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Biogeographical patterns, ecological drivers, and evolutionary mechanisms of plant invasions

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I am submitting herewith a dissertation written by Rafael Dudeque Zenni entitled "Biogeographical patterns, ecological drivers, and evolutionary mechanisms of plant invasions." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Daniel Simberloff, Major Professor

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Biogeographical patterns, ecological drivers, and evolutionary mechanisms of plant invasions

A Dissertation Presented for the
Doctor of Philosophy
Degree
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Rafael Dudeque Zenni
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DEDICATION

To Juliana, Manoela, and Lucas

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ABSTRACT

Understanding and predicting organisms' responses to novel environments is a key issue for global change biology. In this dissertation, I study biogeographical patterns of plant invasions in Brazil, explore some of their ecological drivers, and disentangle the gene-level mechanisms that cause introduced organisms to become successful or failed invaders. I found that, for the invasive flora of Brazil, species were not introduced to new regions at random and that a species' reason for introduction and continent of origin were associated. Asian ornamental and African forage plants are overrepresented, and two families (Poaceae and Fabaceae) dominate the invasive flora of Brazil. To address the reason for the observed patterns, I studied 18 *Pinus* species introduced to Brazil. I found that biotic resistance reduced the rate of spread, but did not prevent invasions from happening. Also, mean values of species traits did not explain which species would have become naturalized or invasive. The number of source populations introduced for each species was the factor that best explained the observed pattern of invasion. These findings indicate that forests might not resist invasion by *Pinus* and support the hypothesis that propagule pressure is a driver of invasions with propagule diversity being a component of this mechanism. Next, I surveyed the ecological literature to explore reasons why invasive species are not always invasive. I found intraspecific variation in invasion success and explanations for this variation: low propagule pressure, abiotic resistance, biotic resistance, genetic constraints, and mutualist release. Finally, to understand mechanisms leading to variation in invasion success, I analyzed the spread of *Pinus taeda* in six forestry provenance trials. I found that range expansions of introduced *P. taeda* resulted from an interaction between genetic provenance and climate and that temperature and precipitation predict the invasive performance of particular provenances. Further, I found genotypes can occupy climate niche spaces different from those observed in their native ranges. Overall, my work demonstrates genotypes respond to climate in distinct ways, and these interactions affect the ability of populations to expand their ranges. The introduction of adapted genotypes is a key driver of naturalization of populations of introduced species.

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INTRODUCTION

Biological invasions are an ecological phenomenon with profound implications for current and future ecological processes (Wardle *et al.*, 2011; Simberloff *et al.*, 2012). Even though the colonization of novel habitats by organisms at the leading edge of their native distribution ranges is a recurrent pattern in nature (Petit *et al.*, 2004), anthropogenic factors have increased manifold the rate, speed, and reach of species dispersal (Blackburn *et al.*, 2011b; Richardson & Rejmánek, 2011). Consequently, introduced species can be found virtually anywhere on the globe. Many of these introduced organisms remain innocuous for some period and eventually die out. Only a small proportion are able to produce fertile offspring and maintain a population, and an even smaller number are capable of spreading far from the point of release, rapidly increasing the range of the population (Blackburn *et al.*, 2011b). Studies of the process of range expansion of introduced species are fundamental for understanding the impacts these organisms can have on populations, communities, and ecosystems of recipient regions (Wardle *et al.*, 2011; Simberloff *et al.*, 2012). Also, the processes involved in organisms' dispersal, naturalization, and invasion can provide insights on a large spectrum of ecological and evolutionary questions, such as species interactions, nutrient and energetic balances, genetic bottlenecks, adaptive evolution and phenotypic plasticity, and hybridization.

Despite the fact that organisms that successfully transit from being casual to become invasive are called "invasive alien species," the invasion is led by a few individuals, possibly only one individual, at the leading edge of the invasion front (Clark *et al.*, 2001). Invasion is likely to happen if at least one individual of the introduced pool can produce a disproportionally large number of viable offspring (i.e., González-Martínez *et al.*, 2006). When more highly successful individuals are present in the source pool, the speed and rate of spread should increase. On the other hand, if none of the individuals of the source pool produce viable offspring, the invasion is sure to fail. This is one reason why some species known to be invasive at some sites fail to invade at other sites. These failures can be caused by biotic resistance, abiotic resistance, genetic effects, and mutualist release. In plants, individual mother plants contribute differentially to future generations, and the distribution of female reproductive success tends to be very skewed. For instance, in *Pinus pinaster* in central Spain, 10% of the trees mothered 50% of the offspring (González-Martínez *et al.*, 2006), and in red oaks in the eastern USA, less than 40% of potential parents were estimated to be the mother of at least one seedling (Moran & Clark, 2011). In both cases, bigger trees were more successful. It is likely that genetic and environmental factors interact to determine an individual's reproductive success. Possessing genes coding for required traits at a given site can be critical for survival, growth, reproduction, and dispersal.

Many hypotheses have been proposed to explain and predict biological invasions (e.g., invasional meltdown, fluctuating resources, enemy release, biotic resistance, ecological stoichiometry, and propagule pressure). While these hypotheses are able to explain many invasions, they fail to explain others (Colautti *et al.*, 2004; Nuñez *et al.*, 2011; Jeschke *et al.*, 2012). Also, many historical factors and species traits have been proposed to explain and predict which species will be invasive (e.g., reason for introduction, relative growth rate, and seed terminal velocity). These traits are successful in explaining why some introduced species invade while others do not, but they fail to explain many other cases (e.g., Zenni & Nuñez, 2013; Zenni & Simberloff, 2013). All of these proposed factors are able to explain invasions to some degree, and they are not mutually exclusive. Hence, in order to find useful generalizations and advance the field beyond the point of case studies, many researchers have proposed theoretical frameworks to explain how organisms advance from introduced to invasive, and to explain the determinants of invasiveness (e.g., Facon *et al.*, 2006; Moles *et al.*, 2008; Catford *et al.*, 2009; van Kleunen *et al.*, 2010; Blackburn *et al.*, 2011a). However, none of them explicitly incorporate variable invasion success at all levels (from individuals to species). Usually, these frameworks focus only at the species level, even though the authors acknowledge invasions happen at the population level. Hence, the role of intraspecific variation in invasions remains elusive, both at the population and individual levels, and new insights in this realm are needed (Zenni & Nuñez, 2013).

For forestry species, a well-established and growing body of literature suggests the main reason for invasion success is the association of a species with humans (i.e., Richardson *et al.*, 1994; McGregor *et al.*, 2012; Procheş *et al.*, 2012). This hypothesis is based on the fact that invasive tree species are often planted in large numbers, repeatedly, and across many different environments. All these factors can greatly increase the chances of naturalization and invasion of non-native species and, by chance alone, it can be expected that the more points of release across different habitats, the greater are the chances of finding the combination of adapted individuals and environment proper for invasion. This is the propagule pressure hypothesis. However, it has also been shown that planting trees in high numbers and for long periods of time does not always result in invasion (Nuñez *et al.*, 2011). Moreover, association with humans is an inherent characteristic of all biological invasions (Simberloff & Rejmánek, 2011; Zenni & Nuñez, 2013). All these factors (introduced in large numbers, repeatedly, and across many different environments) are proxies for ecologically and evolutionary driven processes that are largely untested (Simberloff, 2009; Zenni & Simberloff, 2013).

Dissertation outline

My dissertation is focused on understanding the patterns and process of plant invasions. I use observational and field experiment data to study biogeographical patterns of plant invasions, to explore their ecological drivers, and to disentangle the gene-level mechanisms that cause introduced organisms to become successful or failed invaders.

In chapter 1, I analyzed a dataset of 117 invasive alien plants across 13 habitats in Brazil to identify potential patterns of continent of origin, reason for introduction, and to test the hypotheses that (i) more Eurasian species are invasive in Brazil than species native from other continents, that (ii) more horticultural species are invasive in Brazil than species introduced for other reasons, and that (iii) continent of origin and reason for introduction are associated. I found that significantly more invasive plant species in Brazil are native to Africa and Asia, were introduced for horticulture and forage, and are part of the families Poaceae, Fabaceae, and Pinaceae. I also found a significant association between continent of origin and reason for introduction. In conclusion, the results suggest that the current invasive alien flora of Brazil results from the combination of patterns of recent human migration waves and deliberate species introductions for technological and commercial reasons.

In chapter 2, I quantified invasion at the local scale and compared it with habitat characteristics, propagule size, number of source populations, and species traits. I found that invasive *Pinus* plants were found inside *Araucaria* forest in densities that decreased log-linearly with an increase in native tree density. Number of individuals introduced and number of source populations were strong predictors of naturalization, thus both propagule size and propagule diversity can potentially be driving invasion success. These findings suggest that *Araucaria* forests might not resist invasion by *Pinus* as recently suggested and support the hypothesis that propagule pressure is a fundamental driver of invasions with propagule diversity being a possible component of this mechanism.

In chapter 3, I aimed to find common characteristics between non-invasive populations of known invasive species and evaluated how the study of failed invasions can contribute to research on biological invasions. I found intraspecific variation in invasion success and several recurring explanations for why non-native species fail to invade; these included low propagule pressure, abiotic resistance, biotic resistance, genetic constraints, and mutualist release. In conclusion, I found failed invasions can provide fundamental information on the relative importance of factors determining invasions and might be a key component of several research topics.

In chapter 4, I show that range expansions of introduced *Pinus taeda* result from an interaction between genetic provenance and climate and that temperature and precipitation clines predict the invasive performance of particular provenances. Further, I show that genotypes can occupy climate niche spaces different from those observed in their native ranges and that admixture is not a main driver of invasion. Genotypes respond to climate in distinct ways, and these interactions affect the ability of populations to expand their ranges. While rapid evolution in introduced ranges is a mechanism at later stages of the invasion process, the introduction of adapted genotypes is a key driver of naturalization of populations of introduced species.

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CHAPTER I

ANALYSIS OF INTRODUCTION HISTORY OF INVASIVE PLANTS IN BRAZIL REVEALS PATTERNS OF ASSOCIATION BETWEEN BIOGEOGRAPHICAL ORIGIN AND REASON FOR INTRODUCTION

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RDZ participated in data collection, analyzed the data, and wrote the manuscript.

Abstract

Knowledge of historical factors associated with biological invasions in a region can help identify source regions, vectors and pathways more likely to originate potential invaders as well as prioritize resource allocation for selective prevention and early detection strategies. In Brazil, little is known about the introduction history of many invasive plant species, and analyses of historical factors associated with invasions are lacking. To fill this gap, I used a dataset of 117 invasive alien plants across 13 habitats in Brazil to identify potential patterns of continent of origin, reason for introduction, and to test the hypotheses that (i) more Eurasian species are invasive in Brazil than species native from other continents, that (ii) more horticultural species are invasive in Brazil than species introduced for other reasons, and that (iii) continent of origin and reason for introduction are associated. I found that significantly more invasive plant species in Brazil are native to Africa and Asia, were introduced for horticulture and forage, and are part of the families Poaceae, Fabaceae, and Pinaceae. I also found a significant association between continent of origin and reason for introduction, with more invasive species than average being African forage grasses and Asian agroforestry or ornamental plants. In conclusion, the results suggest that the current invasive alien flora of Brazil results from the combination of patterns of recent human migration waves and deliberate species introductions for technological and commercial reasons. These results can help prioritize invasive species and vectors in prevention, early detection, and control strategies.

Introduction

The alien species pool in a given region depends on historical factors related to the type, intensity, and frequency of human activities in the area (Kueffer 2013). Activities such as forestry, agroforestry, and horticulture are notable for a tradition of moving species well beyond their native ranges (Culley *et al.* 2011; Kull *et al.* 2011; Simberloff *et al.* 2010). Besides the large number of species transported and released via human activity, there is also the selective component where species more prone to succeed in a given area are favoured for cultivation (Culley *et al.* 2011; Essl *et al.* 2010). Consequently, alien species that are able to invade tend to have stronger associations with human activities than non-invasive species (Essl *et al.* 2011; Essl *et al.* 2010). However, a species can be introduced in different places for different reasons or via distinct pathways, which may cause an alien species to become invasive somewhere but to fail to invade somewhere else (Zenni and Nuñez 2013). Therefore, understanding the role of introduction patterns and historical factors in the success or failure of alien species is essential for understanding the process of invasion itself (Blackburn *et al.* 2011; Wilson *et al.* 2009; Zenni and Nuñez 2013).

Introduction patterns and historical factors resulting in the presence of alien species in a given region provide key information for risk management and prevention of potentially harmful introductions. Analyses of these patterns can help identify source regions, vectors and pathways more likely to originate potential invaders and to which more resources should be designated for prevention and early detection (Wittenberg and Cock 2001). For instance, species native to regions with extended dry periods are thought to pose a higher risk of invasion in Australia and Mediterranean regions than species native to other regions (Gassó *et al.* 2010; Pheloung *et al.* 1999), and species native to tropical or subtropical climatic regions are thought to have higher chances of invasion in tropical Pacific Islands (Daehler *et al.* 2004; Kueffer *et al.* 2010) than other species. Knowledge of the historical aspects of species introductions helps disentangle the ecological and human factors associated with invasion success.

Previous studies of invasive alien floras have proposed several biogeographical and anthropogenic factors that affect patterns of invasions. For example, horticultural species become invasive more often than species associated with other human pathways or uses, such as agriculture or biofuel (Richardson and Rejmánek 2011; Speek *et al.* 2011), and species native to the Old World are thought to invade more often than species from other regions (Di Castri 1989; Pyšek 1998). Also, many invasive species in the United States are native to China, which has similar climates and ecogeographic regions (Jenkins and Mooney 2006; Meyerson and Mooney 2007). However, these patterns might vary among regions, and different studies have found different explanations for the studied flora and region. For instance, species origin did not affect observed patterns of occupancy and invasion in Chile (Castro *et al.* 2005), while in Australia most invasive plant species are native to the Americas (Phillips *et al.* 2010). Thus, there is a need to identify the most regionally relevant factors to aid management efforts and for studies in alien floras not yet explored, which can inform studies on global biogeographical patterns of invasions (e.g., Richardson and Rejmánek 2011).

In this study, I present the first database of introduction histories of invasive alien plants in Brazil and the first analyses of historical factors associated with biological invasions. For each of the 117 invasive alien plants in Brazil identified in a previous study (Zenni and Ziller 2011), I gathered data on continent of origin, reason for introduction, and taxonomic classification. I described patterns of introduction history to identify the prevalence of any specific continent of origin or reason for introduction in the invasive flora of Brazil. I tested the hypotheses that (i) Eurasian (Old World) species are more often invasive than species native from other regions, that (ii) horticultural species became invasive more often than do species associated with other human pathways or uses, and that (iii) continent of origin and reason for introduction are associated. I also tested the associations among reason for introduction, continent of origin, and taxonomic classification and number of habitats invaded in Brazil.

Methods

Dataset compilation

I used the data on 117 invasive alien plant species and 13 invaded habitats across Brazil compiled in a previous study (Zenni and Ziller 2011). This dataset contains only records of species spreading beyond the point of introduction. The habitat classification follows the physiognomic-ecological classes described by UNESCO (1973) and habitats are defined based on the structure and composition of a plant community.

For each invasive species I assigned the following information gathered from the I3N Brazil database (IABIN Invasive Species thematic network), the scientific literature, and personal observations: reason for first introduction in Brazil (accidental, agriculture, agroforestry, forage, forestry, land reclamation, or ornamental), continent of origin (South America, Central America, North America, Europe, Africa, Asia, and Australia), and family. The searches were conducted in Google Scholar and Web of Science and included books and articles. The searches were performed using the Latin name of each species and a combination of relevant terms (in English or Portuguese), such as: Brazil, introduction, native range, origin, and use. The list of articles, books, and floras used to build the dataset for this study is available online at the I3N Brazil database (i3n.institutohorus.org.br/www/). See supplemental file “STable” for the full dataset.

Statistical analysis

I used χ^2 goodness-of-fit tests to compare observed and expected numbers of alien invasive species in Brazil with each of the following historical, biogeographical, and taxonomic attributes: continent of origin, reason for introduction, and family. The expected number of invasive species in each category is the mean number of invasive species from all categories ($\sum_{i=0}^{n} K/n$, where K is the number of species in each category and n is the number of categories). In the χ^2 goodness-of-fit test for family I kept only families with more than one species to conform to the assumptions of the test (22 out of the 42 families recorded, Supplemental material).

Using the same data, I built *r* × *c* matrices of continent of origin × reason for introduction, continent of origin × habitats invaded, and reason for introduction × habitats invaded to determine the influence of interacting factors on the distributions of invasive plants. Each cell corresponded to the sum of invasive species with both *r* and *c* attributes. I used Pearson's chi-squared test of independence to explore the relationship of the first matrix, while continent of origin per habitat invaded and reason for introduction per habitat invaded were tested using a likelihood-ratio χ^2 analysis of variance of generalized linear models (GLM) with quasi-Poisson error distribution and log link functions. Finally, I constructed a multi-way contingency table to analyse the relationships among continent of origin, reason for introduction, family, and invaded habitats, also using a likelihood-ratio χ^2 analysis of variance of generalized linear models (GLM) with quasi-Poisson error distribution and log link functions. I used the quasi-likelihood Poisson distribution in those cases because the variance of the response variable was greater than the mean, which could have inflated the probabilities of type I error if a Poisson error distribution were applied (Quinn and Keough 2002). I also used GLM to test the effect of continent of origin, reason for introduction, family, and the interaction terms on the number of habitats occupied by the invasive alien species in Brazil. For these analyses, the package "car" (Fox and Weisberg 2011) built in R 2.15.2 (R Development Core Team 2011) was used (See supplemental file "stats&figs.R" for the annotated codes used to perform the analyses and create the figures).

For all the analyses involving habitats, I removed abundance data and used only occupancy data (presence or absence). This was required owing to the highly heterogeneous sampling effort across the country (Zenni and Ziller 2011). This heterogeneity would add an undesired bias to the analysis performed, potentially hiding more general patterns.

Results

The numbers of invasive species in Brazil originating in each continent differ significantly ($\chi^2 = 84.2$, df = 6, $p < 0.001$; Fig 1). More invasive species are native to Africa (n = 32) and Asia (n = 44), and fewer invasive species are native to Europe (n = 5), North America (n = 7), and other South American countries (n = 4). Australia and Central America contributed 13 and 12 invasive species, respectively. The numbers of invasive species in

Brazil for each reason for introduction also differ significantly ($\chi^2 = 90.350$, df = 6, $p < 0.001$; Fig 2). Most invasive species were introduced for horticulture (n = 50) and forage (n = 19), while many fewer invasive species were introduced for land reclamation (n = 2). Agriculture, agroforestry, and forestry contributed 11 invasive species each. Also, 10 invasive species were introduced accidentally. The numbers of invasive species in Brazil in each family also differ significantly ($\chi^2 = 134.1$, df = 21, $p < 0.001$; Fig. 3). More invasive species belong to the families Poaceae (n = 24), Fabaceae (n = 16), and Pinaceae (n = 6). These three families encompass 39.3% of all the known invasive species in Brazil, while the other 60.7% (n = 71) belong to 39 different families.

Out of the three interaction terms tested, I found the association between continent of origin and reason for introduction to be greater than expected ($\chi^2 = 93.2$, df = 36, $p < 0.001$). More species than expected were introduced from Africa for forage, and more species than expected were introduced from Asia for agroforestry and horticulture (Fig. 4). Regarding the association between reason for introduction, continent of origin, or family and habitat occupancy by invasive species, I found that only horticulture had a significant effect ($p = 0.045$, Fig. 5), with more habitats invaded by species introduced for this reason (Fig. 5). Continent of origin ($p = 0.31$) and family ($p = 0.58$) were not associated with the number of habitats invaded. For the most part, the reason for introduction did not relate to how widespread species are in Brazil, and none of the introduction history attributes evaluated in this analysis could be effectively used to predict how widespread any species would become.

Discussion

The results mostly support the three proposed hypotheses. First, most invasive alien plants in Brazil have Eurasian origin, although Asian species seem to be more successful invaders in Brazil than European species. Asia and Africa are the main sources of invasive alien plants in Brazil. Second, almost half of the invasive alien plants were initially introduced to the country for horticulture, making this the main pathway for the introduction of invasive alien plants. Third, I found a strong association between biogeographic origin and reason for introduction, with invasive alien grasses mostly from Africa and invasive alien horticultural plants mainly from Asia.

Although I found support for the hypothesis that Eurasia is the main source of invasive alien species (Di Castri 1989), the results contradict the proposition that Europe is the main source of invasive plant species (Pyšek 1998). Asian species correspond to about 40% of the invasive species in Brazil, and European species correspond to less than 3% of the invasive flora, against 80% and 58.9% worldwide, respectively (Pyšek 1998). In temperate regions of southern Brazil, European species tend to be more highly represented in the herbaceous alien flora, making up to 40% of some species pools (Schneider 2007), but the number of European invasive species is still low (4 species). The results indicate that, at least for Brazil, Asia is the main source of invasive plants, but a comprehensive dataset of the alien flora of Brazil (including non-invasive) would be required to test this hypothesis fully. Interestingly, South America (and Brazil in great measure) is also the major source of invasive plants in China, contributing 35% of the Chinese invasive plant species pool (Weber *et al.* 2008). Other studies of invasive species in South America failed to support the hypothesis that Eurasian species are more invasive than species from other regions (Castro *et al.* 2005; Delnatte and Meyer 2012), suggesting the number of invasive species from certain regions might be better explained by a sampling artefact than by a biological mechanism of increased invasiveness. In fact, Brazil received more human immigrants from Eurasia than from anywhere else (IBGE 2000), and I can therefore speculate that more plants were introduced from this region than from anywhere else.

Also interesting is the positive association between continent of origin and reason for introduction. It is not clear if this is simply a result of differential introduction efforts (i.e., more Asian species were introduced for horticulture than species native to any other place in the world) or if distinct evolutionary mechanisms in different regions cause different groups of species to have superior competitive abilities. For example, 75% of the invasive grasses in Brazil are from Africa, and previous studies showed African grasses tend to be more tolerant of defoliation than species from other parts of the world (D'Antonio and Vitousek 1992; Simões and Baruch 1991). In these cases, climatic and ecosystem similarities between native and introduced ranges can also be relevant to invasiveness potential. Also, only conifers introduced from North and Central Americas are invasive in Brazil despite the presence of several conifer species native to Europe and Asia (Zenni and Simberloff 2013). However, the association between continent of origin and reason for introduction may not be a pervasive pattern, or might be strongly affected by a sampling effect. For instance, Harris *et al*. (2007) found no association between continent of origin and reason for introduction among exotic vines in Australia.

