interested if the risk of successful seedling establishment might increase under the predicted climate change. Even if vegetative reproduction is the main mode of reproduction for invasive *Fallopia* plants, changes in the distribution and frequency of the taxa or changes in climatic conditions could lead to a shift in the balance between vegetative and sexual reproduction towards more sexual reproduction than we observe today. Sexual reproduction by hybridization is a major force promoting genetic diversity and provides the potential for adaptive evolution in the *Fallopia* complex. In addition, hybridization plays a major role in restoring sexual reproduction in the male-sterile *F. japonica var. japonica* clones. The invasiveness and spread of the exotic *Fallopia* complex could therefore be enhanced by increased sexual reproduction and seedling establishment.

Chapter 5 deals with interactions between *Fallopia* taxa and their antagonists and the influence of hybridization on herbivore resistance. The herbivore resistance of the three hybridizing parental species and the two hybrids of the exotic *Fallopia* complex was studied using a set of herbivores native to Europe with which they share no evolutionary history. We measured leaf palatability of, and herbivore performance on the five exotic *Fallopia* species and on two native plant species (*Rumex obtusifolius*, *Taraxacum officinale*) using three generalist herbivores and one specialist herbivore. Leaf characteristics often associated with palatability, i.e. specific leaf area, leaf dry matter content and C/N ratio, were recorded for all species and related to the feeding response of the herbivores. We also determined leaf palatability of the widespread hybrid *F.* x *bohemica* at the genotype level to assess whether some hybrid genotypes have extreme levels of resistance compared to their parental species. As herbivores can distinguish between hybrid and parental genotypes (Fritz et al. 1998; Fritz et al. 2003), interactions with herbivores may also play an important role in the evolution of hybrids.

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CHAPTER 2

Taxa distribution and RAPD markers indicate different origins and regional differentiation of hybrids in the invasive *Fallopia* complex in central Western Europe

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Abstract

Interspecific hybridization can be a driving force of evolutionary processes during plant invasions by increasing genetic variation and creating novel gene combinations, thereby promoting genetic differentiation among populations of invasive species in the introduced range. We tested for regional genetic structure in the invasive *Fallopia*-complex, consisting of *Fallopia japonica* var. *japonica*, *Fallopia sachalinensis,* and their hybrid *Fallopia* x *bohemica,* in seven regions in Germany and Switzerland using RAPD analysis and flow cytometry. All individuals identified as *F. japonica var. japonica* exhibited the same RAPD-phenotype, while *F. sachalinensis* (11 RAPD-phenotypes for 11 sampled individuals) and *F.* x *bohemica* (24 RAPD-phenotypes for 32 sampled individuals) showed high genotypic diversity. Bayesian cluster analysis revealed three distinct genetic clusters. The majority of *F.* x *bohemica* individuals were assigned to a unique genetic cluster different from those of the parental species, while the other *F.* x *bohemica* individuals had different degrees of admixture to the three genetic clusters. At the regional scale, the occurrence of male-fertile *F. sachalinensis* coincided with the distribution of *F.* x *bohemica* plants showing a high percentage of assignment to both parental species, suggesting that they originated from hybridization between the parental species. In contrast, in regions where male-fertile *F. sachalinensis* were absent, *F.* x *bohemica* belonged to the non-admixed genetic group, indicating multiple introductions of hybrids or sexual reproduction among hybrids. We also found regional differentiation in the gene pool of *F.* x *bohemica,* with individuals within the same region more similar to each other than individuals from different regions.

Introduction

Understanding the dynamics of invasive species and managing them is a major challenge for modern ecology and conservation biology (Mooney and Hobbs 2000). Evolutionary change is often important to our understanding of the ecological dynamics of invasive populations (Lambrinos 2004). There is growing evidence that recently introduced populations often experience rapid evolutionary changes in morphology, behaviour, and life history (Sakai et al. 2001; Lee 2002; Callaway and Maron 2006; Friedmann et al. 2008) sometimes resulting in significant differentiation among populations of invasive species in the introduced range (Prentis et al. 2008). Since the capacity of species to invade may be shaped by evolutionary processes, managers and decision makers can no longer regard alien species as stable genetic entities, without the risk of using inappropriate management strategies.

Two important evolutionary phenomena in plant species are hybridization and polyploidy. Interspecific hybridization between introduced or between introduced and native species have been identified as a potentially important driving force of evolutionary processes in invasions (Abbott 1992; Ellstrand and Schierenbeck 2000; Vila et al. 2000; Hänfling and Kollmann 2002; Lee 2002; Callaway and Maron 2006). Both genotypic and genomic alterations may stimulate invasiveness of newly formed taxa (Ellstrand and Schierenbeck 2000). Hybridization can unload deleterious alleles or fix heterotic genotypes with high vigour and phenotypic plasticity, or create taxa with transgressive phenotypes (Lambrinos 2004). Hybridization may also increase genetic diversity in introduced taxa and provide the genetic material on which selection and genetic drift may act to promote population differentiation. Genetic variation and differentiation of hybrids in an invasive complex may also be promoted by the often complex history of invasion.

Multiple introductions seem to be common in invasions (Dlugosch and Parker 2008), and parental invasive species are likely to exhibit different spatio-temporal invasion dynamics. This results in situations where the patterns of hybridization are complex and provide a huge diversity of genotypes on which selection may act (Ainouche et al. 2003; Rieseberg et al. 2007). Understanding the sources of variation that contribute to evolution in hybridizing invasive species requires detailed examination of the variation of hybridization patterns over the introduced range as well as the examination of the genetic consequences of hybridization events.

The herbaceous rhizomatous perennials, Japanese knotweed (*Fallopia japonica* var. *japonica* (Houtt.) Ronse Decraene, also known as *Reynoutria japonica* and *Polygonum cuspidatum*), giant knotweed (*Fallopia sachalinensis* (F. Schmidt Petrop.) Ronse Decraene) and their hybrid *F.* x *bohemica* (Chrtek et Chrtková) J.P. Bailey form one of the most troublesome invasive species complexes worldwide and are particularly aggressive in Europe and North America (Weber 2003; GISD 2005). The octoploid Japanese knotweed *F. japonica var. japonica* (2n = 88) was introduced into Europe in the 19th century as an ornamental and fodder plant, along with the related tetraploid *F. sachalinensis* (2n = 44). In Europe, plants of *F. japonica var. japonica* are male-sterile, but can be pollinated by *F. sachalinensis*, resulting in the hybrid *F.* x *bohemica*, which exhibits various levels of ploidy, but with the majority of individuals hexaploid in nature (Bailey et al. 2007).

Interspecific hybridization and polyploidization have been important features in the evolution of invasive *Fallopia* in their introduced range (Bailey and Stace 1992; Bailey 2003; Mandak et al. 2003; Tiébré et al. 2007). Recent reviews have made a number of generalizations regarding the pattern and evolutionary consequences of hybridization in invasive *Fallopia* (Bailey and Wisskirchen 2006; Bailey et al. 2007). First, *Fallopia* hybrids are widespread in the introduced range and their abundance has increased since time of introduction. But the variation in the relative frequency of parental and hybrid taxa among different European regions is large (Table 1). Second, hybridization restores sexual reproduction to the populations by generating male and female progeny. Third, hybridization is also probably a major force promoting genetic diversity in the *Fallopia* complex, because *F.* x *bohemica* hybrids are more genetically variable than the parental species.

Apart from these general conclusions, quantifying the variation of hybridization patterns and the resulting genetic structure in *Fallopia* taxa among different European regions remains difficult for different reasons. First, a large part of the potential range of the species has not been explored, especially in the southern part of the parental distribution range, or, where it has been, botanists have had difficulty correctly identifying the plants (Bailey and Wisskirchen 2006). Second, most surveys did not quantify the relative abundance of the different taxa and ploidy levels. Third, genetic markers used by the authors (isozymes, RAPDs and ISSRs) as well as spatial scales (from tens of km to hundreds of km) differed among studies. Thus, the consequences of hybridization on the genetic differentiation of *Fallopia* populations are not clear. Tiébré et al. (2007) found no regional pattern for the *F.* x *bohemica* gene pool over central Belgium. However, the spatial scale considered in this study was not suitable to test the hypothesis of regional differentiation at the European scale. Testing this hypothesis requires studies at larger scales using a hierarchical sampling scheme.

In this paper we test explicitly for a regional genetic structure of the invasive *Fallopia* complex along a north-south transect in western-central Europe. Specifically, we aim, (1) to test for the existence of regional patterns of hybridization, (2) to examine whether genetic differentiation among regions is ongoing at the European scale, and (3) to compare the hybridization and genetic patterns with those in other European regions.

Materials and methods

Plant material and collection sites

Fallopia japonica var. *japonica, F. sachalinensis* and the hybrid between the two species, *F.* x *bohemica*, are clonal plants with erect stems of 1.5 - 4 m height. The plants develop an extensive woody rhizome system and can regenerate easily from small rhizome or shoot fragments (Bailey 2007).

Rhizomes of *F. japonica var. japonica, F. sachalinensis* and *F.* x *bohemica* were collected in September and October 2005 from 132 stands in six different regions in Germany and the Ticino region of Switzerland (Fig. 1).

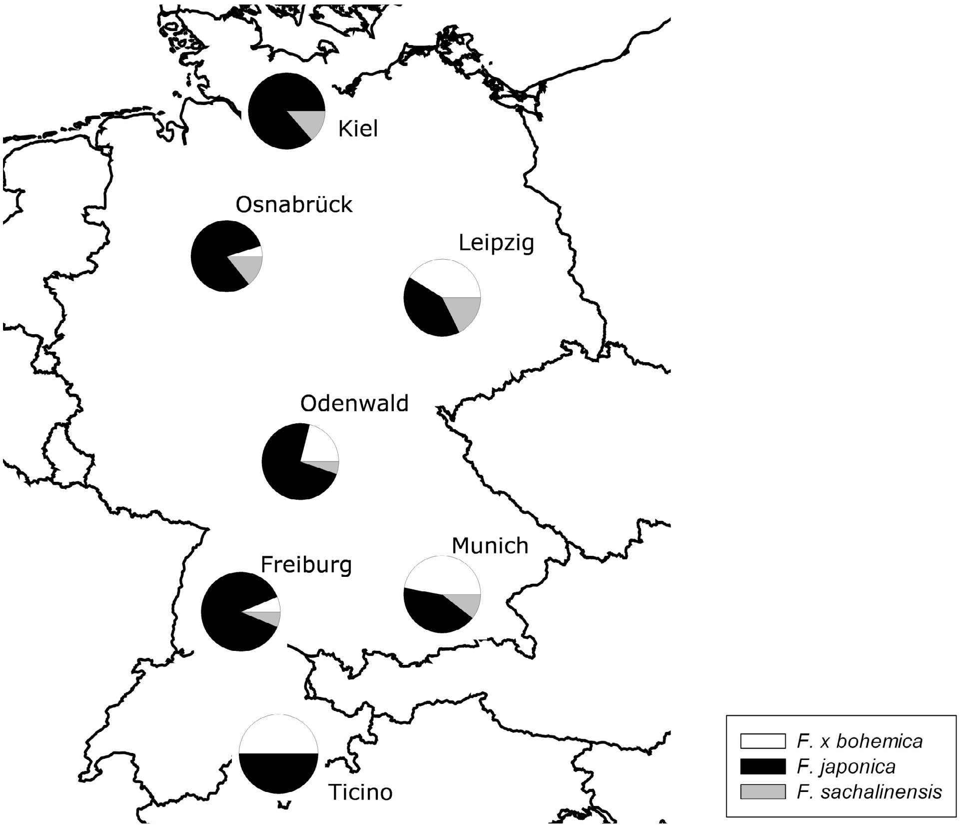

Figure 1: Map showing the sampled regions and the proportional abundance of the taxa within each region.

The area sampled within each region varied from 2500 to 10000 km^2. In each region, plants were collected from ~20 stands selected randomly to obtain a representative picture of the relative abundance of the different taxa. To increase the chance of collecting different clones, the distance between the selected stands was at least 500 m. Because we were interested in genetic variability and hybridization patterns and not in local clonality within stands, only one rhizome from one ramet was sampled in each stand. For each stand GPS data (latitude, longitude and altitude) were recorded. The collected rhizomes were planted into pots in a common garden at the University of Marburg, Germany, and leaf material for the molecular analyses was collected from these plants in October 2005 and for the flow cytometry in April 2006.

Identification of taxa

Taxa were tentatively identified using a combination of morphological and cytological characters as recommended by Tiébré et al. (2007). The morphological identification was based on leaf characters including the presence of trichomes, length and width of the leaves, and the shape of the leaf base and apex (Beerling et al. 1994; Bailey et al. 1995; Bailey et al. 1996). In addition, the ploidy level of the 132 individuals was assessed by flow cytometry following the technique of Tiébré (2007). This technique allows the rapid determination of the relative DNA content of nuclei by measuring the fluorescence of a fluorochrome that specifically binds to DNA (Galbraith et al. 1983). Pieces of fresh, young leaves were chopped with a razor blade, after addition of 500 µL 100 mM sodium hydrogen phosphate at pH 7 and containing 0·5 % Tween 20. After filtration through a 30 µm nylon filter, 500µL of a solution of 5g/L DAPI (fluorochrome) in 100 mM sodium hydrogen phosphate was added. Flow cytometry measurements were performed with a Partec machine (CA3 software 1995) equipped with a UV lamp. The fluorochrome was excited at 340 nm and emitted at 470 nm. A tetraploid individual of *F. sachalinensis* of known chromosome number was used as an internal standard for each measurement. We used the ratio ‘mean fluorescence intensity of sample / mean fluorescence intensity of internal standard’ to assess the ploidy level. For some individuals of all three taxa, Tiébré et al. (2007) had both counted root tip chromosomes and performed flow cytometry. These results could be used to interpret our fluorescence data more accurately.

RAPD analysis

We used RAPD markers to determine the genetic variation and the relationships within and among groups of *Fallopia*. The total genomic DNA of the 132 individuals was extracted using a modified CTAB method (Doyle and Doyle 1990). Fifty mg of young leaves, which had been rinsed with distilled water and dried with silica gel, were used per sample. DNA was quantified by reference to the High DNA mass ladder (Invitrogen) on a 0.6 % agarose gel. For the RAPD-PCR, a reaction mixture of 25 µL per sample was used, containing 10 X buffer (New England Biolabs, Inc), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP (Fermentas GmbH, Germany), 0.4 µM primer, 1 unit *Taq* polymerase (New England Biolabs, Inc.), 0.2 mg/mL BSA (Fermentas GmbH) and 20 ng template DNA. Amplification was performed using a PTC-200 Thermal Cycler (MJ Research: Biozym) programmed for an initial denaturation at 95°C for 2 min; followed by 44 cycles of 20 s at 94°C, 1 min at 36°C, and 1 min at 72°C; and a final extension at 72°C for 10 min. PCR products were run on a 1.8% agarose gel in TAE (Tris-Acetate-EDTA) and stained with 14 µL ethidium bromide. Molecular weights were estimated by reference to a 100 Base-Pair ladder (GeneRuler ^{TM}, Fermentas GmbH).

Eight primers (A10, A19, G06, J12, M10, M15, R11, T07) from the Operon10-mer kit (Operon Biotechnologies GmbH, Cologne/Germany) which produced reproducible and unambiguous polymorphic banding patterns were used for the RAPD analysis. These primers had also been used by Tiébré et al. (2007). The presence or absence of DNA fragments was scored. We genotyped DNA from each individual twice to check for reproducibility among PCR runs. A binary matrix based on polymorphic bands was produced by scoring the presence (1) or absence (0) of DNA fragments manually. For each primer, bands of identical size were considered homologous. Non-reproducible bands were discarded from the data matrix.

Data analysis

A Bayesian-based cluster analysis was performed on the RAPD data using the program Structure 2.1 (Pritchard et al. 2000). The method uses Markov chain Monte Carlo methods (MCMC) to estimate allele frequencies and to assign individuals probabilistically either to distinct gene pools or jointly to two or more gene pools if their genotypes indicate that they are admixed. Because of the dominant marker used, each locus was coded as known for one copy and unknown (coded _9 as recommended in the program) for the other (Pritchard and Wen 2004; Tiébré et al. 2007). To obtain data strictly from the genetic information, we did not use prior information regarding species identity. Analyses were performed under the admixture model. Five independent runs were carried out for each value of K (numbers of clusters assumed) between 1 and 8, with parameters and model likelihood estimated at over 200,000 MCMC iterations following a burn-in period of 50,000 steps. The maximum value of the log likelihood of data [$L(K)$] and the minimum variance of [$L(K)$] associated with each K value were analyzed to identify the number of clusters that best described the data. However, because Evanno et al. (2005) showed that this method does not always correctly estimate K, the ΔK statistic based on the rate of change of $\ln(K)$ between successive values of K was calculated to infer the appropriate number of clusters (Evanno et al. 2005). For each individual, we assessed its mean percentage of membership (q_{mean}) to each of the K genetic clusters based on the four independent runs.

In order to test for isolation by distance for hybrids, spatial autocorrelation statistics were calculated using a relationship correlogram with SPAGEDI version 1.2 (Hardy and Vekemans 2002) to characterize the spatial genetic structure without assumptions about its pattern. The relationship coefficient developed by Hardy (2003) is a measure of pairwise genetic similarity between individuals, adapted to dominant markers. Distance classes (0-100 km, 100-300 km, 300-400 km and >400 km) were chosen to obtain a similar number of pairs of individuals for each distance class. The slope of the regression of the pairwise relationship coefficient on the logarithm of the pairwise geographical distances between individuals was estimated. The significance of the relationship coefficient in each class was tested by permutation tests (10,000 iterations).

Results

Based on morphological identification, the plant material contained 89 putative *F. japonica var. japonica*, 11 putative *F. sachalinensis* and 32 *F.* x *bohemica*. Flow cytometry analyses revealed three levels of ploidy confirming the morphological identification. The fluorescence peak ranging from 0.509 to 0.524 for individuals identified as *F. sachalinensis* corresponded to the tetraploid standard. The fluorescence peaks of *F. japonica var. japonica* individuals ranged from 2.002 to 2.405 indicating that they were octoploid. The fluorescence peaks of individuals identified as *F.* x *bohemica* ranged from 1.502 to 1.684 indicating that these plants were hexaploids. *Fallopia japonica* var. *japonica* was found in all regions studied, *F. sachalinensis* was present in all regions except in the Ticino, and the hybrid was detected in all regions except the region around Kiel. The relative frequency of the three taxa varied strongly among the regions (Fig. 1).

Seventy seven polymorphic RAPD markers were scored, identifying 37 multilocus RAPD phenotypes among the 132 individuals analysed. All the octoploid individuals identified as *F. japonica var. japonica* (*N* = 89) exhibited the same RAPD phenotype. In contrast, each individual (N = 11) of *F. sachalinensis* had a different RAPD phenotype. *F.* x *bohemica* also exhibited a high genotypic diversity with 24 RAPD phenotypes among 32 sampled individuals. One of these RAPD phenotypes was shared by five individuals, four of them from the same region (Leipzig) and one from the Ticino region. Another RAPD phenotype was shared by four individuals in different regions (Osnabrück, Ticino and Munich) and one by two individuals of the Ticino region.

The Bayesian analysis using STRUCTURE indicated the presence of three distinct genetic clusters in the sample of *Fallopia* (Fig. 3). The likelihood of the data gradually increased from K = 2 (LL = -1343.29) to a maximum at K = 5 (LL = -808.55). However, above K=3, Δ*K* was not higher than the inter-run variation of K (Fig. 2).

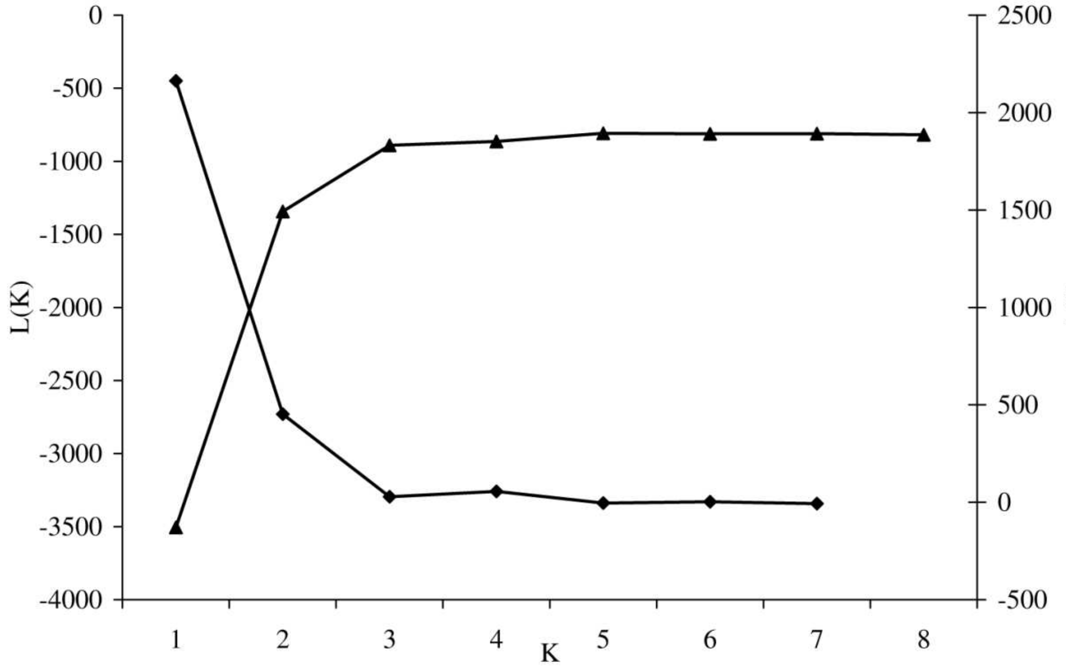

Figure 2: Inference of the number of genetic clusters (K) of *Fallopia* individuals using the model-based clustering method of Pritchard et al. (2000). Mean (± SD) of the log probability of data [L(K)] based on four independent runs (▲) as a function of the value of K and the rate of change in the log probability of data ΔK between successive values of K (♦).

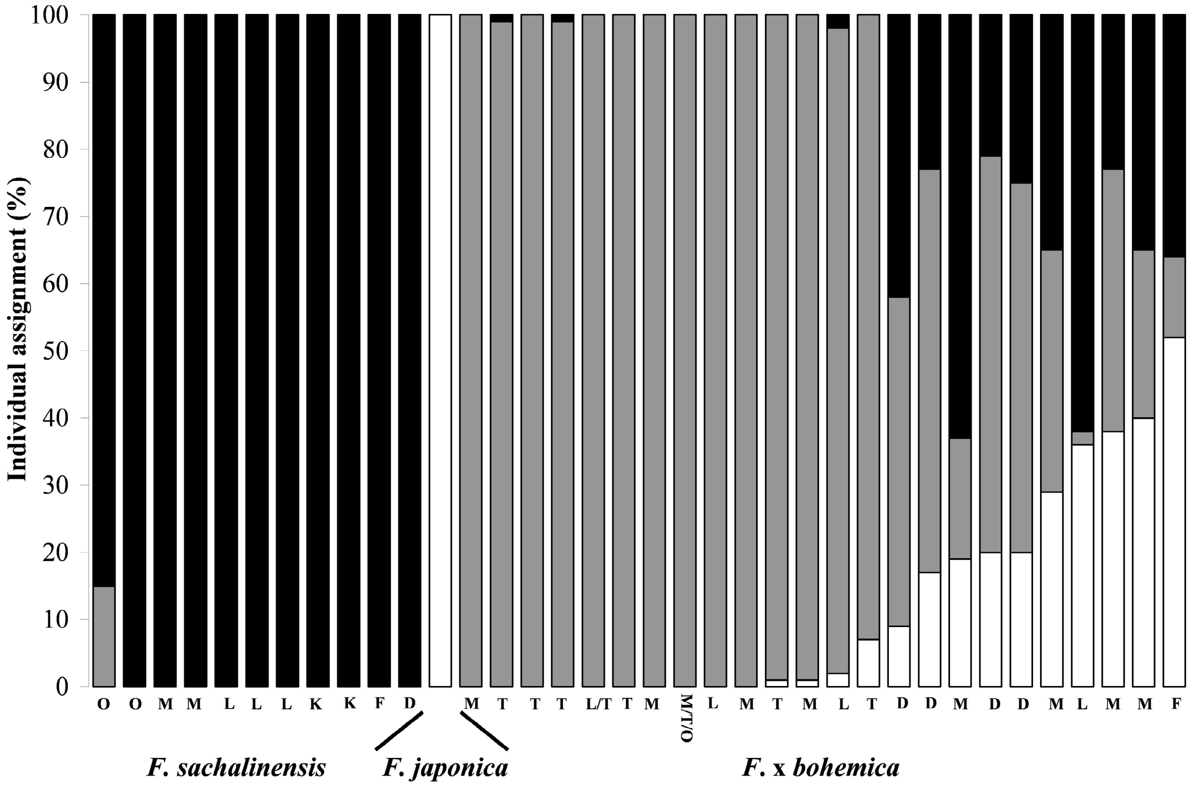

Figure 3: Assignment proportion of the 132 sampled individuals (bars) of three *Fallopia* taxa from western-central Europe to the three genetic clusters (K) detected using the model-based clustering method of Pritchard et al. (2000). Individuals with the same RAPD phenotype are represented only by a single bar. Each colour (white, grey, black) represents a genetic cluster (K). Letters below each bar represent the geographic region of origin (O= Osnabrück, M= Munich, L= Leipzig, K= Kiel, F= Freiburg, D= Odenwald, T= Ticino).

Consequently, the value of K = 3 was retained and the data analyzed for individual assignments. All individuals of *F. japonica var. japonica* were assigned to a single genetic cluster (q = 1.00) (Fig. 3). The individuals of *F. sachalinensis* were also assigned to a single genetic cluster (q = 1.00), except for one that displayed a low level of admixture (q = 0.15) with the genetic cluster dominated by *F.* x *bohemica* individuals (see below).

The situation was more complex for *F.* x *bohemica*. The majority of hexaploid *F.* x *bohemica* individuals were totally (14 out of 29; q = 1.00) or almost totally (another five; q ranging from 93 to 99) assigned to a unique genetic cluster different from those of the parental species. The other *F.* x *bohemica* individuals (10 out of 29) had different degrees of admixture to the three genetic clusters. Assignment to the cluster characteristic for *F.* x *bohemica* ranged from 2% - 60%, to the cluster characteristic for *F. japonica var. japonica* from 9% - 52%, and to the cluster characteristic of *F. sachalinensis* from 21% - 63%. The different patterns of admixture were not randomly distributed among regions. Individual hybrids from the Odenwald region (N = 4) displayed a high admixture to the three genetic clusters with more than 50%

assignment to the cluster typical for *F*. x *bohemica*. In contrast, all individuals from the Ticino region were totally assigned to the genetic cluster typical for *F*. x *bohemica*. Individuals from the Munich region displayed more diversified patterns with about half of the individuals (5/9) assigned to the single genetic cluster typical for *F*. x *bohemica* and the others (4/9) assigned to the three genetic clusters with more than 50% due to the combined genetic cluster of the parental species. In the Leipzig region, six of the individuals were assigned totally or predominantly to a different cluster than the parental species, while a single individual was assigned nearly completely to the clusters of the parental species.