As for the different reasons for introduction, only horticultural use was associated with higher habitat occupancy in Brazil. A recent global review of invasive trees and shrubs also recognized the role of horticulture in the introduction of invasive species in South America and 14 other biogeographic regions (Richardson and Rejmánek 2011). Moreover, most species are invasive in just one or two habitats in Brazil, and not widespread. The low number of widespread invasive species may be explained by the fact that species introductions in Brazil are recent and invasive species may still be in the early stages of spread (Richardson *et al.* 2008; Simberloff *et al.* 2010). However, this interpretation should be viewed with caution, because the dataset used in this study is not comprehensive and results might be skewed owing to incomplete sampling and biased invasive species reports (Zenni and Ziller 2011). Unquestionably, more data are necessary for a reliable conclusion to be reached on this topic.

The three most prominent invasive families (i.e., those with the greatest number of invasive species) in Brazil are among the most invasive families on a global scale (Pyšek 1998). That is, grasses, legumes, and pines tend to be successful invaders in Brazil more often than do other groups of plants, even though there is no convergent morphological, physiological, or ecological trait associated with the increased invasiveness. Success for these families might also be inflated by greater propagule pressure, since these species are commonly introduced in large amounts and over large areas in association with human activities (Kull *et al.* 2011; Richardson 1998; Richardson and Rejmánek 2011; Simberloff *et al.* 2010).

Currently in Brazil, data are available for invasive alien species, but a comprehensive catalogue including aliens more broadly (i.e., invasive, non-invasive, and translocated populations) is still absent. The Brazilian Flora database, which is the most complete and updated virtual herbarium available, listed 673 naturalized alien plant species and 55 cultivated alien plant species in Brazil (http://floradobrasil.jbrj.gov.br/; accessed in August, 2013). Among the 728 alien species of the Brazilian Flora database records, only half (n = 57;

49%) are also among the 117 invasive alien plant species identified for Brazil (Zenni and Ziller 2011). For instance, at least 16 Pinus species are present in different regions of Brazil as cultivated, naturalized, or invasive (de Abreu and Durigan 2011; Zenni and Simberloff 2013), but the Brazilian Flora lists only three Pinus species. The unavailability of comprehensive data on introduced, casual, and naturalized alien plant species in Brazil mandates caution in the interpretation of the results presented. Without data on alien non-invasive plants I could only assume colonization pressure (sensu Lockwood et al. 2009) is equivalent for all regions. Even though this is not ideal, it is probably a reasonable assumption given lists of species compiled in several regional plant catalogues (e.g., Lorenzi 2003; Lorenzi and Matos 2002; Lorenzi and Souza 2001). Another limitation of this study is that omission of invasion failures may inflate the relative importance of historical factors responsible for invasions (Zenni and Nuñez 2013). To advance our knowledge on the relationship between introduction histories and invasion, we need data on invasions at the single introduction-level and for both successful and failed naturalizations and invasions.

In summary, the invasive flora of Brazil shows a distinct association between continent of origin and reason for introduction, and shows that historical factors are important for understanding current patterns of invasion. The results presented in this study can potentially provide valuable insights for early detection and public policy. For example, prioritizing the screening of forage species from Africa and horticultural species from Asia that are already present in the country, but not known to be invading, can help prevent future invasions (e.g., Wilson et al. 2011). Apparently, some introduction pathways resulted in more invasions than others, such as the horticultural trade, and they should be monitored more carefully. However, past introductions and currently naturalized floras may not reflect future introductions and invasion risks (Kueffer 2010). The facts that more than half of the invasive species are currently invading one or two habitats, and that the invasive flora possesses the same characteristics as other alien floras where spread has occurred, suggest that most invasive species are still confined in their original region of initial introduction and will expand their invasive ranges to other habitats with time. An effort to prevent the transit of some species could help minimize these range expansions. Furthermore, this study highlights the necessity of more efforts in collecting and compiling data on alien species in Brazil.

Finally, the results suggest that risk assessments should take into consideration not only biogeographic origin and economic use of species, but also the potential interaction between these two factors.

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Appendix I: Figures

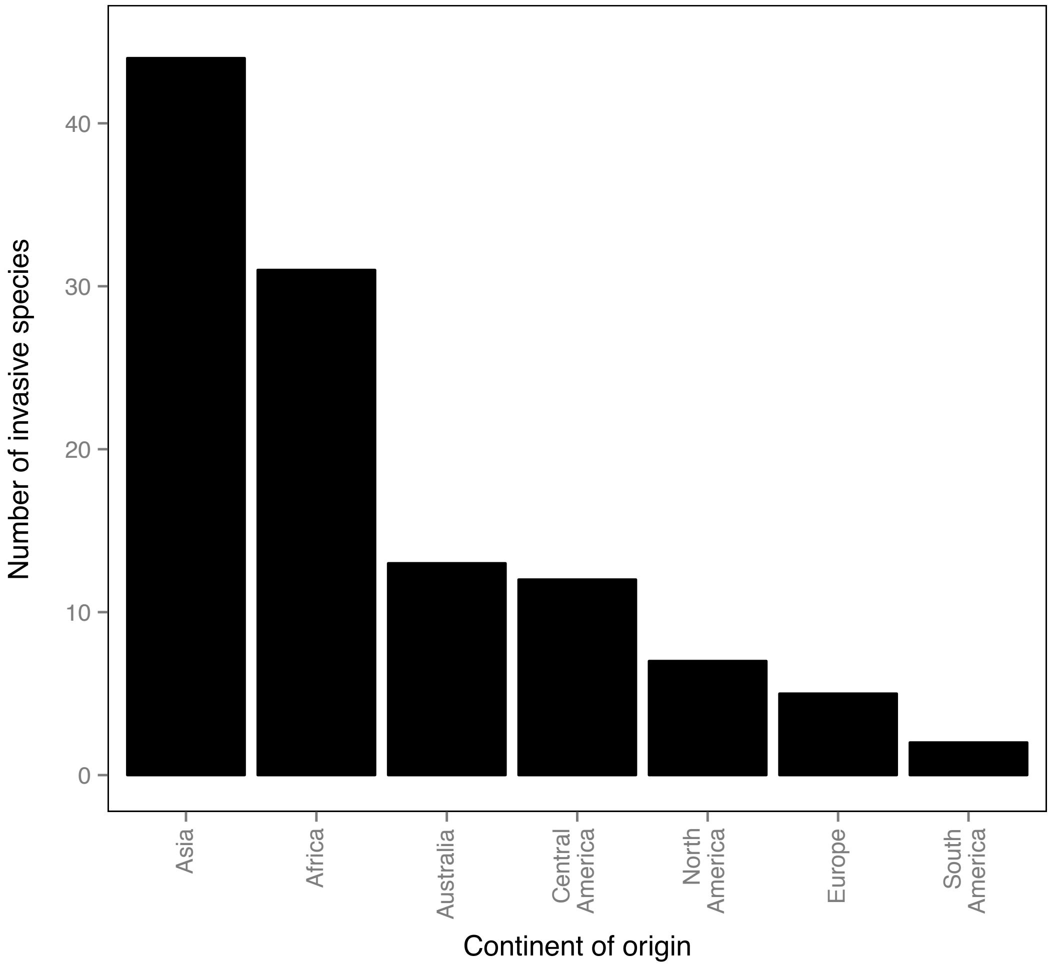

Figure 1-1 Number of invasive species in Brazil originating from each continent.

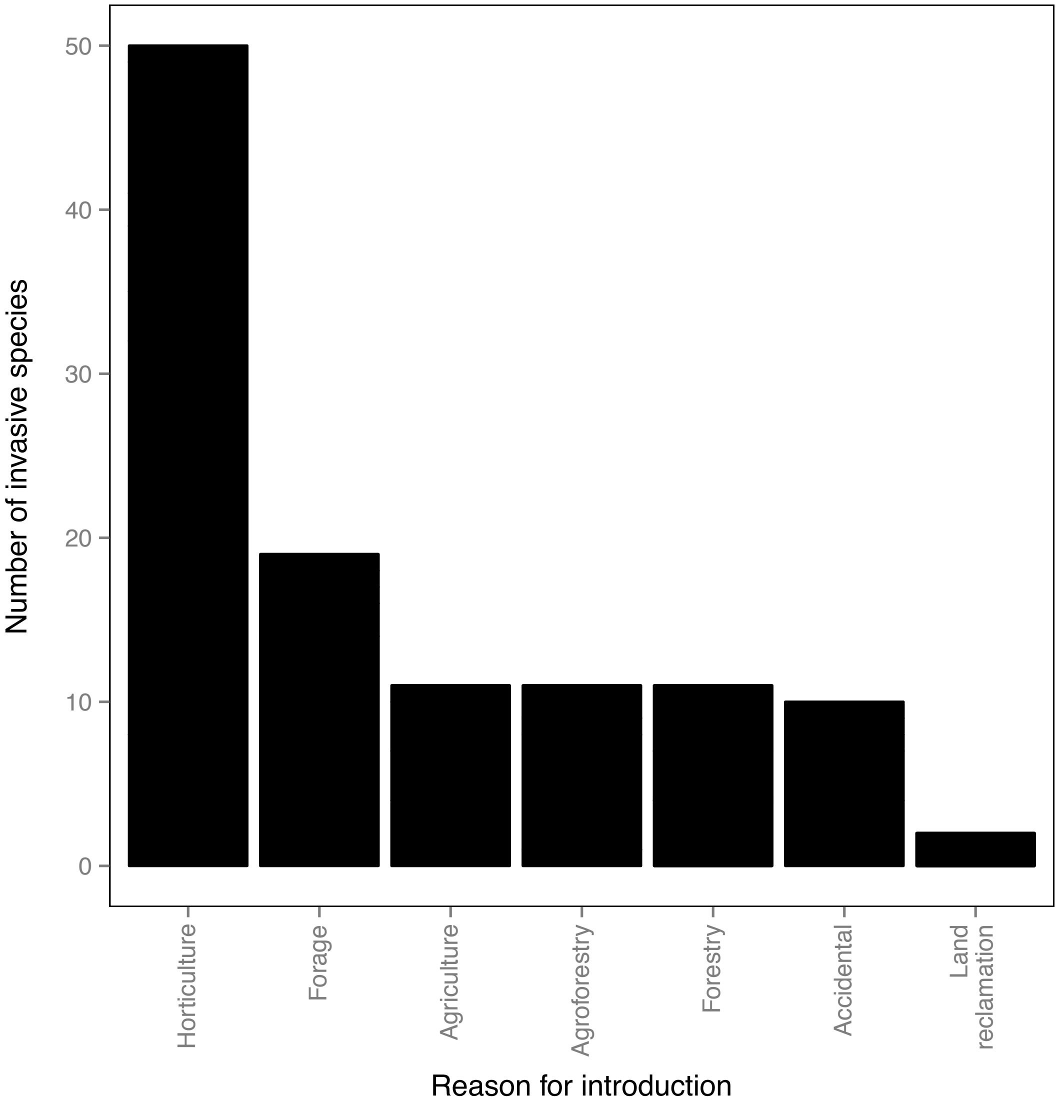

Figure 1-2 Number of invasive species in Brazil introduced for seven different reasons.

These are the reasons for the initial introduction into the country. Once introduced, the species could have spread inside Brazil for other reasons.

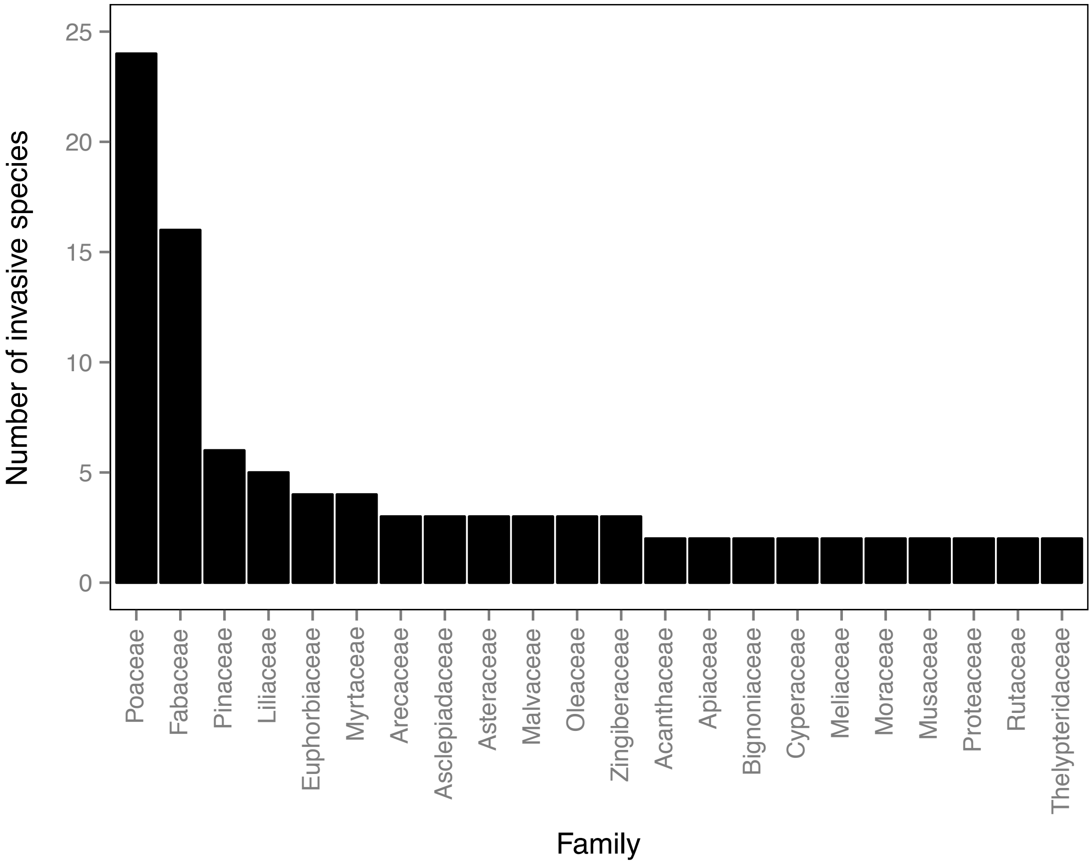

Figure 1-3 Number of invasive species in Brazil belonging to each botanical family. Only families with more than one species are presented.

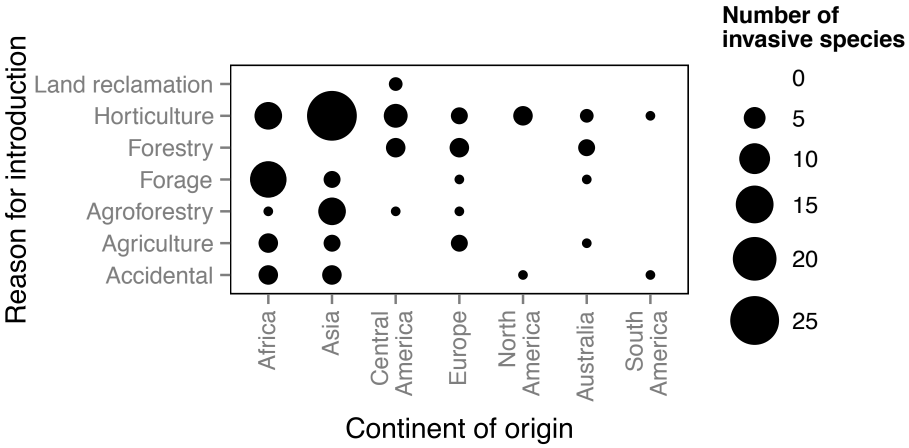

Figure 1-4 Interactions between continent of origin and reason for introduction.

Number of invasive species in Brazil resulted from the interactions between continent of origin and reason for introduction. Interactions that released more invasive species have bigger circles.

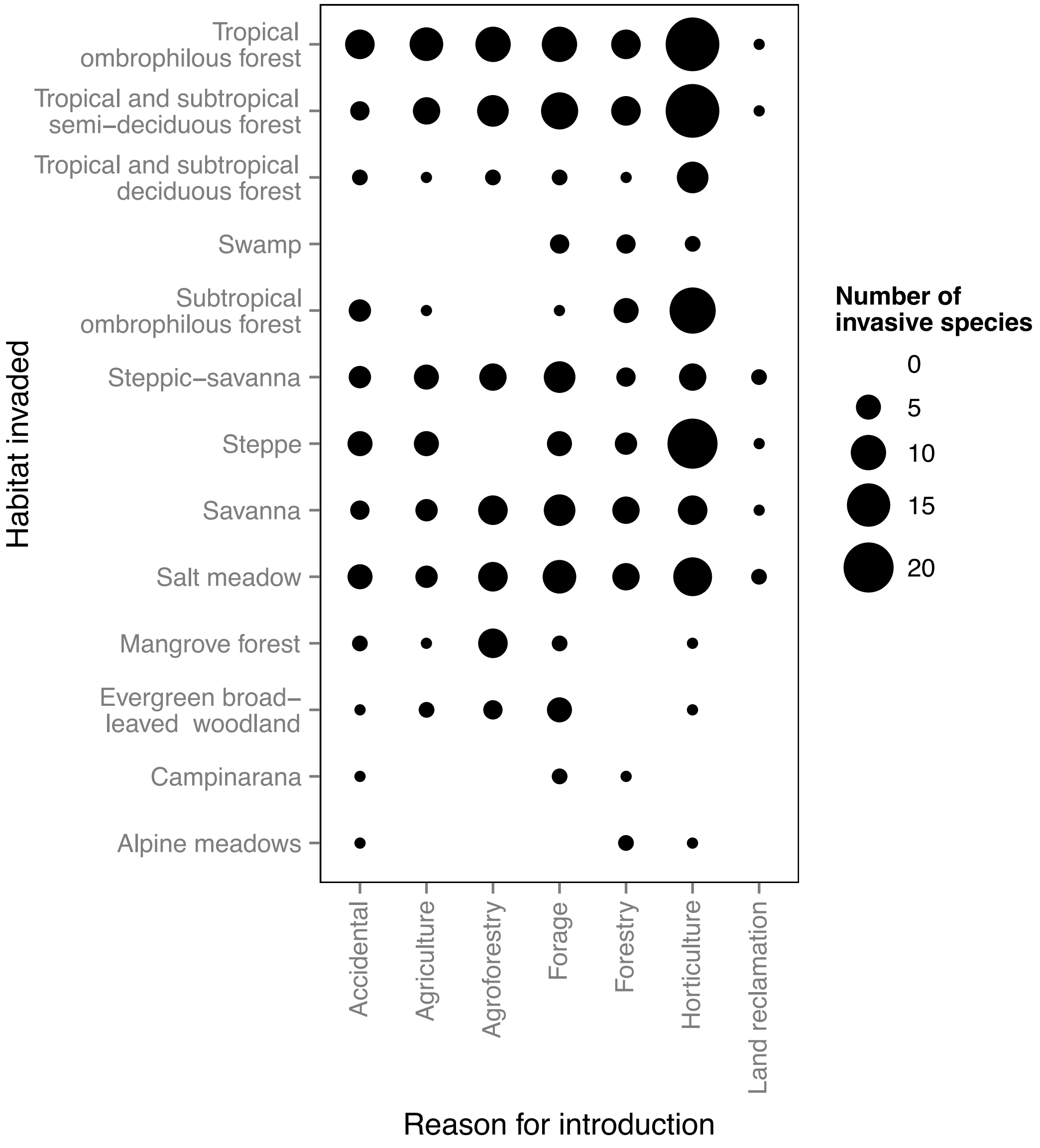

Figure 1-5 Interaction between reason for introduction and habitats.

Occupancy of invasive species in Brazilian natural habitats resulting from each reason for introduction. Reasons for introduction that released more invasive species in a habitat have bigger circles.

CHAPTER II

NUMBER OF SOURCE POPULATIONS AS A POTENTIAL DRIVER OF PINE INVASIONS IN BRAZIL

A version of this chapter was originally published by Rafael D. Zenni and Daniel Simberloff:

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RDZ conduct the field work, analyzed the data, and wrote the manuscript. DS contributed to manuscript revisions.

Abstract

To understand current patterns of *Pinus* invasion in an *Araucaria* forest in southern Brazil, we quantified invasion at the local scale and compared it with habitat characteristics, propagule size, and number of source populations, using generalized linear models. We also compared observed and expected invasive species status based on a previously developed model (Z scores) using chi-square and correlation tests to evaluate the predictability of species status based on their traits. Of the 16 *Pinus* species currently present in the site, three are invasive (*P. elliottii*, *P. glabra*, and *P. taeda*), three are naturalized (*P. clausa*, *P. oocarpa*, and *P. pseudostrobus*), and ten are present only as the originally planted individuals. While *P. taeda* spread the farthest, *P. glabra* had greater overall density, but none of the invasive species has spread more than 250 m in 45 years. Invasive *Pinus* plants were found where forest tree density was below 805 trees ha^{-1}, and invasive *Pinus* density decreased log-linearly with an increase in native tree density. Number of individuals introduced and number of source populations were strong predictors of naturalization, thus both propagule size and propagule diversity can potentially be driving invasion success. Z scores based on species traits did not predict which species would invade in Rio Negro. Our findings suggest that *Araucaria* forests might not resist invasion by *Pinus* as recently suggested and support the hypothesis that propagule pressure is a fundamental driver of invasions with propagule diversity being a possible component of this mechanism.

Introduction

Understanding reasons for invasion successes and failures may provide important insights for basic and applied ecology (Blackburn et al. 2011; Diez et al. 2009). For example, non-native species are expected to be more successful invaders if propagule pressure is high (Simberloff 2009), if the species is adapted to the environment (Nuñez and Medley 2011; Sol 2007), if it evolves new competitive abilities (Dyer et al. 2010), if the habitat offers lower levels of biotic resistance (Fridley et al. 2007; Levine et al. 2004), or if climate in the introduced range is similar to that in the native range (Nuñez and Medley 2011). In addition to the characteristics of the non-native species introduced and the characteristics of the recipient ecosystem, the dispersal pathways and motivations for introduction are also important factors influencing invasion success (Wilson et al. 2009; 2011). For example, many gymnosperms have been introduced throughout the southern hemisphere and currently exhibit great variation in invasion success (Richardson et al. 1994; Simberloff et al. 2010).

Factors that affect invasions play different roles at different stages of the introduction-naturalization-invasion continuum (Blackburn et al. 2011). Also, invasions occur at the population level and different outcomes for the same species should be expected at different places and times (Colautti and MacIsaac 2004). While the ability to cope with the new environment is required for naturalization, overcoming biotic resistance may be crucial only at the spread stage (Dawson et al. 2009). Thus comparisons among casual, naturalized, and invasive species may be at least as important as comparisons between non-invasive and invasive species. Here we define planted non-native species as species able to survive in the new environment but not producing offspring; naturalized non-native species as species able to survive in the new environment and producing consistent offspring only beneath parent plants; and invasive non-native species as species able to survive in the new environment that are producing consistent offspring beneath parent plants and are spreading at least 100 m from the original planting site in 50 years (Richardson et al. 2000).

Propagule pressure is the key mechanism of invasion success in many instances (Colautti et al. 2006; Lockwood et al. 2005; Simberloff 2009). Its importance has been confirmed at spatial scales ranging from 1-m^2 plots (Von Holle and Simberloff 2005), through individual countries (Rejmánek et al. 2005; Křivánek et al. 2006) to continents (Procheş et al. 2012), although a few studies have not found a relationship between naturalization success and propagule pressure (e.g., Nuñez et al. 2011). The two main components of propagule pressure are the number of individuals in a propagule (propagule size) and the number of introduction events (propagule number) (*sensu* Simberloff 2009). These are also the components most commonly explored by studies on propagule pressure (Křivánek et al. 2006; Nuñez et al. 2011; Procheş et al. 2012; Von Holle and Simberloff 2005). However, propagule pressure has a genetic component that is often neglected or underappreciated (Lockwood et al. 2005). Propagule diversity, here defined as the number of source populations comprising a propagule, can increase genetic variation and reduce bottleneck and founder effects (Lockwood et al. 2005) and can increase the likelihood of introducing adapted genotypes (Simberloff 2009).

Pines (*Pinus* spp.) have been present in Brazil since the second half of the 19th century, but large-scale introductions did not start until 1936 when the government began silvicultural experiments with pines. Twelve species are currently grown in commercial plantations and several others are present in experimental stations, resulting in nearly 1.5 million hectares of plantations in seven ecoregions (Simberloff et al. 2010 and references within). While most of this area is continuously managed by foresters in short cycles of 10-25 years, other areas, mostly in government- and university-owned experimental stations, have never been managed since the original plantations were established. Finally, these pine plantations provide great opportunities as natural experiments for investigating how multiple factors (i.e., environments, habitats, and introduction histories) influence the success or failure of invasions across stages of invasion (i.e., establishment, naturalization, and spread) (Richardson 2006).

Characteristics of the environment along with non-native species traits can shape different stages of invasion. For example, pines have greater chances of invasion success in recently disturbed areas (Higgins and Richardson 1998; Osem et al. 2011; Zalba et al. 2008), in regions with climates similar to that of the native range (Boulant et al. 2009; Nuñez and Medley 2011), and in naturally treeless ecosystems (Boulant et al. 2009; Higgins et al. 1996). By contrast, they have lower chances of invasion success when mycorrhizal symbionts are absent (Nuñez et al. 2009), if seed predators are present (Nuñez et al. 2008), and in undisturbed forests (Emer and Fonseca 2010). Propagule pressure, frequently touted as the main single determinant of invasion success (Colautti et al. 2006; Lockwood et al. 2005), has produced contrasting results in predicting pine invasion success (Boulant et al. 2008; Essl et al. 2010; Nuñez et al. 2011; Simberloff et al. 2010). Also, four species traits are related to pine invasion success: mean seed mass, minimum juvenile period, mean interval between large seed crops, and seedling relative growth rate (Grotkopp et al. 2002; Rejmánek and Richardson 1996). Other factors, such as the role of genetic diversity or selective introduction efforts (Simons 2003), have received much less attention, despite evidence that these factors can play major roles in plant invasion (Prentis et al. 2008).

In this study we explored the influence of two components of propagule pressure, propagule diversity (number of source populations) and propagule size (number of individuals introduced), plus habitat and species level traits previously associated with invasiveness, in invasion success or failure of 18 *Pinus* species in an *Araucaria* forest in Rio Negro, southern Brazil (Fig. 1). Rio Negro provides an exceptional opportunity for the study of these factors because of the number of congeneric *Pinus* species introduced at the same time, the breadth of species origins (North and Central Americas, Europe, and Asia), the amplitude of source population origins (Fig. 2), the variability of outcomes (see results), and the relatively few interventions after the trees were planted, which allowed the systems to self-regulate. Therefore, we hypothesized that species producing greater propagule pressure, coming from more source populations, and possessing the traits previously associated with invasiveness (positive Z scores) (Rejmánek and Richardson 1996) would be invasive at the site.

Methods

Study site

Rio Negro Experimental Station (Rio Negro hereafter; 26°03’S, 49°45’W) was established in 1962 on ca. 128 ha with the goal of testing native and non-native tree species with silvicultural potential in southern Brazil. Rio Negro is a mosaic of small tree stands, old fields, secondary forest (*Araucaria* moist forest), and riparian forest (Fig. 1). Among the non-native tree species introduced were 18 *Pinus*, 13 *Eucalyptus*, four *Cupressus*, two *Acacia*, one *Cryptomeria*, one *Grevilea*, one *Melia*, one *Paulownia*, and one *Sequoia* (Appendix 1). The climate is Cfb (subtropical warm temperate), according to the Köppen climate classification, with mean annual precipitation of 1,300 mm uniformly distributed throughout the year. Mean temperatures range from 6° C in the coldest month to 28° C in the warmest month. Mean annual temperature is 17° C with frequent frost during the winter, and elevations are around 900 m above sea level (Santos et al. 2010). The native secondary forest canopy is covered mainly by *Cinnamomum amoenum* (Nees) Kosterm., *C. sellowianum* (Nees & Mart.) Kosterm., *Araucaria angustifolia* (Bertol.) Kuntze, *Mimosa scabrella* Benth., *Prunus brasiliensis* (Cham. & Schltdl.) D. Dietr., *Ocotea pulchella* (Nees & Mart.) Mez, *O. porosa* (Nees & Mart.) Barroso, and *Symplocos tenuifolia* Brand (all native). Also, two graminoid species (Cyperaceae) are highly abundant in the understory.

Eighteen *Pinus* species were planted among the forestry experiments implemented during 1966 and 1967: *Pinus clausa* (Chapm. ex Engelm.) Sarg., *P. echinata* Mill., *P. elliottii* Engelm., *P. glabra* Walter, *P. kesiya* Royle ex Gordon, *P. montezumae* Lamb., *P. oocarpa* Schiede ex Schltdl., *P. palustris* Mill, *P. patula* Schltdl. & Cham., *P. pinaster* Aiton, *P. pseudostrobus* Lindl., *P. radiata* D. Don, *P. rigida* Mill., *P. roxburghii* Sarg., *P. serotina* Michx., *P. strobus* L., *P. taeda* L., and *P. virginiana* Mill. (Table 1). Voucher specimens were deposited in the municipal herbarium of Curitiba/PR (Museu Botânico Municipal de Curitiba). *Pinus pinaster* and *P. radiata* are no longer present in the area for unknown reasons, but at least the former was present until 1987, meaning that plants of *P. pinaster* successfully reached 20 years old at the site (Keinert Junior and Matos 1987). The experiments also included trials of 22 provenances of *P. taeda*, 10 provenances of *P. elliottii*, and two provenances each of *P. glabra* and *P. palustris* (Fig. 2, Appendix 2). The aim of these trials was to find the provenances with fastest growth in the region for silviculture promotion, and all seeds were collected by the U.S. Forest Service from natural stands in the native range of each species (Baldanzi and Araujo 1971; Baldanzi and Malinovski 1976).

Since the implementation of the experiments, very few interventions have occurred in Rio Negro; mostly these consisted of road and access maintenance and occasional measurements of stands. The secondary forest patches have been left undisturbed since 1970, and natural regeneration of native and non-native plants was left unmanaged except for routine roadside maintenance. Fieldwork for this study was conducted in June and July of 2011.

Patterns of pine invasion

To assess invasion for the 16 *Pinus* species we established linear transects from the border of the stands and looked for plants outside plantations (Fig. 1c). Each transect was three meters wide and started at the border of one stand. The number of transects per stand varied depending on stand size, shape, and location, but they were at least 50 m apart and there were at least three transects per stand. Transect length varied from 150 to 300 m depending on the distance from the stand to the border of Rio Negro and on the absence of surveyed plants for at least 100 m. Transects were allowed to overlap only when different species were being surveyed. For all plants found we identified species and measured height and distance from the stand. With these data we were able to estimate the number of plants outside plantations and the distance of spread for each species. Only plants taller than 0.5 m were counted, because smaller plants could not be identified with assurance at the species level.

We surveyed for presence or absence of offspring inside the stands and estimated the number of planted trees present based on counts of number of rows and number of trees per row. Species not forming self-replacing populations (zero offspring) and persisting only by virtue of cultivation were considered as present only as the originally planted individuals ("planted-only") (Fig. 3a). Species sustaining a self-replacing population restricted to the cultivated areas were considered naturalized (Fig. 3b). Species producing offspring consistently and spreading considerable distances from parent plants (> 100 m in < 50 years) were considered invasive (Fig. 4). These definitions follow the terminology for plant invasion ecology proposed by Richardson et al. (2000).

Habitats invaded

To determine which habitats *Pinus* species were invading in Rio Negro, we selected one area of 10 ha surrounded by plantations (Fig. 1c: top line and dash perimeter), set up 25 equidistant plots of variable area, and used the point-quarter method (Krebs 1999) to obtain the total density of trees (excluding *Pinus*). Using the same center point of the point-quarter quadrats, we set up 25 circular plots of 200 m^2 (radius = 8 m) to obtain density of *Pinus* trees. We visually estimated percentage of grass coverage per plot using four classes (0 – 25, 26 – 50, 51 – 75 and 76 – 100 %), and all plants were identified to species.

To explore the effect of native tree density on *Pinus* invasion we calculated tree density in the 200 m^2 plots. To test if the presence of *Pinus* was related to native tree density we performed a Student's t-test ($\alpha = 0.05$) comparing plots with and without *Pinus* and built a general linear model to test for the predictability of *Pinus* presence based on local tree density. In both cases data were log-transformed to accommodate the assumption of normal distributions. We included only plots where *Pinus* was present in the linear model (eight plots). For these analyses we included all *Pinus* species found and did not differentiate among them.

Determinants of naturalization and invasion

To evaluate the role of introduction history in the observed pattern of naturalization and invasion, we tested the effects of propagule size and propagule diversity on species status with generalized linear models (GLM). First, we compared naturalized (invasive or not) and non-naturalized species using a GLM with binomial error distribution and logit link function. Second, we compared planted-only, naturalized, and invasive species using a multinomial distribution and logit link function. Explanatory variables were standardized (mean = 0 and variance = 1) to allow comparisons of models using variables at different scales. We tested six different models: only propagule size, only propagule diversity, and a full model (the two main factors and interaction term), in each case with both binomial and multinomial responses. We then used the Akaike information criterion corrected for small sample sizes (AICc) to find which model was best supported by the data (Johnson and Omland 2004). The GLMs were built and analyzed in R 2.13.0 (R Development Core Team 2011) and the multinomial GLMs were built using the package nnet (Venables and Ripley 2002).

To evaluate the role of species invasiveness in the observed pattern of invasion we compared the observed and expected patterns of invasion. A chi-square test was performed to compare the observed frequency of invasion status with the expected frequency based on the species' Z scores obtained from the literature (Grotkopp et al. 2002; Rejmánek and Richardson 1996). Z scores are calculated by entering mean seed mass (M), minimum juvenile periods (J), and mean interval between large seed crops (S) into a discriminant function developed by Rejmánek & Richardson (1996). Species with positive Z scores would be potentially invasive and species with negative Z scores would be potentially non-invasive. Moreover, species considered to be more invasive are predicted to have higher Z scores. We also performed a Pearson's product-moment correlation test to determine if species that are more invasive at the site (higher frequency of individuals outside plantations) also have higher Z scores.

Results

Patterns of pine invasion

Three species were found to be invasive: *P. elliottii*, *P. glabra* and *P. taeda*. Three species were found to be naturalized: *P. clausa*, *P. oocarpa* and *P. pseudostrobus*. Ten species were found to be planted-only: *P. echinata*, *P. kesiya*, *P. montezumae*, *P. palustris*, *P. patula*, *P. rigida*, *P. roxburghii*, *P. serotina*, *P. strobus* and *P. virginiana* (Table 1). None of the invasive species had spread more than 250 m from the plantation in 45 years (Fig. 4). All species were either restricted to the plantations or spread over 100 m away from them.

While *P. taeda* had spread farther (Fig. 4a), *P. glabra* had greater overall density in the invaded area (Fig. 4b). Densities (excluding absences) varied from 55 to 1,140 plants ha^{-1} for *P. taeda* (mean = 224), from 111 to 3,444 plants ha^{-1} for *P. glabra* (mean = 1,250) and from 33 to 133 plants ha^{-1} for *P. elliottii* (mean = 64). Mean dispersal rates were 5.6 m year^{-1} for *P. taeda*, 3.3 m year^{-1} for *P. glabra* and 2.2 m year^{-1} for *P. elliottii*. We found a total of 195 plants of *P. taeda*, 45 plants of *P. glabra*, and 15 plants of *P. elliottii* that were taller than 0.5 m. We found 488 plants that were less than 0.5 m tall, mostly on roadsides and close to *P. taeda* stands, probably belonging to this species.

Habitats invaded

Plots with *Pinus* have lower forest tree density than plots lacking *Pinus* ($t = 2.48$, $df = 17.8$, $p = 0.012$, Fig. 5a), and *Pinus* abundance decreased with increases in tree density ($r^2 = 0.52$, $F = 6.4$, $p = 0.045$, Fig. 5b). Forest tree density varied from 35 to 1,616 plants ha^{-1}, and *Pinus* were found only in plots with tree densities below 805 plants ha^{-1}. Plots with intermediate native tree densities were colonized only by *P. elliottii* and *P. taeda*, and plots with lower native tree densities and old fields were colonized by all three invasive species, suggesting *P. glabra* might be less shade-tolerant than *P. elliottii* and *P. taeda*. We found no clear trend between understory grass cover and *Pinus* density, with *Pinus* colonizing areas with 0 to 100% grass cover (data not shown).

Determinants of naturalization and invasion

Propagule size and propagule diversity predicted naturalization equally well, and both described the observed patterns of naturalization ($\Delta AICc < 4$; Table 2). However, the model including propagule diversity only was the best to describe the observed patterns of both naturalization and invasion ($\Delta AICc > 4$; Table 2). Propagule size also had high predictive power for naturalization and invasion, but the model had a lower fit than that for propagule diversity. The full models (including main effects and the interaction term) had the lowest fit for the data and performed significantly more poorly than the simpler models (Table 2). However, both variables are also highly correlated ($r = 0.96$, $p < 0.001$), and cannot be interpreted individually.

Based on 15 species' Z scores gathered from the literature (we could not find Z scores for *P. montezumae*, *P. pseudostrobus* and *P. echinata*), 13 species were expected to be invasive (Table 1). However, only three species are invading, which rejects Z score as an accurate predictor of invasion in Rio Negro ($\chi^2 = 57.69$, $p = 3.06\times10^{-14}$), where other factors can be acting to hinder invasions. Also, there is no correlation between species' Z scores and observed patterns of invasion ($r = 0.042$, $p = 0.89$), rejecting the hypothesis that species more invasive at the site also have higher Z scores.

Discussion

Our study lends support to the hypothesis that propagule pressure is a key factor of naturalization and invasion (Crawford and Whitney 2010; Roman and Darling 2007). Our most interesting finding is that whereas propagule size and propagule diversity were related to naturalization success, propagule diversity predicted invasion success better than propagule size did. Therefore, for our study system, both propagule size and propagule diversity could independently explain invasion success. The role of propagule diversity could be related to the presence of greater genetic diversity, adapted genotypes, or formation of novel genotypes by hybridization between previously isolated populations. In our system, all species but one with more than one source population are invasive. This degree of separation between invasive and non-invasive status is likely the cause of the high fit of the propagule diversity models. However, owing to the observational nature of this study, we do not have direct evidence on the role of genetic diversity for the three invasive pines in Rio Negro. Others have similarly suggested a role for genetic diversity. For example, researchers experimenting with waterstriders (*Aquarius najas*, Hemiptera) found that the number of source populations was a key driver of colonization success (Ahlroth et al. 2003), and studies on *Arabidopsis thaliana* showed a direct positive association between number of genotypes and colonization success at the population level (Crawford and Whitney 2010). Moreover, Saltonstall (2002) found that recent increases in distribution and abundance of *Phragmites australis* (Poaceae) in North America were due to a specific genotype previously absent.

Bottleneck effects that might drive small populations to extinction can be reduced with a greater number of source populations (Bossdorf et al. 2005; Lavergne and Molofsky 2007) by increasing the chances of introduction of adapted genotypes (Muirhead et al. 2008; Simons 2003) or by allowing intraspecific hybridization that can create novel genotypes (Ellstrand and Schierenbeck 2000). However, genetic variation per se has limited relevance for determining the adaptive potential of introduced populations if this variation is located in neutral molecular markers (Roman and Darling 2007). In order for genetic diversity to be relevant for countering genetic drift and accommodating environmental stochasticity it must be related to traits associated with fitness (Reed and Frankham 2001; Roman and Darling

2007). In *P. taeda* at least, and probably in many species of *Pinus*, single nucleotide polymorphisms are associated with phenotypic adaptation to environmental gradients (Eckert et al. 2010a; Palle et al. 2010) and this variation is found across populations throughout the native range (Eckert et al. 2010b). Also, in the native range, seed size, seed weight, and seedcoat thickness of *P. taeda* vary by region and affect seedling growth, with seed size decreasing from east to west (Belcher and Karrfalt 1976). In a common garden greenhouse experiment, seed size was the factor that most strongly affected seedling growth rate (Schultz 1997). Moreover *P. taeda* show considerable genetic variation in dormancy and seeds from some populations are practically nondormant (Schultz 1997). Therefore it is not surprising that introducing several geographically distinct populations (Fig. 2) will significantly increase the probability of invasion success. However it is unknown if invasion is caused by one or more source populations pre-adapted to the local environment of Rio Negro, by hybrids of previously disconnected populations, or by all source populations.

The role of propagule size during the naturalization stage in Rio Negro might be related to greater proximity to suitable habitats, greater pollen exchange and seed production, and higher numbers of dispersed seeds. However, only *P. taeda* and *P. elliottii* have distinctly higher numbers of plants in Rio Negro, whereas *P. glabra* is present in roughly the same numbers as many other non-invading species at the site. The fact that *P. glabra* stands are adjacent to an old field with scarce tree cover (Fig. 1) suggests it is likely that many seeds are arriving in a suitable habitat every year, making seed rain equivalent to that of *P. taeda* and *P. elliottii*, which are producing greater absolute numbers of seeds yearly but which also have greater seed losses owing to greater distances from suitable habitats (Fig. 1). However, other species (i.e., *P. kesiya*, *P. clausa*, and *P. radiata*) are also adjacent to this same old field, without producing any signs of seedling establishment. Since many pine species produce serotinous cones, the lack of fire at the site might be affecting the ability of some species to release seeds or establish seedlings.

Z scores did not predict well which species would invade in Rio Negro. This was mostly because the model had a high number of false positives (i.e., species predicted to be invasive that failed to invade), besides the three invasive species at the site being correctly anticipated. However, the model did not have any false negative (i.e., species predicted to fail that successfully invaded), which suggests it may still be useful when more detailed information is not available. One of the possible reasons for the poor performance of the Z score is the fact that most known pine invasions and failures used to build the model were in grasslands and shrublands (Rejmánek and Richardson 1996; Richardson and Rejmánek 2004), while Rio Negro is mostly covered by forest. Therefore, traits associated with successful invasions of open habitats might differ from traits required to invade closed habitats. For instance, shade-tolerance by seedlings might be more relevant for plant survival and growth in a forest than short intervals between large seed crops. If more data on conifer invasions in forests become available, a comparison of traits between the two groups of species might yield new insights.

Pinus elliottii and *P. taeda* are already known to be highly invasive in south Brazil, both in grasslands and degraded or secondary *Araucaria* moist forests (Simberloff et al. 2010; Zenni and Ziller 2011). However, to our knowledge, the Rio Negro case is the first record of invasion by *P. glabra* (cf. Richardson and Rejmánek 2011). The fact that *P. glabra* is not commercially important, and thus plantations outside the native region are small and rare, may be an important factor hindering invasion by this species (Rejmánek and Richardson 1996; Richardson and Rejmánek 2004), but clearly this species has the potential for greater spread and encroachment in the studied region owing to the high density of seedlings currently found (Fig. 4). With more time, invasive saplings will probably mature and start reproducing, thus potentially increasing the rate of spread. On the other hand, *P. oocarpa* and *P. patula* are recorded as invasive in *Araucaria* moist forests in Brazil (Zenni and Ziller 2011), but in Rio Negro they are, respectively, naturalized and planted-only. It is possible that with time these two species will start invading (lag phase), but it is unclear if the current statuses are due to intrinsic reproductive limitations of the plants or limitations in survival imposed by the native community and ecosystem.

Also interesting is the failed naturalization of *P. radiata* and *P. pinaster*, as both species are highly invasive in many temperate and mediterranean-climate regions of the southern hemisphere (Richardson and Rejmánek 2004). This failure may have been caused by inappropriate silvicultural practices (and hence would be completely unrelated to ecological factors) or to the lack of local adaptation (Lonsdale 1999; Richardson and Pyšek 2006).

However, at least *P. pinaster* plants successfully reached 20 years old in the site and then failed to naturalize and invade, suggesting that silvicultural practices (especially seedling production, transportation, and planting) were correct. The reasons for these failures are unclear, but investigation of more attempts of introduction in similar regions could reveal important factors for failure. Other species also known to be naturalized elsewhere according to three recent reviews (Essl et al. 2011; Richardson and Rejmánek 2004; Simberloff et al. 2010) but failing to naturalize and invade in Rio Negro are *P. patula*, *P. rigida*, *P. roxburghii*, *P. strobus*, and *P. virginiana*. The reasons for failure in these cases remain unclear, but possibilities include lack of local adaptation, lack of competitive ability with other forest species, or bottleneck effects owing to small propagule sizes. It is also possible but unlikely that naturalization is happening but we did not record it.