Autocorrelation analysis of the spatial genetic structure of *F*. x *bohemica* individuals revealed a significant negative regression (b = -0.033, P = 0.029) between the relationship coefficient and the logarithm of the spatial distance between individuals. The relationship coefficient (r) values were positive and significantly different from zero (r = 0.073, P = 0.028) in the first distance class (0-100 km) and significant but negative (r = -0.067, P = 0.032) in the second distance class (100-300 km). Relationship coefficient values in the other distance classes were not significantly different from zero (Fig. 4).

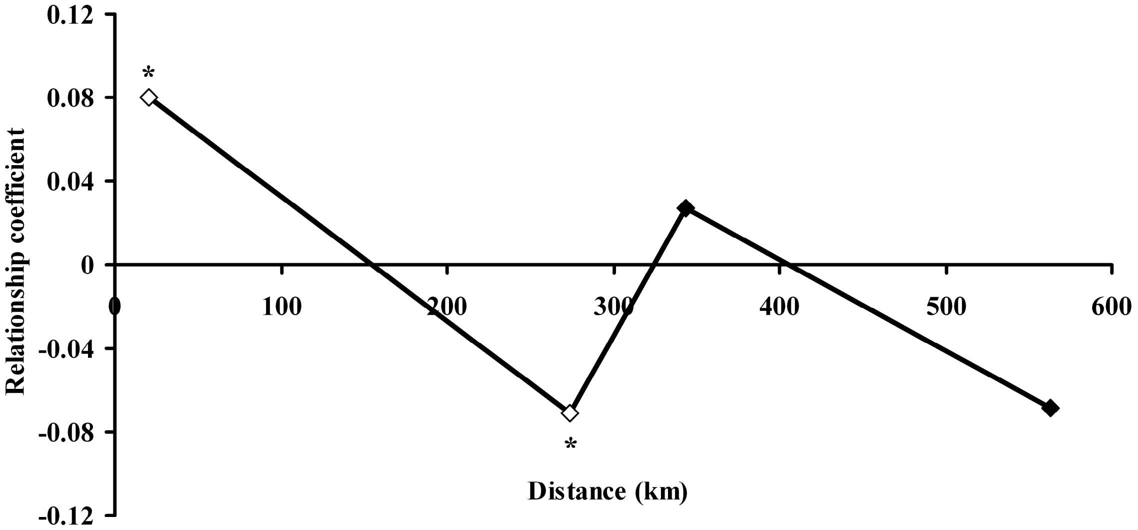

Figure 4: Relationship coefficient correlogram of genetic similarity between *F*. x *bohemica* individuals as a function of their geographic distance (distance classes: 0 - 100 km, 100 - 300 km, 300 - 400 km and > 400 km). Unfilled symbols represent relationship coefficients significantly different from 0 (* P < 0.05).

Discussion

In this study, we demonstrated quantitatively that there is (1) strong variation in hybridization patterns in the invasive *Fallopia* complex among regions separated by hundreds of kilometres over a north-south transect in western central Europe, and, (2) a clear differentiation of genetic pools of *F.* x *bohemica* among regions, with individuals within regions more similar than individuals among regions.

In accordance with studies in Great Britain, the Czech Republic and Belgium (Bailey et al. 1995; Hollingsworth et al. 1998; Hollingsworth 2000; Mandak et al. 2005; Tiébré et al. 2007), we found a single genotype of the most widespread parental species, *F. japonica var. japonica,* over our entire study area. This was the same genotype as observed in Belgium by Tiébré et al. (2007). This strongly suggests that a single introduction of a male-sterile *F. japonica var. japonica* was made, and that this genotype has spread vegetatively throughout western, northern and eastern Europe (Bailey and Conolly 2000). In contrast, we found a high genotypic diversity in the hybrid *F.* x *bohemica* throughout our study area. The level of genotypic diversity found in our study was comparable to that found in Belgium (Tiébré et al. 2007) with the same molecular markers. The genetic variation found in the hybrid *F. x bohemica* may stem from three sources: i) genetic diversity in the parental species *F. sachalinensis*, ii) the genetic segregation of the F1 hybrids, backcrossing to either parent, and iii) multiple introductions of hybrids from the native range.

The occurrence of many different hybrid genotypes suggests that those hybrids originated from sexual reproduction. Seedling recruitment in nature appears to be relatively rare, but has been reported from different parts of Europe (Bailey et al. 1995; Hollingsworth et al. 1998). Tiébré et al. (2007) demonstrated that *Fallopia* seeds can survive winters and germinate in spring. In contrast, in the U.S. seed production of *Fallopia japonica* is high and seeds readily over-winter and can be found in nature (Forman and Kesseli 2003; Grimsby et al. 2007). A low percentage of seedlings surviving and establishing would be sufficient to increase genotypic variation in *F.* x *bohemica* in Europe. For plant species with long lived-genets, even rare events of in situ sexual reproduction of divergent clones can be sufficient to maintain high genetic diversity (Ellstrand and Roose 1987; Gabrielsen and Brochmann 1998).

Prior studies reported a relatively low genetic diversity of the other parental species, *F. sachalinensis*, in Europe (Pashley et al. 2003; Tiébré et al. 2007). In the Czech Republic, Mandak et al. (2005) found a moderately high level of genotypic variation, with 16 male-fertile and male-sterile genotypes among 50 stands, of which one genotype was very common.

Our study also revealed a high genotypic diversity in *F. sachalinensis* across our sampling area, suggesting multiple introductions into Europe. This diversity in the parental species may enhance genetic diversity in the hybrid in regions where RAPD data suggest in situ origin of hybrids (see below).

The high genetic variation in hybrids may provide the material for genetic differentiation among regions due to the combined effects of limited gene flow, genetic drift and local adaptation. However, our results suggest that the genetic differentiation of groups of hybrids among regions stems, in fact, from different hybrid origins. Several scenarios for different hybrid seed origins have been proposed, including seed production by in-situ hybridization among parental species potentially followed by back crosses with hybrids from different generations; independent introductions of hybrid seeds from the native range; and hybrid propagation within the introduced range by exchange of seeds and rhizomes among regions for horticulture (Tiébré et al. 2007).

In the assignment tests using the RAPD data with no prior information on groupings, *F.* x *bohemica* showed a complex pattern of admixture to different gene pools. One group of hybrid genotypes was assigned to a specific genetic pool and not to a mixture between the genetic pools of the putative parents. Using the same assignment test, Tiébré et al. (2007) also found a specific gene pool for *F.* x *bohemica* in Belgium, with little introgression of *F. sachalinensis* into *F.* x *bohemica*. This group of hybrids with no admixture is not likely to consist of first generation hybrids of parental genotypes present in a specific region, unless the hybridization event has led to a rapid structural change at the genome level, a phenomenon that has been shown to be associated with the evolution of polyploid genomes (Ozkan et al. 2001; Salmon et al. 2005). Rather, hybrids with no admixture could have been introduced into a specific region from the native range or other parts of Europe, or they may be the result of ongoing sexual reproduction among hybrids, leading to a gradual loss of the amount of genome identical to those of their parental species. Two more groups were found, with one group of hybrids assigned to a genetic group with a strong contribution of both parental species. This group of admixed hybrids may consist of first generation hybrids. Another group of individuals with a contribution of parental species, but also a high contribution of the specific pool of *F.* x *bohemica* could result from backcrosses of hybrids with the parental species.

An interesting result is that the proportion of the *F.* x *bohemica* groups with different patterns of admixture differs among the regions. An important factor explaining the frequency of these hybrid groups may be the availability of pollen sources due to variation in the presence of parental species. Throughout the study range, *F. japonica var. japonica* was the dominant taxon, but the abundance of *F. sachalinensis* varied considerably among the regions. *Fallopia sachalinensis* plants were more frequent in eastern and northern regions of our sampling area, but were not found in Ticino, neither inside nor outside of our study region (C. Krebs, personal observation). At the regional scale, the occurrence of male-fertile *F. sachalinensis* coincides with the distribution of hybrids of the admixed group (C. Krebs, personal observation). For example, in the region around Munich many *F.* x *bohemica* plants show a high percentage of assignment to both parental species. In this region several *F. sachalinensis* genotypes, both male-sterile and male-fertile, occur. In contrast, in the Ticino region we found no *F. sachalinensis* clone and hybrids with very little admixture. These findings suggest that in regions where *F. sachalinensis* is not sympatric or only the more common male-sterile form occurs, and *F.* x *bohemica* belongs to the non admixed genetic group, the occurrence of the taxon is probably due to multiple introductions from other regions and a high proportion of the genetic variability is caused by sexual reproduction among the hybrids and not by hybridization between the parental species. Alternatively, *F. sachalinensis* might have formerly occurred, hybridized with *F. japonica* and since become extinct.

Our data also clearly illustrate how the relative importance of the different *Fallopia* taxa and patterns of hybridization differs from those in other European areas. The relative abundance of *F. sachalinensis* throughout our entire study area was similar to that reported for the UK and Belgium, but was lower than in the Czech Republic (Table 1). This latter area should be considered a hotspot of distribution for *F. sachalinensis* in its introduced range. The higher abundance in the Czech Republic may be due to the early introduction of *F. sachalinensis* into Eastern Europe, where it is known to have grown in the Botanical Garden of St. Petersburg since 1864, from where it probably started to spread into the west (Bailey and Wisskirchen 2006). We also confirmed that the hybrid *F.* x *bohemica* is widespread in continental Europe, while in Britain *F. japonica var. japonica* is a hundred times more frequent (Table 1). In contrast to other parts of Europe where *F.* x *bohemica* displays different levels of ploidy (aneuploid, 4x, 6x, 8x; see Table 1), all of our hybrids samples were hexaploid. Hence, we confirmed the rarity of the tetraploid hybrid on the continent of Europe compared with the UK. Our results also suggest a South-East - North-West gradient in the distribution of *F.* x *bohemica* within our sampling area, with higher abundance in the south-eastern regions. This pattern does not match findings from other studies which suggest a predominantly northern and western distribution of *F.* x *bohemica* in continental Europe (Bailey and Wisskirchen

2006). However, it has also been pointed out that the apparent absence of hybrids from large areas may be the result of misidentification of *Fallopia* taxa by field botanists, and that, in fact, there is still a lack of information for many European countries regarding the occurrence and distribution of the hybrid (Bailey and Wisskirchen 2006). The highest abundance of hybrids, as compared to that of the parental taxa, was found in the more southern region of Ticino. This supports the idea that *F.* x *bohemica* may be more prone to invade the submediterranean and mediterranean regions than its parents and exhibits a niche that is not only intermediate between its parents but reveals new qualities of independent niche adaptation and range widening (Bailey and Wisskirchen 2006).

In a recent study, Friedman et al. (2008) reported a genetically based latitudinal gradient in cold hardiness in the invasive saltcedar complex (*Tamarix ramosissima*, *Tamarix chinensis*, and hybrids) in North America, with southern hybrid saltcedars more closely related to *T. chinensis* and northern plants more closely related to *T. ramosissima*. According to Friedmann et al. (2008), hybridization is likely to have introduced the genetic variability necessary for rapid evolution of the cline in saltcedar cold hardiness. Similarly, the increased genetic variation in the *Fallopia*-complex by hybridizing and the regionalisation of the genetic pool of hybrids as observed in our study may provide the raw material for adaptive evolution in response to the biotic and abiotic conditions encountered in different regions of Europe, and may ultimately increase the invasiveness of the hybrid *F.* x *bohemica*. Our results also suggest that the potential spread by sexual reproduction should be taken into account when planning management measures against the invasive *Fallopia* complex in Europe.

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Appendix A: Variation in the relative abundance (expressed as proportion of individuals examined) of *F. japonica var. japonica*, *F. sachalinensis* and the hybrid *F.* x *bohemica* in different areas of Europe (data from various studies). For the hybrid *F.* x *bohemica*, the proportion of individuals with different ploidy levels is given in brackets.

Region	*F. japonica var. japonica*	*F. sachalinensis*	*F. x bohemica* (aneuploid, 4x,6x,8x)
British Isles [1]	87%	10%	3% (0, 21, 75, 4)
Belgium [2]	49%	8%	43% (3, 3, 84, 10)
Germany-Switzerland [3]	68 %	8%	24% (0,0,100,-)
Czech Republic [4]	39%	24%	37% (0,2,93,5)

[1] Bailey (2006), [2] Tiébré et al. (2007), [3] Current study, [4] Mandàk et al. (2004).

CHAPTER 3

What affects regional variation in sexual reproduction in the invasive *Fallopia* complex in Europe?

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Abstract

Numerous invasive plant species share the ability of vegetative reproduction. Nevertheless, the efficiency of sexual reproduction may be an important determinant of their invasion success, because recombination can increase genetic variation and create novel genotypes that are better adapted to new environmental conditions. We assessed seed production, seed set, seed germination, flower morphology and pollen viability of the invasive clonal species *Fallopia japonica* var. *japonica, Fallopia sachalinensis* and of their hybrid *Fallopia* x *bohemica* in five European regions and determined the ploidy level of their offspring to potentially infer the pollen donor under natural conditions. In addition, crossing experiments were carried out to assess pollen suitability for intra- and interspecific sexual reproduction. Our study revealed a high level of variation in seed production, flower morphology and pollen viability of the invasive *Fallopia* taxa among and within regions in Europe. The ratio of length of anthers/length of ovary and pollen viability partially explained variation in seed production of *F. sachalinensis* and *F. x bohemica,* but not in the single male-sterile clone of *F. japonica* var. *japonica* detected in Europe. A gradual variation in seed production and pollen viability was found among *F. sachalinensis* and *F. x bohemica* stands, with some stands with male flower morphology producing seeds and having low pollen viability. At the regional level, high seed production of *F. japonica* var. *japonica* was correlated with high ratios of length of anthers/length of ovary of *F. x bohemica*, indicating predominantly male flowers in hybrid stands, which can serve as pollen donor. Seed set in the experimental crosses depended on the pollen donor and was comparable to the highest seed set observed in the field. These results indicate that, beside flower morphology, the local availability of compatible pollen is a key factor contributing to the variation in the seed production of invasive *Fallopia* taxa in Europe. The predicted increase in hybrid abundance is therefore likely to contribute to an increased seed production of invasive *Fallopia* taxa in Europe.

Introduction

One of the most intriguing paradoxes in invasion biology is that during the invasion process, exotic species must initially overcome the perils of isolation before being able to proliferate in new habitats. The mode and the efficiency of reproduction are therefore important determinants of the success of invasive plants in their adventive range, directly influencing the likelihood of establishment and the rate of spread (Kolar and Lodge 2001). The capability for vegetative reproduction is a trait frequently observed among the most successful invasive plants (Baker 1974; Rejmànek 1995; Kolar and Lodge 2001). By clonal propagation, exotic species are less susceptible to stochastic extinction during the establishment phase and can rapidly spread when introduced into regions with favourable environmental conditions (Silander 1985). Recombination through sexual reproduction, on the other hand, can act as a major force in invasion processes by increasing genetic diversity and generating new genotypes that may be better adapted to new environments (Levin 2000). In addition, genetic changes associated with hybridization may induce rapid evolution of newly formed species (Abbott et al. 2000; Ainouche et al. 2003; Soltis et al. 2004) which may be more invasive than their parental species (Ellstrand and Schierenbeck 2000). However, sexual reproduction presents a species with a major challenge: finding suitable mates in the neighbourhood. This presents a particular problem for sessile organisms like plants, especially for obligatory outcrossing species like self-incompatible or dioecious species (Elam et al. 2007). The combination of vegetative and sexual reproduction may be an ideal strategy since both reproduction modes have different advantages (Silander 1985; Crawley 1997; Ceplitis 2001). In plants with both reproduction modes, the contribution of sexual reproduction to invasion success is likely to increase during the invasion process with increasing densities of the invasive plants (Davis et al. 2004; Bailey et al. 2008). Pollen availability and compatibility may determine the level of contribution of sexual reproduction to the invasion success of invasive species with such a dual strategy.

Various taxa of the *Fallopia* complex are considered to be among the most serious exotic weeds in Europe and North America (Beerling et al. 1995; Starfinger and Konold 2003; Weber 2003; GISD 2005) and are capable to alter ecosystem properties (Richardson et al. 2000; Dassonville et al. 2007; Gerber et al. 2008). In the introduced range, vegetative propagation is usually considered to be the main mode of reproduction of exotic *Fallopia* plants because seedling recruitment in nature has only rarely been observed (Beerling et al. 1994; Bailey et al. 1995; Child and Wade 2000; Forman and Kesseli 2003). However, for plant species with long-lived genets, even isolated events of sexual reproduction can be sufficient to maintain high genetic diversity (Ellstrand and Roose 1987; Gabrielsen and Brochmann 1998; Bailey et al. 2008). In North America, interspecific hybridization and introgression in the *Fallopia* complex is common, indicating that sexual reproduction is important both for dispersal and for generating diversity (Forman and Kesseli 2003; Gammon et al. 2007; Grimsby et al. 2007). Recent evidence of high genetic variation and regional differentiation in the hybrid *F.* x *bohemia* suggests that sexual reproduction also occurs in Europe (Bailey et al. 2007; Gammon et al. 2007; Tiébré et al. 2007; Krebs et al. chapter 2). The hybrids seem to grow and spread faster than the parental species (Bimovà et al. 2003) and are consequently suggested to be more invasive than the parental species (Mandàk et al. 2004; Bailey et al. 2007). Hence, while the clonal growth and capability of *Fallopia* taxa to regenerate from small plant fragments is a major factor promoting the spread of this complex, sexual reproduction is likely to contribute to the genetic diversity and invasiveness seen in the introduced range.

In the case of the invasive *Fallopia* taxa, which are dioecious and self-incompatible plants, the challenge of finding compatible mates for sexual reproduction is particularly relevant. The octoploid Japanese knotweed *F. japonica* var. *japonica* (2n = 88) was introduced in the 19th century as an ornamental and fodder plant from eastern Asia. In Europe only a single genotype of *F. japonica* var. *japonica* (octoploid, 2n = 88) with male-sterile flowers has been found so far (Bailey et al. 1995; Hollingsworth et al. 1998; Hollingsworth 2000; Mandàk et al. 2005; Tiébré et al. 2007; Krebs et al. chapter 2). For this reason, the restoration of sexual reproduction requires compatible pollen from congeneric species. *Fallopia sachalinensis* (tetraploid, 2n = 44) is also naturalized but less frequent and several genotypes with male-sterile, male-fertile and hermaphrodite flowers have been recorded from this species in Europe (Mandàk et al. 2005; Bailey et al. 2007; Tiébré et al. 2007; Krebs et al. chapter 2). Apart from spreading vegetatively and forming pure-bred seeds, *F. sachalinensis* can act as a pollen donor for *F. japonica* var. *japonica* (Bailey 1994; Tiébré et al. 2007). The resulting hybrid, known as *F.* x *bohemica* (Chrtek et Chrtkova) J.P. Bailey, shows partial to full fertility, occurs with male-sterile, male-fertile and hermaphrodite plants (Bailey et al. 1996) and is reported to be one of the most frequent pollen donors for *F. japonica* var. *japonica* (Bailey 1994; Tiébré et al. 2007). Moreover, backcrosses with parental species are possible as well as crosses between hybrids (Bailey et al. 2007). *Fallopia* x *bohemica* shows a high genetic variability and varying ploidy levels, with hexaploid hybrids being the most common cytotype (Hollingsworth et al. 1998; Hollingsworth 2000; Pyšek et al. 2003;

Mandàk et al. 2005; Tiébré et al. 2007; Krebs et al. chapter2). Finally, the climbing species *F. baldschuanica* (diploid, 2n = 20), which has hermaphrodite flowers and is planted as an ornamental, also can serve as pollen donor for *F. japonica* var. *japonica* (Bailey 1988; Tiébré et al. 2007). A comparison of several studies performed in different European regions showed that the variation in the relative frequency of parental and hybrid taxa among regions is large (Mandàk et al. 2004; Bailey and Wisskirchen 2006; Tiébré et al. 2007). Recently, a regional genetic structure in the hybrid genotypes was demonstrated in Europe, with different kinds of admixture between the putative parental gene pools, suggesting regional variation in hybridization patterns (Krebs et al. chapter 2).

In comparison to observations made by Conolly in the 1970s (1977), seed production of *F. japonica* var. *japonica* has increased in the past 30 years in the UK (Bailey et al. 2008). This suggests that invasive *Fallopia* stands might experience increased opportunities for sexual reproduction at least in some regions in Europe. To study whether reproductive success within the *Fallopia* complex varies among different parts of the invaded range and among taxa, we assessed the extent of sexual reproduction within the *Fallopia* complex in Western Europe using a hierarchical sampling design. To assess factors influencing reproductive success, we measured female and male fitness traits and germination of offspring from the parental species *F. japonica* var. *japonica* and *F. sachalinensis* as well as of the hybrid *F.* x *bohemica*. In addition, ploidy levels of offspring were analysed to infer the paternal origin of field collected seeds, and crossing experiments were carried out to assess pollen compatibility for intra- and interspecific sexual reproduction.

Material and methods

Study species

The *Fallopia* taxa included in this study were *F. japonica* var. *japonica* (Houtt.) Ronse Decraene (also referred to as *Polygonum cuspidatum* and *Reynoutria japonica*), *Fallopia sachalinensis* (F. Schmidt Petrop.) Ronse Decraene, the hybrid between these species, known as *Fallopia* x *bohemica* (Chrtek et Chrtkova) J.P. Bailey, and the ornamental climbing species *Fallopia baldschuanica* (Regel) Holub (also referred to as *F. aubertii* L. Henry).

In their native range *F. japonica* var. *japonica* and *F. sachalinensis* are dioecious but with hermaphrodite and male–sterile stands (Tanaka 1966; Bailey 1994). In Europe, the breeding system of the exotic *Fallopia* taxa is characterized as dioecious and gynodioecious (male-sterile, male-fertile and hermaphrodite flowers) (Bailey 1994; Beerling et al. 1994) and in North America it has been observed to be subdioecious with male and female flowers on separate plants and males plants with few female flowers, which therefore sometimes set a few seeds (Forman and Kesseli 2003). The plants flower in late August to September or even October (Beerling et al. 1994). The flowers are pollinated by bees, hoverflies and other insects (Beerling et al. 1994). Seed is mature in late October or November and starts germinating in March (Beerling et al. 1994).

Field sampling

Fallopia taxa were sampled in five regions of Western Europe: three regions in Germany (Odenwald, Freiburg and Munich), one in Belgium (area between Mons, Brussels and Namur), and one in Switzerland (Ticino; Table 1; Fig. 1). In each region, sampling was done randomly to get a representative overview of the relative abundance of the different taxa within the region. The minimum distance between stands was 500 m to maximize the probability of collecting different clones, considering the clonality of the species.

In Octobter 2006, seed set was determined for 38 stands of *F. japonica* var. *japonica*, 22 stands of *F.* x *bohemica* and 6 stands of *F. sachalinensis* (Table 1). Most of the stands had previously been characterized based on molecular markers (RAPDs) and flow cytometry by Tiébré et al. (2007) and Krebs et al. (chapter 2) and additional stands were determined based on morphological characters. When possible, seeds were collected for germination experiments and for flow cytometry to assess the ploidy level of seedlings and infer the putative paternal origin of seeds. The seeds were stored in paper bags for two weeks at 4°C and then kept dry at room temperature.

Table1: Number of *Fallopia* stands sampled for analysis of seed production (seeds), germination rate (germ.) and flower morphology (flowers).

Region	*F. japonica*			*F.* x *bohemica*			*F. sachalinensis*		
	seeds	germ.	flowers	seeds	germ.	flowers	seeds	germ.	flowers
Belgium	9	9	8	6	6	1	2		1
Odenwald	9	8	6	3	1	4	1	1	2
Freiburg	10	2	3	3	1	3	1		1
Munich	3	4	3	4	1	2	2	2	3
Ticino	6	1	1	6	4	6	0		

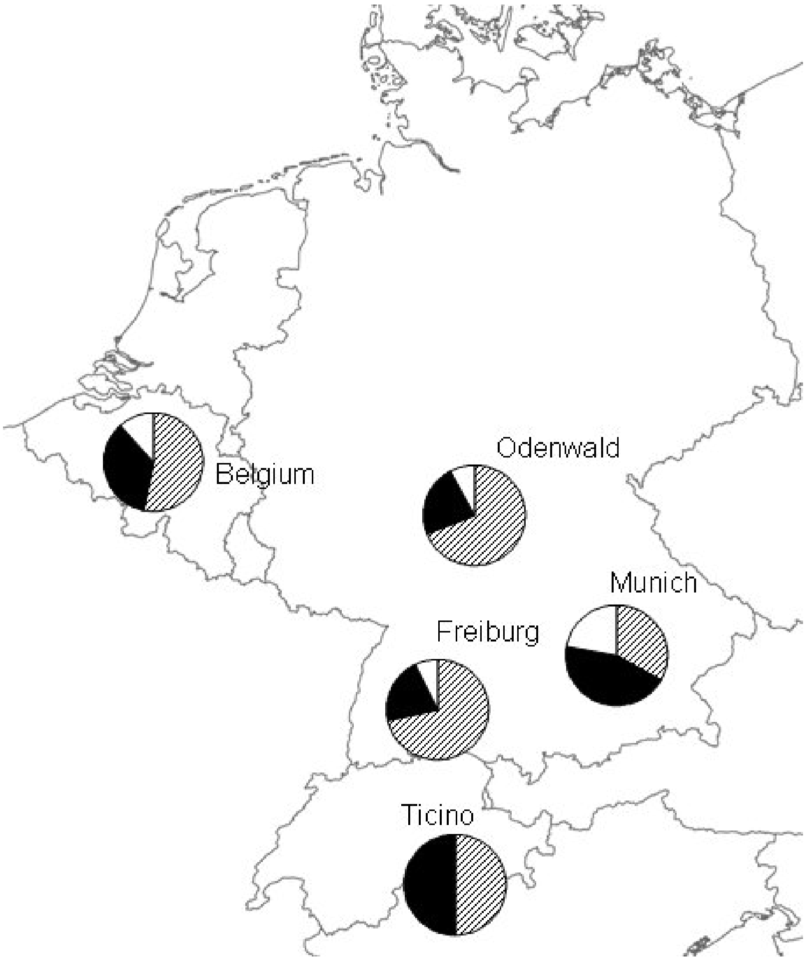

Figure 1: Distribution of the sampled regions and the proportional abundance of the three taxa *F. japonica* var. *japonica* (striped), *F. sachalinensis* (white) and *F.* x *bohemica* (black) within each region.

Seed production

In October 2006, the density of shoots was determined by counting the number of shoots in three 1 m^2 plots in each stand and calculating the mean number of shoots per 1 m^2. In each of the three plots the total number of seeds was counted for one randomly selected shoot and the mean number of seeds per shoot was calculated for each stand. The area covered by each stand (stand size) was estimated by measuring the longest two axes (length and width).