Pinus elliottii was the species exhibiting the least invasive behavior in Rio Negro among the three invasives. This is surprising, because other studies in Brazil found this species to be a prominent invader after approximately the same residence time (Abreu et al. 2011; Abreu and Durigan 2011; Bourscheid and Reis 2010; Zanchetta and Pinheiro 2007; Zenni and Ziller 2011). Potential reasons for this variability are differences in the environment (Rio Negro has a warm temperate climate while Assis has humid subtropical climates), differences in propagule pressure (Rio Negro has 3.5 ha of *P. elliottii* plantations while Itirapina has more than 1,000 ha), differences in community structure and composition (Rio Negro has *Araucaria* forest while Assis and Itirapina have savanna and Florianópolis has short-grass dunes) and presence or absence of specific herbivores, pathogens, or mutualists.

In Rio Negro *Pinus* are colonizing *Araucaria* forest when canopy tree density is ca. 805 plants ha^{-1}, and previous research has shown that this density is common throughout the ecosystem (e.g., Kozera et al. 2006; Negrelle and Leuchtenberger 2004; Rondon Neto et al. 2002). Furthermore, 98% of the plants present in the transects lack any visual sign of pathogen or herbivore attack. These results contradict the claim of Emer and Fonseca (2010) that *Araucaria* forest resists invasion by exotic conifers. Instead it would probably be more appropriate to say that dense forests (> 1000 plants ha^{-1}) create strong light limitation that cannot be overcome by shade-intolerant *Pinus* species unless disturbances create windows of opportunity to invade (Davis et al. 2000). This view is also supported by other studies of pine invasions in *Araucaria* forests, such as *Pinus contorta* invasion in temperate *Araucaria araucana* forests (Peña et al. 2008). Moreover, once *Pinus* invades, it causes decreases in species richness and abundances (Abreu and Durigan 2011; Falleiros et al. 2011), which means that many native species are not able to outcompete *Pinus* if they cannot prevent its naturalization.

Even though we found that propagule size and propagule diversity had strong explanatory power for the observed pattern of *Pinus* in Rio Negro, this study is observational and subject to inherent limitations, such as small and unequal sample sizes and covariation in the dataset. Moreover, this system was originally implemented as a "provenance trial" experiment for silvicultural purposes and was not designed for the type of questions we asked. Another potential limitation is the spatial heterogeneity of the area (e.g., secondary forests, old field, and tree plantations), as each habitat presents different types of barriers for invasion (e.g., Fig. 5) and each species has different traits to interact with these barriers (e.g., results for Z scores). This variability could be benefiting some species more than others. The fact that these plantations are relatively young (< 50 years) might also be limiting, because some of the observed patterns could be due to lag phases. However, all species seemed to have reproductively mature plants in the stands and clear signs of cone production, which means seeds are likely being produced and released.

As regards Rio Negro, it appears that invasions are to a great extent driven by anthropogenic disturbance and selective introduction efforts. While the former is caused by decreases in tree density in *Araucaria* forest owing to deforestation and by providing limiting resources to *Pinus* growth and survival not available in undisturbed forest (Emer and Fonseca 2010), the latter are due to careful selection of promising species and provenances for silviculture (i.e., fast growth, lack of major pathogens, great tolerance to disturbance, abundant seed production, and easy reproduction). Not surprisingly, desirable attributes for forestry are also present in many invasive plants (Grotkopp et al. 2002; Muth and Pigliucci 2006; Procheş et al. 2012; Pyšek et al. 2009; Pyšek and Richardson 2007). Therefore, it is possible species traits are playing a role in the invasion patterns observed in Rio Negro even though we did not detect it. Other traits might be involved (i.e., shade-tolerance and serotiny), or values of mean seed mass, minimum juvenile periods, and mean interval between large seed crops for Rio Negro differ significantly from the values used by Rejmánek andRichardson (1996) to calibrate the discriminant function.

Our study with 16 *Pinus* species provides insights into the role of propagule diversity as a key component of propagule pressure and into the role of habitat characteristics and species traits in invasion success. Our most important finding is that propagule diversity described patterns of naturalization and invasion, and it did so better than propagule size. This is one of the few studies to observe this pattern outside of an experimental set-up. Our study also shows that pine invasions are not restricted to treeless habitats, and that prediction of invasiveness based solely on species traits may not be useful for single introduction cases since a wide range of factors may determine invasion success. We reason that propagule diversity should be explicitly incorporated in models and frameworks of propagule pressure and hope that future work will expand our findings to explore the relative importance of different mechanisms of propagule diversity in invasion success, such as novel genotypes and preadaptation. We also hope our findings can be useful to research aiming to prevent and manage biological invasions.

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Appendix II: Tables and Figures

Table 2-1. *Pinus* species introduced in Rio Negro, their status in Rio Negro determined after field surveys (see methods) and following the classification proposed by Richardson *et al.* (2000), their status as naturalized anywhere else in the world (Essl et al. 2011; Richardson and Rejmánek 2004), residence time in Rio Negro gathered from historical records kept with the Rio Negro administration, propagule size measured as number of plants present inside plantation determined from field surveys and historical records, Z scores published in the literature (Grotkopp et al. 2002; Rejmánek and Richardson 1996), native location of the species based on Critchfield & Little (1966) and Hurrell & Bazzano (2007), and propagule diversity measured as number of source populations introduced from each species (Fig. 2) according to historical records kept with the Rio Negro administration and to Baldanzi and Araújo (1971). Voucher specimens were deposited in the municipal herbarium of Curitiba/PR (Museu Botânico Municipal de Curitiba).

***Pinus* species**	**Status in Rio Negro**	**Naturalized elsewhere**	**Residence time**	**Propagule size**	**Z score**	**Native location**	**Propagule diversity**
P. echinata Mill.	Planted-only	no	44	50	NA	Southeast USA	1
P. kesiya Royle ex Gordon	Planted-only	yes	44	120	9.45	Southeast Asia	1
P. strobus L.	Planted-only	yes	44	50	3.46	Eastern North America	1
P. virginiana Mill.	Planted-only	yes	44	50	10.02	Central east North America	1
P. patula Schltdl. & Cham.	Planted-only	yes	44	10	7.3	Southern North America	1
P. montezumae Lamb.	Planted-only	no	44	110	NA	Southern North America and northern Central America	1
P. palustris Mill.	Planted-only	no	44	128	-6.36	Southeast USA	2
P. rigida Mill.	Planted-only	yes	44	15	1.49	Northeast USA	1
P. roxburghii Sarg.	Planted-only	yes	44	20	-2.37	South-central Asia	1
P. serotina Michx.	Planted-only	yes	44	100	10.85	Southeast USA	1

Table 2.1. Continued.

Pinus **species**	**Status in Rio Negro**	**Naturalized elsewhere**	**Residence time**	**Propagule size**	**Z score**	**Native location**	**Propagule diversity**
P. elliottii Engelm.	Invasive	yes	45	5500	4.33	Southeast USA	10
P. glabra Walter	Invasive	no	44	100	7.02	Southeast USA	2
P. taeda L.	Invasive	yes	45	7500	3.41	Southeast USA	22
P. pseudostrobus Lindl.	Naturalized	no	44	100	NA	Southern North America and northern Central America	1
P. clausa (Chapm. ex Engelm.) Sarg.	Naturalized	no	44	40	9.7	Southeast USA	1
P. oocarpa Schiede ex Schltdl.	Naturalized	no	45	50	6.5	Southern North America and Central America	1
P. pinaster Aiton	Absent	yes	44	0	7.46	Western Europe	1
P. radiata D. Don	Absent	yes	44	0	9.27	Southwest USA	1

Table 2-2. Statistics of the models analyzed for probabilities of naturalization and invasion in Rio Negro, Brazil

Response	**Factor**	**χ^2**	**df**	**ρ**	**AICc**
Naturalization	Propagule size	4.5141	1	0.03362	21.579
	Propagule diversity	4.9011	1	0.02684	21.19201
	Propagule size	0.00049	1	0.9824	27.90463
	Propagule diversity	0.38748	1	0.5336	
	Propagule size × Propagule diversity	0.00018	1	0.9894	
Naturalization and invasion	Propagule size	9.0418	2	0.01088	32.08239
	Propagule diversity	13.194	2	0.001364	27.92974
	Propagule size	0.4688	2	0.79104	51.8993
	Propagule diversity	4.6215	2	0.09919	
	Propagule size × Propagule diversity	0.4967	2	0.78009	

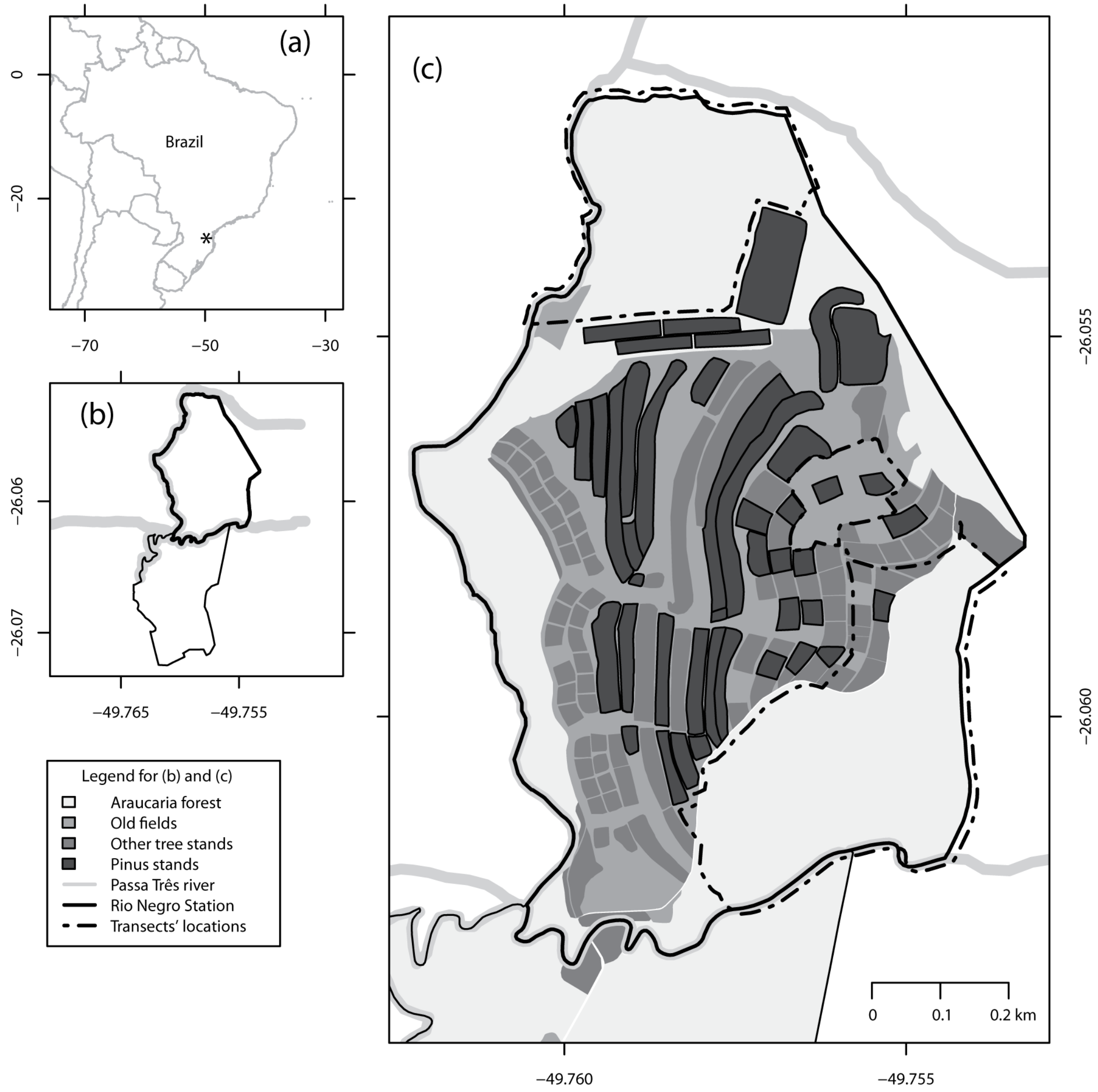
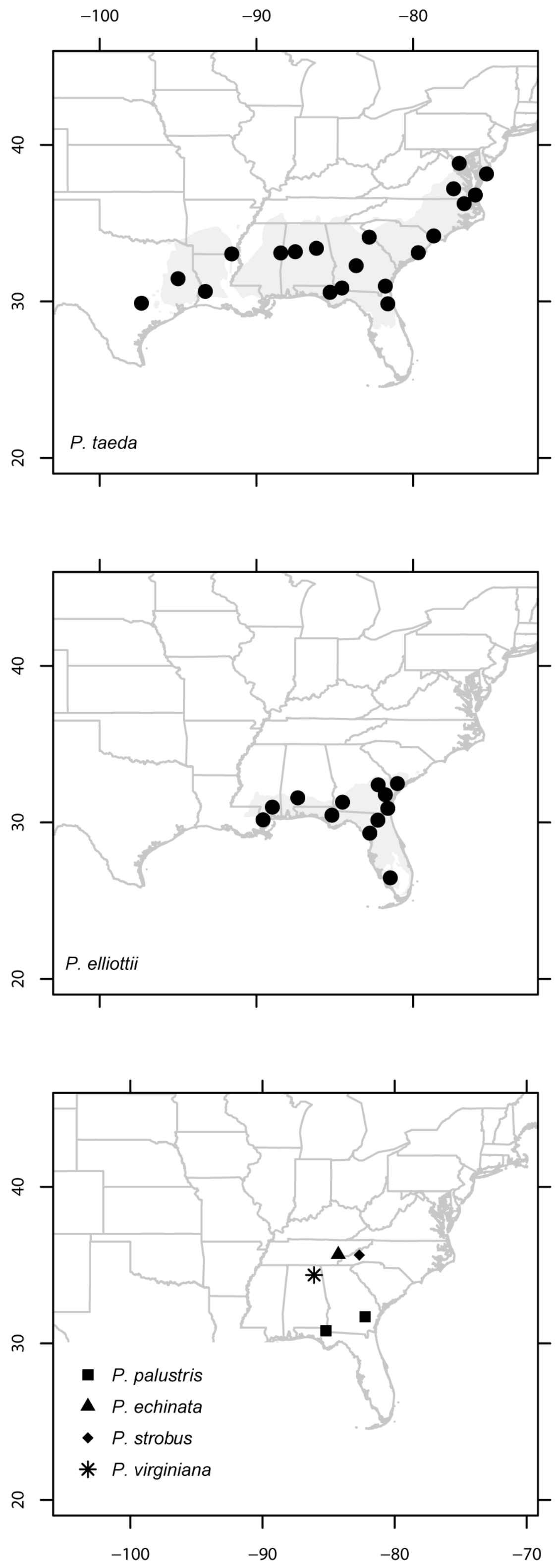


Figure 2-1 map of Rio Negro with major vegetational features.

Rio Negro Experimental Station is located in southern Brazil (A), and for this study we focused on the area north of the Passa Três river (B). Major vegetational features are secondary *Araucaria* forest, *Pinus* stands, old fields, and plantations of other native and exotic tree species (C).

Figure 2-2 Locations of the source populations of *Pinus taeda* L., *P. elliottii* Engelm., *P. palustris* Mill., *P. echinata* Mill., *P. strobus* L., and *P. virginiana* Mill.

These source populations were selected for the provenance experiments implemented in 1966 and 1967 (see methods for a description of the experiments and Appendix 2 for more detail on the source populations). For *P. taeda* and *P. elliottii* the source population selection covers the full native range (in gray) of the species (Critchfield and Little 1966).



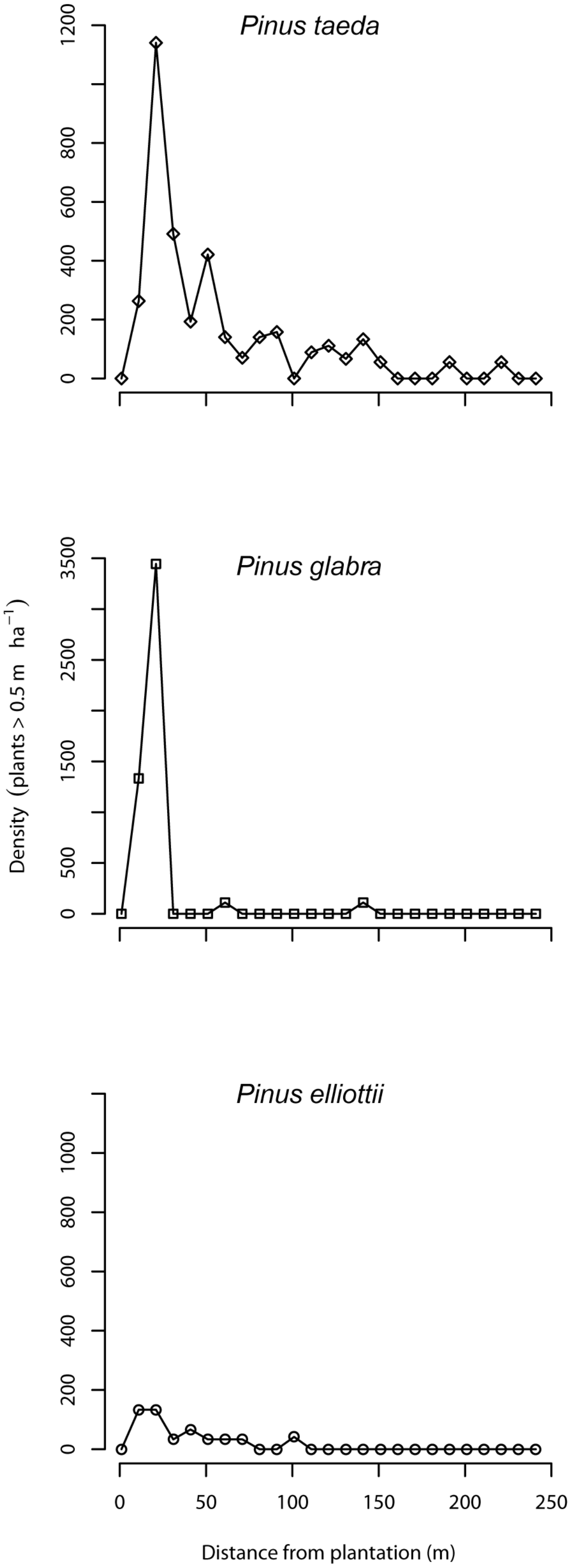
−100
−90
−80
40
30
20
P. taeda
40
30
20
P. elliottii
40
30
20
P. palustris
P. echinata
P. strobus
P. virginiana
−100
−90
−80
−70

Figure 2-3 Example of casual and natualized species in Rio Negro.

Planted-only species are species able to survive in the new environment but not producing offspring, such as *Pinus kesyia* Royle ex Gordon (a); naturalized non-native species are species able to survive in the new environment and producing offspring consistently only beneath parent plants (b); and invasive non-native species are species able to survive in the new environment that are producing offspring consistently beneath parent plants and spreading at least 100 m from the plantation, such as *Pinus taeda* L. Terminology for this study followed Richardson *et al*. (2000).

Figure 2-4 Transects established from the border of the *Pinus* stands and extending for up to 300 m provide information on presence of plants outside plantations as well as relative densities for invading species.

Pinus taeda L. spread over 200 m in 45 years and reached densities of 1,200 plants ha^{-1}. *Pinus glabra* Walter spread almost 150 m in 44 years and reached densities of 3,500 plants ha^{-1}, but high densities are restricted to the border of the stands. *Pinus elliottii* Engelm. is the invasive species with least spread and encroachment currently, having spread around 100 m and never in densities higher than 200 plants ha^{-1}.


Pinus taeda
1200
800
600
400
200
0
Pinus glabra
3500
2500
1500
500
0
Density (plants > 0.5 m ha^{-1})
Pinus elliottii
1000
800
600
400
200
0
0
50
100
150
200
250
Distance from plantation (m)

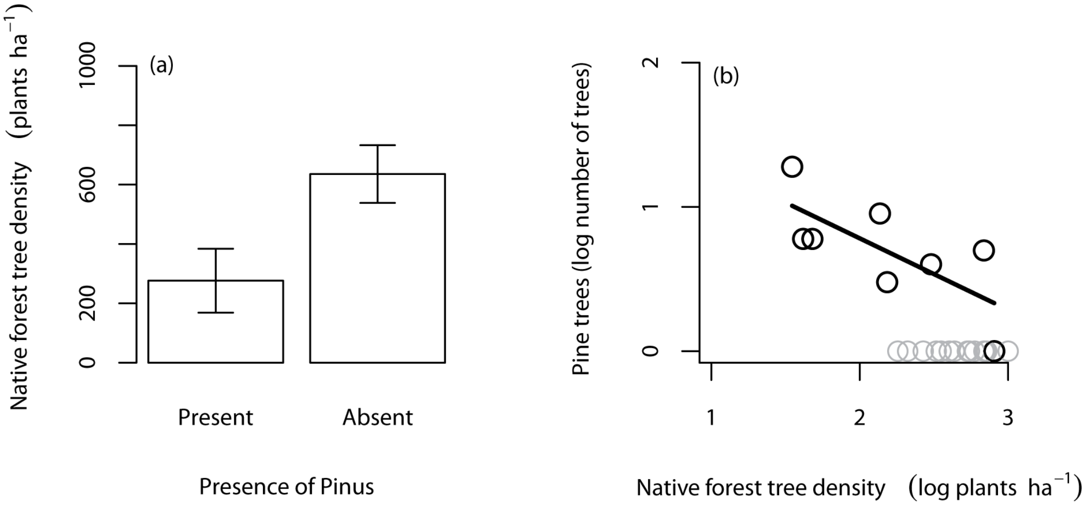


Figure 2-5 Pine invasion in *Araucaria* forest.

(a) Mean and standard error of native forest tree densities in quadrats successfully colonized by *Pinus* and in quadrats where *Pinus* was not found. Native forest tree densities are lower in quadrats where *Pinus* was found (t = 2.48, df = 17.8, p = 0.012). (b) Log-log linear relationship between *Pinus* density and native forest tree density. *Pinus* density decreases with increases in forest tree density (r^2 = 0.52, F = 6.4, p = 0.045). Black points are quadrats with *Pinus* and grey dots are quadrats without *Pinus*. Only quadrats with *Pinus* were used in the linear model.

CHAPTER III

THE ELEPHANT IN THE ROOM: THE ROLE OF FAILED INVASIONS IN UNDERSTANDING INVASION BIOLOGY

A version of this chapter was originally published by Rafael D. Zenni and Martin A. Nuñez:

Zenni, R. D., & Nuñez, M. A. (2013). The elephant in the room: the role of failed invasions in understanding invasion biology. *Oikos, 122*, 801-815.

RDZ designed the study, did the literature survey, analyzed the data, and wrote the first draft of the manuscript. MAN contributed substantially to manuscript revisions.

Abstract

Most species introductions are not expected to result in invasion, and species that are invasive in one area are frequently not invasive in others. However, cases of introduced organisms that failed to invade are reported in many instances as anecdotes or are simply ignored. In this analysis, we aimed to find common characteristics between non-invasive populations of known invasive species and evaluated how the study of failed invasions can contribute to research on biological invasions. We found intraspecific variation in invasion success and several recurring explanations for why non-native species fail to invade; these included low propagule pressure, abiotic resistance, biotic resistance, genetic constraints, and mutualist release. Furthermore, we identified key research topics where ignoring failed invasions could produce misleading results; these include studies on historical factors associated with invasions, distribution models of invasive species, the effect of species traits on invasiveness, genetic effects, biotic resistance, and habitat invasibility. In conclusion, we found failed invasions can provide fundamental information on the relative importance of factors determining invasions and might be a key component of several research topics. Therefore, our analysis suggests that more specific and detailed studies on invasion failures are necessary.

Introduction

Historically the field of invasion biology has focused on the study of species that successfully invaded (i.e., invasive alien species) after introduction to a new range, and during the past decades invasion biologists have collected numerous case studies of successful invasions (MacIsaac et al. 2011, Richardson and Pyšek 2008). This focus on successful invaders helps us understand their overall importance as a threat to global biodiversity and why certain introduced species become invasive. However, most species introductions are not expected to result in invasion (Kowarik 1995, Williamson and Fitter 1996a) and species that are invasive in one area can be non-invasive elsewhere (e.g., Simberloff et al. 2002). Even though the fact that most introductions do not result in invasions is generally accepted (Blackburn et al. 2011, Lockwood et al. 2005), we still lack a comprehensive understanding of failed invasions. It is clear that failures are not part of the mainstream research on invasive species, as can be observed in many of the most important books in the discipline (Davis 2009, Lockwood et al. 2007, Richardson 2011, Sax et al. 2005, Simberloff and Rejmánek 2011).

After individuals of a species are released within a new range, invasion failure can occur during any stage of the invasion continuum (Blackburn et al. 2011). Populations can be incapable of surviving, reproducing, or maintaining a sustainable population, and therefore they cannot invade (failure to naturalize). In other instances, populations may naturalize and not spread, also failing to invade (failure to invade after naturalization). Different mechanisms can operate at each stage; populations can either stagnate in a stage previous to invasion or recede to earlier stages, up to the point of local or regional extinction (Simberloff and Gibbons 2004, and references therein). Often, failure to naturalize is unknown and difficult to detect (especially for unintentional introductions), while failure to invade after naturalization is more commonly observed (e.g., Phillips et al. 2010).

For this study, we reviewed the literature and searched for cases where a non-native species that is a known invader in one habitat or region has failed to invade a differing region or habitat or at a different time. We only considered cases of intraspecific variation in invasion success. Even though studies of species that never invaded can produce informative results, comparisons of invasive and non-invasive populations of a given species may be more likely to determine the cause of current failure (Blackburn et al. 2011). If a species has never been documented as invasive there may be many non-exclusive causes.

Assessment of the published reports on failed invasions

We conducted different searches to collect cases of failed invasions. Given that this is not a research topic, it cannot be expected that summaries, titles, or key words would adequately sample and locate many cases of failed invasions. Therefore, we conducted extensive searches by querying academic search engines (ISI Web of Science and Google Scholar) using combinations of the key words introduction, naturalization, invasion, invasive, fail, and failure. We also searched the reference lists and citations received by the papers identified in the search. Complementary, we searched mentions for failures in global catalogues of naturalized species (i.e., Lever 1987, 1996, 2003, Long 2003). Experts in the field also helped identify cases of failed invasions. We included 76 cases where there was intraspecific variation in invasion success across continents, local habitats, or time frames (Table 1). We did not aim for a complete list of cases, but instead we hoped to provide examples that illustrate the extent of invasive species failures. We grouped the examples based on hypotheses that were proposed to explain these failures and compared the number of times where a hypothesis for the failed invasion was only suggested, the number of times a proposed hypothesis was suggested and tested, and the number of times where no factor was suggested (Fig. 1).

Factors associated with invasion failure

From the 76 reported species with invasive and non-invasive populations (Table 1), we found five distinct factors suggested as reasons for invasion failures: propagule pressure, abiotic resistance, biotic resistance, genetic constraints, and mutualist release (Fig. 1). We found taxonomic and geographic biases in reports of invasion failures and these biases are also present in Table 1. Reports of failed invasions for trees and terrestrial vertebrates abound, while cases of failure for herbaceous plants and arthropods (except biocontrol insects) are scarce. Also, there are many more reports for failures in Europe, Oceania, and USA. We found very few cases for Africa and Asia. We lack formal explanations for these biases; although they can be partially explained by unequal introduction effort and history of attention to species' introductions (Nuñez and Pauchard 2010). In most cases, only one mechanism for failures was suggested, and 11 studies tested the proposed factors. One striking result is that two-thirds of the cases presented (48), lack explanation for invasion failures. Abiotic and biotic resistances were found to be commonly associated with failures, but in very few cases these factors were experimentally or statistically tested. Below we present the evidence available for the factors we found are associated with failures to invade.

Failed invasions and propagule pressure

Current theory predicts that increased propagule pressure increases the likelihood of invasion, which has been proposed as the main determinant of invasion success (Colautti et al. 2006, Lockwood et al. 2005, Simberloff 2009). With few individuals, species can fail to naturalize because of demographic stochasticity (e.g., lack of mate encounters or pollen outcrossing). However, some small populations do naturalize and fail to invade after naturalization for various reasons that are unrelated to initial propagule pressure (Boyce 1992, Simberloff and Gibbons 2004). For example, on Isla Victoria (Argentina) propagule pressure did not explain the current invasion failure of 18 non-native tree species known to be invasive elsewhere (Nuñez et al. 2011, Simberloff et al. 2010). Also, invasive populations of *Pinus radiata* in Australia are scarce, despite being widely planted (Williams and Wardle 2007), while in South Africa and New Zealand, where *P. radiata* was extensively planted during the 19th and 20th centuries, invasive populations are common (Richardson 1998, Simberloff et al. 2010). In Argentina, *P. radiata* is well established in some regions but fails to establish in others, and in southern Brazil and Uruguay plantations of *P. radiata* exist but there is no record of naturalized populations outside plantations (Simberloff et al. 2010, Zenni and Simberloff 2013).

Failed invasions and abiotic resistance

The ability to cope with abiotic factors in the introduced range might determine the survival and reproductive capacities of non-native organisms, and the environmental suitability of the introduced range seems to be crucial for naturalization success (Blackburn and Duncan 2001, Menke and Holway 2006, Moyle and Light 1996). Abiotic factors act strongly at the naturalization stage, prior to invasion, because they affect the survival of introduced individuals prior to reproductive maturity (Castro et al. 2002, Moyle and Light

1996). Also, different factors can operate at different scales. While climatic variables such as mean annual temperature and precipitation are mostly macroclimatic factors, soil moisture and depth can vary locally. Abiotic resistance may be the strongest mechanism causing invasions to fail in some regions (Blackburn and Duncan 2001).

Abiotic factors are key determinants of invasion success or failure of non-native fish species in California streams and estuaries (Moyle and Light 1996). The rainbow trout (*Oncorhynchus mykiss*), one of the most widely introduced and invasive fish species (Welcomme 1985), varies from highly successful to failed invader in the USA (Fausch et al. 2001). Similarly, the bluegill (*Lepomis macrochirus*) failed to invade freshwater systems in California (Meffe 1991) even though it successfully invaded streams in Japan (Nakao et al. 2006) and Korea (Kawamura et al. 2006). Invasion failures for these populations could be related to stream free-flow (Meffe 1991). Several studies with plants also have reported variation in invasion success of introduced populations. For example, *Prunus serotina* is unable to invade waterlogged and calcareous soils, whereas it successfully colonizes well-drained, nutrient-poor soils in northern France (Closset-Kopp et al. 2011). Also, the naturalization success of non-native plants in coastal dunes of California is related to exposure of the different sites to wind (Lortie and Cushman 2007). Nitrogen-fixing plants may fail to invade when phosphorus is limited since nitrogen fixation requires high availability of this nutrient (González et al. 2010, Vitousek 1999). As for invertebrates, cooler and wetter climate determined where dung beetles populations failed to naturalize in Australia (Duncan et al. 2009), and local soil moisture correlated with Argentine ants (*Linepithema humile*) local abundances in California (Menke and Holway 2006).

Failed invasions and biotic resistance

Community factors can locally prevent populations of non-native species from invading. Resident species cover (Levine 2000), competition (Crawley et al. 1999), or predation (Nuñez et al. 2008) can play key roles in determining a community's resistance to invasion. For example, thousands of colonies of the Sardinian bumblebee, *Bombus terrestris sassaricus,* were introduced in southern France for crop pollination between 1989 and 1996, but after 1998 no feral workers or hybrids between the introduced subspecies and the native subspecies were observed. The failure is probably due to competition with the three native subspecies existing in the region (Ings et al. 2010). By contrast, in Argentina, Chile, Japan, and New Zealand, *B. terrestris* has become an invasive species of increasing concern (Morales 2007). The success of the nonnative *B. terrestris* in Japan is related to its greater reproductive capacity and greater competitive ability in comparison with native bumblebees (Matsumura et al. 2004). Biotic resistance also seems to play an important role in invasion failure of populations of several *Pinus* species across a number of ecosystems predicted to be climatically suitable for these species (Bustamante and Simonetti 2005, Nuñez et al. 2011). Plant communities dominated by woody species, like forests and shrublands, seem to be more resistant to invasion by pine trees than other communities, like grasslands and dunes (Richardson et al. 1994). Also, many non-native populations thrive only in constantly disturbed sites (e.g., roadsides and pastures) and fail to invade undisturbed habitats. For example, the South African lovegrass (*Eragrostis plana*) currently invades more than two million hectares in Brazil but only in degraded or overgrazed steppes (Zenni and Ziller 2011). Another example is the climbing asparagus (*Asparagus scandens*), which has a patchy distribution in New Zealand, mainly in disturbed forest remnants near urban areas (Timmins and Reid 2000). Probably these non-native species are not able to thrive under competition in the native communities where they were introduced. However, it remains unclear if biotic resistance can deter invasions completely or if it only slows the invasion process.

Failed invasion and genetic constraints

Genetic factors could affect invasion success and different genetic lineages can exhibit different levels of invasiveness. The grasses *Phragmites australis* and *Phalaris arundinacea* in North America are good examples. The former is a macrophyte native to North America that over the last century has expanded into tidal and non-tidal wetlands, displacing native vegetation (Chambers et al. 1999). The expansion is due to the introduction of a non-native genetic lineage that exhibits greater rates of photosynthesis and greater rates of stomatal conductance, which allows the exotic lineage to outcompete native lineages of *P. australis* and native vegetation (Mozdzer and Zieman 2010, Saltonstall 2002). *Phalaris arundinacea* is also a native wetland grass in North America that became invasive after previously isolated non-native genotypes combined to create a novel genotype (Lavergne and Molofsky 2007). Likewise, population genetic diversity influences colonization success of the weedy herb *Arabidopsis thaliana* more than population density (Crawford and Whitney 2010). However, we could find no study exploring the role of genetics in invasion failures or comparing genetic characteristics between successful and unsuccessful populations. Although a genetic bottleneck is commonly argued to be one of the main reasons why introductions fail (Simberloff 2009), empirical evidence is missing or too biased towards cases of successful invasions, a fact that impedes the understanding of this factor as a limit to invasion.

Failed invasions and the lack of mutualists

Many species rely on mutualisms to grow or reproduce and will not successfully naturalize and invade until their mutualistic partner arrives (Richardson et al. 2000). For example, a lack of mycorrhizal fungi limited invasion by non-native trees in Patagonia (Nuñez et al. 2009), and non-native fig species were not invasive in Florida until their specific wasp pollinators arrived (McKey and Kaufmann 1991, Nadel et al. 1992, Ramirez and Montero 1988). Leguminous plants, which depend on mutualisms with root-nodule bacteria (rhizobia), may also fail to naturalize if the introduced population is small and if rhizobia density is low (Parker 2001), or if the co-evolved rhizobia strains from the native range are not co-introduced (Rodríguez-Echeverría et al. 2012). Given that many plant species rely on facilitation for their survival (e.g., for pollination, dispersal, and growth), and that sometimes mutualisms can be highly specialized, it is possible that numerous failed invasions are caused by the lack of a mutualist in the new habitat (Richardson et al. 2000). Contrary to the "enemy release" mechanism of invasion success (Keane and Crawley 2002), "mutualist release" can be one key mechanism of failure for populations of invasive species with obligatory mutualists. On the other hand, co-invasions seem to be common and many mutualists are generalists (Dickie et al. 2010, Rodríguez-Echeverría et al. 2012).

When is it important to know about failure and when is it not?

In this study, we report many species that successfully invaded somewhere and also failed to invade somewhere else, and this intraspecific variation in invasion success occurs across habitats as well as continents (Table 1). Yet, most studies of invasions rely on invasion successes only. For instance, the most common approach to study the determinants of invasiveness is to compare invasive vs. non-invasive species in a given, usually fairly large and heterogeneous, region (Diez et al. 2009, Van Kleunen et al. 2010). Also, studies on species potential invasive ranges mostly use invasion data only (Elith et al. 2006). The assumption that species can only be assigned to the invasive or non-invasive categories pose serious limitations to the interpretation of results in broader contexts, especially if spatial scale and heterogeneity are not clearly taken into account. Some research questions might require information about failed invasions more than others, and sometimes very different results can be obtained if failures are considered or are ignored. We have identified six research topics for which incorporating intraspecific variation in invasion success can help improve current understanding. Below, we describe these areas and suggest ways to incorporate failed invasions.

Historical factors associated with invasions

Several authors have pointed out historical factors (i.e., factors associated with human decision or activities and not with the biology of the species) such as dispersal pathways, reason for introduction, and propagule pressure, play important roles in invasion success (Harris et al. 2007, Wilson et al. 2009). For example, cultivation is generally agreed to be one of the most important dispersal pathways for invasive plants because the propagation of species increases propagule pressure and the cultivated species benefits from human-assisted long distance dispersal (Huang et al. 2010, Von Der Lippe and Kowarik 2007). However, it is also known that the numbers of species introduced through different dispersal pathways vary greatly (Richardson and Rejmánek 2011), and most studies on the topic include only records of naturalization and invasion (Harris et al. 2007, Huang et al. 2010). Omission of the failures can inflate the relative importance of historical factors responsible for many failed invasions. For example, forestry is considered an important pathway for tree invasions because many species introduced for forestry became invasive (Essl et al. 2010, Simberloff et al. 2010), even though in several cases plantations of the same species repeatedly fail to naturalize (Carrillo-Gavilán and Vilà 2010, Mortenson and Mack 2006, Nuñez et al. 2008). To improve our understanding of the relative importance of historical factors in invasion success, the next step is to explicitly include records of failed invasions in the analyses (e.g., Gravuer et al. 2008).

Small numbers of individuals might fail to invade owing to chance or idiosyncratic factors. However, high propagule pressure by itself cannot guarantee invasion success, although it certainly can increase the likelihood. Propagule pressure should be considered a null hypothesis in studies of invasions, and if it does not explain patterns of successes and failures, other mechanisms should be considered (Colautti et al. 2006, Lockwood et al. 2005, Simberloff 2009). Learning why introductions with abundant propagules (i.e., unlikely to go extinct because of demographic stochasticity) fail to naturalize and invade can further our understanding of invasions because they would not only demonstrate which historical factors contribute to invasions but also their relative strengths. It is not clear yet if certain dispersal pathways are more important because they truly promote invasion more often than others, or if they simply were more often used and had more opportunities to transport and release a successful invader.

Species distribution models

Studies of the potential distributions of invasive populations, or species distribution models (SDM), often use known presence records of the invasive species, both in the native and introduced ranges. Most SDMs generate pseudo-absences, in place of true absences, to predict the areas species could potentially occupy (Elith et al. 2006, Phillips et al. 2006). Pseudo-absences are points in the environmental layers of the model where the species is not known to be present and are used to simulate areas where the species is absent (Zaniewski et al. 2002). The lack of records of true absences is an important caveat in model accuracy because of several uncertainties generated by pseudo-absences (Elith et al. 2006); SDMs do not verify the species does not occur at “absence” locations, or that a species could not potentially thrive if introduced or dispersed to the “absence” point. For potential distribution models of invasive species, records of failed invasions represent true absences that might significantly improve model calibration and validation and decrease the uncertainties surrounding the predictions (Duncan et al. 2009, Václavík and Meentemeyer 2009). If a species was introduced to a place and did not thrive there, and local extinction is not attributable to demographic stochasticity, this is key evidence for poor fit to the site, which can potentially cause important changes in model outcomes. Since many widely used species distribution models require presence and absence data (e.g., GAM, GLM, and MAXENT), replacing pseudo-absences with true absences will clearly improve the predictive model (Fig. 2).

Species traits and invasiveness

Comparisons of invaders and non-invaders help elucidate the role of species traits in invasions (Hayes and Barry 2008). However, to learn if a trait increases the chances for a species to invade, it is key to test if the lack of this trait is involved in failed invasions. Herbert G. Baker, in his 1965 seminal paper (Baker 1965), did not systematically include failures, which was a source of later criticism of the "ideal weed" hypothesis. Many species possessing traits considered unfavorable invade and many other species with traits considered favorable fail to invade (Williamson and Fitter 1996b). Moreover, traits often exhibit considerable intraspecific variation and the optimal trait value is context-dependent. It is possible that a better approach would include quantitative analysis of mean trait values between invasive and non-invasive populations. Stoichiometry-based mechanisms have been also suggested as possible reasons for invasion failures, but these hypotheses remain largely untested. Under this mechanism, only individuals meeting their nitrogen and phosphorous demands would thrive, and invasion would happen when the non-natives are able to acquire these nutrients more efficiently than the natives (González et al. 2010). Without a detailed account of failed invasions, studies can overestimate the importance of traits in invasions and hide potential differences among traits that might be intrinsically related to invasiveness (e.g., length of juvenile period) (Rejmánek and Richardson 1996) and traits that might be important only in specific circumstances (e.g., shade tolerance) (Emer and Fonseca 2010).

Biotic resistance

From the examples drawn from the literature, we found biotic resistance may prevent naturalized populations from invading. Even though some evidence suggests that high levels of predation are sometimes unable to prevent spread and encroachment of populations of non-native species (Maron and Vila 2001), competition and predation can strongly affect offspring survival and population growth of non-natives (Levine et al. 2004, Pearson et al. 2011). The existing literature on the importance of biotic resistance in invasion failures is limited. Currently, we do not know when biotic resistance causes invasion of introduced populations to fail because most experiments use species that have already overcome the naturalization barrier in the studied system (Levine et al. 2004, Maron and Vila 2001). For example, many studies on biotic resistance focus on comparisons between “weak” and “strong” invaders (Pearson et al. 2011) or between invasive and native species (Blaney and Kotanen 2001). More powerful tests of the role of biotic resistance would include known invaders that are failing to invade in the studied system (i.e., Nuñez et al. 2008).

Genetic effects

To understand if genetic factors determine invasion outcomes, it can be important to consider failed invasions. For example, failure may be important for understanding the role of genetic diversity, hybridization, and other factors associated with the genetic structure of non-native populations that affect invasions (Hardesty et al. 2012). Incorporating failures in studies of genetic processes related to invasions might be especially important when populations undergo sudden changes in behavior (e.g., from innocuous to aggressive colonizer), since these changes can be associated with admixture, novel genotypes, or adaptation and help explain variation in invasiveness and evolution of increased competitive ability (EICA). Also, invasion failures can certainly be valuable in studies of genotype-by-environment interactions in introduced ranges, because intraspecific comparisons between successes and failures could help elucidate mechanisms producing fitness variations in different environments using empirical studies (Lee 2002). Finally, genetic data for failures can improve our understanding of factors typically associated with invasion failures but with little direct evidence supporting their importance, such as bottlenecks (Fridley et al. 2007, Roman and Darling 2007).

Studies on invasibility and invasiveness

Ignoring failed naturalizations can also result in erroneous predictions about invasibility of habitats or about the invasiveness of certain taxa. For example, previous studies based only on successful naturalizations show islands as inherently more invasible than continents (Lonsdale 1999). However, when successful and failed naturalizations are taken into account, overall rates of naturalization between islands and continents did not differ (Diez et al. 2009). If failures were ignored, the probability of success would have been overestimated for most species (Diez et al. 2009). Even well-established patterns, such as the tens rule (Williamson 1996), are impossible to test given the lack of reports on failed invasions and the bias to report only successful invasions (Rodriguez-Cabal et al. 2012). Without solid data on failed invasions, it is hard to detect if some taxa are intrinsically more invasive than others or if some habitats are more invasible than others.

When it may not be important to consider failed invasions

The absence of studies of failed invasions may not be problematic for several areas of research. For example, studying the impact of invasive species is a key question in conservation biology, and understanding failed invasions may be of little significance. Also, it may not be relevant to know about failed invasions when comparing attributes in the native vs. introduced ranges of species (Hierro et al. 2005).

Discussion

After reviewing many cases of species that exhibit invasive and non-invasive populations, it is clear that failed invasions are a common outcome of species introductions and that species show intraspecific variation in invasion success (Table 1). We found five mechanisms associated with failures: low propagule pressure, abiotic resistance, biotic resistance, limited or inappropriate gene pool, and lack of mutualists (Fig. 1). If studies do not take into account the number of introduction attempts and intraspecific differences between invasive and non-invasive populations, the estimates of intrinsic invasiveness of a species may be biased. Moreover, failed invasions may be one key component for understanding and controlling invasive populations, because understanding what makes a species that is highly invasive elsewhere fail to invade can be crucial to improve its effective control. Understanding when and why populations of invasive species fail to invade is as important as understanding when and why they invade.