To estimate fruit set, the total number of flowers and seeds were counted on ten randomly selected axes (one branch within panicle) per stand in October 2006, except in Belgium, where total number of seeds and number of axes (one branch within panicle) was counted on three shoots per stand and the total number of fully developed flowers on three axes per stand. On each axis, the ratio of the number of ripe fruits to the number of developed flowers was calculated. As there is only one ovule per flower, fruit set was equivalent to seed set. Number of flowers per shoot was calculated by dividing number of seeds per shoot by seed set.

Germination

In May 2007, germination was determined for all stands with a sufficient number of seeds available (> 60 seeds; Table 1). In early May, seeds were placed into Petri dishes filled with moist sand and kept in an incubator at 20°C with a 16/8 hour day/night cycle to let them germinate. For each stand 20 seeds each were placed into three Petri dishes. The number of germinated and non germinated seeds was recorded in each Petri dish every second day during six weeks until no further seedlings developed. During the experiment seedlings were removed from the Petri dishes four days after their emergence to prevent mould.

Flower morphology and pollen viability

To determine the sex of flowers and pollen viability, flowers were collected in different stands in different regions in September 2007 (Table 1). In each stand at least three axes with flowers were chosen randomly and put in glass vials in 70% ethyl alcohol, sealed with parafilm and stored until determination. For six flowers per stand the length of the ovary and length of the anthers were measured and the ratio between length of anthers and ovary was calculated. This ratio can be used to assess the sex of flowers. If it is > 0.5, flowers tend to be male and if < 0.5, to be female. Mature stamens of six flowers per stand were crushed in a drop of Alexander solution (Jahier 1992) on a microscope slide and the pollen examined at X

200. Pollen was considered viable and potentially fertile if it appeared normal in shape and if the protoplasm was stained in red-violet and the pollen walls in blue (Jahier 1992). The percentage of viable pollen grains was calculated on the basis of 100 observations when sufficient pollen grains were present. In case of insufficient number of pollen grains, the total number of pollen was checked.

Ploidy level of seedlings

According to the availability of seeds, and to infer the putative paternal origin of seeds, the ploidy level was determined for 17 stands (12 *F. japonica* var. *japonica* and 5 *F.* x *bohemica*). These stands were not chosen randomly to cover a range of stands with different levels of seed production. In autumn 2007, 50 seeds per stand were placed into plastic boxes filled with common garden soil and the seedlings were grown in a greenhouse. Ploidy level of seedlings was measured by flow cytometry using the first two leaves of the young seedlings as described in Tiébré et al. (2007) and using the same *F. sachalinensis* clone (2n = 66) as internal standard.

Crossing experiments

Cut branches were used for the experiments following Bailey (personal communication). Shoots with flower buds of *F. japonica* var. *japonica*, *F. sachalinensis* and the hybrid between these two species, *F.* x *bohemica*, were collected in September 2006 in the region around Munich and the Odenwald region, Germany (Appendix A). RAPD analyses and flow cytometry had previously been used to confirm the different species, hybrids and their different RAPD patterns (Krebs et al. chapter 2). Shoots of at least 1 cm diameter were used to make sure that shoots had enough nutrients to survive during the experiment. The cut shoots were put into labelled plastic bottles filled with water and kept in a laboratory. The water was changed regularly. All leaves and open flowers were removed, so that just the flower buds remained on the shoots. The buds were covered with polythene bags, which were perforated slightly for ventilation. There were at least five shoots with several flower panicles from each stand. For the pollinations, flowers with ripe anthers (male flowers) were collected in Petri dishes and dried for an hour. At the end of September 2006, hand pollinations were carried out by dabbing male flowers with ripe anthers on female flowers with a ripe ovary. This was repeated at least three times with different male flowers to make sure that pollen was transferred.

Five *F. japonica* var. *japonica* clones from different sites were used as pollen acceptors (female flowers; Appendix A). However, although *F. japonica* var. *japonica* was collected from different sites, they all represented the same genotype. For *F.* x *bohemica,* eight RAPD genotypes and for *F. sachalinensis,* four RAPD genotypes were included as pollen acceptors. As pollen donor three *F. sachalinensis* and five *F.* x *bohemica* RAPD genotypes were used. All possible crossings between the taxa were carried out at each pollen acceptor stem using different genotypes of each taxon as pollen donor. Each taxon was pollinated with different genotypes of each possible taxon, but not each genotype with each genotype. Number of stems was recorded and stems were marked to keep track of different crossings. In addition, some flowers were left non-pollinated as control and a test for selfing was set up for *F. sachalinensis* and *F.* x *bohemica* by using flowers on the same individual stem as pollen donor and pollen acceptor. Non-pollinated flowers that were used as controls were removed from the stems. The number of pollinated flowers for each crossing combination was counted. The stems were kept in the water bottles until seeds turned brown and the fruiting perianth had been developed. Two weeks after pollination, the number of developed seeds was counted for each crossing combination. Five weeks after pollination, matured seeds were counted again for each crossing combination, removed, dried and stored in paper bags for two weeks at 4°C and then in a dry atmosphere at room temperature. Five months later, all seeds obtained from the crossings were placed on moistened sand in Petri dishes and kept in an incubator at 20°C with a 16/8 hour day/night cycle. To avoid fungal infection the perianth was removed. If there were enough seeds for the different crossing combinations, seeds were distributed between three Petri dishes. The germination experiment was carried out for five weeks to make sure that all viable seeds germinated. Germinating seeds were counted four times during the experiment and again at the end. The proportion of seeds germinating was calculated for each crossing combination.

A similar crossing experiment was carried out with *F. baldschuanica* in September 2007. Although the hybrid between *F. japonica* var. *japonica* and *F. baldschuanica*, *F.* x *conollyana,* is not common in nature, *F. baldschuanica* could be an important pollen donor for *F. japonica* var. *japonica* (Bailey 2007, Tiébré et al. 2007). Flowers of *F. baldschuanica* were collected in the Odenwald region of Germany and near Bern and Delémont, Switzerland, kept at 2°C and used as pollen donor. *Fallopia baldschuanica* was pollinated by three different *F.* x *bohemica* RAPD phenotypes and by *F. baldschuanica* (selfing) and some flowers were left unpollinated as control. *Fallopia sachalinensis* could not be used as a pollen donor because there was no plant material with male flowers available at that time. The resulting seeds were placed into Petri dishes and the experiment was stopped after 40 days after no germination had been observed.

Statistical analyses

The effects of plant taxon, region and stand on the number of seeds per shoot, number of flowers per shoot, on germination, and on the ratio length of anthers/length of ovary were analysed by ANOVAs with the variation among stands as the error term for taxon, region and their interaction. In addition, for number of seeds per shoot an ANCOVA was carried out with shoot density (number of shoots/m^2) and stand size as covariates, also using variation among stands as the error term for taxon, region and their interaction. To meet the assumptions of statistical procedures, i.e. homoscedasticity and normal distribution of residuals, number of seeds per shoot and shoot density were $\log_{10}$ +1, the ratio length of anthers/length of ovary was $\log_{10}$ and germination was arcsin-squareroot transformed.

The effect of plant taxon, region and stand on seed set (number of seeds/number of flowers) was analysed by analyses of deviance. The effect of plant taxon, region and the interaction were tested against the variation among stands. This analysis of seed set was carried out without the Belgian stands because the number of seeds was assessed in a different way compared to the other regions.

Pollen viability was analysed in two steps. First, analyses of deviance were carried out to explore the effects of taxon, region and stand on pollen viability (yes/no). Then, samples with viable pollen were analysed by ANOVAs. In both analyses, the effect of plant taxon, region and the interaction were tested against the variation among stands.

An analysis of deviance was used to analyse the effect of pollen acceptor (*F. japonica* var. *japonica* and *F.* x *bohemica*) and pollen donor (*F.* x *bohemica* or *F. sachalinensis*) on the seed set of *F. japonica* var. *japonica* and *F.* x *bohemica*. The effect of pollen acceptor was tested against shoot, pollen donor against the interaction pollen acceptor x pollen donor and the interaction pollen acceptor x pollen donor against the interaction pollen donor x shoot. The percentage of pollination success was calculated. Seed set in the crossing experiment with *F. baldschuanica* was analysed using ANOVA with pollen acceptor and shoot as factor. The effect of pollen acceptor was tested against shoot.

The relationships between number of seeds per shoot and shoot density, number of seeds per shoot and number of flowers per shoot, length of anthers and length of ovary, number of seeds per shoot and the ratio length of anthers/length of ovary, number of seeds of

F. japonica var. *japonica*, ratio length of anthers/length of ovary of *F.* x *bohemica* and number of average frost days and number of seeds per shoot were analysed by regressions. All analyses were carried out using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Seed production

The number of seeds per shoot varied highly significantly among stands, ranging from 0 to 865.77 seeds per shoot. Among stands the total number of seeds per stand varied from 0 to 70 300 000. Both region and the interaction region x taxon explained a significant amount of the variation found among stands (Table 2). *Fallopia japonica* var. *japonica* had the highest seed number per shoot in Munich and the Odenwald region and the lowest in Ticino, while *F.* x *bohemica* had the highest seed number per shoot in Freiburg and Ticino and the lowest in the Odenwald region (Fig. 2A). Shoot density had a significant effect on the number of seeds per shoot when used as a covariate (Table 2). Number of seeds per shoot increased with increasing shoot density ($r = 0.224$, $F_{1,192} = 10.12$, $P < 0.01$). On the other hand, the stand size had no significant effect on number of seeds per shoot when used as a covariate ($F_{1,50} = 0.15$, $P = 0.70$).

Table 2: Results of analyses of covariance of the effect of taxon, region and stand on number of seeds per shoot using shoot density as covariate. ***, P < 0.001 and **, P < 0.01

Source of variation	SS	df	MS	F	
Shoot density	8.40	1	8.40	25.13	***
Taxon	4.29	2	2.14	2.01	
Region	18.03	4	4.51	4.23	**
Taxon x Region	39.82	7	5.69	5.33	***
Stand	54.42	51	1.07	3.19	***
Residual	42.80	128	0.33		
Total	167.76	193			

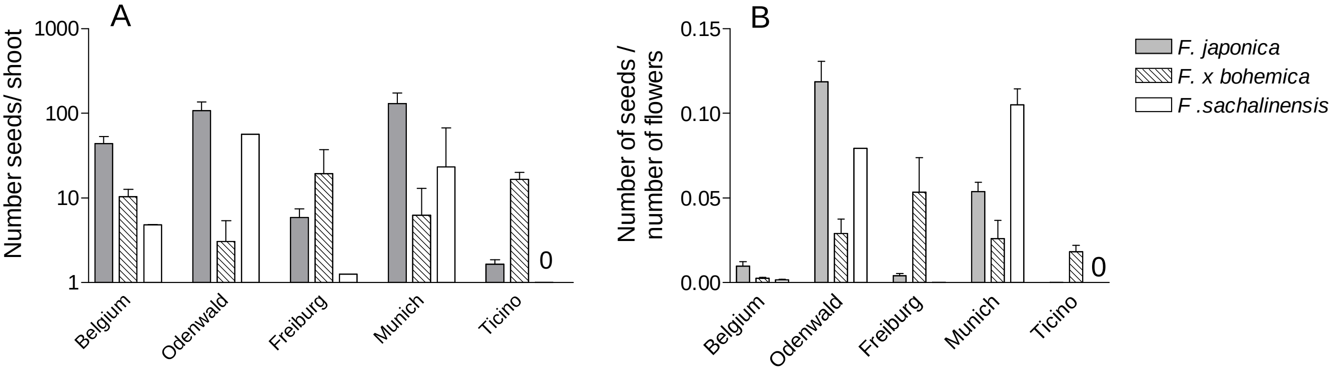

Figure 2: (A) Number of seeds per shoot (log-scale), (B) number of seeds/number of flowers (per axe) of the three taxa *F. japonica* var. *japonica, F. sachalinensis* and *F. x bohemica* per region. No *F. sachalinensis* stands were sampled in Ticino. Means + 1 SE.

We observed a highly significant effect among stands on seed set (Table 3) with seed set varying between 0 and 0.21 among stands. The taxon and the region and their interaction also had a significant effect on the seed set (number of seeds per axe/number of flowers per axe; Table 3). *Fallopia japonica* var. *japonica* had the highest seed set in the Odenwald region and the lowest in Ticino. *Fallopia* x *bohemica* had the highest in Freiburg and the lowest in Ticino (and Belgium, not included in analyses). *Fallopia sachalinensis* had the highest seed set in Munich and no seed set in Freiburg (Fig. 2B).

Table 3: Deviance analysis for the effect of taxon, region and stand on seed set (number of seeds per axe/number of flowers per axe). Belgian data were excluded from the test because of differences in measurement. ***, P < 0.001; **, P < 0.01 and *, P< 0.05

	Deviance	df	Mean Deviance	Quasi-F	
Taxon	62.47	2	31.24	3.28	*
Region	399.75	3	133.25	13.98	***
Taxon x Region	241.70	5	48.34	5.07	**
Stand	352.77	37	9.53	6.40	***
Residual	1581.83	353	1.49		

Taxon ($F_{2,35}$ = 2.51, P = 0.10), region ($F_{4,35}$ = 1.87, P = 0.14) and stand ($F_{35,93}$ = 1.45, P = 0.07) had no significant effect on the number of flowers per shoot (number of flowers per axe/seed set). Number of flowers per axe was not correlated with number of seeds per axe at the stand level, neither when combining the *Fallopia* taxa (r = 0.264, $F_{1,45}$ = 3.37, P = 0.07), nor when analysing them separately (*F. japonica* var. *japonica*: r = 0.22, $F_{1,27}$ = 1.37, P =

0.25; *F*. x *bohemica*: r = 0.342, $F_{1,12}$ = 1.59, P = 0.23; *F. sachalinensis*: r = 0.59, F_{123} = 1.08, P = 0.40). Mean number of seeds per shoot over regions was not correlated with average number of frost days per region (r = 0.579, $F_{1,3}$ = 1.52, P = 0.31).

Germination

The stand effect was highly significant ($F_{28,83}$ = 4.95, P < 0.001) and germination rates varied from 0 to 0.95 among stands. For seeds from *F. baldschuanica* no germination was observed. Germination did not significantly differ among the taxa ($F_{2,28}$ = 2.27, P = 0.12) from which seeds had been collected. The region ($F_{4,28}$ = 6.79, P < 0.001) had a significant effect on the germination of the seeds. The highest germination was recorded for seeds from Belgium and the lowest for seeds from Ticino (Fig. 3).

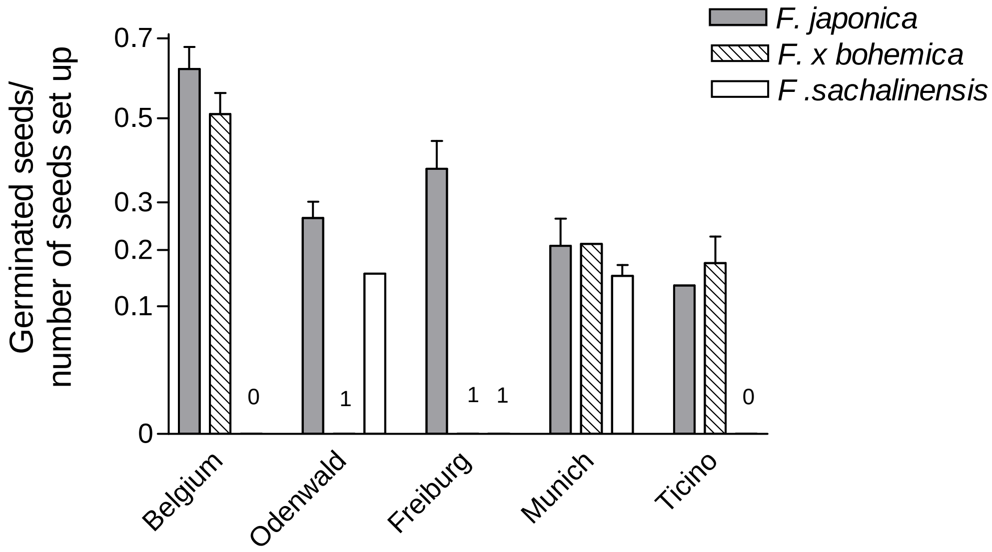

Figure 3: Germination of seeds (germinated seeds/number of seeds set up) collected from the three taxa *F. japonica* var. *japonica*, *F. x bohemica* and *F. sachalinensis* per region. Data were arcsin-squareroot-transformed. Numbers indicate number of stands, where seeds had been collected. Means + 1 SE.

Flower morphology and pollen viability

Stand had a significant effect on the ratio length of anthers/length of ovary ($F_{38,261}$ = 10.84, P < 0.001). Among stands the ratio length of anthers/length of ovary varied between 0.193 and 1.174. Within stands highest range of variation was from 0 to 1.26. Taxon ($F_{2,38}$ = 15.84, P < 0.001) and region ($F_{4,38}$ = 4.28, P < 0.01) also had a highly significant effect on the ratio length of anthers/length of ovary. The ratio showed very little variation among regions for *F.*

japonica var. *japonica*, whereas differences were observed among regions for the other two species (Fig. 4). *Fallopia* x *bohemica* showed the highest ratio length of anthers/length of ovary with values higher than 0.50 in Belgium, Odenwald and Munich while lower than 0.50 in Freiburg and Ticino (Fig. 4). For *F. sachalinensis* the lowest value was observed in Freiburg and the highest in Munich (Fig. 4). Length of anthers was negatively correlated with length of ovary (r = -0.197, $F_{1,438}$ = 17.70, P < 0.001). The mean number of seeds per shoot decreased with increasing mean ratio length of anthers/length of ovary when analysing *F. sachalinensis* and *F. x. bohemica* stands jointly (r = -0.345, $F_{1,37}$ = 5.002, P = 0.03; Fig. 5). The correlation was also significant for hexaploid *F.* x *bohemica* stands alone (r = -0.717, $F_{1,11}$ = 10.61, P = 0.009, Fig. 5), but not for *F. sachalinensis* stands (r = -0.434, $F_{1,4}$ = 0.93, P = 0.39, Fig. 5). At the regional level, the mean number of seeds per shoot of *F. japonica* var. *japonica* increased significantly with increasing mean ratio length of anthers/length of *F.* x *bohemica* stands (r = 0.903, $F_{1,3}$ = 13.32, P = 0.04; Fig. 6).

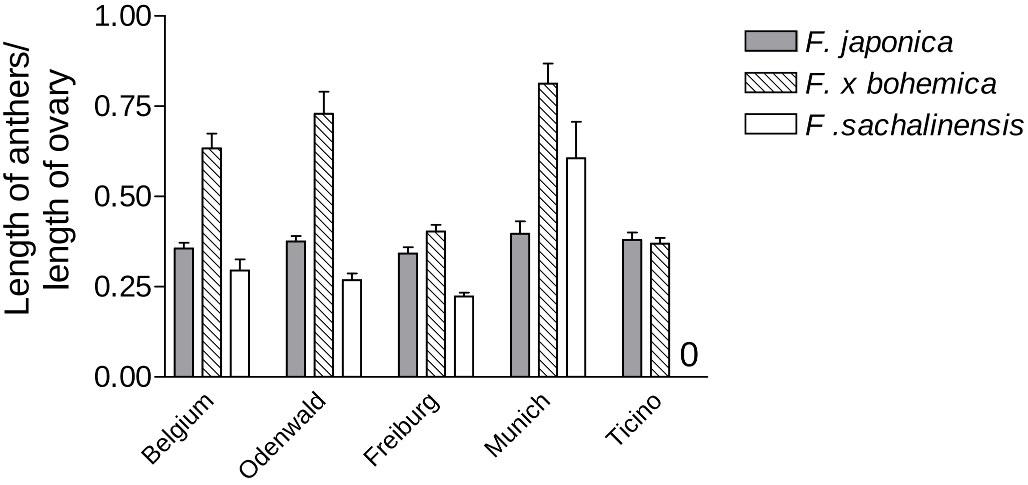

Figure 4: Ratio length of anthers/length of ovary of *F. japonica* var. *japonica*, *F.* x *bohemica* and *F. sachalinensis* in the different regions. No *F. sachalinensis* stands were sampled in Ticino. Means + 1SE.

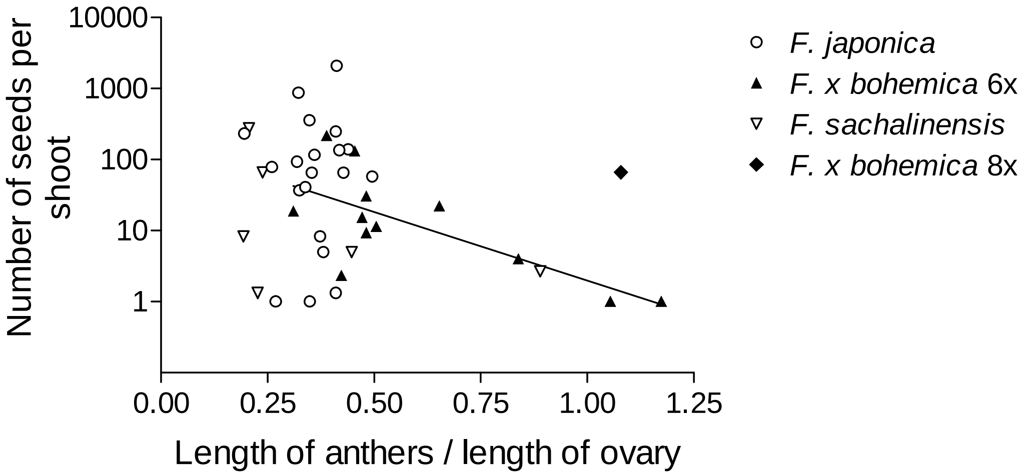

Figure 5: Relationship between length of anthers/length of ovary and number of seeds per shoot (log-scale). Regression line for *F.* x *bohemica* is shown.

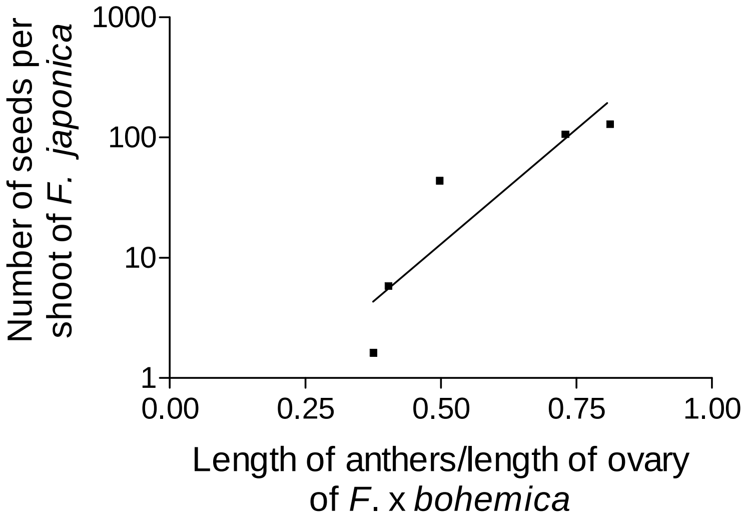

Figure 6: Relationship between length of anthers/length of ovary of *F.* x *bohemica* and number of seeds per shoot of *F. japonica* (log-scale). Regression line is shown.

Taxon (Quasi-$F_{2,38}$ = 20.41, $P < 0.001$) and region (Quasi-$F_{4,38}$ = 6.86, $P < 0.001$) had a significant effect on pollen viability. Stands of *F. japonica* var. *japonica* had no viable pollen (0/117), while for *F.* x *bohemica* the highest number of stands with viable pollen was recorded (52/127, *F. sachalinensis*: 45/60; Fig. 7). For *F.* x *bohemica,* highest pollen viability was observed in Ticino and the lowest in Freiburg (Fig. 7). When pollen viability was analysed excluding samples without viable pollen, taxon ($F_{1,10}$ = 7.17, $P < 0.05$) and stand ($F_{10,74}$ = 5.61, $P < 0.001$) had a significant effect. Pollen viability among the stands varied between 0 and 90% and within stands the highest range was from 0 to 88%. No fertile pollen was detected on any of the sampled *F. japonica var. japonica* stands. The ratio length of anthers/length of ovary was not correlated with pollen viability of *F. sachalinensis* (r = 0.483, $F_{1,9}$ = 2.74, P = 0.13) and *F.* x *bohemica* stands (r = 0.414, $F_{1,19}$ = 3.94, P = 0.06).

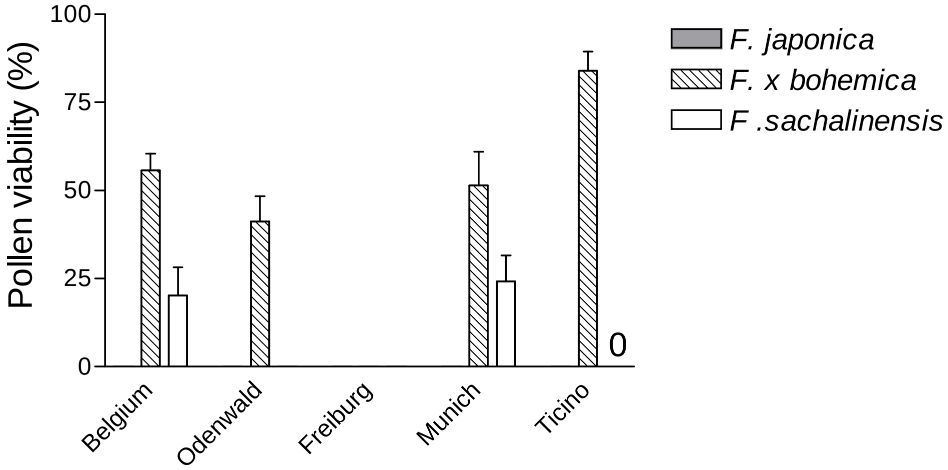

Figure 7: Pollen viability (%) of the three taxa *F. japonica* var. *japonica*, *F. x bohemica* and *F. sachalinensis* per region. Pollen viability in Freiburg was 0% for all flowers examined. No *F. sachalinensis* stands were sampled in Ticino. Means + 1 SE.

Ploidy level of seedlings

In Belgium, the large majority of seedlings from *F. japonica* var. *japonica* resulted from crosses with irregular or unreduced pollen of octoploid and hexaploid hybrids (87%). All other possible combinations also were observed, but at lower frequencies (Table 4).

Table 4: Ploidy level and putative male parent of seedlings from seeds collected from *F. japonica* var. *japonica*, and *F.* x *bohemica*. Frequency of ploidy levels are given for the five regions. Crosses between *F. japonica* var. *japonica* and unreduced or irregular pollen from the octoploid and hexaploid *F.* x *bohemica* could not been discriminated based on flow cytometry, as well as crosses where unreduced maternal gametes are involved and backcrosses of the octoploid *F.* x *bohemica* (Tiébré et al., 2007).