Despite the importance of understanding invasion failures, there are key aspects to consider when determining if an exotic species truly failed to invade. For instance, a long residence time is sometimes necessary for the species to overcome a lag phase (Caley et al. 2007, Crooks 2011), and, in fact, many non-native populations do experience a delay between introduction and the first instance of invasion (Daehler 2009, Kowarik 1995, Simberloff et al. 2010). Some cases indicated in the literature as failed invasions could be of a species undergoing a lag phase. However, in many cases the populations are established for several decades and still have not invaded. With increased residence time, it is possible that site conditions may change, that other genotypes able to trigger invasion will arrive, or that populations may evolve, allowing the species to invade. Some examples of niche evolution suggest that this can be the case (Fitzpatrick et al. 2007, Medley 2010). Even if a population's invasive status changes because of ecosystem changes or evolutionary dynamics, it is still important to understand why under the current circumstances the population is not invading.

After reviewing the current literature, we identified two main gaps. First, the data on failed invasions are circumstantial and not easily accessible; and second, comprehensive comparisons of successful and failed invasions, especially comparisons at the same stage of invasion (e.g., before or after naturalization) are still rare. Long-term monitoring and early detection programs are probably good sources of information for identifying and tracking species introductions and variations in population size that could lead to local extinction or invasion. Also, the literature has many anecdotal notes of regions where populations of invasive species are not invading and comparative studies between these introduced ranges could be made. For instance, the biological control literature has kept excellent records of successful and failed introductions (i.e., Julien and Griffiths 1998). In many cases, the type of data needed to be collected to address questions on failed invasion can be the same as data collected to answer questions on successful or potential invasions. Each question and hypothesis will demand different types of data, but information on date of arrival, number of individuals initially present, number and origin of source populations, type and reason for introduction, and genetic variation can be fundamental for studies of failures. With these data available, researchers would be able to draw strong inferences about the importance and strength of the mechanisms proposed to predict and explain the outcome of species introductions. Ideally, researchers would start collecting data on introduced populations just after the introduction or first detection, especially for populations of species invasive elsewhere.

Invasion biology is a science with many biases and constraints because species are never introduced from a random sample and they are not introduced to random places. The taxonomic and geographic biases of introduced species, donor regions, and recipient habitats complicate many analyses. Ignoring failed invasions may hinder our understanding of the process of invasion, especially for some research topics such as species distribution modeling and analyses of historical factors associated with invasions. The limited number of studies on failed invasions has already provided some important insights to invasion biology, and more studies on failed invasion can only promote a deeper understanding of the invasion process.

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Appendix III: Tables and Figures

Table 3-1 List of 76 species with known invasive and non-invasive populations (sensu Blackburn et al. 2011). When available, the factor suggested for failure of the non-invasive populations was included. The level of detail provided for locations of invasive and non-invasive populations vary according to the data available in the literature. We added an "(?)" after some proposed factors when it was not explicitly suggested in the citation, but it was implied in the discussion. Species marked with * were introduced as biological control agents.

Group	Species (common name)	Examples of invasive populations	Examples of non-invasive populations	Reason for failure of non-invasive populations	References
ALGAE					
Seaweed	*Fucus serratus* (toothed wrack)	North America (east cost and/or estuarine)	North America (east cost and/or estuarine)		Ruiz et al. 2000
ANIMALS					
Amphibian	*Alytes obstetricans* (common midwife toad)	Great Britain, Netherlands	Poland		Kraus 2009
	Bufo marinus (cane toad)	American Samoa, Antigua (second introduction attempt in the 1950s), Australia, Barbados, Bermuda, Japan (Ogasawara and Ryukyu Islands), Philippines, USA (Florida: after 1955, Hawaiian Islands, Louisiana)	Anguilla, Antigua (first introduction attempt in 1934), Barbados, Cook Islands, Cuba, Dominica, Egypt, Mascarene Islands, Taiwan, Thailand, USA (Florida: before 1955)	Abiotic resistance (?)	Kraus 2009, Lever 2003
	Osteopilus septentrionalis (Cuban tree frog)	Anguilla, Antigua, Bahamas, Costa Rica, Puerto Rico, Saint Barts, USA (Florida), Virgin Islands (British and USA)	Canada (Ontario), Curaçao, Dominica, USA (Colorado, Maryland, Virginia)		Kraus 2009
	Rana catesbeiana (American bullfrog)	Brazil, Colombia, England, Italy, Peru, Puerto Rico, Spain, USA (Arizona, California, Colorado, Hawaii, Montana), Venezuela	Belgium, Italy, Netherlands, Portugal, USA (Massachusetts, North Dakota)	Abiotic resistance	Kraus 2009, Lever 2003
	Xenopus laevis (African clawed frog)	Ascension Island, Chile, France, Great Britain, Italy (Sicily),	USA (Colorado, Florida, Massachusetts,		Kraus 2009

Table 3-1. Continued.

Group	**Species (common name)**	**Examples of invasive populations**	**Examples of non-invasive populations**	**Reason for failure of non-invasive populations**	**References**
		Japan, Mexico, USA (Arizona, California)	North Carolina, Texas, Virginia, Wisconsin)		
Bird	*Acridotheres tristis* (common myna)	Australia, Hong Kong, Madagascar, Mauritius, New Zealand (North Island), South Africa	New Zealand (South Island), Tasmania (early 1900s)		Lever 1987
	Alauda arvensis (skylark)	Australia, New Zealand, USA (Hawaii)	USA (Continental)		Sol 2000
	Coturnix chinensis (king quail)	Australia	New Zealand, USA (continental, Hawaii)		Sol 2000
	Perdix perdix (grey partridge)	USA (west of Allegheny mountains)	Australia, New Zealand, USA (continental: east of Allegheny mountains, Hawaii)	Abiotic resistance (?)	Lever 1987, Sol 2000
	Streptopelia decaocto (Eurasian collared dove)	Czech Republic, New Zealand, USA (continental)	Australia, USA (Hawaii)		Sol 2000, Šefrová and Laštůvka 2005
	Sturnus vulgaris (European starling)	Australia, Canada (after 1917), Jamaica, New Zealand, South Africa, USA (after 1920)	Canada (earlier introductions in 1875 and 1889), Russia (Buryat Republic), USA (earlier introductions in 1872 and 1897)		Lever 1987
	Zenaida macroura (mourning dove)	Bermuda, USA (Florida)	South Africa		Lever 1987
Freshwater fish	*Carassius auratus* (goldfish)	England	Belgium		Copp et al. 2005, Copp et al. 2007
	Lepomis macrochirus (bluegill)	Japan and Korea	USA (west)	Abiotic resistance (?)	Kawamura et al. 2006, Meffe 1991, Nakao et al. 2006
	Micropterus dolomieui (smallmouth bass)	South Africa, Sweden (south), Vietnam	Austria, Finland, Germany, Japan, Mariana Islands, Uganda	Abiotic resistance (?)	Lever 1996
	Micropterus salmoides (largemouth bass)	Europe (south and center), South Africa	Brazil, England		Copp et al. 2007, Olds et al. 2011, Schulz and Leal

Table 3-1. Continued.

Group	**Species (common name)**	**Examples of invasive populations**	**Examples of non-invasive populations**	**Reason for failure of non-invasive populations**	**References**
					2005
	Oncorhynchus mykiss (rainbow trout)	USA	USA	Abiotic resistance (?)	Fausch et al. 2001, Welcomme 1985
	Oreochromis mossambicus (Mozambique tilapia)	China (south), Hong Kong, India, Nicaragua	Bangladesh, Ecuador, Egypt, South Korea	Abiotic resistance (?)	Lever 1996
	Pseudorasbora parva (stone moroko)	Many regions in Europe	Belgium, Lithuania, United Kingdom (ponds and lakes)		Copp et al. 2005, Copp et al. 2007, Witkowski 2009
Insect	*Anoplolepis gracilipes* (yellow crazy ant)	Australia, Christmas Island, Indonesia, Malaysia, Papua New Guinea, Seychelles, Solomon Islands, USA (Hawaii)	New Zealand	Abiotic resistance (?)	Gerlach 2004, Hoffmann and Saul 2010, Holway et al. 2002, Lester 2005
	Bombus terrestris sassaricus (Sardinian bumblebee)	Argentina, Chile, Japan, New Zealand	France (south)	Biotic resistance (?)	Ings et al. 2010, Morales 2007
	*Ceutorhynchus litura**	Canada, USA	New Zealand	Propagule pressure	Julien and Griffiths 1998
	*Procecidochares utilis**	Australia, China, India, New Zealand, South Africa, USA (Hawaii)	Thailand		Julien and Griffiths 1998
	*Rhinocyllus conicus**	Canada	Australia, South Africa		Julien and Griffiths 1998
	*Rhopalomyia californica** (Coyote Bush)	Australia (after second introduction in 1982)	Australia (first introduction in 1969)	Propagule pressure	Julien and Griffiths 1998
	Solenopsis invicta (red fire ant)	Puerto Rico, USA (southeast), Virgin Islands	New Zealand		Holway et al. 2002, Lester 2005, McGlynn 1999
	Solenopsis papuana (Papuan thief ant)	USA (Hawaii)	New Zealand		Holway et al. 2002, LaPolla et al. 2000, Lester 2005
	*Trichosirocalus horridus**	Canada (British Columbia, Ontario), USA	Argentina		Julien and Griffiths 1998
	*Tyria jacobaeae**	Canada, New Zealand, USA (west	Australia (six release attempts	Biotic resistance	Julien and Griffiths 1998

Table 3-1. Continued.

Group	**Species (common name)**	**Examples of invasive populations**	**Examples of non-invasive populations**	**Reason for failure of non-invasive populations**	**References**
		coast)	were made)		
	Wasmannia auropunctata (electric ant)	Galápagos, New Caledonia, USA (Hawaii)	New Zealand	Abiotic resistance (?)	Clark et al. 1982, Le Breton et al. 2003, Lester 2005
Mammal	*Capra hircus* (goat)	Australia (all States and territories except the northern territory), Ascension Island, Canary Islands, Desertas, Galápagos, Saint Helena, USA (Channel Islands)	Australia (Northern Territory), Costa Rica, Crozet Archipelago, Falkland Islands	Biotic resistance (?)	Letts 1964, Long 2003
	Castor canadensis (North American beaver)	Argentina (Tierra del Fuego), Chile, Russia	Europe (central)		Lizarralde et al. 2004, Novillo and Ojeda 2008, Nummi 2006, Pastur et al. 2006
	Cervus axis (axis deer)	Argentina, Australia, Yugoslavia (Istria)	Australia (Tasmania), Brazil, France, Java		de Vos et al. 1956, Long 2003
	Macropus rufogriseus (red-necked wallaby)	New Zealand, Tasmania (Maria Island)	Czech Republic (Poděbrady), Germany	Biotic resistance (?)	Long 2003
	Mustela vison (mink)	Denmark, Finland, Iceland, Norway, Scandinavia, Sweden	Chile (lake Todos los Santos), Europe (central), Netherlands		Long 2003
	Oryctolagus cuniculus (European rabbit)	Argentina and Chile (Beagle Channel, Tierra del Fuego)	Argentina and Chile (Beagle Channel, Tierra del Fuego)	Biotic resistance (?) and abiotic resistance (?)	de Vos et al. 1956, Long 2003
	Ovis ammon (mouflon)	USA (Hawaiian Islands: Lanai)	USA (Hawaiian Islands: Hawaii, Kauai)		Long 2003
	Sus scrofa (wild boar)	Argentina, Falkland Islands (Malvinas), New Zealand (Ruapuke), South Africa (Cape Peninsula), USA (Hawaii)	Argentina, Australia, Puerto Rico, USA		Long 2003, Novillo and Ojeda 2008
Mollusc	*Crassostrea gigas* (pacific oyster)	North America (west cost and/or estuarine)	North America (west cost and/or estuarine)		Ruiz et al. 2000

Table 3-1. Continued.

Group	**Species (common name)**	**Examples of invasive populations**	**Examples of non-invasive populations**	**Reason for failure of non-invasive populations**	**References**
	Crassostrea virginica (Atlantic oyster)	North America (west cost and/or estuarine)	North America (west cost and/or estuarine)		Ruiz et al. 2000
	Ilyanassa obsoleta (eastern mudsnail)	North America (west cost and/or estuarine)	North America (west cost and/or estuarine)		Ruiz et al. 2000
	Laternula marilina (littoral spoon clam)	North America (west cost and/or estuarine)	North America (west cost and/or estuarine)		Ruiz et al. 2000
	Littorina littorea (common periwinkle)	North America (east coast)	North America (southwest coast)	Abiotic resistance (?)	Chang et al. 2011
	Mercenaria mercenaria (hard clam)	North America (west cost and/or estuarine)	North America (west cost and/or estuarine)		Ruiz et al. 2000
	Neotrapezium liratum (quadrate trapezium)	North America (west cost and/or estuarine)	North America (west cost and/or estuarine)		Ruiz et al. 2000
	Ostrea edulis (European flat oyster)	North America (west cost and/or estuarine)	North America (west cost and/or estuarine)		Ruiz et al. 2000
	Venerupis philippinarum (Japanese littleneck)	North America (west cost and/or estuarine)	North America (west cost and/or estuarine)		Ruiz et al. 2000
Reptile	*Agama agama* (common agama)	Comoros, USA (Florida)	Malta, Spain		Kraus 2009
	Anolis sagrei (brown anole)	Cayman Islands, Grenada, Jamaica, Mexico, Taiwan, USA (Alabama, Florida, Georgia, Hawaii, Louisiana, Texas)	Canary Islands, USA (Ohio, South Dakota, Tennessee, Virginia)		Kraus 2009
	Bradypodion pumilum (cape dwarf chameleon)	Namibia	Greece		Kraus 2009
	Gallotia galloti (tenerife lizard)	Canary Islands	Madeira		Kraus 2009
	Gekko gecko (tokay gecko)	Martinique, USA (Florida, Hawaii)	Australia, Guam, New Zealand		Kraus 2009
	Trachemys scripta (red-eared slider)	Brazil, France, Spain	Sweden	Abiotic resistance (?)	Cadi et al. 2004, Lever 2003, Perez-Santigosa et al. 2008
PLANTS					
Fern	*Asparagus scandens*	New Zealand (disturbed forest)	New Zealand (other habitats)	Biotic resistance (?)	Timmins and Reid 2000

Table 3-1. Continued.

Group	**Species (common name)**	**Examples of invasive populations**	**Examples of non-invasive populations**	**Reason for failure of non-invasive populations**	**References**
	(climbing asparagus)				
Grass	*Eragrostis plana* (South African lovegrass)	Brazil (degraded and overgrazed steppes)	Brazil (steppes)	Biotic resistance (?)	Zenni and Ziller 2011
	Phalaris arundinacea (reed canarygrass)	North America	North America	Genetic effects	Chambers et al. 1999
	Phragmites australis (common reed)	North America (past 100 years)	North America (> 100 years)	Genetic effects	Chambers et al. 1999
Herb	*Echium vulgare* (blueweed)	Australia, Chile, South Africa	USA (California)		Marcel Rejmánek (personal communication)
Tree	*Abies grandis* (grand fir)	Great Britain	Ireland, Sweden		Richardson and Rejmánek 2004
	Afrocarpus falcatus (sickle-leaved yellowwood)	South Africa	Australia (north and southwest)		Richardson and Rejmánek 2004
	Cryptomeria japonica (Japanese cedar)	Azores	Argentina (Patagonia: Isla Victoria), Brazil, Germany, Hawaii, New Zealand		Richardson and Rejmánek 2004, Simberloff et al. 2002, Zenni and Simberloff 2012
	Larix decidua (European larch)	Czech Republic, Great Britain, New Zealand	Argentina (Patagonia: Isla Victoria), Canada (Newfoundland), Ireland, Lithuania, USA (New England, New York)		Richardson and Rejmánek 2004, Simberloff et al. 2002
	Melia azedarach (chinaberry)	Argentina, Brazil (deciduous forests, mostly on basaltic soils), South Africa	Africa (east), Brazil (other soils and habitats), USA (California)		Marcel Rejmánek (personal communication)
Tree	*Myrica faya* (fayatree)	USA (Hawaii: nitrogen limited, phosphorous rich ecosystems)	USA (Hawaii: phosphorous limited ecosystems)	Abiotic resistance	González et al. 2010, Vitousek 1999
	Pinus caribaea (Caribbean pine)	Australia (northeast), Brazil (central), New Caledonia	Brazil (south), Puerto Rico, USA (Hawaii), Venezuela		Richardson and Rejmánek 2004, Simberloff et al. 2010
	Pinus contorta (lodgepole pine)	Australia (north and southwest), Chile,	Argentina (Patagonia: Isla	Mutualist release	Langdon et al. 2010, Richardson

Table 3-1. Continued.

Group	Species (common name)	Examples of invasive populations	Examples of non-invasive populations	Reason for failure of non-invasive populations	References
		Great Britain, Ireland, New Zealand, Sweden	Victoria), Russia, Sweden		and Rejmánek 2004, Simberloff et al. 2010
	Pinus elliottii (slash pine)	Argentina, Australia (north and southwest), Brazil, USA (Hawaii), South Africa	New Zealand		Richardson and Rejmánek 2004, Simberloff et al. 2010, Zenni and Simberloff in press
	Pinus halepensis (Aleppo pine)	Argentina (eastern), Australia (south, Victoria), Israel, New Zealand, South Africa	Argentina (Patagonia: Isla Victoria), Brazil, USA (California)		Richardson and Rejmánek 2004, Simberloff et al. 2010
	Pinus nigra (Austrian pine)	Czech Republic, New Zealand	Argentina (Patagonia: Isla Victoria)		Bellingham et al. 2004, Křivánek et al. 2006, Simberloff et al. 2002
	Pinus radiata (Monterey pine)	Australia, Chile (forest edges), New Zealand, South Africa	Brazil, Chile (forest interiors)	Biotic resistance (Chile)	Simberloff et al. 2010, Williams and Wardle 2007, Zenni and Simberloff in press
	Pinus taeda (loblolly pine)	Argentina, Australia (north, southwest and Queensland), Brazil, South Africa, USA (Hawaii)	Brazil (interior of dense forests), New Zealand, Zimbabwe	Biotic resistance (Brazil)	Emer and Fonseca 2010, Richardson and Rejmánek 2004, Simberloff et al. 2010, Zenni and Simberloff in press
	Prunus serotina (black cherry)	France (well-drained, nutrient-poor soils)	France (waterlogged and calcareous soils)	Abiotic resistance	Closset-Kopp et al. 2011
	Pseudotsuga menziesii (Douglas-fir)	Argentina, Austria, Bulgaria, Chile, Czech Republic, Great Britain, New Zealand (open habitats)	Germany, Ireland, New Zealand (forests) USA (New York)		Richardson and Rejmánek 2004, Von Holle et al. 2003
	Thuja plicata (western redcedar)	Great Britain	Argentina (Patagonia: Isla Victoria), New Zealand, Poland		Richardson and Rejmánek 2004
Shrub	*Acacia paradoxa* (kangaroo thorn)	Australia (Victoria), South Africa (Western Cape), USA	Chile, Israel		Zenni et al. 2009

Table 3-1. Continued.

Group	**Species (common name)**	**Examples of invasive populations**	**Examples of non-invasive populations**	**Reason for failure of non-invasive populations**	**References**
		(California)			

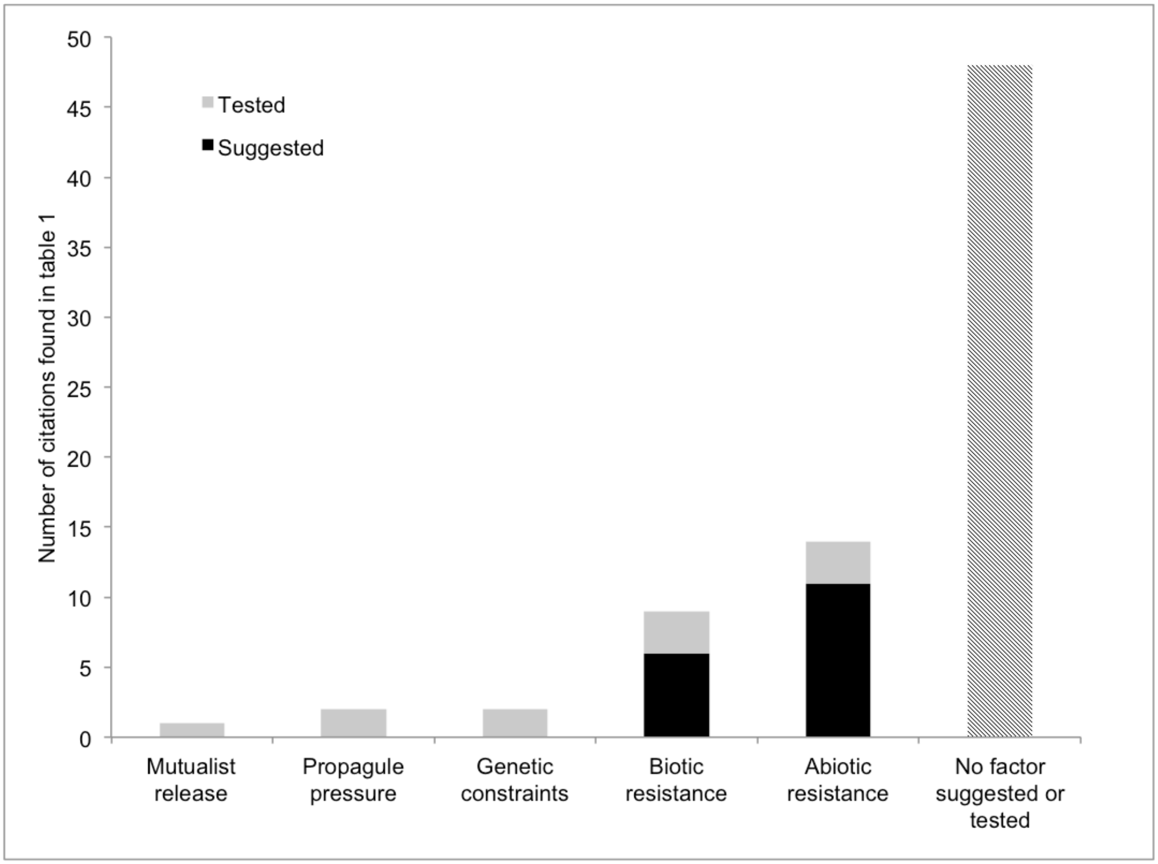

Figure 3-1 We summarized from table 1 the factors proposed to explain failed invasions, and counted the number of times each factor was suggested or tested.