Maternal parent	Female gamete	Putative male parent	Male gamete	Ploidy levels of seedlings	Number of observed seedlings/ total number of seedlings				
					Belgium	Odenwald	Freiburg	Munich	Ticino
F. japonica	$x = 44$	*F. baldschuanica*	$x = 10$	$2n = 54$	3/92	11/45	2/13	0/20	0/20
$2n = 88$	$x = 44$	*F. sachalinensis*	$x = 22$	$2n = 66$	3/92	31/45	0/13	0/20	0/20
	$x = 44$	*F. sachalinensis*	irregular pollen	$2n = 44 - 66$	6/92	3/45	0/13	6/20	17/20
	$x = 44$	*F. x bohemica*	$x = 33$	$2n = 77$	68/92	0/45	11/13	10/20	3/20
	$x = 44$	*F. xbohemica*	unreduced or irregular pollen	$2n = 110$					
	$x = 44$	*F. x bohemica*	unreduced or irregular pollen	$2n = 110 - 132$	12/92	0/45	0/13	4/20	0/20
	unreduced $x = 88$	unknown	unknown	$2n = 110 - 132$					
F. x bohemica	$x = 33$	*F. x bohemica*	$x = 33$	$2n = 66$	19/71	0	2/13	0/7	0/9
$2n = 66$	unreduced $x = 66$	unknown	unknown	$2n = 77- 121$	52/71	0	11/13	7/7	9/9

For seeds collected on *F.* x *bohemica*, 27% resulted from crosses with putative *F.* x *bohemica*, while the other 73% exhibited high ploidy levels from 2n = 77 to 2n = 121 indicating crossings between unreduced maternal gametes and unknown paternal pollen or regular crosses with the octoploid hybrids. In the Odenwald region, the majority of seeds from *F. japonica* var. *japonica* potentially resulted from crosses with regular or irregular pollen of *F. sachalinensis* (76%). Crosses with putative *F. baldschuanica* were the only other combination observed (24%). In Freiburg, the large majority of seedlings from *F. japonica* var. *japonica* probably resulted from crosses with irregular or unreduced pollen of octoploid and hexaploid hybrids (85%) and crosses with *F. baldschuanica* were the only other combination observed (15%). For seedlings from seeds collected on *F.* x *bohemica* in the Freiburg region, 15% resulted from crosses with putative *F.* x *bohemica*, while the other 85% exhibited high ploidy levels from 2n = 77 to 2n = 121. In Munich, 50% of seedlings potentially resulted from crosses with irregular or unreduced pollen of octoploid and hexaploid hybrids, 30% from regular crosses with *F. sachalinensis,* and 20% from unreduced maternal gamete and unknown paternal pollen or regular crosses with the octoploid hybrid species. Seeds collected on *F.* x *bohemica* in the region of Munich exhibited very high ploidy levels indicating non-reduction of the maternal gametes. In Ticino, the main putative pollen donor was *F. sachalinensis*, but crosses with irregular or unreduced *F. x bohemica* were also possible (15%). For seedlings from seeds collected on *F.* x *bohemica*, 100% exhibited very high ploidy levels indicating unreduced gametes.

Crossing experiments

The pollen acceptor (*F. japonica* var. *japonica*, *F.* x *bohemica* and *F. sachalinensis*) and the pollen donor (*F.* x *bohemica* and *F. sachalinensis*) had a highly significant effect on seed set (Table 6). Seed set of *F. japonica* var. *japonica* was higher than seed set of the hybrid *F.* x *bohemica*, independent of pollen donor (Table 5). However hybrids used as pollen acceptor were mainly male clones. In the analyses we excluded the combination *F. sachalinensis* x *F.* x *bohemica*, because only one shoot gave seeds. Many shoots of *F. sachalinensis* died during the experiment. *Fallopia sachalinensis* was a better pollen donor than the hybrid *F.* x *bohemica.* No self pollination was observed and unpollinated flowers had no seed set. When *F. baldschuanica* was used as pollen donor, the pollen acceptor had no significant effect on seed set ($F_{2,7}$ = 0.37, P = 0.71). When comparing results of the two crossing experiments, seed set on *F. japonica* var. *japonica* was lower with *F. baldschuanica* as pollen donor than with *F.* x *bohemica* and *F. sachalinensis* (Table 5). Seed set on *F.* x *bohemica* was higher with pollen donor *F. baldschuanica* than with pollen donor *F.* x *bohemica* and *F. sachalinensis* (Table 5).

Table 5: Means of seed set (%, ratio number seeds/number of flowers pollinated) for pollen acceptor (*F. japonica* var. *japonica, F.* x *bohemica, F. sachalinensis* and *F. baldschuanica*) and pollen donor (*F. sachalinensis, F. baldschuanica* and *F.* x *bohemica*) with ± SE.

Pollen acceptor	Pollen donor	Seed set (%)	± SE (%)	Number of shoots
F. japonica	*F.* x *bohemica*	8.37	2.90	17
F. japonica	*F. sachalinensis*	31.23	6.91	18
F. x *bohemica*	*F.* x *bohemica*	0.40	0.40	22
F. x *bohemica*	*F. sachalinensis*	0.68	0.40	21
F. sachalinensis	*F.* x *bohemica*	2.50	2.50	10
F. sachalinensis	*F. sachalinensis*	0.00	0.00	8
F. japonica	*F. baldschuanica*	4.69	2.32	6
F. x *bohemica*	*F. baldschuanica*	14.64	7.84	8
F. sachalinensis	*F. baldschuanica*	6.90	4.42	5

Table 6: Results of an analysis of deviance with a logit link and binomial errors of the effect of pollen donor (*F.* x *bohemica* or *F. sachalinensis*) on the seed set of *F. japonica* var. *japonica* and *F.* x *bohemica* (pollen acceptor). Pollen acceptor was tested vs. shoot, pollen donor vs. pollen donor x pollen acceptor and pollen donor x pollen acceptor vs. pollen donor x shoot. The combination *F. sachalinensis* x *F.* x *bohemica* was not included in the analyses because it concerned only one shoot. ***, P < 0.001

	Deviance change	df	Mean Deviance	Quasi-F	P
Pollen acceptor	347.25	1	347.25	28.58	***
Shoot	327.95	27	12.15	37.61	***
Pollen donor	149.15	1	149.15	50.21	***
Pollen donor x Pollen acceptor	4.43	1	4.43	1.49	
Pollen donor x Shoot	77.12	26	2.97	9.19	***
Residual	852.24	2635	0.32		

The pollen acceptor (*F. japonica* var. *japonica* or *F.* x *bohemica*; Quasi-$F_{1,31}$ = 1.83, P = 0.19) and pollen donor (*F. sachalinensis* or *F.* x *bohemica*; Quasi-$F_{1,31}$ = 1.63, P = 0.21) had no significant effect on the germination of the seeds. The interaction pollen acceptor x pollen donor was also not significant (Quasi-$F_{1,31}$ = 0.33, P = 0.57). For seeds pollinated by *F. baldschuanica* no germination was observed.

Discussion

Our study revealed a very high level of variation in seed production and other reproductive traits among *Fallopia* stands in Europe. Total seed production of stands varied from no seed production at all to some 70 million seeds per stand. While the size of stands did not explain the variation in reproductive success, we found a positive correlation between the density of stems and the number of seeds per shoot, which might be explained by increased pollinator attraction. Number of flowers produced per axe predicted not the number of seeds produced per axe.

The significant effect of stand on the ratio length of anthers/length of ovary and on pollen viability of *F. sachalinensis* and *F.* x *bohemica* points towards different sex of flowers and different degrees of male fertility among stands within taxon, which in turn is likely to affect variation in seed set (Bailey 1994). A high ratio length of anthers/length of ovary indicates male-fertility, as evidenced by the decreasing number of seeds with increasing ratio. The hybrid *F.* x *bohemica* showed a strong negative correlation between number of seeds and the ratio length of anthers/length of ovary suggesting that sex of a stand is a key determinant of seed production by the hybrid under natural conditions.

The results indicate a high proportion of putative male hybrids among stands of *F.* x *bohemica.* However, hybrid stands with a high mean ratio length of anthers/length of ovary, indicating male-fertile flowers, also produced a few seeds, but less than female *F. x bohemica* stands. Within some of these putative male *F.* x *bohemica* stands, we observed a high variation in the ratio length of anthers/length of ovary indicating that some flowers are female, which explains the production of a few seeds. This is consistent with observations from the U.S. where most of the hybrid plants seem to have a subdioecious system with mainly male functioning flowers, but also a few female flowers (Forman and Kesseli 2003; Grimsby et al. 2007). Variation in floral morphology within plant individuals is also known from other plant species. Hermaphrodite flowers of *Muntingia calabura* produce a wide and continuous range of floral forms: at one extreme flowers have a large pistil and few stamens and at the other extreme there are flowers with a very reduced pistil and many stamens (Bawa and Webb 1983).

Variation in the ratio length of anthers/length of ovary in *Fallopia* is based on variable sizes of both the male and female flower characteristics (Vanderhoeven, unpublished results). Our sampling of flowers is only a snapshot and flower morphology could vary depending on time of collections and other external factors. Bailey observed that the anther filaments on *F. japonica* var. *japonica* can elongate as a result of treatment with certain herbicides and hermaphrodite flowers can have more ovary development towards the end of the season (Bailey, personal communication).

Taking sex of flowers into account there still remains unexplained variation in seed production in invasive *Fallopia* species in Europe. This is most evident in *F. japonica* var. *japonica* where significant variation in seed production was found, despite the fact that all flowers are female. Seed set of *F. japonica* var. *japonica* in crossing experiments was comparable to the highest seed set observed for this species in the field. This suggests that there is limitation of pollen either in terms of quantity or quality. It is worth noting that most studies considering sexual reproduction in the *Fallopia* complex characterized male-fertility vs. male-sterility of clones on the only approximate morphology of flowers without taking the pollen viability, a very pertinent character, into account (but see Tiébré et al., 2007). In our study we found high variation in pollen viability within and among stands of putative males of *F.* x *bohemica*. In the Ticino region, for example, the mean ratio length of anthers/length of ovary of *F.* x *bohemica* was similar to values observed for *F. japonica* var. *japonica,* which would lead to female assignment. However, pollen viability was higher than 80% and flowers produced a significant amount of pollen (Vanderhoeven, unpublished results). Besides, two distinct regions with similar regional hybridization patterns like Odenwald and Freiburg differ in the pollen viability of their hybrids, which could also influence the contrasting seed production we observed. In Freiburg, for example, pollen of *F.* x *bohemica* was not viable, which might explain the low seed production on *F. japonica* var. *japonica* plants in this region.

Experimental crosses performed in the present study revealed that the seed set was dependent of the pollen donor involved in crosses indicating that pollen suitability differs among potential pollen donors. A large range of ploidy levels was observed among offspring of the sampled *Fallopia* stands, sometimes with very high ploidy levels suggesting unreduced or irregular gametes. The results of the ploidy analysis indicate that in the Odenwald and the Ticino region, *F. sachalinensis* is a main pollen donor for this species. In contrast, the main pollen donor for *F. japonica* var. *japonica* in the other regions appears to be *F. x bohemica*. In the crossing experiment pollination of *F. japonica* var. *japonica* by *F. sachalinensis* and *F.* x *bohemica* resulted in relatively high seed set. However, it remains to be shown how easily the paternal ploidy level can be traced back from ploidy analysis of *Fallopia* offspring. For example, ploidy analysis of offspring, collected from *F. japonica* var. *japonica* stands in Ticino, suggest that the main pollen donor is *F. sachalinensis*. However, *F. sachalinensis* is rare in Ticino. Bailey found that chromosome numbers of plants produced from seed bear absolutely no resemblance to established plants growing in the wild (Bailey 2008). The correlation between seed production of *F. japonica* var. *japonica* and flower morphology of *F.* x *bohemica* at the regional level suggests that *F.* x *bohemica* is a more important pollen donor for *F. japonica* var. *japonica* than one would assume based on the ploidy analysis of the offspring.

Every trait measured in the study, except number of flowers per shoot, showed significant variation among the regions investigated, indicating that mechanisms operating at the regional level explained some of the variation observed in reproductive traits in the invasive *Fallopia* complex. Regional effects may arise from the different relative frequency of *Fallopia* taxa involved in hybridization. The different regions we investigated in our study are characterized by varying proportions of the taxa. Moreover, the variation in flower morphology and pollen viability as indicator of sex varied among and within regions indicating that different distribution and frequencies of male and female *F. sachalinensis* and *F.* x *bohemica* stands may also contribute to regional variation in seed production. In general, density of pollen donors is known to be correlated with availability of pollen (Davis et al. 2004). Especially in dioecious plants the distribution and frequency of male and female plants has an important influence on seed set (Graff 1999). In our study, we found overall more male *F.* x *bohemica* than female hybrids and male *F. sachalinensis*. For example in the region of Freiburg, where seed production was low, the lowest number and proportion of male hybrids and no male *F. sachalinensis* were recorded. In this region and in Ticino, where seed production of *F. japonica* var. *japonica* was also low, the ratio length of anthers/length of ovary of *F.* x *bohemica* had low values, indicating predominance of putative female hybrids. In regions with a high seed production on *F. japonica* var. *japonica*, the ratio length of anthers/length of ovary of *F.* x *bohemica* also was high.

Differences in climatic conditions may furthermore influence the fitness of clones. The plants require a hot dry summer and a frost-free late autumn for high seed production. Frost or even a cold spell is known to cause extensive flower drop (Beerling et al. 1994). Investigated areas span a large range of climatic conditions from the Belgian atlantic climate to dry continental in more eastern regions of Germany and submediterranean conditions in Ticino. Differences in the number of frost days may partly be responsible for the differences in traits we observed among regions. However, no significant correlation was found between average number of frost days and mean number of seeds per shoot over the regions, but this may be at least partly explained by the low power of this analysis.

Analyses of fitness related traits revealed a significant region x taxon interaction, indicating that the fitness of parental species and the hybrid varied depending on the environment. In both the Freiburg and Ticino regions, the hybrid *F.* x *bohemica* had higher seed set and produced more seeds per shoot than the parental species, while in Belgium, Odenwald and Munich, the opposite trend was observed. This was also demonstrated for other species through field experiments. For example, Bleeker & Matthiès (2005) focussing on German hybrid zones between invasive *Rorippa austriaca* and the native *R. sylvestris* showed that the fitness of hybrids relative to that of their parental species varied depending on the environment they were growing in. Such an idiosyncratic effect between *Fallopia* taxa and environment should be further experimentally investigated in common garden experiments.

Differences in pollinator behaviour or composition among regions might also contribute to variation in seed production, considering that *Fallopia* taxa are characterized by a late flowering period. For example, it has been hypothesized that the increase in seed production by *F. japonica* var. *japonica*, witnessed in the UK in the 1990s (Bailey et al. 2008), is the result of changing patterns of pollinator behaviour. Flower-constant pollinators, which are required when pollen sources are some distance apart, might need time to adapt to this new nectar source (Beerling et al. 1994).

The three taxa *F. japonica* var. *japonica*, *F. sachalinensis* and *F.* x *bohemica* significantly differed in their seed set, pollen viability and in the ratio length of anthers/length of ovary. The seed set of *F.* x *bohemica* was lower than that observed for both parental species, which is consistent with results from the crossing experiments and other studies on *Fallopia* taxa (Bailey 1994; Tiébré et al. 2007). The low maternal fitness of *F.* x *bohemica* could be due to the high proportion of male stands observed in our study and also reported by Bailey (1994). Even if male *F.* x *bohemica* plants do not produce high amounts of viable seed, the hybrids might be one of the most frequent pollen sources for *F. japonica* var. *japonica* beside *F. sachalinensis* (Bailey 1999). The high proportion of male *F.* x *bohemica* plants we found in our sampling might be a major component of the invasive success of *Fallopia* by promoting sexual reproduction which may increase genetic variability and lead to adaptation to new and different environments. If the proportion of hybrid plants will increase with further hybridization events, the opportunities for sexual reproduction could be even enhanced, contributing to an overall increase in the invasiveness of the *Fallopia* complex. Moreover, it is worth noting that it is not obligatory for hybrids to display a reproductive advantage compared to the progenitors for being successful. In the invasive *Fallopia* complex, highest regeneration ability was observed for the hybrid compared to the parental species (Bimovà et al. 2003). This argument is sometimes used to justify the current or to predict the future expansion of hybrids in Europe (Mandàk et al. 2004; Bailey et al. 2007). A synergistic combination of clonal growth and sexual reproduction is besides conceivable. One advantage of vegetative clonal growth is the increase in attraction of pollinators by increased floral display (Harder and Barrett 1996; Honnay and Jacquemyn 2008), which might explain the positive correlation we observed between the density of stems and the number of seeds per shoot.

Conclusion

While vegetative regeneration is considered as the main mode of reproduction of exotic *Fallopia* plants, there is growing evidence that sexual reproduction contributes to its invasion process (Krebs et al. chapter 2). Overall we found high variation in seed production in *Fallopia* stands, with the majority of seeds being viable. The ratio length of anthers/length of ovary and pollen viability partially explained variation in seed production of *F. sachalinensis* and *F. x bohemica*. However, sex of flowers obviously could not explain variation in the seed production of *F. japonica* var. *japonica*, since so far only one genotype with female flowers has been recorded from this species in Europe. A gradual variation in seed production and pollen viability was found among *F. sachalinensis and F. x bohemica* stands, with some stands with male flower morphology producing seeds and having low pollen viability. At the regional level, high seed production of *F. japonica* was correlated with high ratios length of anthers/length of ovary of *F. x bohemica*, indicating predominantly male flowers in hybrid stands serving as pollen donor. These results indicate that the local availability of compatible pollen is a key factor contributing to the variation in the seed production of invasive *Fallopia* taxa in Europe, and that regional variation in seed production is influenced by the distribution and frequency of male and female plants. The hybrid *F.* x *bohemica* showed no overall fitness advantage over its parental species in terms of the number of flowers per axe and the seed set. Nevertheless, the predicted increase in abundance of the hybrid *F. x bohemica* is likely to further enhance pollen availability and thereby promote sexual reproduction in the invasive *Fallopia* complex in Europe. Currently, seedling recruitment under natural conditions appears to be rare, but seeds can remain viable over winter and successfully germinate in spring (Krebs et al. chapter 4). A multiple site experiment suggests that recruitment and growth of seedlings will increase under the climate change predicted for large parts of Europe (Krebs et al. chapter 4). It remains to be shown whether the observed variation in seed production correlates with the current level of seedling recruitment in the *Fallopia* complex across Europe, or whether it should be considered as a potential for future levels of seedling recruitment of *Fallopia* taxa in the context of climate change.

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Appendix A: Taxon, origin (region and number of sampled stands) and sex of flowers of the plant material used as pollen acceptor and pollen donor in the crossing experiment. Sex of flowers was determined by pollen viability and flower morphology (ratio length of anthers/length of ovary, ect.).

Pollen acceptor (♀)				Pollen donor (♂)			
Taxon	Region	No. of stands	Sex	Taxon	Region	No. of stands	Sex
F. japonica	Odenwald	6	female	*F. x bohemica*	Odenwald	3	male
F. x bohemica	Odenwald	1	male/hermaphrodite	*F. x bohemica*	Munich	2	male
F. x bohemica	Odenwald	3	male	*F. x bohemica*	Munich	1	male/hermaphrodite
F. x bohemica	Munich	2	male/hermaphrodite	*F. sachalinenis*	Munich	1	hermaphrodite
F. x bohemica	Munich	2	male	*F. sachalinenis*	Munich	1	male
F. x bohemica	Munich	1	hermaphrodite	*F. sachalinenis*	Munich	1	male/hermaphrodite
F. sachalinenis	Odenwald	1	female				
F. sachalinenis	Munich	1	hermaphrodite				
F. sachalinenis	Munich	1	male				
F. sachalinenis	Munich	1	male/hermaphrodite				

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CHAPTER 4

Seedling establishment of invasive *Fallopia* taxa in Europe under different climatic conditions

With Urs Schaffner, Marco Moretti and Diethart Matthies

Abstract

In Europe, invasive *Fallopia* taxa spread mainly by vegetative means, despite the fact that female plants produce a lot of viable seeds. We assessed the likelihood of successful seedling establishment and thus spread by sexual reproduction of exotic *Fallopia* taxa in Europe and investigated whether the risk of seedling establishment might increase under the predicted climate change. Seeds of *Fallopia japonica* var. *japonica*, *Fallopia sachalinensis* and their hybrid *Fallopia* x *bohemica* were sown in autumn at six different sites from Northern Germany to Southern Switzerland in a standardized potting soil. We found that a large amount of seeds of all three *Fallopia* taxa remained viable throughout the winter, and between 25 and 75% germinated in the following spring at the different field sites. Most of the seedlings established successfully and survived until the following autumn, but performance during the first season varied significantly among regions and among mother taxa, with offspring of *F. japonica* var. *japonica* showing the highest performance. The lowest seedling emergence and performance were recorded at the coldest site and highest values at the most southern site under climatic conditions that closely match those predicted for large parts of northern Europe. While those plants which had high performance during the first season continued to grow during the second season, plants with low performance during the first season did not grow further during the second season. Our results suggest that under the predicted climate change, sexual reproduction and successful establishment of *Fallopia* seedlings might increase in Europe in the future. Increased levels of sexual reproduction could further promote the spread, increase the genetic variability and stimulate the invasiveness of the *Fallopia* complex.

Introduction

Biological invasions and climate change are two important components of global change (Dukes and Mooney 1999; Mack et al. 2000; Mooney and Hobbs 2000). Because of the considerable potential of biological invasions to alter ecosystems and their processes (Vitousek 1990), it is important to assess the future threats posed by invasive species and to assess if they will become more widespread (Willis and Hulme 2002). Climate change can play a fundamental role in the invasion process (Dukes and Mooney 1999; Mooney and Hobbs 2000; Willis and Hulme 2002), and it is thus important to investigate if climatic change is likely to increase the success of invasive species in their introduced range (Beerling 1993).

Altered environmental conditions due to climate change are likely to favour invasive species by promoting their establishment, growth, competitive ability or reproduction in the introduced range (Dukes and Mooney 1999; Menzel 2003; Thuiller et al. 2007). Exotic species may particularly profit from climate change if the new climatic conditions become more like those the exotic species experienced in their native range (Hellmann et al. 2008), or more generally, if climatic factors currently limiting their spread will become more favourable. For example, in the invasive *Impatiens glandulifera* and *Heracleum mantegazzianum,* both germination and growth in height increased with increasing temperatures in their introduced range in Europe (Willis and Hulme 2002). An increase in temperature due to climate change is therefore likely to further promote the spread of these two species in Europe both along latitudinal as well as altitudinal clines. For Europe increases in temperature, changes in precipitation regimes, milder winters and a lengthening of the growing season have been predicted (Alcamo et al. 2007). In most regions, especially at northern latitudes, warming will be more pronounced in winter than in summer and specifically at northern latitudes the mean annual precipitation will increase (Alcamo et al. 2007). At the moment the actual climate in the South of Switzerland corresponds to that predicted for large parts of Northern Europe with warmer summers, milder winters and heavier rain fall (Alcamo et al. 2007).

Japanese knotweed (*Fallopia japonica* var. *japonica* (Houtt.) Ronse Decraene, also known as *Reynoutria japonica* and *Polygonum cuspidatum*), giant knotweed (*Fallopia sachalinensis* (F. Schmidt Petrop.) Ronse Decraene) and their hybrid *F.* x *bohemica* (Chrtek et Chrtková) J.P. Bailey form one of the most troublesome invasive species complexes worldwide (Weber 2003; GISD 2005) and are capable of altering ecosystems (Vitousek 1990). The exotic

Fallopia taxa, native to eastern Asia, are widespread in most European countries (Beerling et al. 1994). *Fallopia japonica* var. *japonica* plants (*Fallopia japonica* hereafter) are highly vulnerable to late spring and early autumn frosts and are known to prefer areas with high precipitation (Beerling et al. 1994). The northern distribution limit of *F. japonica* in Europe appears to be controlled by the length of growing season and the minimum temperature (Beerling 1993). Distribution models, calculated on the basis of increasing temperature caused by global climate change, predict an expansion of the range of *F. japonica* in Northern Europe (Beerling 1993; Beerling 1994).

For successful invasion the mode of reproduction is an important determinant, directly influencing the likelihood of establishment and the rate of spread (Daehler and Strong 1994; Kolar and Lodge 2001). Vigorous clonal growth, but also the capability to reproduce sexually, which generates genetic variation, are frequent attributes of successful invaders (Pyšek et al. 2003).

In their native range *Fallopia* plants reproduce by sexual propagation and by vegetative regeneration (Tanaka 1966; Maruta 1976). In Europe, vegetative regeneration is usually considered as the main mode of reproduction of exotic *Fallopia* plants (Beerling et al. 1994; Bailey et al. 1995; Child and Wade 2000). Seedling survival and establishment of *Fallopia* in nature has relatively rarely been observed (Beerling et al. 1994; Bailey et al. 1995; Hollingsworth et al. 1998; Child and Wade 2000; Forman and Kesseli 2003; Tiébré et al. 2007). However, there is growing evidence that sexual reproduction also occurs in the introduced range (Bailey et al. 2007; Tiébré et al. 2007). In North America, interspecific hybridization and introgression has been shown to be common occurrences, indicating that sexual reproduction in the *Fallopia* complex is important for dispersal and especially for generating diversity (Forman and Kesseli 2003; Gammon et al. 2007; Grimsby et al. 2007).

In Europe, recent evidence for high genetic variation and regional differentiation in the hybrid *F.* x *bohemia* suggests that sexual reproduction also occurs in Europe (Bailey et al. 2007; Gammon et al. 2007; Tiébré et al. 2007). *Fallopia* plants can produce large numbers of viable seed in the introduced range (Bailey 1994; Beerling et al. 1994; Bram and McNair 2004; Tiébré et al. 2007) with up to several million seeds within one stand (Krebs et al. chapter 3), experimental studies show that seeds are able to survive the winter (Tiébré et al. 2007) and high germination rates have been reported under containment (Bailey 1994; Bailey et al. 1995; Alberternst 1998; Forman and Kesseli 2003). In the USA, seeds are able to overwinter and germinate under natural conditions (Forman and Kesseli 2003). However, germination has been found to be reduced after a winter cold period in experimental studies

(Forman and Kesseli 2003; Tiébré et al. 2007) and it has been proposed that seedlings of *Fallopia* are sensitive to frost and a cold humid winter (Beerling et al. 1994). In the native range seedling survival and dry weight of seedlings have been reported to be lower in higher altitudes due to lower temperatures and a shorter growing season (Maruta 1983; Beerling et al. 1994).

Increases in global temperature, milder winters and a lengthening of growing season due to the predicted climate change might result in increased germination rates, as well as higher establishment and survival of *Fallopia* seedlings. Low seedling establishment in the introduced range might be due to unsuitable climate conditions in many regions of Europe. Predicted and observed changes in mean temperature are most pronounced in winter and early spring (Sparks and Menzel 2002), which is the most important period for germination and seedling establishment. With increasing temperature, more frost-free days and extended growing seasons sexual reproduction and establishment of exotic *Fallopia* seedlings might therefore increase. For example, seedlings have been reported from Eastern Germany after several years without early frosts (Kosmale 1981; Alberternst 1998).

In the *Fallopia* complex sexual reproduction is probably not the most important means of spread, but it may nevertheless play an important role in the invasion process. Through frequent sexual events and hybridization new genotypes with new traits could originate, which could be better competitors and more successful invaders (Stebbins 1969; Arnold and Hodges 1995; Vilà and D`Antonio 1998). An increased genetic variability may facilitate adaptation to altered environmental conditions due to climate change. Even rare events of sexual reproduction can be sufficient to maintain high genetic diversity (Ellstrand and Roose 1987; Gabrielsen and Brochmann 1998).