Black bars represent instances where the factor was proposed, but not tested, and grey bars represent instances where the factor was experimentally or statistically tested. The dashed bar indicates mentions to failed invasions from table 1 where a possible driver of failure was not suggested.

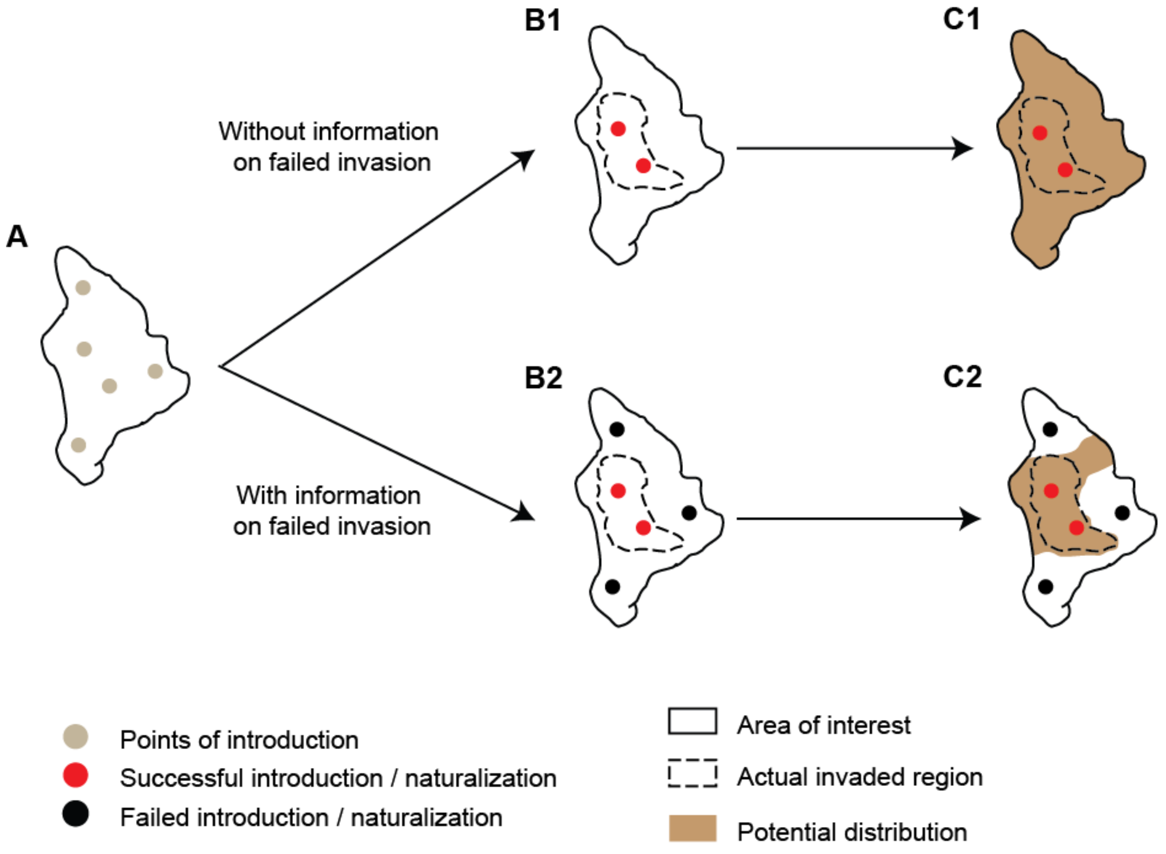

Figure 3-2 Information on failed invasions is important for predicting potential distributions of invasive species within an area of interest (e.g., bioclimatic, biogeographical or geopolitical regions).

Given (A), several introduction events, it is expected that (B) some introductions will not thrive (black dots) while others may invade (red dots), forming an invaded area (dashed area). If the data on the failed naturalizations / invasions are lacking (B1), it would be easy to misestimate the invasive species potential distribution (C1), and it would be impossible to distinguish from a more accurate model (C2). However, if data on failed naturalizations / invasions exist (B2) and failures are because of deterministic causes, it becomes feasible to subtract unsuitable regions from the potential area based on the failures and obtain a more accurate prediction (C2).

CHAPTER IV

RAPID EVOLUTION AND RANGE EXPANSION OF AN INVASIVE PLANT ARE DRIVEN BY PROVENANCE-ENVIRONMENT INTERACTIONS

A version of this chapter was originally published by Rafael D. Zenni, Joseph K. Bailey, and Daniel Simberloff:

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RDZ designed the study and did all the field and laboratory work. RDZ and JKB analyzed the data. RDZ wrote the manuscript. JKB and DS contributed to manuscript revisions.

Abstract

To improve our ability to prevent and manage biological invasions, we must understand their ecological and evolutionary drivers. We are often able to explain invasions after they happen, but our predictive ability is limited. Here we show that range expansions of introduced *Pinus taeda* result from an interaction between genetic provenance and climate and that temperature and precipitation clines predict the invasive performance of particular provenances. Further, we show that genotypes can occupy climate niche spaces different from those observed in their native ranges and, at least in our case, that admixture is not a main driver of invasion. Genotypes respond to climate in distinct ways, and these interactions affect the ability of populations to expand their ranges. While rapid evolution in introduced ranges is a mechanism at later stages of the invasion process, the introduction of adapted genotypes is a key driver of naturalization of populations of introduced species.

Introduction

In recent years, great advances have been made to improve our understanding of biological invasions. We can now shortlist ecological and evolutionary factors and organismal traits contributing to invasion success (Moles *et al.* 2008; Van Kleunen *et al.* 2010b; Colautti & Barrett 2013). However, even though we are now competent at explaining how and why many biological invasions happened, we are largely unable to predict invasive range expansions. Two probable explanations for this limitation are the predominant focus on species-level variation, whereas invasions occur at intraspecific levels (Petit 2004; Zenni & Nuñez 2013), and the heavy reliance on correlative instead of mechanistic models (Peterson & Vieglais 2001; Broennimann *et al.* 2007). Studies of the process of range expansion of introduced species are fundamental for understanding impacts these organisms can have on populations, communities, and ecosystems of recipient regions (Wardle *et al.* 2011; Simberloff *et al.* 2012). Also, understanding and predicting organisms' responses to novel environments is a key issue for global change biology. Human-mediated introductions can provide valuable insights on how organisms respond to climate change and novel interactions (Hampe & Petit 2005; Caplat *et al.* 2013).

A biological invasion is likely to happen if high-performance genotypes exist in the introduced pool and produce a disproportionate fraction of offspring that, in turn, repeat the parental reproductive performance. Empirical evidence shows that individual mother plants contribute differentially to future generations (González-Martínez *et al.* 2006) and that some genotypes have higher reproductive output in favorable conditions (Matesanz & Sultan 2013). However, invasiveness, defined as the invasion capacity of a taxon, is often considered a species-level trait that materializes only when certain environmental requirements are met (Richardson & Pyšek 2006). Moreover, despite the general trend of individual-level variation in reproductive trait values, no major theoretical framework characterizing how organisms advance from introduced to invasive, or what determines invasiveness, explicitly incorporates intraspecific variation (van Kleunen *et al.* 2010a; Blackburn *et al.* 2011). Genotypes performing well in introduced environments can result from past evolution in the native range or evolution in the novel habitat (Colautti & Barrett 2013; Felker-Quinn *et al.* 2013). Consequently, some populations adapt to the novel environment, or are adaptively more plastic, and spread, whereas others may not have the same adaptations or have them in lower frequencies and hence fail to invade (Zenni & Nuñez 2013; Zenni *et al.* 2014). Also, genetic constraints may help explain why propagule diversity increases the chances of invasion for a species (Zenni & Simberloff 2013).

For this study, we measured the invasive range expansion of *Pinus taeda* (loblolly pine) genetic provenances in six locations along an 850 km north-south transect covering about 6° of latitude in southern Brazil (Fig. 1). In each location *P. taeda* was introduced in 1973 as part of a forestry provenance trial experiment (common garden, hereafter). The common gardens are replicated parallel introductions; thus, the propagule pressures, residence times, and genetic material introduced are identical for all locations. Because *P. taeda* is long-lived, has multi-generational populations, reproduces early (five years) and yearly, and is wind-dispersed with viable seed dispersal distances of less than 20 m (Vitorino *et al.* 2013), we could track changes in frequencies of provenances in each naturalized population over multiple generations. This generational progress of the invasion front over 40 years, fully replicated in six locations, allowed us to study changes in allele frequencies from the introduced pool to the leading edge of the invasion front in response to selective pressures posed by the environment of each introduced location.

We hypothesized that local adaptation that had occurred during millennia in the native range would affect the invasive potential of genotypes in the introduced range. We predicted that genetic provenances would successfully invade only at locations with abiotic conditions similar to those of the provenances' native range. We also tested the hypothesis that admixture between previously isolated populations could stimulate the evolution of invasiveness (Ellstrand & Schierenbeck 2000). We provide strong evidence that invasive range expansions of *P. taeda* are mediated by provenance-climate interactions that would not be expected based on the climate of the native range alone, but that provenance-level invasion can be explained based on annual temperature and precipitation of the introduced location. Also, *P. taeda* invasions are led by single-provenance descendants likely containing genetic variation that conveyed higher fitness in the introduced environments, and not by admixed plants.

Methods

Study system

Pinus taeda has been introduced to many regions and is invasive in several of them (Simberloff *et al.* 2010). The species is native to the southeastern United States, ranging from the Lost Pines in central Texas to Delaware with a discontinuity along the Mississippi River Valley (Critchfield & Little 1966) (Fig. 1). Original range limits are well defined by isoclines of annual actual evapotranspiration (Schultz 1997). The species is moderately genetically differentiated among populations east and west of the Mississippi River Valley and has increased levels of admixture for populations on the Gulf Coastal Plain (Wells *et al.* 1991). This pattern is consistent with the hypothesis of dual Pleistocene refugia, which helps explain differences in growth, disease resistance, and concentrations of secondary metabolites among populations (Eckert *et al.* 2010a). In the native range, *P. taeda* shows considerable genetic variation in seed dormancy and need for pre-chilling (Schultz 1997). Seed size, weight, and coat thickness vary regionally and affect seedling growth. Seed size decreases from east to west. Loblolly populations also exhibit phenological differences and different degrees of seasonal drought resistance, fungal disease resistance, and net photosynthesis (Schmidtling 2001) Also, recent association analysis depicts large allele frequency differences among populations that are correlated with geography, temperature, growing degree-days, precipitation, and aridity (Eckert *et al.* 2010b). Several of the above-mentioned traits are associated with increased invasiveness at the species level in other studies (Van Kleunen *et al.* 2010b).

We studied six common gardens in Brazil located at the Santa Maria Experimental Farm (53.92°W 29.66°S; hereafter “SM”), São Francisco de Paula National Forest (50.38°W 29.43°S; hereafter “SFP”), Três Barras National Forest (50.32°W 26.19°S; hereafter “TB”), Rio Negro Experimental Station (49.76°W 26.05°S; hereafter “RN”), Irati National Forest (50.57°W 25.36°S; hereafter “IR”), and Capão Bonito National Forest (48.51°W 23.88°S; hereafter “CB”). Each common garden was planted with 29 or 32 seed sources of which 20 were present in all gardens (Shimizu & Higa 1981). The seed sources constitute a seed lot collected from between 5-10 trees of a natural stand (not planted) in a specific location in the native range of *P. taeda* (Fig. 1A). In each Brazilian common garden, seed sources were planted in randomized blocks with four repetitions – a total of 144 trees from each seed source. Over the years, each common garden and its surroundings received circumstantial and haphazard management. In June and July 2012, all seed sources were still represented by at least 10 trees at any given site, but the mean number of trees per provenance per site is usually higher.

Since introduction, the common gardens have produced spreading naturalized populations (naturalized populations). Whereas in some locations loblolly expanded ~78 m from the common garden (SFP), in other locations (TB) range expansion was ~ 450 m. This variation is likely because of local vegetation cover (e.g., forest and old field), topography, and wind patterns. The common gardens were considered parallel replicated introduction pools resulting in identical propagule pressures and residence times for these six locations. Several reasons make loblolly pine common gardens an ideal system to examine evolutionary and ecological aspects of genotypic-level range expansions (Zenni *et al.* 2014): (i) the correlation of climate with seed source performance of loblolly, as well as large-scale genomic resources available for this species (Eckert *et al.* 2010a); (ii) its distribution across climatically diverse environments in both the native and introduced ranges; and (iii) the multitude of association genetic studies identifying genes underlying quantitative traits (Eckert *et al.* 2010a; Eckert *et al.* 2010b).

Data collection

We haphazardly sampled 50 loblolly plants taller than 1.3 m from each naturalized population using equidistant transects starting at the edge of the common garden and ending 50 m after no more loblolly plants were found (300 samples in total). We avoided going beyond 500 m from the common garden owing to the increased chance of sampling trees coming from different (unknown) seed sources. Transects were 20 m apart and the number of transects per common garden varied according to stand shape. For each plant we collected green needles or cambium tissue for genetic work, and we measured size of the plant and how far it was located from the common garden edge. Plant material (*ca*. 100 mg of dry weight) was immediately stored in 2 ml tubes containing silica gel. Tubes were stored at -20° C until extraction, and saturated silica gel was replaced when necessary until the material dried. Only plants taller than 1.3 m were sampled because *P. taeda* is very similar to *Pinus elliottii* (also present at some locations) at earlier stages, and sometimes it is impossible to separate them correctly based solely on visual cues.

We choose one site (IR) to collect DNA samples from seed sources. We did this because all experiments commenced with the same seed lots, so genetic material is identical at all sites. Using a leather punch, we extracted one disk of cambium tissue measuring 2.5 cm in diameter and ~ 2 mm thick (*ca*. 100 mg of dry weight) from between 8-10 plants of each seed source (288 samples in total). Cambium tissue was sliced off the bark and wood using scalpel and forceps. The disk was processed as described above. All equipment (gloves, forceps, scalpel, and leather punch) was sterilized between extractions.

We extracted genomic DNA from pine needles and cambium tissue using the DNeasy Plant kit (QIAGEN®) following the manufacturer's protocol. After extraction, samples were sent to the Genotyping and Sequencing Core at the University of California Los Angeles to be genotyped for 96 single nucleotide polymorphisms (SNPs) using Fluidigm® SNPtype Assays. We used a subset of the 3,084 SNPs used by Eckert et al. (2010a). We selected the 96 SNPs that were the most informative for population structure based on the statistics implemented on Infocalc 1.1 (Rosenberg *et al.* 2003) and that conformed to SNP Assay primer design standards (sequence length, presence of neighboring SNPs, and percentage of C/G content). Details for the SNPs can be found at http://dendrome.ucdavis.edu/DiversiTree/. The SNP call rate threshold was 65%. We removed two SNPs (SNP_216801 and SNP_219848) from all analyses owing to very low call rates and call confidence for them. Some samples were duplicated to test SNP call accuracy; all duplicated samples showed consistent calls.

We obtained climatic variables from the Worldclim database (Hijmans *et al.* 2005). Variables are at 30 arc-seconds (~ 1 km^2) resolution and correspond to current (1950-2000) climatic conditions. From these data we also calculated potential evapotranspiration and aridity indexes for all locations, but these variables were not used because they were highly correlated with mean annual temperature and annual precipitation. We obtained soil type data from the Oak Ridge National Laboratory global soil type dataset (Post & Zobler 2000).

Population structure and assignment of invasive plants to provenances

To determine the proportional ancestry of each individual plant in all six naturalized populations, we built two models using the Bayesian model-based clustering method implemented in the software STRUCTURE (Pritchard *et al.* 2000), following guidelines proposed by Porras-Hurtado *et al.* (2013). Our first model aimed to group seed sources into discrete genetic clusters (provenances). In this model, we tested the existence of one through 25 provenances using a model that accounted for the existence of admixture between populations and correlated allele frequencies (clustering model, hereafter). Parameters alpha (relative admixture levels between populations) for each potential provenance and lambda (distribution of allele frequencies) were estimated from the data. We ran 20 iterations for each potential provenance with a 100,000 burn-in period and 300,000 MCMC repeats after burn-in.

The optimal number of provenances was determined using the ad hoc statistic Δ*K* described in Evanno *et al.* (2005) calculated in Structure Harvester (Earl & von Holdt 2012). Our second model (assignment model, hereafter) aimed to assign each individual loblolly plant growing outside the common gardens (naturalized populations) to one provenance or more identified in the clustering model. The assignment model also accounted for admixture between populations and correlated allele frequencies. However, in this model, we set alpha and lambda parameters according to estimates calculated by the clustering model instead of asking the model to estimate them directly from the data (alpha = 0.0782 and lambda = 0.4744). Provenance plants were used as learning samples for updating the inferred proportion of ancestry (q_k) of plants from the naturalized populations. We did this using the POPFLAG and USEPOPINFO options in STRUCTURE. We also used the PFROMPOPFLAGONLY function to ensure allele frequency estimates would depend only on learning samples and set MIGPRIOR at 0.01 to allow for some misclassification of learning samples. We ran 30 iterations of the optimal number of provenances. All STRUCTURE runs were done at the Bioportal of the University of Oslo (www.bioportal.uio.no). The iterations of inferred proportion of ancestry for the optimal number of provenances of the clustering model and the iterations of population assignments of the assignment model were permuted using the Greedy algorithm of the CLUMPP software to average replicates of each model run (Jakobsson & Rosenberg 2007).

To support our choice of three genetically distinct provenances in the introduced loblolly pool, we calculated provenance genetic differentiation (F_{ST}) between all pairs of provenances using GenoDive 2.0b25. For biallelic markers (such as SNPs) F_{ST} is appropriate as calculated and no standardization is necessary (Meirmans & Hedrick 2011). However, our pre-selection of SNPs with high informativeness scores may increase F_{ST} estimates compared to other studies on conifers that use randomly selected markers.

Propagule pressure

We tested the propagule pressure hypothesis at all sites using a permutation linear model with proportion of each provenance in the introduced pool nested within site as independent variables and q_k as dependent variable. We performed post-hoc pairwise comparisons using Tukey's HSD test.

Genetic admixture

We considered individuals with $0.3 < q_k < 0.7$ to be admixed. These would include both two- and three-provenance hybrids. There are no standards for these cutoff thresholds, but simulation studies indicate that first generation hybrids should have $q_k = 0.5$ (Vähä & Primmer 2006). We counted the total number of admixed individuals in each naturalized population and the number of admixed individuals for each possible admixture combination. We built a generalized linear model with Poisson error distribution to test if total number and number of each type of admixture differed among locations. We also compared distributions of admixed and non-admixed plants along the naturalization gradient using the non-parametric Mann-Whitney test to see if admixed plants were more invasive than non-admixed plants (i.e., were more frequent than non-admixed plants at the leading edge far from the source pool).

Provenance-level adaptation to climate

To characterize the climate of each seed source location, we used the 19 bioclimatic variables extracted from the WorldClim plus soil type as factors in two redundancy analyses (RDA) (i) to evaluate how much allelic variation in the native range was explained by environment, and (ii) to evaluate the provenance association with climate and/or soil type. One RDA used the 94 SNPs as the community matrix, bioclimatic and soil variables as constraining variables, and seed sources' latitude and longitude as conditioning variables. Using this formulation, we removed the effect of spatial correlation from the model (Legendre & Legendre 2012). Another RDA used q_k values as the community matrix while constraining and conditioning variables were as in the first RDA. We checked for collinearity between predictor variables using the variance inflation factor (VIF) method. Because most climatic variables were highly correlated, we kept only mean annual temperature and annual precipitation for the remainder of the analyses. We tested for significance of the RDA model using an anova-like permutation test with 10,000 permutation steps. To find if climates of native and introduced ranges were comparable, we did a hierarchical cluster analysis of all sites (Brazilian introduced locations and US seed sources) based on the first five components of a principal component analysis (Fig. S1).

Provenance-by-environment interactions during invasive range expansion

To test if q_k in naturalized plants differed within and between locations we used a permutation linear model in which we nested provenance ancestry coefficients within location. Second, to explore how climate may function as a selective agent during range expansion of loblolly provenances in introduced regions, we constructed a linear model with permutation tests and tested how inferred proportions of ancestry from each provenance varied as a factor of distance from the introduction point. The genetic clusters are characterized by allele frequencies at each the 94 loci (Pritchard et al. 2000). Thus, by looking at changes in q_k we are, by definition, looking at changes in allele frequencies. The farther away a plant was found from the common garden, the more likely it would be the offspring of a previously established generation and less related to common garden plants, which creates a gradient of selection in which adapted genotypes are more likely to survive, grow, reproduce, and contribute to the invasive range expansion. We normalized distances between each plant and the common garden to fall between 0 and 1 so slopes are comparable across sites and used normalized distance as the independent variable in the model. Positive values for the slope (β) mean alleles of a provenance are becoming more abundant in the population as invasion progresses, negative values mean alleles of a provenance are less abundant as invasion progresses, and a value of zero means the allelic contribution of a provenance does not change as invasion progresses. Next, we used the slope estimate for each provenance across each site as response variable for a permutation model testing direction of the slope as a result of mean annual temperature and annual precipitation ($\alpha = 0.1$). This approach can be interpreted as a genotype-by-environment test of introduced provenances during the spread of an invasive plant.

Results

Population structure and assignment of invasive plants to provenances

Clustering of seed sources resulted in three genetic provenances (Fig. 1). Most individuals showed high probability of belonging to only one provenance, even though plants from the same seed location sometimes did not cluster together. The western provenance consists mostly of plants west of the Mississippi discontinuity in Texas (Texas provenance, hereafter), another provenance consists mostly of plants from the southeastern coastal plain (coastal provenance, hereafter), and a third provenance consists mostly of plants from east of the Mississippi Gulf region in Alabama, Louisiana, and Georgia (central provenance, hereafter). F_{ST} between coastal and central provenances is 0.05, between coastal and Texas provenances is 0.21, and between central and Texas provenances is 0.16, showing moderate to high between-provenance genetic differentiation. Of the 288 plants genotyped (29 seed sources), 27 plants were assigned to the Texas provenance (9.6%), 164 plants to the coastal provenance (56.8%), and 97 plants to the central region provenance (33.6%). Thus, although at all sites equivalent numbers of plants were introduced from each seed source, genetic clustering revealed distinct effective propagule sizes for each provenance. The assignment of plants in the naturalized populations to their ancestral provenance lineage revealed all possible combinations of ancestry coefficients exist in the naturalized populations of all six locations. Some plants are pure descendants of each provenance, but many plants show admixture among provenances (Fig. 1C).