So far, little is known about the extent of sexual reproduction and its contribution to the spread of the *Fallopia* complex in Europe. The aim of this study was to assess the likelihood of successful seedling establishment and thus spread by sexual reproduction of the exotic *Fallopia* species and their hybrids in Europe. More specifically, we aimed to (a) assess whether there is regional variation in the success of germination and seedling establishment across Europe, and (b) whether climatic conditions predicted for large parts of Europe under current climate change scenarios are likely to enhance seedling recruitment in the invasive *Fallopia* complex.

Material and Methods

Study species

Seeds used in this study were collected from the mother taxa *Fallopia japonica* var. *japonica*, *F. sachalinensis* and the hybrid between these species, known as *F.* x *bohemica*. All three taxa are clonal plants with erect stems of 1.5 - 4 m height and develop an extensive woody rhizome system and flower in late summer. Seeds mature in October, November and start germinating in March (Beerling et al. 1994).

In Europe only a single genotype of *F. japonica* var. *japonica* with male-sterile flowers has been found so far (Bailey et al. 1995; Hollingsworth et al. 1998; Hollingsworth 2000; Mandàk et al. 2005; Tiébré et al. 2007; Krebs et al. chapter 2). This naturalized and widely distributed species can thus only reproduce vegetatively (Hollingsworth 2000). *Fallopia sachalinensis* is also naturalized but is found less frequently. This species may produce male-sterile, hermaphrodite and male-fertile flowers (Mandàk et al. 2005; Bailey et al. 2007; Tiébré et al. 2007) and can act as a pollen donor for *F. japonica* var. *japonica* (Bailey 1994; Tiébré et al. 2007). The resulting hybrid, *F.* x *bohemica* (Chrtek et Chrtkova) J.P. Bailey, which shows partial to full fertility, may be either male-sterile or male-fertile (Bailey et al. 1996) and is also a pollen source for the *Fallopia* complex (Bailey 1994; Bailey et al. 2007; Tiébré et al. 2007). The hybrid grows and spreads faster than its parental species (Bimovà et al. 2003) and it has been suggested that it is more invasive than its parental species (Mandàk et al. 2004).

The experiment

In October 2006, seeds were collected in 15 populations of *F. japonica* var. *japonica*, four of *F. sachalinensis* and three of *F.* x *bohemica* in the regions Munich, Odenwald, Freiburg and Kiel in Germany, the Ticino area of Switzerland and in Belgium (Table 1). Seeds were collected from populations whose taxonomic status had previously been identified using a combination of morphological and cytological characters as recommended by Tiébré et al. (2007). A preliminary morphological identification was based on leaf characters including the presence of trichomes, length and width of the leaves, and the shape of the leaf base and apex (Beerling et al. 1994; Bailey et al. 1995; Bailey et al. 1996). Plant material from all populations was studied using molecular markers (RAPDs) and flow cytometry by Tiébré et al. (2007) and Krebs et al. (chapter 2).

Table 1: Number of stands of *F. japonica* var. *japonica*, *F. sachalinensis* and *F.* x *bohemica* from which seeds were sampled in different regions.

Country	Region	*F. japonica*	*F. sachalinensis*	*F. x bohemica*
Belgium		3		
Germany	Kiel	1		
Germany	Odenwald	7	1	1
Germany	Freiburg	1	1	
Germany	München	3	2	1
Switzerland	Tessin			1

The distance between the selected populations was at least 500 m to increase the chance of collecting different clones in each region. Due to this sampling we do not have information for the next possible pollen donor. However, we assessed the ploidy level of offspring, which can be used to infer the putative paternal origin in natural crosses, for four *F. japonica* var. *japonica* stands also included in this study and additional stands of *F. japonica* var. *japonica* and *F.* x *bohemica*. Ploidy level of seedlings was measured by flow cytometry as described in Tiébré et al. (2007) and Krebs et al. (chapter 3). Because plants of the sampled populations of *F.* x *bohemica* produced mostly male flowers the seed production of *F.* x *bohemica* was often very low, which is consistent with results from crossing experiments (Krebs et al. chapter 3). Therefore, we could only include few populations of *F.* x *bohemica* in the experiment. Moreover, populations of *F. sachalinensis* were scarce. Seeds were sampled randomly and stored cool and dry until the start of the experiment.

At the end of November 2006 seeds were sown into pots at six different sites from Northern Germany to Southern Switzerland (Table 2). When site names are used in the tables of analyses, names of sites are indicated only with their first letter. These sites cover a wide range of climatic conditions, including a cold site at high altitudes (Soubez) and a site with insubric climate (Cadenazzo). The insubric area of southern Switzerland is characterized by a moist, warm, temperate climate. While precipitation is low in winter, heavy rainfall occurs in summer (Moretti et al. 2006). All sites are within the range of the distribution of the *Fallopia* taxa in Western Europe and populations of *Fallopia* exist in the area. The insubric climate in the south of Switzerland corresponds to that predicted in future for large parts of Germany with warmer summers, milder winters and heavier rain fall (Alcamo et al. 2007).

All the growing sites were open and situated either in botanical gardens or at easily accessible locations. At each site five replicate pots with seeds from each of the 22 stands were set up. Into each pot 40 seeds were sown. The same pots (Plantpak penta 5, 13 x 9.6 cm, 850 ml, Hermann Meyer, Deutschland) and the same potting soil (CMI Pflanzerde, OBI Merchandise-Center GmbH, Wermelskirchen, Germany) were used at each site. The seeds were covered with c. 1 cm of soil and the pots were placed into enclosures (2 m x 2 m) filled c. 30 cm high with saw dust. At the beginning of the experiment the pots were watered regularly. During winter the pots were not watered and from spring until autumn only during dry periods. When the seedlings started to emerge, molluscicide pellets (Neudorff, Emmerthal, Germany) were used to prevent herbivory by slugs. From March 2007 onwards seedling emergence was recorded weekly. At the end of May 2007 the number of seedlings, the height, number of leaves and length and width of the largest leaf were recorded for each plant and the seedlings were thinned to three per pot. At the end of September 2007 plants were counted again and the height, number of leaves, length and width of the largest leaf and the diameter of the stem of each plant were recorded.

Table 2: Sites were *Fallopia* seeds were sown and seedlings grown and their climatic characteristics. [1]Climatic data for Soubez were not available, instead values for La Chaux-de-Fonds (1018 m a.s.l.) and for Bellelay (930 m a.s.l.) are given for mean temperature and precipitation, respectively. Both towns are also in the Jura mountains at a similar altitude.

Site	Latitude (north)	Longitude (east)	Altitude (m a.s.l.)	Mean annual temperature (°C)	Annual precipitation sum (mm)	Average number of frost days
D-Lüneburg	53°13\`42.8\`\`	10°24\`12.7\`\`	17	8.7	715	71
D-Marburg	50°48\`02.8\`\`	8°48\`25.6\`\`	280	8.0	640	69
D-Freiburg	48°00\`55.7\`\`	7°48\`27.5\`\`	280	10.2	800	76
CH-Delémont	47°22\`22.9\`\`	7°19\`32.3\`\`	415	8.0	960	93
CH-Soubez	47°17\`29.5\`\`	7°14\`09.4\`\`	950	5.8[1]	1360[1]	150
CH-Cadenazzo	46°09\`37.0\`\`	8°55\`52.0\`\`	211	12.0	1700	99

In Lüneburg, Freiburg and Soubez all plants were harvested above ground and the root systems carefully washed free of soil. The plant material was dried for 48 h at 60ºC and weighed. Plants at the other three sites were not harvested to determine the survival of the plants over winter. In mid May 2008, the surviving plants were counted in each pot and at the beginning of June the height, number of leaves, length and width of the largest leaf, and the stem diameter were measured for each plant. In July 2008, the remaining plants were harvested and their biomass determined as described above. For Lüneburg, Marburg, Freiburg and Delémont climate data (temperature and precipitation) were obtained from public climate stations (Deutscher Wetterdienst, MeteoSchweiz) and additional data (temperature) was provided for Marburg from “Hessisches Landesamt für Umwelt und Geologie”, for Lüneburg from “Verein Naturschutzpark Lüneburger Heide”. For Soubez climate data was provided by J. M. Carnal and for Cadenazzo from “Agroscope”.

Statistical analyses

For the analyses means per pot were calculated for each measurement. The effect of mother taxon and site on germination and measures of plant size were analysed by ANOVAs. Mother taxon and site were considered to be fixed factors, whereas stand of origin was considered to be a random factor. According to the rules for the analysis of mixed models (Zar 1996), the effect of mother taxon was tested against the variation among stands, those of site and the mother taxon by site interaction against the site by stand interaction, and the remaining terms against the residual. The effect of site was partitioned into the orthogonal contrasts Soubez vs. other sites (i.e. the coldest site vs. the remaining) and Cadenazzo vs. the rest (i.e. the warmest vs. the others). For plants in the second growing period only data from three sites were still available and the effect of site was partitioned into Cadenazzo vs. Marburg and Delémont. To meet underlying assumptions of statistical procedures, i.e. homoscedasticity and normal distribution of residuals, all data were log-transformed, except for germination data, which were arcsin-squareroot-transformed and survival, which were not transformed. Winter survival from 2007 to 2008 was studied by analyses of deviance.

The relationships between climate data (temperature, precipitation and number of frost days per year) and germination, and measures of plant performance were analysed by regression. We used the average temperature from January until April 2007 (average winter temperature), average annual temperature in 2007, precipitation sum from January until April in 2007 (winter precipitation sum), and annual precipitation sum of 2007 (Appendix A). For performance of seedlings in autumn the regression was carried out with average temperature from April until September 2007, (average summer temperature) and precipitation sum from April until September 2007 (summer precipitation sum; Appendix A). The relationship between height of seedlings in 2007 and height in 2008 was calculated by regression.

For offspring from each mother taxon separately, variance components were calculated to assess how much of the variation in height is explained by population of origin. To test if the morphology of offspring from each mother taxon varied among sites, the ratios leaf length/leaf width (leaf shape) of seedlings in autumn were computed, log-transformed and analysed by using one-way ANOVAs. The effect of site was tested against the interaction site x population of origin.

Results

Seedling emergence

Seeds of all taxa and from all stands germinated at all sites. Seedling emergence did not differ among mother taxa, but differed strongly among the stands of seed origin (Table 3). Seedling emergence differed among the sites, but emergence also depended on the specific combination of stand of origin and site (significant interaction between stand of origin and site in Table 3). The highest seedling emergence was recorded in Delémont, followed by Cadenazzo, and the lowest emergence was found in Soubez (Fig. 1B). Seedlings in Soubez differed significantly in their emergence from all other sites (contrast S vs. rest in Table 3; Fig. 1B) and seedlings in Cadenazzo significantly from Freiburg, Marburg, Delémont and Lüneburg (contrast C vs. F, M, D, L in Table 3; Fig. 1 B). Mean seedling emergence in Cadenazzo varied among stands of origin from 0.01 to 0.73, in Delémont from 0.03 to 0.77, in Lüneburg from 0 to 0.66, and in Soubez from 0 to 0.08.

Mean seedling emergence per site was not significantly correlated with average temperature in 2007 (r = 0.43, $F_{1,4}$ = 0.93, P = 0.39), average winter temperature (r = 0.32, $F_{1,4}$ = 0.46, P = 0.54), annual precipitation sum in 2007 (r = -0.09, $F_{1,4}$ = 0.03, P = 0.87) and winter precipitation sum (r = -0.37, $F_{1,4}$ = 0.62, P = 0.48). Number of frost days did not explain differences among sites in seedling emergence (r = -0.46, $F_{1,4}$ = 1.08, P = 0.36).

Table 3: Results of analyses of variance of the effect of mother taxon, stand of origin and site on seedling emergence (seedlings emerged per seed sown) from seeds of *F. japonica* var. *japonica*, *F. x bohemica* and *F. sachalinensis.* S: Soubez, C: Cadenazzo, F: Freiburg, M: Marburg, D: Delémont and L: Lüneburg. ***, P < 0.001; **, P < 0.01.

Source of variation	SS	df	MS	F	
Mother taxon	3.23	2	1.62	2.04	
Stand of origin	15.08	19	0.79	55.45	***
Site	8.39	5	1.68	31.11	***
S vs. other sites	4.60	1	4.60	49.00	***
C vs. (F, M, D, L)	0.40	1	0.40	11.89	**
Rest	3.39	3	1.13	80.71	***
Mother taxon x Site	0.27	10	0.03	0.51	
Site x Stand of origin	5.02	93	0.05	3.77	***
Residual	6.67	466	0.01		
Total	38.67	595			

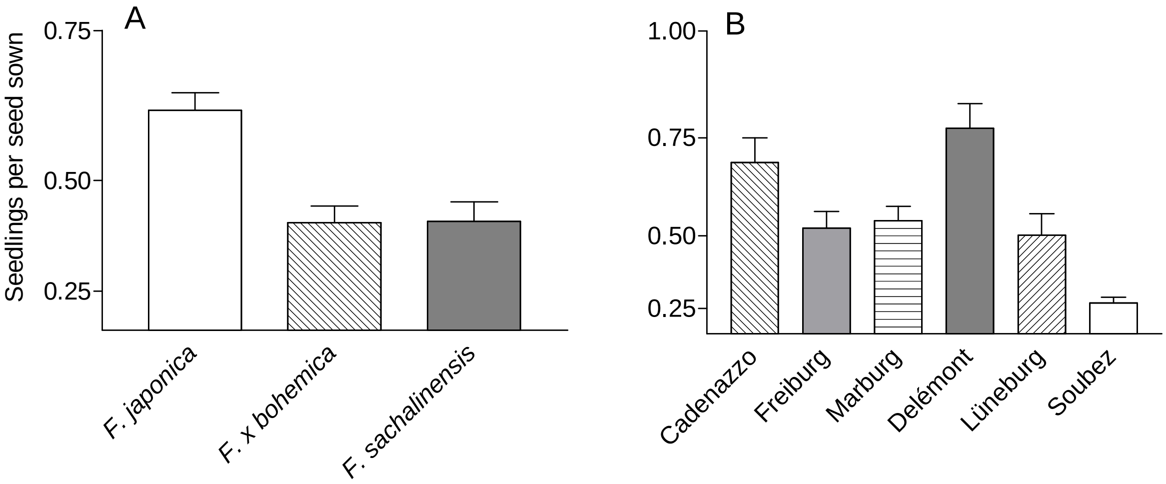

Figure 1: The proportion of seeds producing a seedling for seeds collected from *F. japonica* var. *japonica*, *F. sachalinensis* and *F.* x *bohemica*. (A) Means over the sites for the three taxa, (B) means over the taxa for the six sites. Means + 1 SE. Note angular scale for seedling emergence.

Early seedling establishment

Mother taxon, stand of origin and site had a significant effect on height, leaf length and width of the largest leaf of the seedlings in May 2007 (Table 4). But measures of plant size also depended on the specific combination of stand of origin and site (significant interaction between stand of origin and site in Table 4). The tallest seedlings with largest leaves were seedlings grown from seeds collected from *F. japonica* (Fig. 2A and C). The shortest seedlings were offspring from *F. x bohemia* (Fig. 2A and C). Seedlings grown from seeds collected from *F. sachalinensis* had larger leaves than seedlings from *F.* x *bohemica* (Fig. 2A and C). The shortest seedlings with the smallest leaves grew in Soubez, which differed significantly in their height and leaf length from seedlings from all other sites (contrast S vs. other sites in Table 4; Fig. 2B and D). The tallest seedlings with largest leaves were recorded in Cadenazzo, which differed significantly from seedlings in Freiburg, Marburg, Delémont and Lüneburg (contrast C vs. F, M, D, L in Table 4; Fig. 2B and D).

Table 4: Analyses of variance for the effect of mother taxon, stand of origin and site on the performance of seedlings in May 2007 grown from seeds from *F. japonica* var. *japonica*, *F. x bohemica* and *F. sachalinensis*. For abbreviation see Table 3. ***, P < 0.001; **, P < 0.01 and *, P < 0.05.

	Height			Leaf length		
Source of variation	df	F		df	F	
Mother taxon	2	12.63	***	2	10.17	**
Stand of origin	21	2.31	**	21	2.10	**
Site	5	14.60	***	5	40.29	***
S vs. other sites	1	36.41	***	1	179.26	***
C vs. (F, M, D, L)	1	11.74	**	1	34.15	***
Rest	3	7.74	***	3	19.71	***
Mother taxon x Site	10	1.21		10	1.71	
Site x Stand of origin	96	1.63	**	96	1.32	*
Residual	437			436		
Total	571			570		

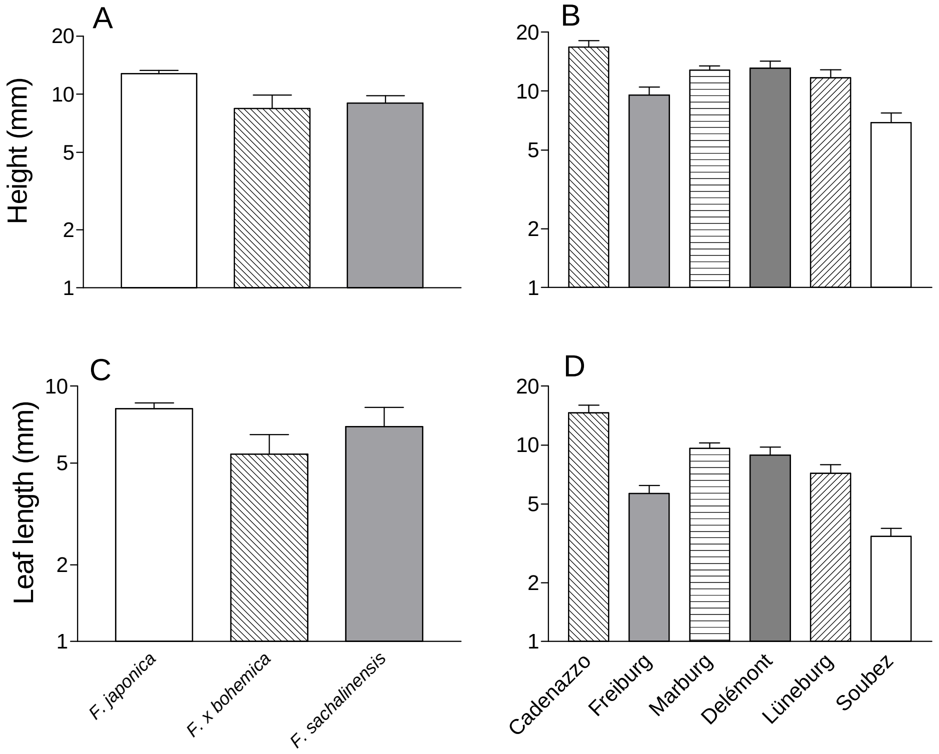

Figure 2: Height (log-scale) of offspring from *F. japonica* var. *japonica*, *F. x bohemica* and *F. sachalinensis* in May 2007. (A) Means over sites for the three taxa, (B) means over taxa for the six sites. Leaf length (log-scale) of offspring from *F. japonica* var. *japonica*, *F. x bohemica* and *F. sachalinensis* in May 2007. (C) Means over sites for the three taxa, (D) means over taxa for the six sites. Means + 1 SE

The mean height of the seedlings varied in Cadenazzo among stands of seed origin from 7.2 to 32.4 mm, in Lüneburg from 2.9 to 22.9 mm and in Soubez from 3.8 to 11.2 mm. Leaf length and width were highly correlated with each other (r = 0.99, $F_{1,570}$ = 34257, P < 0.001). The mean height of the seedlings at a site was not significantly correlated with average temperature in 2007 (r = 0.63, $F_{1,4}$ = 2.63, P = 0.18), average winter temperature (r = 0.42, $F_{1,4}$ = 0.86, P = 0.41), annual precipitation sum in 2007 (r = -0.11, $F_{1,4}$ = 0.05, P = 0.84) and winter precipitation sum (r = -0.24, $F_{1,4}$ = 0.25, P = 0.65). Height of seedlings was not correlated with number of frost days (r = -0.51, $F_{1,4}$ = 1.38, P = 0.31).

For offspring from each mother taxon it was tested how much of the variation was explained by site and stand of origin. Within *F. japonica* 24.5% of the variance in height of seedlings was due to site, 3.8% to stand of origin and 5.7% to the interaction site x stand of origin. Site ($F_{5,67}$ = 18.64, P < 0.001) and stand of origin ($F_{17,312}$ = 2.50, P < 0.01) were significant, but not the interaction between site and stand of origin ($F_{67,312}$ = 1.33, P = 0.06). Within *F.* x *bohemica* 19.7% of the variance in height of seedlings was due to site, 0% to stand of origin and 14.2% to the interaction site x stand of origin. The effect of site ($F_{5,17}$ =

2.99, P < 0.05) and the interaction site x stand of origin ($F_{17,61}$ = 2.02, P < 0.05) were significant, but not stand of origin ($F_{5,61}$ = 0.42, P = 0.84).

Within *F. sachalinensis* variance in height of seedlings was not explained by site and stand of origin. 2.3% of the variance was due to the interaction site x stand of origin. Site ($F_{5,10}$ = 0.81, P = 0.57), stand of origin ($F_{5,64}$ = 1.02, P = 0.46) and the interaction ($F_{10,64}$ = 1.25, P = 0.28) were not significant.

Establishment at the end of the first growing period

The mother taxon had a significant effect on height, leaf length and number of leaves, of the plants in October 2007 (Table 5). The tallest plants with the highest number of leaves (5.43 ± 0.7) and largest leaves (23.35 cm ± 1.39) developed from seeds collected from *F. japonica*, the second largest were those with *F. sachalinensis* (leaf length: 19.57 cm ± 3.23) as the mother plant and the shortest with smallest leaves (16.26 cm ± 2.19) developed from seeds collected from *F.* x *bohemica* (Fig. 3A). Offspring from *F. sachalinensis* had a lower number of leaves (2.08 ± 0.28) than *F.* x *bohemica* (3.49 ± 0.62).

Stand of origin and site had a significant effect on all measured traits (Table 5). The shortest plants with smallest leaves (4.74 mm ± 0.51) were recorded from Soubez (Fig. 3B). Plants in Soubez differed significantly from plants at all other sites (contrast S vs. other sites in Table 5). The tallest plants with the largest leaves were recorded for Cadenazzo (54.64 mm ± 3.74; Freiburg: 5.84 mm ± 0.56, Marburg: 37.04 mm ± 2.96, Delémont: 5.82 mm ± 0.48, Lüneburg: 21.55 mm ± 0.86; Fig. 3B). These plants differed significantly from plants in Freiburg, Marburg, Delémont and Lüneburg (contrast C vs. F, M, D, L in Table 5). Mean height of the plants varied among stands of seed origin in Cadenazzo from 42.7 to 213.8 mm, in Marburg from 7.8 to 114.8 mm and in Soubez from 3.2 to 22.4 mm. Plants from Cadenazzo had the highest number of leaves (16.04 ± 3.17), plants growing in Delémont the lowest number (0.76 ± 0.08, Freiburg: 1.51 ± 0.11, Marburg: 5.84 ± 0.36, Lüneburg: 3.01 ± 0.12, Soubez: 1.81 ± 0.13). The interaction site x stand of origin was significant for number of leaves and leaf length (Table 5). For leaf length the interaction between site and mother taxon was also significant (Table 5). Height was highly correlated with leaf length and width, number of leaves and diameter (r = 0.91, $F_{5,240}$ = 218.11, P < 0.001), diameter with height (r = 0.54, $F_{1,518}$ = 208.88, P < 0.001), leaf length with width (r = 0.97, $F_{1,518}$ = 9326, P < 0.001), width with height (r = 0.85, $F_{1,518}$ = 1316, P< 0.001). The above-ground biomass of the plants in Lüneburg, Freiburg and Soubez differed significantly among the sites ($F_{2,36}$ =

201.73, P < 0.001). The mother taxon had no significant effect on the biomass ($F_{2,19} = 3.40$, P = 0.06). The plants in Lüneburg had the highest biomass (8.8 mg ± 0.66) and the plants in Soubez the lowest (0.997 mg ± 0.38; Freiburg: 1.276 mg ± 0.21).

Table 5: Analyses of variance for the effect of mother taxon, stand of origin and site on height, leaf length and number of leaves of plants grown from seeds collected from *F. japonica* var. *japonica*, *F. x bohemica* and *F. sachalinensis*at at the end of the first growing season in October 2007. For abbreviation see Table 3. ***, P < 0.001; **, P < 0.01 and *, P < 0.05.

		Height		Leaf length		No. of leaves	
Source of variation	df	F		F		F	
Mother taxon	2	20.27	***	8.72	**	8.85	**
Stand of origin	19	2.06	**	3.95	***	3.05	***
Site	5	80.75	***	134.63	***	454.47	***
S vs. other sites	1	139.38	***	117.98	***	35.89	***
C vs. (M,F, D, L)	1	75.05	***	402.99	***	83.65	***
Rest	3	52.39	***	190.38	***	130.19	***
Mother taxon x Site	10	1.45		2.46	*	1.19	**
Site x Stand of origin	91	1.16		1.71	***	1.45	
Residual	392						
Total	519						

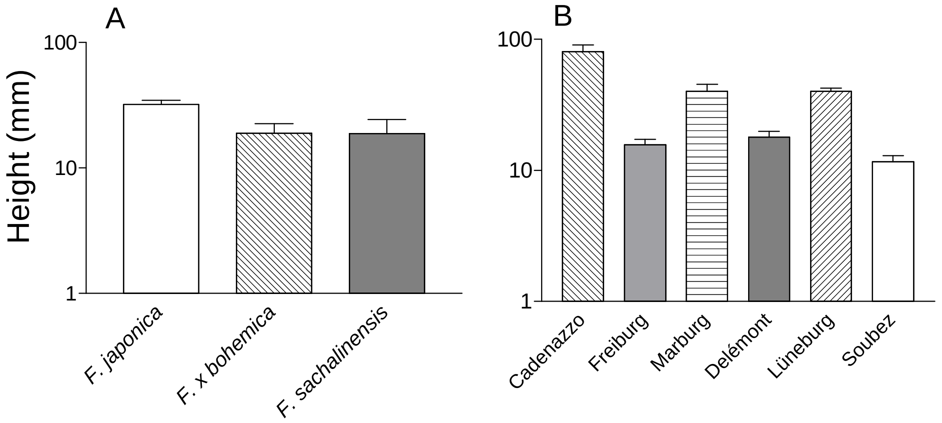

Figure 3: Height of plants (log-scale) grown from seeds collected from *F. japonica* var. *japonica*, *F. x bohemica* and *F. sachalinensis* at the end of the first growing season in October 2007. (A) Means over sites for the three taxa, (B) means over taxa for the six sites. Means + 1 SE.