Propagule pressure

Propagule sizes did affect the relative ancestry coefficient frequencies of the naturalized populations, and this effect was mediated by introduced location ($F_{17,882} = 5.01$, $p < 0.001$). However, contrary to expectation, the largest propagule sizes did not result in the greatest frequencies of provenance ancestry in the invasive plants (Table 1). A post-hoc Tukey test indicated the Texas provenance is overrepresented, whereas the coastal provenance is underrepresented in three naturalized populations (CB, IR, and SFP). The central and coastal provenances did not show differences in observed mean q_k for all locations (Table 1; appendix S1).

Genetic admixture

The number of admixed individuals in naturalized populations varied greatly by location, ranging from about half the sampled plants down to 10% of the plants ($\chi^2 = 88.9$, $p < 0.001$). Admixtures of the three provenances or between Coastal and Central provenances were rare (mean = 2 and 6, respectively), whereas admixtures between Texas and Central provenances and between Texas and Coastal provenances were common (mean = 8.3 and 6, respectively; Fig. 2A). However, distributions of spread distances of admixed plants are the same as or lower than that of non-admixed plants (Mann-Whitney $W > 50$ and $p > 0.05$ for all locations; Fig. 2B-G; Table 2). Thus, we cannot reject the null hypothesis that the two groups have identical spread rates.

Provenance-level adaptation to climate

In the native range, climate explains 14.4% ($r^2 = 0.14$) of the variation in allele frequencies among seed sources and 24.1% ($r^2 = 0.24$) of the variation in provenance genetic structure. Climate factors ($F_{1,245} > 1.3$, $p < 0.05$), but not soil type ($F_{1,245} = 1.22$, $p = 0.11$), explained variation in allele frequencies and provenance genetic clustering (Appendix S1). In the introduced locations in Brazil, we identified four climatic clusters (Fig. 1B) that are distinct from the climatic clusters in the native range (Fig. S1). These relationships between climate and allele frequencies and provenance genetic structure indicate that climate is a selective agent for loblolly pine in its native range, leading to provenance-level genetic divergence resulting from local adaptation.

Provenance-by-environment interactions during invasive range expansion

As expected based on the provenance-by-climate interaction found for the native range, in the introduced ranges loblolly pine provenances had distinct genetic contributions to the genotypes of the naturalized populations at the different locations ($\chi^2 = 60.018$, $p < 0.001$, Fig. 1C), and the inferred proportion of ancestry in naturalized plants varied by location and distance from the point of introduction (Fig. 3). The Texas provenance had positive slopes in

IR (β = 0.28, p = 0.02) and SM (β = 0.34, p = 0.01), negative slopes in CB (β = -0.26, p = 0.03) and TB (β = -0.14, p = 0.09), and flat slopes in RN (p = 0.26) and SFP (p = 1). The coastal provenance had negative slopes in IR (β = -0.39, p = 0.01), SM (β = -0.29, p = 0.08), and SFP (β = -0.23, p = 0.05), a positive slope in CB (β = 0.3, p = 0.08), and flat slopes in RN (p = 1), and TB (p = 0.42). Finally, the central provenance showed a positive slope in TB (β = 0.29, p = 0.06) and RN (β = 0.22, p = 0.08) and flat slopes in all other sites (p > 0.1). Plants at the leading edge of the invasion front had different provenance ancestry coefficients than plants at the trailing edge and in the introduced pool (Fig. 3), which is consistent with the idea that climate functions as a selective gradient for introduced populations causing rapid evolution during invasive range expansion.

Our statistical model to test the changes in frequencies of provenance ancestries along the invasion gradients as an effect of temperature and precipitation confirms the provenance-environment interactive nature of the invasive range expansion patterns. Higher mean annual temperatures negatively affected invasiveness of the Texas provenance, positively affected invasiveness of the Coastal provenance, and did not affect invasiveness of the Central provenance ($r^2 = 0.55$; p = 0.02, 0.06, and 0.5, respectively). By contrast, higher annual precipitation positively affected invasiveness of the Texas provenance, negatively affected invasiveness of the Coastal provenance, but did not affect invasiveness of the Central provenance ($r^2 = 0.49$; p = 0.04, 0.08, and 0.82, respectively). In the full model, both mean annual temperature and annual precipitation affected provenance invasiveness ($r^2 = 0.84$, full model p = 0.1; interaction term p = 0.04). Strikingly, the temperature and precipitation ranges where provenances were more invasive did not match the values from their native ranges (Fig. 4). The Texas provenance seems to have higher fitness and be most invasive in regions with mean annual temperatures below 16.5° C and annual precipitations above 1,500 mm, whereas the coastal provenance appears to have higher fitness and be most invasive where mean annual temperature is above 19° C and annual precipitation is below 1,300 mm.

Discussion

Our study provides strong evidence that provenance-by-environment interactions are a major force driving invasions, which supports our initial hypothesis that evolutionary history is a key mechanism driving naturalization patterns of *P. taeda*. Genetic constraints likely limit the ability of provenances to expand in unfavorable introduced habitats. This adaptive mechanism was strong enough to overcome important differences in propagule pressure. Moreover, we found that it is possible to predict invasive potential of provenances using temperature and precipitation isoclines given the linear clinal variation in provenance-climate interactions (Fig. 4). Interestingly, a recent study also found that temperature and precipitation were important factors causing niche evolution of genetic lineages of the invasive plant *Phragmites australis* (Guo *et al.* 2013). Taken together, these results counter the idea that patterns of genetic structure and diversity emerging during invasive range expansions are caused mainly by genetic drift (e.g., Schulte *et al.* 2013). Instead, it shows that natural selection can produce rapid evolutionary changes in introduced populations, leading towards local adaptation, and potentially resulting in the evolution of invasiveness (Colautti & Barrett 2013).

Surprisingly, we found that provenances are more invasive in climate niche spaces distinct from those of the native range (Fig. 4). This is evidence that provenances can occupy climate niche spaces very different from those observed in their native ranges (Broennimann *et al.* 2007; Guo *et al.* 2013). This fact implies that the sole use of climate variables from the native range to predict the potential invasive range of species may be misleading. This result also suggests that niches could be inferred more precisely at the genotype level. Furthermore, we found partial support for the hypothesis that introductions encompassing different source populations can increase the likelihood of invasion success (Zenni & Simberloff 2013). In this case, genetic variance per se does not explain invasion success, but by introducing propagules from numerous populations, foresters increased the probability of introducing provenances adapted to the introduced regions – a classic sampling effect.

A common claim in invasion science is that genetic admixture can stimulate the evolution of invasiveness in plants (Ellstrand & Schierenbeck 2000). Yet, empirical evidence to support this assertion is limited. In our case, many of the invasive plants are indeed admixed between provenances (Fig. 2). However, our interpretation of these results is that admixture does not increase invasiveness of loblolly pine plants because admixed plants are not overrepresented at the invasion leading edge, and because there is no correlation between abundance of admixed plants and distance of spread. Instead, these results support our hypothesis that individuals descending from a particular provenance exhibit greater invasiveness in favorable conditions, and they are also consistent with the hypothesis of high-performance genotypes (Matesanz & Sultan 2013).

Both the coastal and Texas provenances responded strongly to the selective forces posed by the introduced locations (Fig. 3). However, the central provenance showed the exact opposite trend, responding to selection in only two of the six introduced locations. We lack a definite explanation for this pattern, but it is possible the central provenance is more plastic than the coastal or the Texas provenances; or it possesses intermediate traits from both coastal and Texas provenances, since it evolved in the center of the current native range of *P. taeda* (Fig. 1). Lastly, there is the possibility that the central provenance is experiencing introgressive hybridization with the Texas provenance (Fig. 2).

Biotic interactions are also an important factor in invasion successes and failures (Zenni & Nuñez 2013). For instance, pines may not be able to invade in the absence of mycorrhizal symbionts (Nuñez *et al.* 2009) or under strong competition for light (Zenni & Simberloff 2013). Currently, we have no evidence of how biotic interactions might affect invasive potential of individuals and populations other than at the species level. Also, this study did not evaluate phenotypic traits and we do not know how the detected changes in allele frequencies over the course of the range expansion may have resulted in phenotypic changes as well. Given that some of the markers used in this study are positioned at functional genes related to drought tolerance (Eckert *et al.* 2010b), we expect phenotypic changes leading towards higher frequencies of adaptive traits at the leading edge of the invasion front.

In summary, our results constitute a unique empirical demonstration of fine-scale rapid evolution during invasive range expansions that are largely determined by provenance-environment interactions. Also, the fully replicated landscape-level characteristics of this study provided a powerful empirical test of abiotic determinants of invasive range expansion at the gene level. Further, our novel approach reduced the effect of confounding factors that pervade invasion studies (i.e., sampling bias, residence time, and propagule pressure), allowing direct comparisons among invasive ranges. We are aware of several other large-scale long-term provenance trials (e.g., common garden experiments) using species planted well outside their native ranges as well as in their native ranges (Gundale *et al.* 2013; Zenni *et al.* 2014). Thus, we believe our approach can be replicated in different systems and would greatly enhance the understanding of the evolution of invasiveness at the gene level. The use of putatively functional markers that have adaptive significance may also have helped produce clearer results regarding the rapid evolutionary change we observed. Moreover, our study can help researchers outline mechanistic approaches (e.g., provenance-level common garden experiments) to predict the invasive potential of genotypes at specific locations. These predictions would certainly aid pre-border screening of potential invaders. Taken together, our findings suggest that to understand patterns of invasive range expansions and to improve the ability to predict these events it will help to work at intraspecific levels and to test the potential of range expansion of genotypes under a specific set of conditions.

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Appendix IV: Tables and Figures

Table 4-1 Mean inferred proportion of ancestry (q_k) and standard error for each provenance at each location. First row shows the initial relative propagule pressure for each provenance. Bold values indicate provenances that are significantly overrepresented ($p < 0.05$) and italic values indicate provenances that are underrepresented in the naturalized populations according to a Tukey post-hoc test.

Location	Provenance (q_k)		
	Texas	Coastal	Central
Source pool	0.096	0.336	0.568
CB	**0.32±0.04**	0.33±0.05	*0.35±0.04*
IR	**0.36±0.04**	0.33±0.05	*0.32±0.05*
RN	0.08±0.02	**0.5±0.06**	0.43±0.06
SFP	**0.38±0.04**	*0.27±0.04*	*0.35±0.05*
SM	0.2±0.04	0.33±0.05	0.48±0.05
TB	0.17±0.03	0.39±0.06	0.45±0.06

Table 4-2 Mean distance of spread (normalized distance) of admixed and non-admixed plants at each location (±SD) and the results for the Mann-Whitney test comparing the distributions of spread distance of admixed and non-admixed plants at each location (high p values indicate both groups have the same mean).

Location	Spread of admixed plants	Spread of non-admixed plants	W	p
CB	0.16±0.15	0.3±0.3	202	0.98
IR	0.57±0.28	0.52±0.24	329	0.28
RN	0.27±0.18	0.44±0.22	58	0.89
SFP	0.35±0.26	0.3±0.26	345	0.27
SM	0.56±0.3	0.4±0.28	308	0.07
TB	0.16±0.25	0.34±0.33	147	0.94

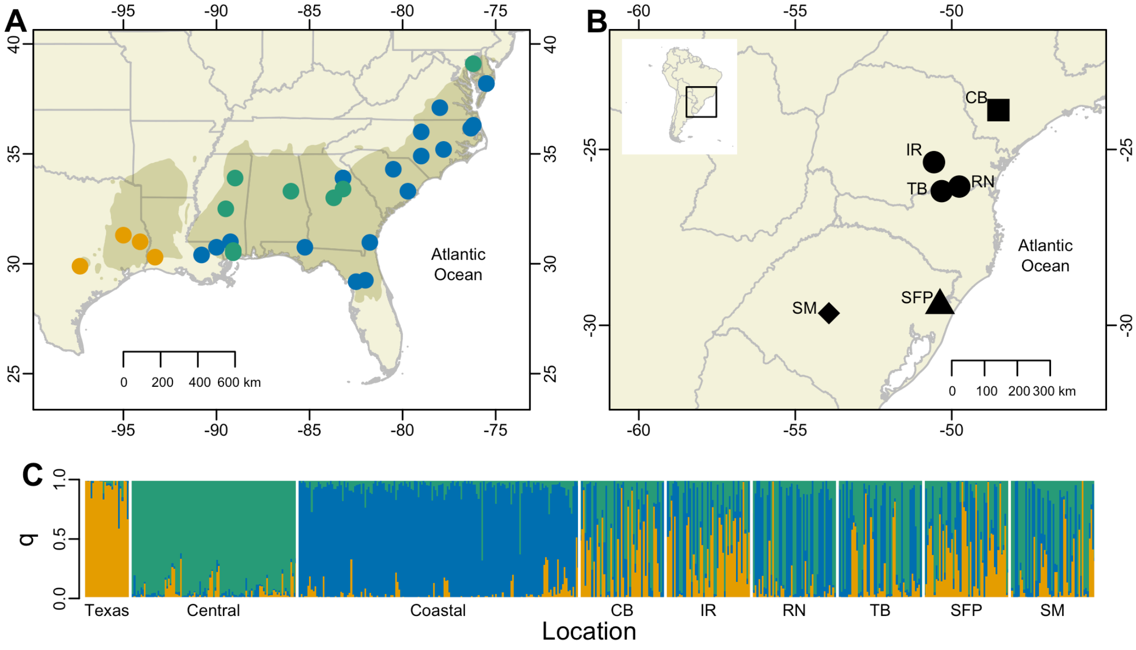

Figure 4-1 The native and introduced ranges of *P. taeda* used for this study, and the genetic clustering of provenances and invasive individuals.

(A) Seed sources for the parallel introductions were taken from 32 locations spanning the entire native range of *P. taeda* (brown area). (B) These seed sources were planted in six common gardens, in a fully replicated experiment, spanning a latitudinal gradient of 850 km and encompassing four climatic clusters (dots of different shapes). (C) The seed sources represent three distinct genetic clusters (Texas, Central, and Coastal) that have distinct contributions to the genomes of plants in the naturalized populations (CB, IR, RN, TB, SFP, and SM).

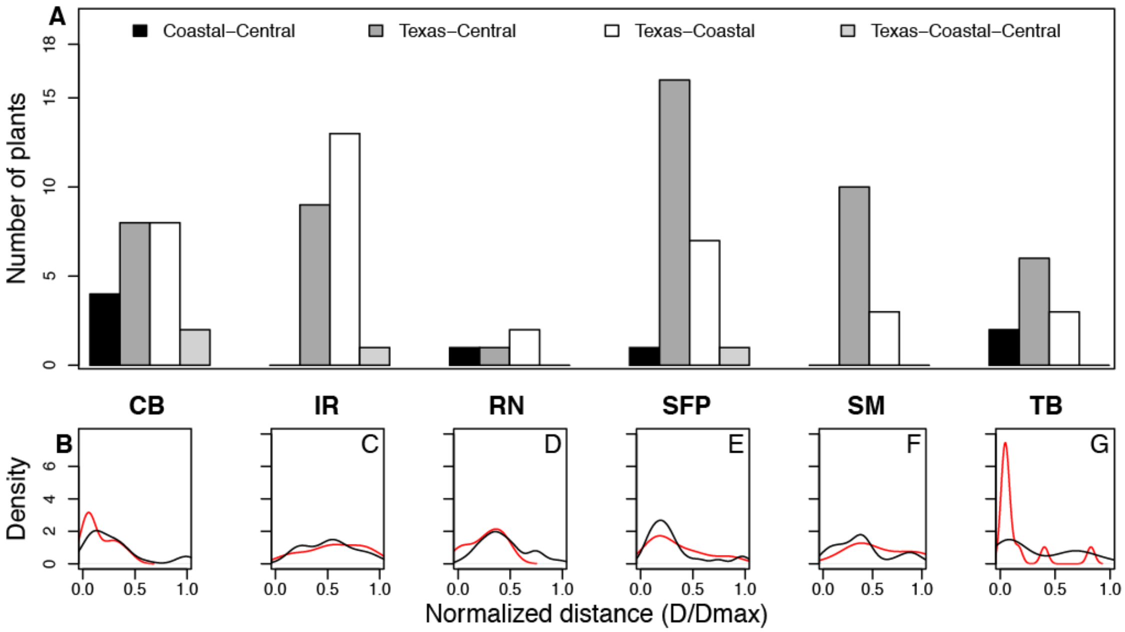

Figure 4-2 Although genetically admixed plants are common, they are not at the leading edge of the invasion front.

(A) Bar plot of number of genetically admixed plants at each location shown by type of admixture. (B-G) Density frequency distributions of genetically admixed (red lines) and non-admixed plants (black lines) at each location. Bars and line plots are paired by location. Plants were considered admixed when $q_k \approx 0.5$ for two provenances or $q_k \approx 0.3$ for the three provenances. The distribution of spread distances of admixed plants is the same as or lower than that of non-admixed plants ($p > 0.05$ for all locations; appendix S1).

Fig. 3

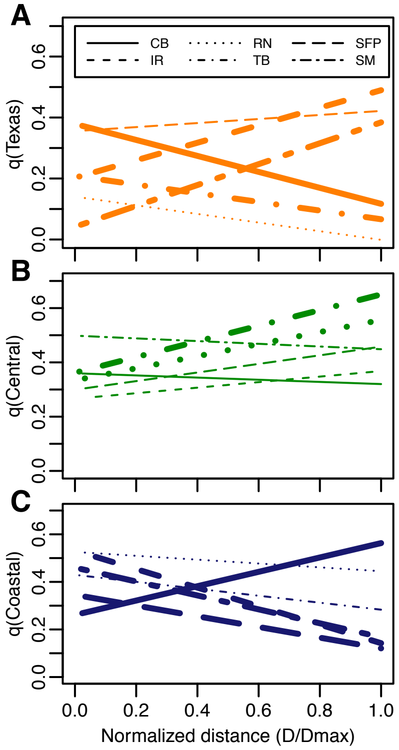

Figure 4-3 During *P. taeda* range expansion selective pressures affect the invasive potential of provenances, and this is mediated by provenance-by-environment interactions.

Bold lines represent slopes statistically different from zero ($\alpha = 0.1$). (A) Alleles from the Texas provenance become more abundant in the invasion leading edge at IR and SM and less abundant at CB and TB. (B) Alleles from the Central provenance become more abundant in the invasion leading edge at TB and less abundant at RN. (C) Alleles from the coastal provenance become more abundant in the invasion leading edge at CB and less abundant at IR, SM, and SFP. Normalized distance is the proportional distance that each plant is located from the common garden in relation to farthest plant sampled at each location.

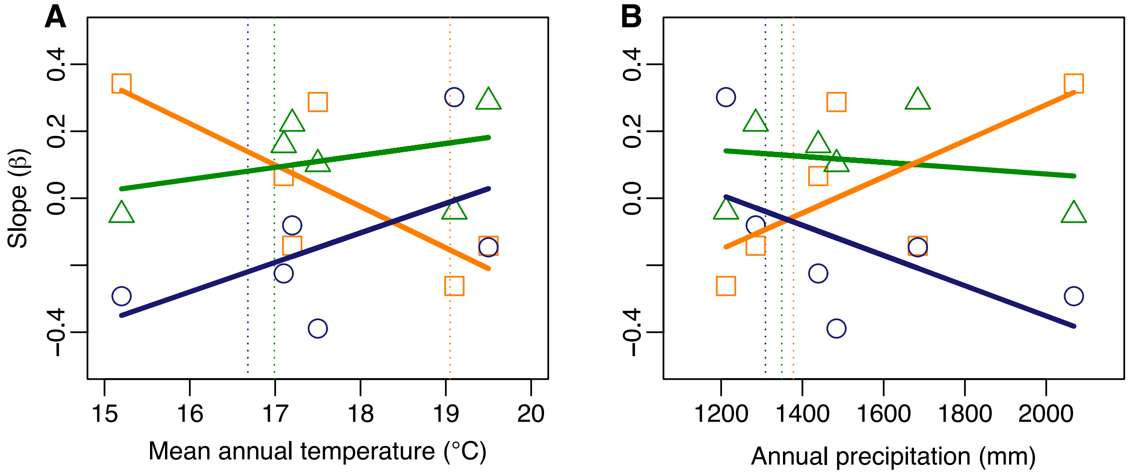

Figure 4-4 *Pinus taeda* provenances exhibit variation in invasion potential that is mediated by climate.

The increasing (positive slopes) and decreasing (negative slopes) contributions of provenances to the invasive plants during each range expansion are linearly affected by (A) mean annual temperature and (B) annual precipitation. While the Texas provenance is invasive in colder and wetter locations (orange squares and solid lines), the coastal provenance is invasive in warmer locations (blue circles and solid lines). The central provenance (green triangles and solid lines) is not affected by climate as strongly as are the other provenances, but its invasive potential is higher in warmer locations. Interestingly, provenances are not more invasive in locations with temperature and precipitation more similar to those of their native ranges (dotted lines).

CONCLUSION

My dissertation studied the patterns and processes involved in the invasive range expansion of introduced non-native plants. My work showed that introduction of non-native species is a selective process that affects subsequent invasive potential of introduced organisms. Moreover, is showed that the invasion process is better understood at the genotype- and population-levels, and not at the species-level. Taken together, this research highlights the importance of understanding an organism's ecological and evolutionary histories occurred prior to introduction, and the importance of humans in selecting specific genotypes for introduction.

VITA

Rafael D. Zenni was born in Curitiba, Brazil on March 8th 1982. He attended elementary and high school in Curitiba, graduating in 1999. In 2005 he received a forest engineering degree from the Federal University of Paraná. After graduation he worked as a consultant in several environmental projects and as an applied scientist for the South American Invasive Species Program at the The Nature Conservancy. In 2008, he moved to South Africa where he received a BSc. Honours degree in Botany from the University of Stellenbosch with a study of the invasiveness of *Acacia paradoxa* in South Africa. Back in Brazil, he worked for the *O Boticário* Foundation on restoration and payment for ecosystem services projects. In 2010 he started the PhD program in Ecology and Evolutionary Biology at the University of Tennessee, Knoxville. The Doctoral degree was awarded in May 2014.