The mean height of plants was most closely correlated with average temperature at the sites in 2007 (r = 0.60, $F_{1,4}$ = 2.30, P = 0.20), but due to low statistical power this relationship was not significant. The correlations with other climatic variables were lower: with average winter temperature (r = 0.35, $F_{1,4}$ = 0.54, P = 0.51), with average summer temperature (r = 0.54, $F_{1,4}$ = 1.67, P = 0.27), with annual precipitation sum in 2007 (r = -0.18, $F_{1,4}$ = 0.14, P = 0.73), with winter precipitation sum in 2007 (r = -0.11, $F_{1,4}$ = 0.04, P = 0.84) and with summer precipitation sum (r = 0.05, $F_{1,4}$ = 0.01, P = 0.92). Height of plants was not correlated with number of frost days (r = -0.49, $F_{1,4}$ = 1.24, P = 0.33).

The site at which the plants were grown influenced the morphology of the *Fallopia* plants. The ratio leaf length/leaf width (leaf shape) of offspring from *F. japonica* and *F.* x *bohemica* differed significantly among the sites (Table 6). For plants, which originated from *F. japonica,* the effect of stand of origin and the interaction site x stand of origin was also significant (Table 6). For seedlings from *F. japonica* the ratio varied among stands of origin from 1.2 to 1.5. In Cadenazzo leaf shape varied from 1.07 to 2.35, in Marburg from 1.34 to 2.4, Lüneburg from 1.32 to 1.65, in Freiburg from 1.07 to 1.44, in Delémont from 1.08 to 1.44 and in Soubez from 1.05 to 1.37.

For offspring from each mother taxon it was tested how much of the variation was explained by the population of origin. Within *F. japonica* 53.9% of the variance was due to site, within *F.* x *bohemica* 59.7%, and within *F. sachalinensis* 40.3%.

Table 6: Results of analyses of variance of the effect of site and stand of origin on the ratio leaf length/leaf width (leaf shape) for offspring of *F. japonica* var. *japonica*, *F.* x *bohemica* and *F. sachalinensis* in October 2007. ***, P < 0.001 and **, P < 0.01.

	F. japonica			*F. x bohemica*			*F. sachalinensis*	
	df	F		df	F		df	F
Site	5	16.54	***	5	5.08	**	5	3.02
Stand of origin	14	2.55	**	3	0.83		2	2.28
Site x Stand of origin	67	1.60	**	14	1.15		9	0.15
Residual	280			47			46	
Total	366			69			62	

Winter survival

Average winter survival for the *Fallopia* taxa ranged from 51 - 75%, and was not significantly affected by the mother taxon (Quasi-$F_{2,13}$ = 0.18, P = 0.84), the site (Quasi-$F_{2,13}$ = 0.06, P = 0.95) nor by the interaction between them (Quasi-$F_{4,13}$ = 0.15, P = 0.96; analysis of deviance).

Growth in the second growing period

In 2008 only plants at Marburg, Delémont and Cadenazzo were still available. In June 2008 the height, number of leaves, leaf length, and above- and below-ground biomass did not differ between offspring of the three *Fallopia* taxa, but differed between the populations of origin and between the three sites (Table 7). For leaf length the interaction mother taxon x site was significant and for the above- and below-ground biomass the interaction site x population of origin (Table 7).

Highest means for all variables were recorded in Cadenazzo, where plants differed significantly from plants in Marburg and Delémont (contrast C vs. other sites in Table 8; Fig. 4). The lowest means were recorded in Delémont (Fig. 4). Height was strongly correlated with diameter (r = 0.89, $F_{1,202}$ = 806.42, P< 0.001), height with leaf width (r = 0.95, $F_{1,204}$,= 1983, P< 0.001), and leaf length with width (r = 0.99, $F_{1,203}$ = 6459, P< 0.001). The height of seedlings in October 2007 was a good predicator of the height of plants in June 2008 (r = 0.78, $F_{1,59}$ = 88.76, P < 0.001).

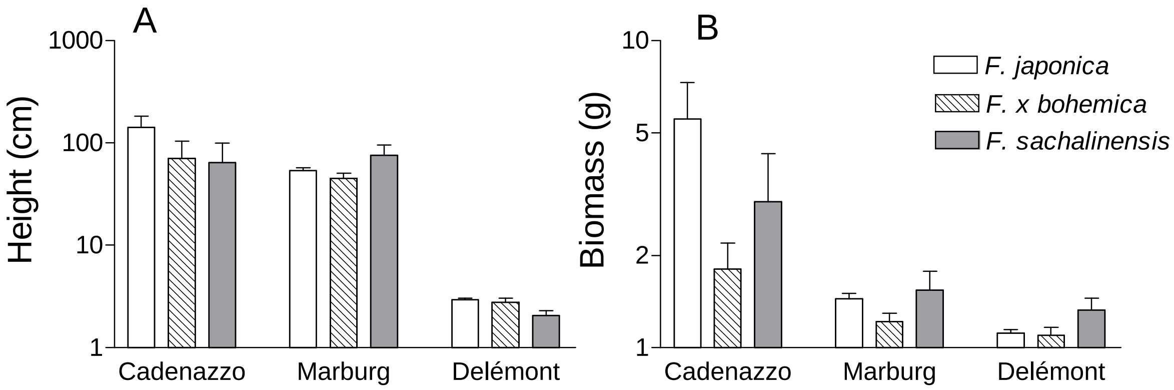

Figure 4: (A) Height (log-scale) and (B) above-ground biomass (log-scale) of plants grown from seeds collected from *F. japonica* var. *japonica, F. sachalinensis* and *F.* x *bohemica* at the three sites Marburg, Delémont and Cadenazzo in the second growing period in June 2008. Means + 1 SE.

Table 7: Analyses of variance for the effect of mother taxon and site on height, leaf length, leaf width, diameter, above- and below-ground biomass of offspring from *F. japonica* var. *japonica*, *F. x bohemica* and *F. sachalinensis* in the second growing period in June 2008. C: Cadenazzo. ***, P < 0.001; **, P < 0.01 and *, P < 0.05.

	Height			Leaf length			No. of leaves			Above-ground biomass			Below-ground biomass		
Source of variation	df	F		df	F		df	F		df	F		df	F	
Mother taxon	2	0.42		2	0.23		2	0.45		2	1.00		2	1.07	
Stand of origin	20	2.83	***	20	5.92	***	20	2.04	**	20	3.67	***	20	3.31	***
Site	2	272.40	***	2	1223.53	***	2	46.69	***	2	21.69	***	2	37.01	***
C vs. other sites	1	66.10	***	1	187.53	***	1	40.91	***	1	20.99	***	1	35.64	***
Rest	1	506.03	***	1	1293.53	***	1	38.58	***	1	3.95	*	1	11.11	**
Mother taxon x Site	4	1.08		4	4.29	**	4	1.49		4	1.10		4	0.83	
Site x Pop. of origin	33	1.37		33	0.79		36	1.22	.	37	2.71	***	37	2.09	**
Residual	142			139			144			217			218		
Total	203			200			208			282			283		

Discussion

In our study seeds of the invasive *Fallopia* species *F. japonica*, *F. sachalinensis* and of their hybrid *F.* x *bohemica* successfully survived the winter outdoors and germinated in the following spring. Seedlings grown from collected seeds from all three taxa established at all sites in spring and during the first vegetation period and a high percentage of plants survived the first winter. These results demonstrate that exotic *Fallopia* plants have the ability to reproduce sexually in addition to the vegetative reproduction in the introduced range. This is also supported by other studies, which have reported a high production of viable seed (Bailey 1994; Beerling et al. 1994; Bram and McNair 2004; Tiébré et al. 2007), seed capacity to overwinter and germinate outdoors in the introduced range (Forman and Kesseli 2003; Tiébré et al. 2007) and high germination rates (Bailey 1994; Bailey et al. 1995; Alberternst 1998; Forman and Kesseli 2003; Tiébré et al. 2007). Alberternst (1998) found at least one seedling in the Black Forest which survived the winter.

As far as we know, this is the first study in Europe, which investigates seedling recruitment outdoors under natural conditions over a longer period. Our study shows that seedlings can survive more than one vegetation period. However, our results also indicate that seedlings need a critical size to grow and develop beyond seedling stage. Offspring in Delémont was still alive in the second growing period, but most of the plants were small and had stopped growing. The decrease in height of plants in Delémont in the second growing period in comparison to the first year of growth, suggests that these plants would have not survived a third vegetation period. In contrast, seedlings in Cadenazzo, which performed well in the first growing season, showed also a high increase in height and biomass in the second growing period. Since at all sites soil in the pots was prevented to dry out completely by additional watering, the low performance at some sites can not be caused by drought. Performance in the first year seems to determine if seedlings survive and establish, while winter survival appears to be less important. During the first growing season only seedlings, which may reach a critical size can develop, while seedlings too small in size become stunted. Even if seeds are able to survive European winter and germinate and seedlings establish during the first vegetation period, most of the plants could be still too small to survive more than one winter. From the native range seedling size is known to be critical for establishment and it has been reported that seedlings must grow beyond a critical size, to form perennating-buds, for full freezing resistance and winter survival capacity (Maruta 1983; Maruta 1994).

Offspring from all three *Fallopia* taxa showed the ability to germinate and establish. However, mother taxon had a significant effect on seedling growth, with seedlings from seeds collected from *F. japonica* showing the highest performance. Highest seedling emergence also was recorded for seeds collected from *F. japonica* var. *japonica*. In the second growing period there was still the tendency of a higher performance of offspring from *F. japonica* var. *japonica* in comparison to the other taxa, but the effect of mother taxon was not signficant. While offspring from *F. sachalinensis* and *F.* x *bohemica* showed similar performance in the first growing period, offspring from the hybrid *F.* x *bohemica* was smaller and had a lower biomass than that from *F. sachalinensis* in the second growing period. The higher performance of offspring from *F. japonica,* has also been found in other experiments with seeds collected in the field and from crossing experiments (Krebs unpublished).

Due to the fact that only one male-sterile genotype of *F. japonica* occurs in Europe, offspring of exotic *Fallopia* plants mainly originate from hybridization among the *Fallopia* taxa. For example, one of the rare observations of established seedlings was in the Black Forest in Germany, and all of these seedlings had been identified as hybrid plants between *F. japonica* and *F. sachalinensis* (Alberternst 1998). All seeds produced by *F. japonica* are hybrids. Due to the infrequent distribution of *F. sachalinensis* (Tiebre 2007, Krebs et al. chapter 2) the majority of seeds from *F. sachalinensis* might also originate from hybridization. As pollen donor within the *Fallopia* complex serve *F. sachalinensis* and the Russian wine *F. baldschuanica* (Regel) Holub (also referred to as *F. aubertii* L. Henry) and moreover, backcrosses of the hybrid with the parental species and crosses between the hybrids are also possible (Bailey et al. 2007). Therefore, performance of offspring from *F. japonica, F. sachalinensis* and *F. x bohemica* may significantly depend on the compatibility of pollen from other *Fallopia* taxa. Assessment of ploidy levels of *Fallopia* seedlings suggest that in *F. japonica* stands *F. sachalinensis* appears to be the main pollen donor with low contribution of pollen of *F. baldschuanica,* while offspring from *F. x bohemica* seems mainly result from crosses with *F. baldschuanica.* Results from crossing experiments showed, that offspring of *F. japonica* from crosses with *F. sachalinensis* performed better than offspring from crosses with pollen donor *F. x bohemica* (Krebs et al. chapter 3). However, in the crossing experiments performance of offspring from *F. japonica* was in general higher than that of offspring from *F. sachalinensis* and *F.* x *bohemica,* indicating that the taxon of the mother plant has a stronger impact on fitness of the offspring than the taxon of the pollen donor.

Because most of the offspring within each *Fallopia* taxon may result from hybridization with several other *Fallopia* taxa whereas possible pollen donors are the same for each taxon, the significant differences in the performance of offspring of the three *Fallopia* taxa seem to be largely determined by the identity of the mother plant. This effect of the maternal parent on fitness of offspring has also been found for hybrids in other plant species (Campbell and Waser 2001; Burgess and Husband 2004; Kimball et al. 2008). In most flowering plants cytoplasmic genes, which have the potential to influence fitness, are maternally inherited (Kimball et al. 2008).

Seedling performance differed not only among offspring of different mother taxa, there was also a high variation among offspring from different stands of seed origin. The significant effect of stand of origin on seedling emergence and performance, even if corrected for the effect of mother taxon, indicates that pollen sources differ within mother taxon. Especially for seeds collected from *F. japonica* the variation among stands of origin was very high while in separate analyses for each mother taxon, stand of origin was only significant for *F. japonica*. While high variation in offspring of the genetic variable *F. sachalinensis* and the hybrid *F.* x *bohemica* with its high genetic variation and different hybrid stages (F1, F2, backcrosses) would be not surprising, it is striking that offspring from the genetically uniform mother plant *F. japonica* (Bailey et al. 1995; Hollingsworth et al. 1998; Hollingsworth 2000; Mandak et al. 2005; Tiébré et al. 2007) reveals this high variation among offspring from different stands of origin. These results suggest that pollen sources may differ among stands, leading to the variation in performance of offspring from different *F. japonica* stands. In our study most of the plants were still too small to identifiy the paternal taxon on morphological characters. However, numbers of chromosomes available for seedlings (see Krebs et al. chapter 3) and observed differences in morphology among offspring from one mother taxon in another experiment with seeds of some of the stands also used in this study (Krebs unpublished), indicate as well different pollen donors. The distribution of male *F. sachalinensis* and *F.* x *bohemica*, which serve as pollen donor, differ among regions and even locally (Krebs et al. chapter 2), which may lead to different hybridization patterns and different hybrid offspring.

Seedling emergence and performance differed significantly among sites with different climatic conditions. Statistical power was low due to the small number of sites, however, there was at least a trend of increased growth with higher temperatures and lower number of frost days. The lowest seedling emergence and lowest performance was recorded in Soubez, the coldest site at the highest elevation, where seedling emergence and performance differed significantly from all other sites. Highest seedling performance and a high germination success were observed in Cadenazzo, the most southern site with highest temperature for the winter period and highest average temperature in 2007. Seedling emergence and performance at this site differed significantly from Freiburg, Marburg, Delémont and Lüneburg, sites with higher temperature than Soubez. In Europe, this species seems to occupy broadly the same climatic range as in its native range in Southeast Asia (Beerling et al. 1995) and the results indicate that the climate in Europe provides suitable conditions for seedling establishment. The region around Freiburg, where seedlings have been found (Alberternst 1998), was in our study not particular suitable in comparison to the other sites. This indicates that seedling establishment could also occur in other regions in Europe. In our study a more suitable climate can be found in Southern Switzerland (Canton Ticino) where our study site Cadenazzo is located and where seedlings showed highest performance. One reason why so far no seedlings have been observed in the field in this region, might be due to a lack of compatible mates (Krebs et al. chapter 3), since *F. sachalinensis* seems to be absent in this region so far (Krebs et al. chapter 2; Moretti, personal communication). The results of our study suggest that the importance of sexual reproduction and the additional spread by seed of the exotic *Fallopia* taxa could increase with global warming and milder winters due to higher germination and survival rates. However, further experiments are required to study this, since the power in our study was low.

Temperature, especially in late winter and early spring, is known to be important for seedling recruitment for many plant species (Dzwonko and Gawronski 2002; Sparks and Menzel 2002; Walther 2003) and has been found to determine the germination and performance of other invasive plant species in the introduced range. For example, for invasive *Heracleum mantegazzianum* plants temperature was positively correlated with mean germination rate and plant height of invasive *Impatiens glandulifera* and *Heracleum mantegazzianum* increased with increasing temperature (Willis and Hulme 2002).

For Europe a warming between 1 and 5.5ºC has been predicted (Alcamo et al. 2007). The actual climate in the South of Switzerland corresponds to that predicted for large parts of Germany with warmer summers, milder winters and heavier rain fall (Alcamo et al. 2007). Cadenazzo, located in the South of Switzerland and the most southern site in our study, is on average 2ºC warmer than Lüneburg, the most northern site and Soubez on 1000 m elevation. The higher seedling emergence and performance found in Cadenazzo in comparison to seedlings at the colder sites indicate that seedling recruitment could be enhanced with further increasing temperature and lengthening of freeze-free periods. Milder winters for example could not only increase germination and survival rates of seedlings of the exotic *Fallopia* complex, but would also mean longer growing seasons, that could lead to higher seed production and seed quality (Hellmann et al. 2008).

Even if precipitation has been reported to be less connected with seedling recruitment than temperature (Dzwonko and Gawronski 2002), the trend of higher seedling emergence and performance with lower precipitation sum indicates that precipitation also influence the germination success and seedling performance of exotic *Fallopia*. High precipitation during winter could for example lead to high soil moisture, which can have a negative effect on survival and germination of *Fallopia* seeds due to fungal infestation of seeds (Bailey et al. 1995; Bailey 2001; Bailey 2003; Tiébré et al. 2007). High soil moisture in winter was observed in Soubez and Freiburg, where low seedling emergence was recorded. However, the set up of our study makes it difficult to discuss the influence of differences in precipitation on seedling establishment, at least for older seedlings, due to additional watering in dry periods during the vegetation period.

Even if in the exotic *Fallopia* complex sexual reproduction is not the main mode of reproduction so far, the balance between vegetative and sexual reproduction, which affects the genetic diversity and the long-term survival of plant populations (Callaghan and Jonasson 1995), could shift towards more sexual reproduction than observed today in response to climate warming (Aerts et al. 2006).

Beside climatic factors like a negative effect of cold humid winter, the low seedling establishment observed in the field in the introduced range could be also due to other factors, for example to unsuitable soil conditions (Bailey et al. 1995), resource limitation (Forman & Kesseli 2003), or a lack of suitable habitats (Pyšek and Prach 1993). Strong competition of seedlings for example underneath well established *Fallopia* stands might be another important factor (Forman and Kesseli 2003) and Forman & Kesseli (2003) suggest that seedling establishment has just not been detected in the field because of searching only in *Fallopia* infested areas where competition is too high. Because the majority of seeds fell near the maternal clone (Tiébré et al. 2007) only a few seeds might be able to reach suitable microsites for germination. Establishment is restricted to open, bare ground (Beerling 1993) due to the high shade-intolerance of exotic *Fallopia* plants (Beerling et al. 1994; Forman and Kesseli 2003).

Limited availability of pollen, for example resulting from low densities of pollen donors and skewed sex ratios (Burke and Arnold 2001; Davis et al. 2004; Taylor and Hastings 2005; Elam et al. 2007) can also lead to reduced seed production and thus recruitment (Beerling 1993; Bailey et al. 1995), mainly confined to species with animal pollinators (Lamont et al. 1993). Finding compatible mates is especially challenging for obligatory outcrossing plant species, such as those that are self-incompatible or dioecious (Elam et al. 2007) like plants of the exotic *Fallopia* complex (Bailey 1994; Beerling et al. 1994). We found for example in regions where exotic *Fallopia* plants had a high seed set and hybrids a high genetic variability, higher densities of male *F. sachalinensis* and male *F.* x *bohemica* plants than in regions with low seed set (Krebs et al. chapter 2 and 3).

Conclusion

High seed set, high germination rates, winter survival capacity of seedlings and the occurrence of many different genotypes of the hybrid *F.* x *bohemica* provide evidence that sexual reproduction and successful seedling establishment of the invasive *Fallopia* taxa occur in the introduced range. By the establishment of new hybrid plants, which can act as pollen donors and replace the missing male *F. japonica* plants in Europe, sexual reproduction might be promoted and increases genotypic variation in the invasive *Fallopia* complex (Hollingsworth 2000; Tiébré et al. 2007). By the origin of novel genotypes, which may have an increased adaptation potential in the introduced range (Stebbins 1969; Arnold and Hodges 1995; Ellstrand and Schierenbeck 2000; Rieseberg et al. 2007), sexual reproduction may enhance the invasion success of the *Fallopia* complex in Europe. Only a small number of established seedlings per year can already be sufficient to increase the genetic diversity (Ellstrand and Roose 1987). Moreover, the hybrid of *F. japonica* and *F. sachalinensis, F.* x *bohemica* has a higher regeneration potential (Bimova et al. 2003), which could additionally enhance the spread also by vegetative reproduction. With an increase in the proportion of hybrids, from which male-fertile plants are reported to be more frequent than male-sterile (Bailey 1994; Beerling et al. 1994), the density of pollen sources necessary for sexual reproduction would also increase leading to increased opportunities for sexual reproduction.

The importance of sexual reproduction and the risk of successful seedling recruitment could increase with further increasing temperatures, milder winters and lengthening of growing season predicted for the climate change in Europe. Results of this study and observations of a remarkable increase in fruit set on *Fallopia* plants in the UK during the last 30 years (Bailey et al. 2008), where the rate of warming has been greater than at any other time during the past 1000 years (Houghton et al. 2001), support this notion. Increased sexual reproduction, mainly by hybridization, may give the possibility of enhanced invasiveness of exotic *Fallopia* plants and additional dispersal by seed could increase the spread. With an increasing density of male *F.* x *bohemica* plants the opportunity for sexual reproduction could be even further enhanced.

For the development of control and management strategies against invasive *Fallopia* plants in Europe the importance of sexual reproduction should not be underestimated particularly in the face of global warming (Forman and Kesseli 2003; Tiébré et al. 2007)

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Appendix A: Temperature (temp.) and precipitation (precip.) at the growing sites in 2007, sorted by temperature.

Site	Average Temp. 2007 (Cº)	Precip. sum 2007 (mm)	Winter temp. (Cº)	Winter precip. (mm)	Summer temp. (Cº)	Summer precip. (mm)
CH-Cadenazzo	12.3	1244.7	8.57	162.2	18.30	962.6
D-Freiburg	11.13	837.1	8.20	187.9	16.42	531.5
D-Marburg	10.48	798.8	7.45	384.1	15.72	487.2
CH-Delémont	10.37	1118.9	7.28	227.4	15.79	724.2
D-Lüneburg	10.03	869.9	6.63	244.2	14.71	465.8
D-Soubez	9.93	1360	7.06	286.0	15.19	862

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CHAPTER 5

Herbivore resistance of invasive *Fallopia* species and their hybrids

With Esther Gerber, Diethart Matthies and Urs Schaffner

Abstract

Hybridization has been proposed as a mechanism by which exotic plants can increase their invasiveness. By generating novel recombinants, hybridisation may result in phenotypes that are better adapted to the new environment than their parental species. We experimentally assessed resistance of the parental species *Fallopia japonica var. japonica*, *Fallopia sachalinensis* and *Fallopia baldschuanica*, the two hybrids *Fallopia x bohemica and Fallopia conollyana* as well as the common European plants *Rumex obtusifolius* and *Taraxacum officinale* to four native European herbivores, the slug *Arion lusitanicus,* the moth *Noctua pronuba*, the grasshopper *Metrioptera roeselii* and the beetle *Gastrophysa viridula.* Leaf area consumed and relative growth rate of the herbivores differed significantly between the *Fallopia* taxa and the native species as well as among *Fallopia* taxa, and was partly influenced by interspecific variation in leaf morphology and physiology. *Fallopia japonica* var. *japonica*, the most abundant *Fallopia* taxon in Europe, revealed the highest level of resistance against all herbivores tested. The level of resistance of the hybrids varied depending on the herbivore species tested; in most of the cases, the hybrids resembled the more susceptible parent, followed by hybrids with intermediate levels of resistance. Genotypes of the hybrid *F. x bohemica* varied significantly in the leaf area consumed, but never exceeded that of the parental species. In general, exotic *Fallopia* taxa were more resistant to the European herbivores than the two native plant species, suggesting that both parental and hybrid *Fallopia* taxa largely escape from herbivory in their introduced range in Europe.

Introduction

Hybridization is a widespread feature of plants and an important mechanism in their speciation and evolution (Stebbins 1969; Ellstrand et al. 1996). Gene combinations resulting from hybridization may result in phenotypes that have a level of tolerance to environmental conditions that is intermediate to those of the parental species or similar to one of the parents (Schwarzbach et al. 2001; Whitney et al. 2006). Moreover, hybridization and subsequent segregation can generate novel recombinants that are better adapted to certain environments than either of their parental species and may therefore be favoured by natural selection (Stebbins 1969; Rieseberg 2003). For example, colonization of extreme habitats by sunflowers was made possible by interspecific hybridization and subsequent selection on extreme or “transgressive” hybrid phenotypes (Rieseberg 2003). Hybridization may entail also a change in ploidy level, which by itself is considered to be an important mechanism in plant evolution (Soltis et al. 2000; Thompson et al. 2004). Polyploids tend to be more successful than their diploid ancestors due to a wider ecological range, better colonizing abilities, increased rate of adaptation, masking of deleterious alleles or increased heterozygosity (Levin 1983; Soltis et al. 2000).

A major selective force affecting the evolution of plant traits is herbivory (Marquis 1992). Herbivores can distinguish between hybrid and parental genotypes (Fritz et al. 2003) as well as among different hybrid genotypes (Fritz et al. 1998), indicating that interactions with herbivores may also play an important role in the evolution of hybrids. Hybrids inherit resistance traits from both parental species, but the overall resistance of hybrids against natural enemies depends on the expression and interaction of these traits (Fritz et al. 1999). Often, the level of herbivore resistance in hybrids was found to be intermediate to that of their parental species (Boecklen et al. 1990; Aguilar et al. 1992; Fritz et al. 1996) or to resemble that of one of the parental species. In the latter case the more common pattern is that hybrid resistance resembles that of the more susceptible parent (Fritz et al. 1996). However, some hybrids were also found to be more (Boecklen et al. 1990; Fritz et al. 1994; Fritz et al. 1996; Whitham et al. 1999) or less resistant to herbivores than their parental species (Boecklen et al. 1990; Fritz et al. 1996).

Polyploidization has also been shown to affect interactions with herbivores (Nuismer et al. 2001; Münzbergova 2006). Plants with different ploidy levels differ in their plant secondary metabolism, in life history traits as well as in morphology, all of which might affect their palatability and the response of herbivores (Levin 1983; Thompson et al. 1997).

In general, plants with higher ploidy levels tend to be more resistant to herbivores than plants with lower ploidy levels, which may contribute to the success of polyploids (Levin 1983).

Recently, it has been suggested that hybridization and polyploidization may act as a stimulus for the evolution of increased invasiveness in exotic plant species (Ellstrand et al. 2000; Müller-Schärer et al. 2004). Support for this hypothesis comes from a study by Gaskin and Schaal (2002) documenting extensive hybridization in North America between two exotic *Tamarix* species that are geographically isolated in their native Eurasian range, with the hybrid being the most invasive taxon. Polyploid plant species have been found to be overrepresented among invasive species in regional floras (Verlaque et al. 2002), indicating that polyploidy promotes the establishment and spread of exotic plant species.

The enemy release hypothesis (ERH) posits that plant species, on introduction to an exotic region, experience a reduced top-down regulation by natural enemies, allowing them to increase in distribution and abundance (Keane & Crawley 2002). However, while introduced plant species loose interactions with natural enemies from their native range, they are exposed to a new set of enemies from the resident community in the introduced range (Mitchell et al. 2006). It has been proposed that most introduced plant species do not become invasive because they are vulnerable to generalist herbivores that are native to the introduced range and with which they share no evolutionary history (Parker et al. 2005). In line with the biotic resistance hypothesis, introduced non-invasive plants were found to interact with more new pathogens or experience more herbivory than introduced invasive plant species (Cappucino et al. 2005). If introduced plant species are vulnerable to generalist herbivores, and hybrids are more resistant to this evolutionary new set of herbivores than their parental species, the invasion success of hybrids might surpass that of parental species.

Several taxa of the genus *Fallopia*, originating from East-Asia, are among the most troublesome invasive weeds worldwide (Weber 2003). In the introduced range, hybridization occurs among three of the introduced species, i.e. the octoploid *F. japonica* var. *japonica* (Houtt.) Ronse Decraene (also referred to as *Polygonum cuspidatum* or *Reynoutria japonica*) (hereafter *F. japonica*), the tetraploid *F. sachalinensis* (F. Schmidt Petrop.) Ronse Decraene, and the diploid *F. baldschuanica* (Regel) Holub (also referred to as *F. aubertii* L. Henry). The hybrid between *F. japonica* and *F. sachalinensis*, *Fallopia* x *bohemica* (Chrtek et Chrtkova) J.P. Bailey, is predominately hexaploid, is frequently found in Europe (Hollingsworth 2000; Pyšek et al. 2003; Tiébré et al. 2007) and is considered to be more invasive and to spread faster than its parental species (Pyšek et al. 2003) . Hybridization between *F. japonica* and *F. baldschuanica* (Tiébré et al. 2007) results in the *pentaploid* hybrid *Fallopia* x *conollyana* J.P. Bailey, which so far has only rarely been reported from Europe (Bailey et al. 2007).

Here we set out to assess variation in herbivore resistance among the three hybridizing parental species and the two hybrids of the exotic *Fallopia* complex against a set of herbivores native to Europe with which they share no evolutionary history. We measured palatability of the leaves of the five exotic *Fallopia* species and of two native plant species, *Rumex obtusifolius* L. and *Taraxacum officinale* Web., to three generalist herbivores and one specialist herbivore and determined herbivore performance. Both native plant species are common species, and *R. obtusifolius* belongs to the same family as the *Fallopia* taxa. To assess whether some hybrid genotypes have extreme levels of resistance compared to their parental species, we also determined leaf palatability of the widespread hybrid *F.* x *bohemica* at the genotype level. Leaf characteristics that are often associated with palatability, i.e. specific leaf area, leaf dry matter content and C/N ratio (Elger and Willby 2003; Schädler et al. 2003), were recorded for all species and compared with the feeding response of the herbivores.

Material and Methods

Study species

The *Fallopia* taxa used in this study include the species *F. japonica, F. sachalinensis* and *F. baldschuanica*, and the two hybrids *F.* x *bohemica* and *F.* x *conollyana*. *Fallopia baldschuanica* is a climbing plant, whereas the other taxa are rhizomatous perennial geophytes with erect stems. The plants are native to eastern Asia and were introduced into Europe as ornamentals in the 19th century, where *F. japonica* and *F. x bohemica*, and to a lesser extent also *F. sachalinensis*, became highly invasive (Weber 2003; GISD 2005). Exotic *Fallopia* species are especially a problem along rivers, where dense stands displace native plants and fauna (Gerber et al. 2008) and increase the risk of soil erosion and flooding (Child et al. 2000).

A single genotype of *F. japonica* (octoploid, 2n = 88) with male-sterile flowers has been identified in Europe (Hollingsworth 2000; Mandàk et al. 2005; Tiébré et al. 2007). Similarly, only a single genotype of *F. baldschuanica* (diploid, 2n = 20) with hermaphrodite flowers was apparently introduced into Europe (Bailey 1994; Tiébré et al. 2007), but further investigations are warranted. In contrast, several genotypes of *F. sachalinensis* (tetraploid, 2n = 44) with either male-sterile or male-fertile flowers occur in Europe (Mandak et al. 2005;

Tiébré et al. 2007), and this species can therefore act as a pollen donor for *F. japonica* (Bailey 1994; Tiébré et al. 2007). Genetic analyses indicated that the resulting hybrid *F.* x *bohemica* (hexaploid, 2n = 66) has a higher genetic diversity in Europe than both parental species (Mandak et al. 2005; Bailey et al. 2007; Tiébré et al. 2007). Only one individual of *F.* x *conollyana* (pentaploid, 2n = 54) has ever been found established in nature and there is no information available on flower structure of this hybrid (Bailey 2007).

In the experiments, leaf material was used from one genotype each of *F. japonica, F. baldschuanica* and *F.* x *conollyana,* from five genotypes of *F. sachalinensis* and 15 genotypes of *F.* x *bohemica.* Plants of *F. japonica, F. sachalinensis* and *F.* x *bohemica* were grown from rhizomes which had been collected at field sites in Germany and Switzerland in September 2005. These plants had previously been genotyped using RAPD analyses and the number of chromosomes had been determined by flow cytometry (Krebs et al. in prep.). *Fallopia baldschuanica* and *F.* x *conollyana* were obtained from collections held at the CABI Europe – UK Centre, Egham, England. All *Fallopia* plants were grown in pots filled with standard potting soil in a common garden in Delémont, Switzerland. In addition to the introduced *Fallopia* taxa, two native species, *Rumex obtusifolius* (Polygonaceae) and *Taraxacum officinale* (Asteraceae), were included in the experiment for comparison. Leaves of these species were collected each time when required from ten different individuals near Delémont, Switzerland. No molecular analyses were carried out with these two plant species. However, because sampled individuals were separated by a minimum distance of one meter, we assumed that each plant represented a different genotype.

We used four invertebrate herbivores for the experiments: the slug *Arion lusitanicus* L. (Pulmonata: Arionidae), the moth *Noctua pronuba* L. (Lepidoptera: Noctuidae), the grasshopper *Metrioptera roeselii* Hag. (Saltatoria: Tettigoniidae), and the chrysomelid beetle *Gastrophysa viridula* Deg. (Coleoptera: Chrysomelidae). The first three species are generalists, while the beetle is a specialist feeding only on Polygonaceae (Mohr 1966). *Arion lusitanicus* was introduced from southern Europe into central and northern Europe. Today the species is widespread and found in moist habitats all over Europe (Weidema 2006). The host range of *A. lusitanicus* includes more than 100 plant species from various families (Weidema 2006). Slug herbivory was observed on introduced *Fallopia* taxa in the field on rare occasions (Krebs, personal observation). The moth *N. pronuba* is abundant throughout Europe and occurs on waste land, cultivated land and in gardens (Carter et al. 1986). The caterpillars feed on a wide range of herbaceous plants and grasses. Larvae of Noctuid moths have been found feeding on leaves of *F. japonica* in nature; however, no identifications to the species level have been made (Beerling et al. 1994). The grasshopper *M. roeselii* is a common grassland species throughout Europe and feeds on a wide range of herbaceous plants and grasses (Bellmann 1993). No information is available on whether they also accept exotic *Fallopia* as host plants in nature. The chrysomelid *G. viridula* is common in open habitats and along water courses throughout Europe. This leaf beetle feeds only on members of the Polygonaceae, in particular on *Rumex* species (Mohr 1966). Adults and larvae have been found feeding on leaves of *F. japonica* (Beerling et al. 1994). Occasionally, partial defoliation of exotic *Fallopia* by *G. viridula* has been recorded in the UK, particularly after heavy skeletonization of neighbouring *Rumex obtusifolius* plants (Shaw et al. 2002).

Leaf characteristics

Leaf traits like specific leaf area (SLA), leaf dry matter content (LDMC) and C/N ratio have been shown to affect plant resistance to herbivores and their performance (Elger et al. 2003; Schädler et al. 2003).To assess leaf characteristics of the plant taxa used in the experiments, leaves were collected from all plants used in the bioassays in July 2007. Leaf area and fresh and dry leaf mass were determined to calculate leaf dry matter content (LDMC; leaf dry mass as a proportion of fresh mass) and specific leaf area (SLA; leaf area/leaf dry mass [cm^2 g^{-1}]). Additional leaf material for the analysis of the C/N ratio was collected from the same plants in July 2007 and frozen at minus 20°C. Prior to analysis, samples were defrosted and dried at 40°C for 48 h. Leaves were ground (0.25 mm) to obtain a homogeneous powder and dried again at 40°C. The leaf powder of exotic *Fallopia* plants had to be pooled per clone to have a sufficient amount for the analysis. The carbon and nitrogen content of the leaves was determined with a FlashEA 1112 elemental analyzer (Thermo Fisher Scientific Inc., Milano, Italy).

Plant resistance and herbivore performance

We assessed resistance of our study species both in terms of leaf area removed by the herbivores as well as in terms of herbivore performance (Strauss and Agrawal 1999; Strauss et al. 2002). Herbivores were reared individually on leaves from a single *Fallopia* plant throughout the experiment. Leaves of the native European species were collected each time from different individuals.

Arion lusitanicus. In early July 2007, slugs were collected near Delémont, Switzerland and kept for 48 hours without food at 12°C. On 6 July, 104 slugs were weighed and individually placed into plastic cups (7 cm diameter, 8 cm height) that were lined with moistened filter paper. Each slug received fresh leaf material from one of the seven plant taxa (see Table 1 for details). Depending on leaf size, whole leaves or leaf sections of c. 100 cm^2 were offered. Before they were offered to the slugs, the leaves were scanned and their area was determined. The cups were closed with a perforated plastic lid and kept at 12°C with a 16/8 hour day/night cycle. Every third day, the residuals of leaves were removed and scanned and fresh leaves were placed into the cups. Every second day all slugs were washed and weighed, and placed back into the cups until the end of the experiment on 2 August. Individuals which died during the experiment were replaced.

Noctua pronuba. In early September 2007, eggs of *N. pronuba* were collected near Delémont, Switzerland, and the hatched caterpillars were reared on *Trifolium pratense* L. at 20°C with a 16/8 hour day/night cycle. On 17 September, 90 second instar caterpillars were weighed and placed individually into Petri dishes of 9 cm diameter lined with moistened filter paper. *Fallopia* material was field collected from the same populations as the rhizome material for experiments with the other herbivores (see above), because leaves of the plants kept in pots in the common garden were senescent. Shoots with green leaves were collected shortly before the start of the experiment, kept at 2ºC in plastic bags and regularly moistened. Leaf discs of 2.5 cm diameter were cut from fresh leaf material of the seven plant taxa, avoiding large veins and the mid-rib, and placed into the Petri dishes (see Table 1 for details). Since feeding on *R. obtusifolius* and *T. officinale* was high, two leaf discs were provided in each Petri dish. During the experiment, the caterpillars were kept under the same conditions as when initially reared on *T. pratense*. Leaf discs were changed and scanned and caterpillars weighed every second day until the end of the experiment on 1 October. Individuals which died during the experiment were replaced.

Metrioptera roeselii. In June 2008, *M. roeselii* individuals were collected in the Chasseral region, Switzerland and kept on a mixed diet of native herbs for one day. On 20 June 2008, 84 individuals were weighed and placed into cups of 5 cm diameter and 10 cm height containing moistened cotton pads. Leaf discs with a diameter of 2 cm were cut from fresh leaf material of the seven plant taxa, avoiding large veins and the mid-rib, and placed into cups (see Table 1 for details). Cups were kept at ~24°C with a natural day/night cycle. Leaf discs were changed every second day and scanned and the grasshoppers were weighed daily until the end of the experiment on 24 June 2008.

Gastrophysa viridula. In May 2008, adult beetles were collected near St. Brais, Switzerland, kept at 20°C with a 16/8 hour day/night cycle and adults and hatching larvae were regularly provided with *R. obtusifolius* as food. On 26 May 2008, 135 first instar larvae were weighed and placed individually into 9 cm Petri dishes lined with moistened filter paper. Leaf discs of 2 cm diameter were cut from fresh leaf material of the seven plant taxa, avoiding large veins and the mid-rib, and added to the Petri dishes (see Table 1 for details). Since feeding on *R. obtusifolius* was high, two leaf discs were provided from that species. Petri dishes were kept at 20°C with a 16/8 hour day/night cycle. Until the end of the experiment on 2 June 2008, larvae were weighed daily and leaf discs changed every second day and scanned.

The area of the leaves collected in all experiments was determined using image analysis (Scion image, Alpha 4.0.3.2., Scion Corp., Frederick, Maryland), and leaf area consumed per day was calculated for each plant taxon. Herbivore performance was calculated as the slope of a linear regression of natural log herbivore fresh weight against time.

Table 1: Number of replicates per genotype of the various plant taxa and number of genotypes used in the experiments with each herbivore. [1]The number of genotypes of *R. obtusifolius* and *T. officinale* is not known, because no genetic analyses were carried out.

Plant taxon	Herbivore			
	A. lusitanicus	*N. pronuba*	*M. roeselii*	*G. viridula*
F. sachalinensis	3 x 5	4 x 3	4 x 3	5 x 5
F. x bohemica	3 x 13	4 x 5	4 x 8	5 x 12
F. japonica	10 x 1	10 x 1	8 x 1	10 x 1
F. x conollyana	10 x 1	10 x 1	8 x 1	10 x 1
F. baldschuanica	10 x 1	10 x 1	8 x 1	10 x 1
R. obtusifolius	10[1]	10[1]	8[1]	10[1]
T. officinale	10[1]	10[1]	8[1]	10[1]

Statistical analyses

One-way analyses of variance (ANOVA) were carried out to test for differences among the taxa in leaf dry matter content (LDMC), specific leaf area (SLA) and C/N ratio. The following non-orthogonal contrasts were chosen a-priori (Quinn et al. 2002): *Fallopia* vs. non-*Fallopia* species, *Fallopia* hybrids vs. non-hybrids, *F.* x *bohemica* vs. parental species (*F. japonica* and *F. sachalinensis*) and *F.* x *conollyana* vs. parental species (*F. japonica* and *F. baldschuanica*). The effect of plant taxon on leaf area consumed per day and on relative growth rate (RGR) was analysed for each herbivore species using analysis of covariance (ANCOVA) with taxon as fixed factor and with the same a-priori contrasts as above, and with either initial herbivore mass or one of the leaf traits (SLA, LDMC and C/N ratio) as covariate.

For *A. lusitanicus, N. pronub*a and *G. viridula*, regressions between log-mass of each herbivore and number of days the experiment had run were carried out and the slope of the regression was used as an estimate of the relative growth rate (RGR) of each herbivore species. Individuals which had died during the experiment and had been replaced, were excluded from analyses of RGR, when less than four measurements of their biomass were made. Due to high mortality of the individuals during the experiment, no analysis of RGR was made for *M. roeselii*. To meet underlying assumptions of statistical procedures, i.e. homoscedasticity and normal distribution of residuals, data for leaf area consumed were log-transformed (log +1). Since the specialist beetle *G. viridula* does not feed on *T. officinale*, this plant species was not included in the ANCOVAs calculated for *G. viridula*. For *M. roeselii*, undamaged samples were excluded from the analyses because many individuals did not feed at all during tests. For this species we conducted a supplementary analysis of deviance to explore the effect of taxon, and the contrasts *Fallopia* vs. non-*Fallopia* species, *Fallopia* hybrid vs. non-hybrid, *F.* x *bohemica* vs. parental species and *F.* x *conollyana* vs. parental species on the probability of feeding.

The effect of genotype on leaf area damaged and on RGR was analysed for *F.* x *bohemica* and *F. sachalinensis* using one-way ANCOVA with genotype as factor and with either initial herbivore mass or one of the leaf traits (SLA, LDMC and C/N ratio) as covariate.

To analyse how much of the differences among the plant taxa in leaf area consumed was due to differences in leaf traits, ANCOVAs were carried out for each herbivore with SLA, LDMC or the C/N ratio as covariate (see above) and the proportion of the taxon effect that could be explained by the effect of the covariate (in terms of sums of squares) was calculated. All analyses were carried out using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Leaf characteristics

The leaf characters LDMC and C/N ratio were strongly positively correlated with each other ($r = 0.90$), SLA and CN ratio ($r = -0.75$) as well as SLA and LDMC strongly negatively ($r = -0.84$; all $P < 0.001$). All leaf characters differed significantly among the taxa (SLA: $F_{6,186} = 213.01$; LDMC: $F_{6,186} = 51.41$; C/N ratio: $F_{6,28} = 11.89$; all $P < 0.001$). The two native species had a 146 % higher SLA, a 38 % lower LDMC and a 98 % lower C/N ratio than the exotic *Fallopia* taxa (SLA: $F_{1,186} = 340.03$, $P < 0.001$; LDMC: $F_{1,186} = 85.18$, $P < 0.001$; C/N ratio: $F_{1,28} = 15.39$, $P < 0.001$; Fig. 1). *Rumex obtusifolius* leaves had the highest value for SLA and the lowest for LDMC of all species tested, and *F. japonica* var. *japonica* leaves the lowest value for SLA and highest for LDMC (Fig. 1). The C/N ratio was highest for *F.* x *bohemica* and lowest for *R. obtusifolius* (Fig. 1). The SLA of the two hybrids was 12 % higher and their LDMC 11 % lower than those of the parental species (SLA: $F_{1,186} = 4.96$, $P < 0.05$; LDMC: $F_{1,186} = 8.77$, $P < 0.01$; Fig. 1).

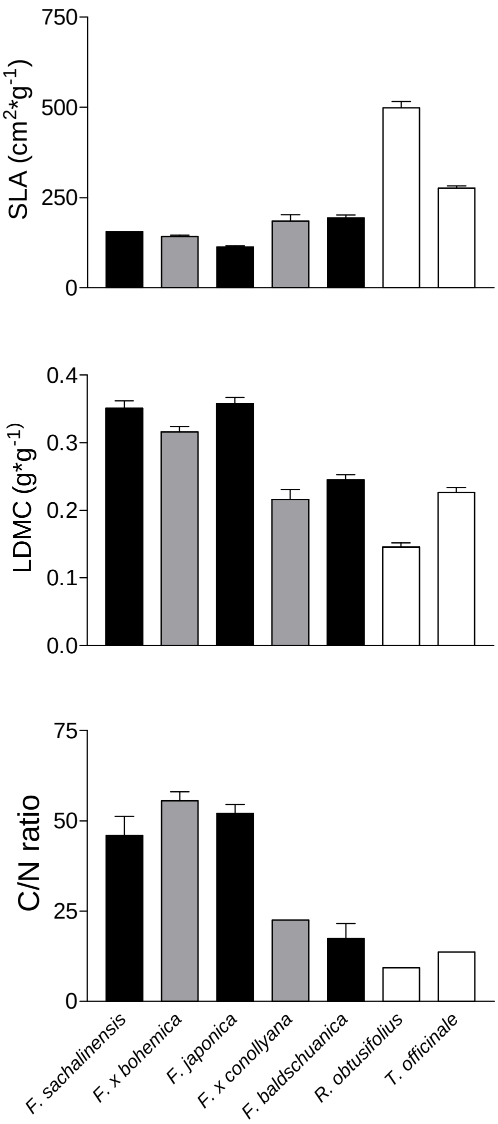

Figure 1: Leaf dry matter content (LDMC), specific leaf area (SLA) and C/N ratio of three exotic *Fallopia* species (*F. sachalinensis, F. japonica* var. *japonica* and *F. baldschuanica*), their hybrids (*F.* x *bohemica* and *F.* x *conollyana*) and two native species (*Rumex obtusifolius* and *Taraxacum officinale*). Data are means +1 SE.

Interspecific variation in plant resistance and herbivore performance

The initial mass of herbivores had no significant effect on leaf area consumed for all herbivores (all $P > 0.051$) and was therefore not included as covariate in further analyses. In contrast, all leaf traits had a significant effect ($P < 0.05$) on leaf area consumed per day by the four herbivores, with the exception of SLA for *M. roeselii* (see Table 3). The mean leaf area consumed per day increased with increasing SLA and decreased with increasing LDMC and C/N ratio. The SLA explained 53% (*N. pronuba*), 47% (*A. lusticanicus*), 9% (*M. roeselii*) and 4% (*G. viridula*) of the variation in leaf area consumed, LDMC 37% (*A. lusticanicus*), 32% (*N. pronuba*), 20% (*G. viridula*) and 20% (*M. roeselii*), and the C/N ratio 58% (*A. lusticanicus*), 28% (*N. pronuba*), 25% (*M. roeselii*) and 3% (*G. viridula*).

Leaf area consumed by all herbivores varied strongly among the plant taxa (Fig. 2, Table 2), irrespective of whether the leaf trait SLA was included as a covariate in the analysis or not. Analyses that included the traits LDMC or C/N-ratio gave similar results. All herbivores (with the exception of *G. viridula*) consumed more leaf area of the native species *R. obtusifolius,* and particularly *T. officinale,* than of the exotic *Fallopia* taxa. *Arion lusitanicus* consumed 94%, *N. pronuba* 66%, and *M. roeselii* 61% more leaf area of the two native species than of the exotic *Fallopia* species. However, in the case of *M. roeselii,* this was only due to strong feeding on *T. officinale,* as the native *R. obtusifolius* was not well accepted as a food plant. The beetle *G. viridula,* a specialist on Polygonaceae, consumed 78% more of the native *R. obtusifolius* than of the exotic *Fallopia* taxa.

Among the exotic taxa, the lowest leaf area consumed was consistently recorded on *F. japonica* var. *japonica* (Fig. 2) and most leaf area consumed on either *F. baldschuanica* (*A. lusitanicus, M. roeselii* and *G. viridula*) or *F. sachalinensis* (*N. pronuba*; Fig. 2). Leaf area consumed on hybrids tended to be intermediate to that on parental species. However, leaf area consumed by *A. lusitanicus* was significantly higher on parental than on hybrid species (parents: 0.134 ± 0.034 cm^2, hybrids: 0.023 ± 0.009 cm^2; contrast “hybrid vs. parents” in Table 2) and leaf area consumed by *M. roeselii* was significantly lower on parental species than on hybrid species (parents: 0.072 ± 0.122 cm^2, hybrids: 0.094 ± 0.014 cm^2; contrast “hybrid vs. parents” in Table 2).

Table 2: Results of analyses of covariance of the effect of taxon on leaf area consumed per day by *A. lusitanicus*, *N. pronuba*, *M. roeselii* and *G. viridula*. Specific leaf area (SLA) was used as covariate. Data for leaf area consumed were log-transformed. ***, P < 0.001; **, P < 0.01 and *, P < 0.05.

Source of variation	*A. lusitanicus*			*N. pronuba*			*M. roeselii*			*G. viridula*		
	df	F		df	F		df	F		df	F	
SLA	1	227.44	***	1	99.18	***	1	0.74		1	6.09	*
Taxon	6	42.76	***	6	16.44	***	6	6.78	***	6	22.08	***
Fallopia vs. natives	1	166.59	***	1	42.97	***	1	23.85	***	1	103.69	***
Hybrid vs. parents	1	10.93	**	1	1.79		1	5.67	*	1	0.03	
F. x *bohemica* vs. parents	1	8.51	**	1	20.93	***	1	3.68		1	0.12	
F. x *conollyana* vs. parents	1	4.65	*	1	0.09		1	2.14		1	0.08	
Residual	96			82			55			132		
Total	103			89			62			139		

Moreover, leaf area consumed on *F.* x *bohemica* differed significantly from that on parental species for *A. lusitanicus* and *N. pronuba,* but in contrasting ways: it resembled that of the more resistant parent *F. japonica* var. *japonica* for *A. lusticanicus* (Fig. 2A) and that of the more susceptible parent *F. sachalinensis* for *N. pronuba* (Fig. 2B) and *M. roeselii* (Fig. 2C). Leaf area consumed by *G. viridula* was intermediate to those of the parental species (Fig. 2D). Leaf area consumed on *F.* x *conollyana* differed significantly from that of the parental species for *A. lusitanicus* (contrast "*F.* x *conollyana* vs. parental species" in Table 2). Leaf area consumed on *F.* x *conollyana* tended to be intermediate to that on its parental species for *A. lusitanicus* and *M. roeselii,* but closer to that on the more susceptible parent *F. baldschuanica* for *N. pronuba* and *G. viridula* (Fig. 2). Results of the ANCOVAs for leaf area consumed with the leaf traits LDMC and C/N ratio as covariates were similar to the results of the ANCOVA with SLA as covariate (Table 2).

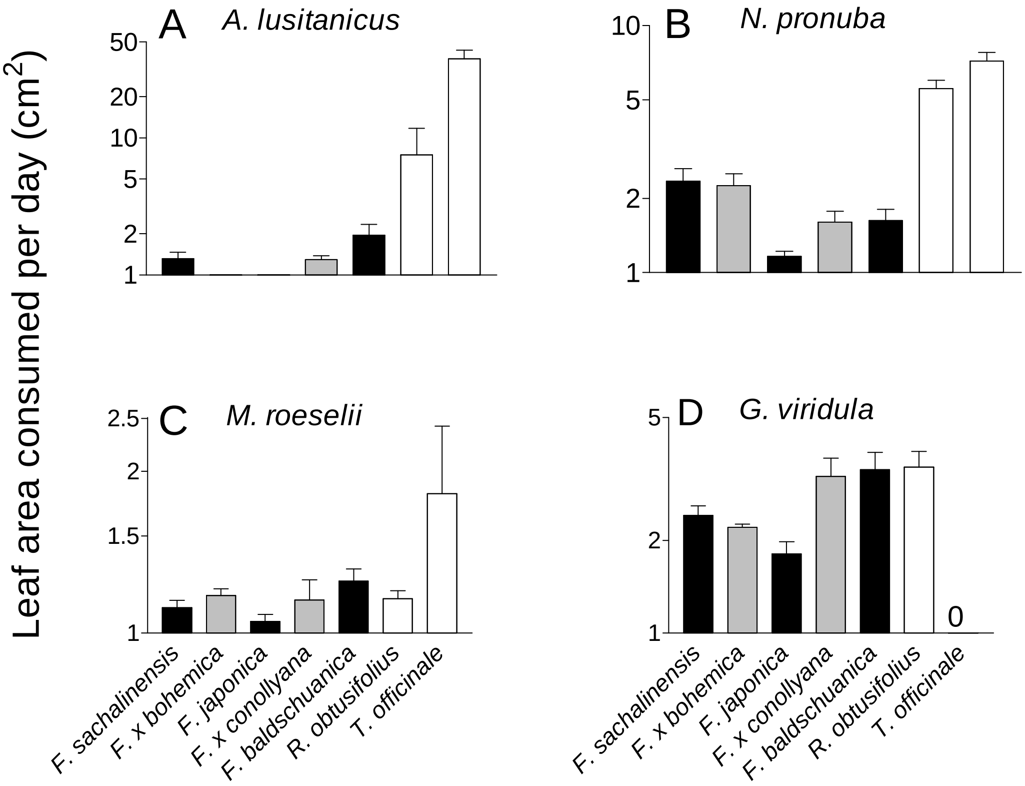

Figure 2: Leaf area consumed per day (log-scale) by (A) *A. lusitanicus*, (B) *N. pronuba*, (C) *M. roeselii* and (D) *G. viridula*. Open bars indicate native species, black bars *Fallopia* parental species and gray bars their hybrids. Data are means +1 SE.

Table 3: Results of analyses of covariance of the effect of taxon on the relative growth rate of *A. lusitanicus*, *N. pronuba* and *G. viridula*. Specific leaf area (SLA) was used as covariate. ***, P < 0.001 and **, P < 0.01.

Source of variation	*A. lusitanicus*			*N. pronuba*			*G. viridula*		
	df	F		df	F		df	F	
SLA	1	12.86	**	1	60.94	***	1	58.66	***
Taxon	6	21.66	***	6	6.49	***	6	15.50	***
Fallopia vs. natives	1	104.11	***	1	22.30	***	1	45.47	***
Hybrid vs. parents	1	0.82		1	0.25		1	2.85	
F. x *bohemica* vs. parents	1	0.47		1	3.18		1	0.15	
F. x *conollyana* vs. parents	1	1.71		1	0.41		1	13.21	**
Residual	74			67			129		
Total	81			74			136		

The covariates SLA (Table 3), LDMC and C/N ratio (results not shown) had a significant effect on the RGR of the three herbivores included in the analysis. The RGR increased with increasing SLA and decreased with increasing LDMC and C/N ratio. In accordance with the results for leaf damage, plant taxa also had a highly significant effect on the RGR of the herbivores (Table 3). The RGR of herbivores on exotic *Fallopia* taxa was significantly lower than on native species, but there were no differences between hybrid *Fallopia* and their parents (Fig. 3). *Arion lusitanicus* had a negative RGR on all plant taxa but *T. officinale* (Fig. 3A). For all herbivores, all other contrasts tested were non-significant, except for the contrast “*F.* x *conollyana* vs. parents” for *G. viriduala* (Table 3).

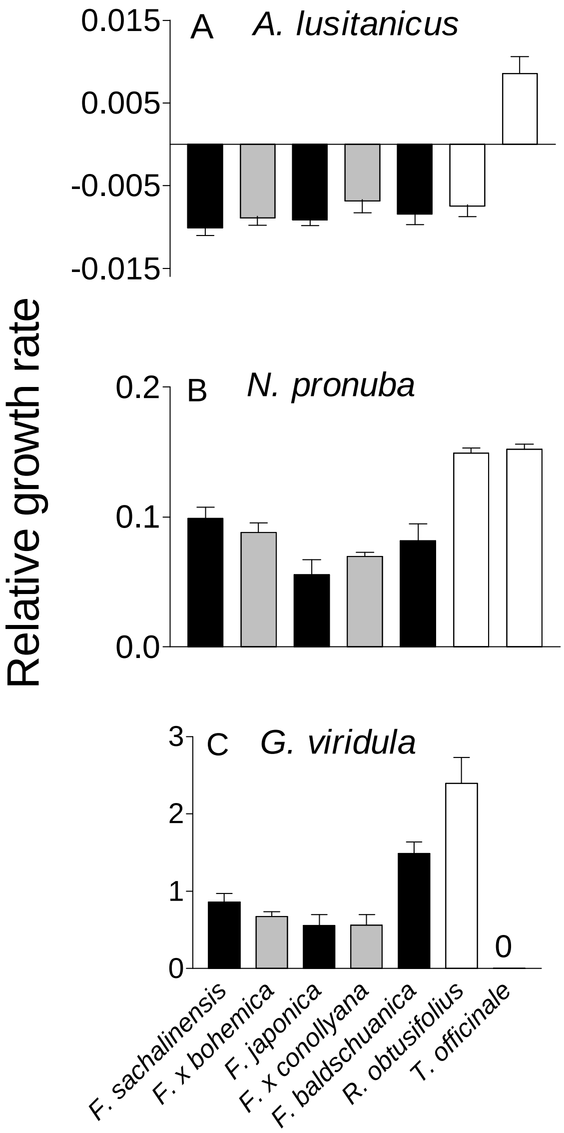

Figure 3: Relative growth rate of (A) *A. lusitanicus*, (B) *N. pronuba* and (C) *G. viridula* on the different *Fallopia* taxa and native species. Relative growth rate was calculated as the slope of linear regression of log-transformed herbivore fresh weight against time. Open bars indicate native species, black bars the parental *Fallopia* species and the gray bars the *Fallopia* hybrids. Data are means +1 SE.

Plant resistance of and herbivore performance on different genotypes of the hybrid F. x bohemica and of F. sachalinensis

Leaf area consumed differed significantly among the *F.* x *bohemica* genotypes for *G. viridula* ($F_{10,48} = 2.61$, $P < 0.05$; Fig. 4D), but not for *A. lusitanicus* ($F_{11,26} = 0.81$, P = 0.63; Fig. 4A), *N. pronuba* ($F_{3,20} = 0.81$, P = 0.50; Fig. 4B) and *M. roeselii* ($F_{6,18} = 2.19$, $P = 0.09$; Fig. 4C). Among *F. sachalinensis* genotypes, leaf area consumed differed for *N. pronuba* ($F_{1,12} = 7.97$, $P < 0.05$), but not for *A. lusitanicus* ($F_{3,10} = 2.25$, P = 0.145), *M. roeselii* ($F_{1,7} = 1.77$, P = 0. 23) and *G. viridula* ($F_{3,20} = 0.73$, P = 0.548). The covariate SLA was only significant for *N. pronuba* with *F.* x *bohemica* ($F_{1,20}$ = 32.71, P < 0.001) and *F. sachalinensis* ($F_{1,12}$ = 15.47, P < 0.01), and for *M. roeselii* with *F. x bohemica* ($F_{1,18}$ = 9.96, *P* < 0.01). LDMC was only significant for *M. roeselii* ($F_{1,18}$ = 12.676, P < 0.01) with *F.* x *bohemica* and for *N. pronuba* with *F. sachalinensis* ($F_{1,12}$ = 9.21, P < 0.05). Among *F.* x *bohemica* genotypes, RGR differed significantly for *G. viridula* ($F_{10,48}$ = 6.82, *P* < 0.001), but not for *A. lusitanicus* ($F_{11,15}$ = 1.25, P = 0.34) and for *N. pronuba* ($F_{3,14}$ = 0.43, P =0.74). Among *F. sachalinensis* genotypes, RGR differed for *N. pronuba* ($F_{1,12}$ = 7.76, P < 0.05), but not for *A. lusitanicus* ($F_{3,9}$ = 1.18, P = 0.37) and *G. viridula* ($F_{3,20}$ = 0.094, P = 0.96). The covariate SLA was significant for *N. pronuba* with *F.* x *bohemica* ($F_{1,14}$ = 28.50, P < 0.001) and with *F. sachalinensis* ($F_{1,12}$ = 30.45, P < 0.001), and for *G. viridula* with *F.* x *bohemica* ($F_{1,48}$ = 6.28, P < 0.05). The covariate LDMC was significant for *N. pronuba* with *F.* x *bohemica* ($F_{1,14}$ = 15.56, P < 0.01) and *F. sachalinensis* ($F_{1,12}$ = 20.91, P < 0.01).

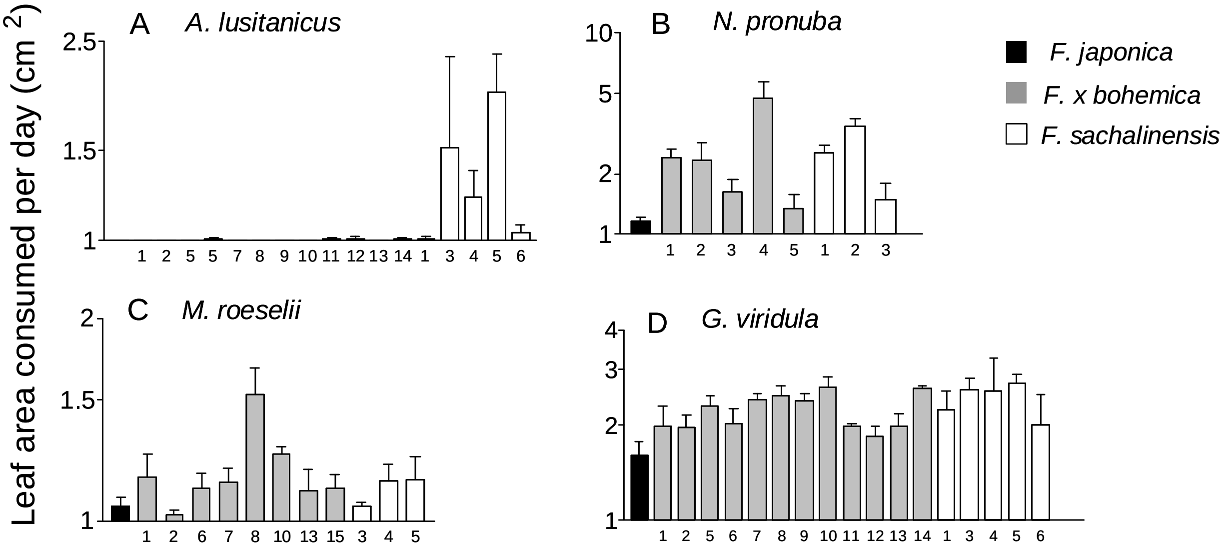

Figure 4: Leaf area consumed per day (log-scale) by (A) *A. lusitanicus*, (B) *N. pronuba*, (C) *M. roeselii* and (D) *G. viridula*. Numbers indicate different genotypes of *F.* x *bohemcia* and *F. sachalinensis*. Grey bars indicate different *F.* x *bohemica* genotypes, black bars the parental species *F. japonica* and white bars the parental species *F. sachalinensis*. Data are means +1 SE.

Discussion

The factor taxon explained a significant amount of the variation in leaf area consumed and in herbivore performance in all analyses with the four herbivore species, irrespective of whether the herbivore’s initial biomass or a leaf trait was included as a covariate or not. We will therefore first discuss the a-priori contrasts within the factor taxon, and then discuss leaf traits, resistance to herbivores and herbivore performance.

Differences in plant resistance and herbivore performance between the species of the Fallopia complex and two native plant species

For all four herbivores tested, the leaf area consumed on exotic *Fallopia* taxa was significantly lower than that on the native species. These results are consistent with field observations in the introduced range; *Fallopia* species in Europe are hardly attacked by native herbivores (Beerling et al. 1994), while *Fallopia* species can be substantially damaged by herbivores in their native range in Japan (Kawano et al. 1999; Shaw et al. 2002). To what extent generalists native to the introduced range are able to exploit a new food source may vary strongly among introduced plant species. While the ERH predicts that generalist herbivores have a great impact on the native competitors than on the introduced plant species, the biotic resistance hypothesis (BRH) states that resistance to native enemies in the new range will limit the establishment and spread of most of the introduced plant species (Parker et al. 2005). In our bioassays, we only included two native plant species, which does not allow for a robust comparison between native plant species and exotic *Fallopia* species in terms of their resistance against and suitability for native European herbivores. However, in other studies *Rumex* species ranked as low-quality (Cates et al. 1975) and *T. officinale* as medium-quality host plants for European generalists (Rathcke 1985). We therefore suggest that the results from our bioassays and from field observations (Beerling 1994; Krebs, personal observations) are in line with the predictions of the ERH, indicating that in Europe the exotic *Fallopia* taxa exhibit higher biotic resistance to native generalist herbivores than native plant species.

Invasive plants may contain secondary compounds that are novel for the introduced range (Cappucino et al. 2006). Novel compounds can repel or have toxic effects on naïve herbivores and make the plants better defended against resident natural enemies than native plants or exotic plants that have no novel chemistry (Callaway et al. 2000; Callaway et al. 2004; Cappucino et al. 2006; Siemens et al. 2007). Plants of the *Fallopia* complex possess numerous plant secondary compounds such as polyphenolics and alkaloids (Siemens et al. 2007), some of which are known to play an important role in the defence against generalist herbivores (Kawano et al. 1999; Cappucino et al. 2006). It is currently unkown whether some of the secondary metabolites found in invasive *Fallopia* taxa are indeed new to the invaded habitats in Europe.

Differences in plant resistance and herbivore performance between parental and hybrid Fallopia taxa

Depending on the herbivore species tested, we found different patterns of herbivore resistance in the hybrids compared to the parental *Fallopia* species. The level of plant resistance to *A. lusitanicus* was significantly lower in hybrids than in parental species. The overall difference in resistance between hybrids and parental species to this herbivore was largely due to the strong differences between *F. x bohemica* and its parental species, *F. japonica* and *F. sachalinensis*. In contrast, with *M. roeselii* the level of plant resistance was significantly higher in hybrids than in parental species. Significant differences in herbivore resistance between the hybrid *F.* x *bohemica* and its parental species *F. japonica* and *F. sachalinensis* were also found for *N. pronuba*. However, no overall differences in herbivore resistance between hybrids and parental species were found in the bioassay with *N. pronuba* and *G. viridula*. In general, the hybrid *F.* x *bohemica* tended to resemble the more susceptible parent. This pattern is in agreement with previous studies suggesting that, when the parental species differ in their herbivore resistance, resistance of the hybrid plants more often resemble that of the susceptible parent (Fritz et al. 1996; Messina et al. 1996; Whitham et al. 1999) . This dominance towards susceptibility suggests that plant resistance traits are often recessive (Fritz et al. 1999), or that plant factors, responsible for host-plant recognition, are the influential traits and inherited dominantly (Fritz et al. 1999).

Fallopia x *conollyana* showed a tendency for intermediate herbivore resistance in comparison to the parental species, since only with *A. lusitancius* the contrasts between *F.* x *conollyana* and its parental species were significant. Although not as frequent as dominance towards susceptibility, intermediate responses have also been repeatedly found in hybrid plants (Boecklen et al. 1990; Aguilar et al. 1992; Fritz et al. 1999). If the expression of plant resistance is dosage-dependent, then an additively inherited trait is likely to lead to intermediate plant resistance in cases where one parental species is resistant and the other susceptible (Fritz et al. 1996).

In our study, herbivore resistance varied depending on the herbivore species used. Variable responses of herbivores on the same hybrids have also been observed in other studies in which multiple herbivores were tested (Boecklen et al. 1994; Fritz et al. 1994; Fritz et al. 1996; Messina et al. 1996; Fritz et al. 1998). Plant resistance is known to be specific to herbivores (Fritz et al. 1998; Fritz et al. 1999). Different herbivores may be affected by different resistance traits or respond differently to the same trait (Fritz et al. 1998; Fritz et al. 1999). However, the results show that even if the responses are variable, herbivores are able to detect differences in palatability induced by hybridization between hybrids and parental species (Hjälten 1997).

The *Fallopia* taxa have different ploidy levels, ranging from diploid to octoploid : *F. japonica* is octoploid, *F. sachalinensis* tetraploid, *F.* x *bohemica* hexaploid, *F. baldschuanica* diploid and *F.* x *conollyana* is pentaploid. Halverson et al. (2007) found that five insect gallmakers attacked diploid, tetraploid and hexaploid *Solidago altissima* plants in the field, but the ploidy subjected to highest attack varied both across herbivores within sites and across sites within herbivores. This may be due to the fact that cytotypes typically differ in cell size, relative growth rates and other life-history traits that may influence resistance to herbivores or herbivore performance (Ramsey et al. 2002). Polyploids often have lower growth rates than diploids and are more resistant to herbivores than diploids due to increased quantities of alkaloids, terpenes or other secondary compounds in comparison to diploids (Levin 1983). The results of our study are in line with this pattern; leaf damage tended to decrease with increasing ploidy level of the *Fallopia* taxa, with the octoploid *F. japonica* showing the highest and the diploid *F. baldschuanica* the lowest resistance against European herbivores.

The different levels of resistance against natural enemies might contribute to the different invasion success of the *Fallopia* taxa. For many invasive plants a negative correlation between herbivory and invasiveness has been observed (Carpenter et al. 2005). For the three invasive taxa *F. japonica*, *F.* x *bohemica* and *F. sachalinensis*, the leaf area consumed by native herbivores in this experiment was negatively correlated with their relative abundance in Europe. *Fallopia japonica*, the most abundant taxon in Europe, showed the highest resistance against generalists, whereas the least common taxon *F. sachalinensis* showed a low resistance. Field observations also suggest that herbivore feeding occurs more frequently on *F. sachalinensis* than on *F. japonica* and *F.* x *bohemica* (Krebs, personal observation). However, although *F. japonica* is still the most frequent taxon in Europe, *F.* x *bohemica* spreads faster and seems to be more invasive than its parental species (Pyšek et al.

2003). Our results suggest that the higher invasiveness of *F. x bohemica* compared to *F. japonica* is not due to higher herbivore resistance. Moreover, the immense differences in abundance of the two hybrids *F.* x *bohemica* and *F.* x *conollyana* cannot be explained by different levels of resistance either. This and the generally low herbivore densities on *Fallopia* plants suggest that differences in the susceptibility to herbivores among the taxa are not responsible for the differences in their invasiveness in Europe.

Genetic variation in plant resistance and herbivore performance in the hybrid F. x bohemica and in F. sachalinensis

In our study, resistance against the herbivore *G. viridula* varied significantly among the different genotypes of *F.* x *bohemica*. However, we found no evidence for genotypes with transgressive levels of herbivore resistance. Genetic variation in insect responses to hybrids was also found in other studies (Fritz et al. 1992; Craig et al. 2000; McGuire et al. 2006), resulting in different damage levels under field conditions (McGuire et al. 2006). Fritz et al. (1998) found significant genetic variation in herbivore resistance among naturally occurring *Salix* hybrid genotypes.

Morphological traits, nutrient content or concentrations of secondary compounds can differ within hybrid species (O`Reilly-Wapstra et al. 2007). In our study, the SLA of *F.* x *bohemica* genotypes was positively and the LDMC negatively correlated with leaf area consumed by *A. lusitanicus* and *M. roeselii*. *Fallopia* x *bohemica* genotypes with a lower herbivore resistance in comparison to other genotypes seem to have similar leaf traits like *F. sachalinensis*, like lower leaf toughness and higher water content, which make them more susceptible than genotypes with leaf traits more similar to those of *F. japonica*. However, the variation among genotypes in leaf area consumed was still significant even if leaf traits were included as covariates in the analysis, indicating that the variation cannot be completely explained by variation in these leaf traits.

We have no information on the genetic status of the *F.* x *bohemica* genotypes used in this study (F1, F2 or backcross). The genetic status of hybrids can influence the resistance to herbivores (Craig et al. 2000; McGuire et al. 2006) and influence insect preference (Fritz et al. 1992). In a *Populus* complex F1 hybrids and first backcrosses were resistant against insect attack, but with an increasing degree of introgression their susceptibility increased (Paige et al. 1989) In our study the variation among *F.* x *bohemica* genotypes might be partly attributed to different levels of backcrosses.

Leaf traits and herbivore resistance

Differences in leaf traits may contribute to differences in herbivore resistance among the exotic *Fallopia* taxa and the native plants and result in a lower herbivore attack on *Fallopia*. In general, leaves with low palatability tend to contain more carbon-based secondary compounds such as phenolics and lignin (Schädler et al. 2003), are tougher (Choong et al. 1992), have higher LDMC (Elger et al. 2003; Schädler et al. 2003), lower SLA and a higher C/N ratio (Choong et al. 1992; Elger et al. 2003; Schädler et al. 2003).The frequent invasive taxa *F. japonica, F. sachalinensis* and *F.* x *bohemica,* had a low specific leaf area and high leaf dry matter content and C/N ratio, suggesting that a low water content and physical defence like high leaf toughness and lignification influence the palatability of their leaves in comparison to leaves of the native species. However, the differences in palatability between the *Fallopia* taxa and the native species were still significant if adjusted for differences in leaf traits. This suggests that other factors like novel secondary compounds may also contribute to the high resistance of *Fallopia* to European generalist herbivores.

Conclusions

Interspecific hybridization among invasive *Fallopia* species affect herbivore resistance and the performance of generalist herbivores native to the invaded range, but the effects vary both between hybrid taxa and among herbivore species. In most of the cases, the hybrids tended to resemble the more susceptible parent, followed by hybrids with intermediate levels of resistance and those resembling the more resistant parent. None of the hybrid genotypes revealed resistance levels that were more extreme than those of the parental species. Because the herbivore resistance of the hybrid *F.* x *bohemica* resembles more that of the susceptible parent, its high invasion potential is apparently not due to a higher resistance against herbivores than the parental species. Rather, our bioassays indicate that exotic *Fallopia* taxa are generally well defended against European herbivores, which is mirrored by the generally low attack rates observed in the field. The mechanisms underlying the inter- and intraspecific variation in herbivore defence in the *Fallopia* complex and its generally high resistance against European herbivores require further investigations.

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CONCLUSIONS

Even if vegetative spread has been reported to be the main mode of reproduction in the invasive *Fallopia* complex, the high seed set, high germination rates and the capacity of seedlings to survive the winter in the field suggest that sexual reproduction and successful seedling establishment of the invasive *Fallopia* taxa occurs under favourable conditions in Europe. The high genetic variability and regional differention of the widespread hybrid between *F. japonica* var. *japonica* and *F. sachalinensis*, *F.* x *bohemica*, with its increasing abundance since time of introduction, provides further evidence that sexual reproduction occurs and that at least a few seedlings survive and establish. Sexual reproduction is mainly possible by interspecific hybridization among the invasive *Fallopia* taxa, since only male-sterile and genetically uniform plants of *F. japonica* var. *japonica* occur in Europe. Hybridization also restores sexual reproduction by generating male and female progeny. If hybridization is frequent and widespread parental species and their hybrids may form one reproductive complex in Europe.

In the *Fallopia* complex sexual reproduction might not be important for the main spread and dispersal. Nevertheless, it may play an important role in the invasion process. Through frequent sexual events and hybridization new genotypes with new traits could originate, which could be better competitors and more successful invaders (Stebbins 1969; Arnold and Hodges 1995; Vilà and D`Antonio 1998). In our studies we found that interspecific hybridization can affect and alter herbivore resistance, which is one of the most important determinants of invasiveness. Hybridization is probably a major force promoting genetic diversity in the *Fallopia* complex, producing novel gene combinations on which natural selection can act (Anderson 1949; Stebbins 1969). An increased genetic variability may facilitate adaptation to new and different environmental conditions for example altered by climate change. Even rare events of sexual reproduction can be sufficient to maintain high genetic diversity (Ellstrand and Roose 1987; Gabrielsen and Brochmann 1998). Moreover, rare hybridization events may thus result in a permanent addition of highly invasive genotypes and may be all that is necessary to make a hybrid lineage invasive (Pyšek et al. 2003). By increasing genetic variation in the *Fallopia* complex and by the origin of novel genotypes, which may have an increased adaptation potential in the introduced range (Stebbins 1969; Arnold and Hodges 1995; Rieseberg et al. 2007), sexual reproduction gives a considerable evolutionary potential and may enhance the invasive success of the *Fallopia* complex in Europe.

The reported higher invasiveness of the hybrid *F.* x *bohemica* in comparison to its parental species might be a result of hybridization events and selection of new genotypes with extreme traits (Mandàk et al. 2005). In our study, resistance against the herbivores varied among the different genotypes of *F.* x *bohemica*, but we found no evidence for genotypes with extreme levels of herbivore resistance. However, there is evidence that for other traits, like regeneration capacity, extreme genotypes of hybrids exist (Pyšek et al. 2003), which might contribute to the higher invasion potential of *F.* x *bohemica* in comparison to the parental species (Bimovà et al. 2003). Overall, the hybrids tended to be more similar to their susceptible parental species. This suggests that there is rather a selection for genotypes with higher competitive ability, than high herbivore resistance due to the lack of enemies in the introduced range. Overall, our studies indicate that exotic *Fallopia* taxa are generally well defended against European herbivores, which might contribute to their invasion success in Europe.

Our studies indicated different hybridization patterns among regions in Europe. Moreover, we found different degrees of admixtures between the parental genomes according to the regions, from hybrid genotypes showing both parental contributions to genotypes assigned to a unique genetic cluster different from those of the parental species. Differences in the distribution and frequency of exotic *Fallopia* taxa and of male and female clones as well as different degrees of male fertility within taxon might be of importance for these regional hybridization patterns. The regionalisation of the genetic pool of hybrids as observed in our study may provide the raw material for adaptive evolution in response to the biotic and abiotic conditions encountered in different regions of Europe, and may ultimately increase the invasiveness of the hybrid *F.* x *bohemica*. Our study revealed a very high level of variation in traits related to sexual reproduction of exotic *Fallopia* among regions and stands in Europe. It remains to be shown, though, whether the variation in seed production correlates with the current level of seedling recruitment in the *Fallopia* complex across Europe, or whether it should be considered as a potential for future levels of seedling recruitment of *Fallopia* taxa in the context of climate change.

In our study, we found overall more male *F.* x *bohemica* than female clones, which can act as pollen donor and replace the missing male *F. japonica* var. *japonica* plants in Europe. This high proportion of male *F.* x *bohemica* plants may be a major component of the invasive success of *Fallopia,* since the availability of compatible pollen donors seems to limit seed production. If the proportion of hybrid plants increases with further hybridization events, the density of pollen donors necessary for sexual reproduction would also increase leading to enhanced opportunities for sexual reproduction. Moreover, the hybrid *F.* x *bohemica* has a higher regeneration potential than the parental species (Bimovà et al. 2003), which could additionally enhance the spread by vegetative reproduction and increase the proportion of hybrids.

Even if sexual reproduction in the exotic *Fallopia* complex is not the main mode of reproduction, the balance between vegetative and sexual reproduction could shift towards more sexual reproduction in response to climate warming than we observe today. The predicted climate for large parts of the study region is similar to the current climate in the Ticino area, which is characterized as moist and warm-temperate climate with heavy rainfalls during summer and low precipitation in winter. We observed a very high seedling emergence and performance in Ticino. This suggests that the importance of sexual reproduction, the additional spread by seed and the risk of successful seedling recruitment of the exotic *Fallopia* taxa could increase under the climate change predicted for Europe.

The Convention on Biological Diversity (CBD), signed by almost all countries of the world, states under article 8h to “prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species”. Exotic *Fallopia* taxa are among the most serious exotic weeds in Europe, causing high ecological and economic costs and are not easy to control or eradicate (Child and Wade 2000; Reinhardt et al. 2003; Gerber et al. 2008). The fact that exotic *Fallopia* plants are not attractive for native herbivores has a strong influence on diversity of the fauna inside *Fallopia* stands (Topp et al. 2007; Gerber et al. 2008). Invasiveness of the *Fallopia* complex may be even enhanced in the future by more frequent hybridization events due to higher frequency of compatible mates and increased sexual reproduction and successful seedling establishment due to climate warming. An increased evolutionary potential may lead to highly invasive genotypes and adaptation to new enviroments and thus enhanced spread and a range widening (Bailey and Wisskirchen 2006). These risks make it important to understand the genetics and ecology of invasive *Fallopia* taxa and the role of hybridization in their invasion process to aid the development of more effective management strategies. Our results imply that the potential spread by sexual reproduction should not be underestimated when planning control and management strategies against the invasive *Fallopia* complex in Europe, particularly in the face of global warming (Forman and Kesseli 2003; Tiébré et al. 2007).

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"The scientist does not study nature because it is useful;
he studies it because he delights in it, and he delights in it
because it is beautiful.
If nature were not beautiful, it would not be worth knowing,
and if nature were not worth knowing, life would not be
worth living."

Jules Henri Poincare

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Erklärung

Ich versichere, dass ich meine Dissertation

"Hybridization in the invasive *Fallopia* complex and its influence on sexual reproduction and herbivore resistance"

selbständig, ohne unerlaubte Hilfe angefertigt und mich dabei keiner anderen als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe.

Die Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

Ort / Datum

Christine Krebs