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EDITORIAL

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Advancing research on alien species and biological invasions

Halting Biological Invasions in Europe: from Data to Decisions. A message from NEOBIOTA 2012

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Introduction

The NEOBIOTA conferences initiated by the European Group on Biological Invasions represents a forum for exchange of ideas and discussion of topics related to biological invasions as well as an interface between science, application and policies (Kowarik and Starfinger 2009). The 7th NEOBIOTA conference (http://neobiota2012.blogspot.com.es/), held in Pontevedra (Spain) from 12–14 September 2012, brought together 288 participants (ecologists, conservationists, representatives of governmental agencies and stake-holders), from 24 European countries and 9 non-European ones (namely, Australia, Brazil, Canada, Chile, Colombia, New Zealand, South Africa, United States and Venezuela).

Keynote lectures offered a substantial look and provided new perspective at the topic. Darren Kriticos (CSIRO Ecosystem Sciences, Australia) presented the recent advances in bioeconomic techniques for pest risk assessment that can inform efforts to prevent the spread of IAS to new regions, or to manage their subsequent spread in the new environment and addressed frontier issues for the application of these techniques more broadly under current and future climates. Gregory Ruiz (Smithsonian Institute, USA) gave an overview on how non-native species richness for invertebrates changes along the latitudinal gradient and on mechanisms that may explain the observed pattern in marine systems highlighting their relevance for evaluating effects on coastal marine

ecosystems and advancing management strategies to limit invasion impacts. Dave Richardson (Centre of Invasion Biology at the University of Stellenbosch, South Africa) gave a global snapshot of the current status of alien tree invasions and the problems they cause; reviewed some approaches and strategies that have evolved to deal with invasive alien trees in different parts of the world; and offered some ideas on potentially useful strategies for developing more effective and sustainable strategies for management.

Oral and poster sessions, structured around 4 main topics (ecology, evolution, impacts and management of biological invasions) provided the framework for in-depth debates on theoretical and applied aspects dealing with biological invasions, and wider discussions on what can be done to halt the problem. Around 290 scientific contributions covering a wide range of organisms and ecosystems addressed the current state of knowledge about interactions of climate change with biological invasions, modelling the success of alien species, species traits conferring invasiveness, biotic and environmental control of biological invasions, genetics and evolution of introduced and native populations, ecological impacts, new tools for prevention and early detection of invasive species, routes, pathways and vectors of invasions, risk analysis and control and eradication of invasive species.

At the end of the conference participants called once again the attention on the progressing and escalating threats posed by invasive alien species in Europe and suggested that immediate cooperative, specific policies are needed if we are to have any chance to halt biodiversity loss. In accordance with the Neobiota 2012 slogan "Halting Biological Invasions in Europe: from Data to Decisions" which emphasizes the need of bridging science and policy to deal effectively with invasive alien species, a resolution entitled "Time to act! Biological invasions need a strong European legal framework urgently!" was adopted by participants and later delivered to European Authorities (see Suppl. material 1).

The scientific research is contributing significantly to the management of invasive alien species and our understanding of the complexity of the processes underlying biological invasions. But, there remain challenges and questions which require novel approaches, multidisciplinarity, new techniques from different fields and cooperative efforts both in theoretical and applied research. Some of them are approached in this Special Issue.

There is a strong interest to understand why some species are successful in invading new environments and others not. Several hypotheses have been proposed to explain invasion success on the basis of intrinsic biological traits and extrinsic eco-evolutionary differences between the native and introduced ranges. However, their applicability is up for debate in the scientific arena.

Within this framework Colautti et al. (2014a) provide a novel way to quantify the invasiveness of species taking into account (i) interspecific differences in performance among native and introduced species within a region, and (ii) intraspecific differences between populations of a species in its native and introduced ranges. The authors demonstrate how inter- and intraspecific comparisons using field surveys can improve testing of the major hypotheses of invasion success, and highlight the need for large-scale

sampling efforts quantifying simple performance measurements in a large number of populations across entire native and introduced distributions.

Large-scale research networks can be a powerful tool to fill this gap permitting the collection of spatially extensive ecological data using minimal resources. Therefore, Colautti et al. (2014b), aiming at establishing *Alliaria petiolata* as a model species for plant invasion biology, introduce the Global Garlic Mustard Field Survey, a coordinated distributed field survey to collect performance data and germplasm from this species across its entire distribution. They describe constraints on protocol design and implementation, summarize the extent of participation, outline results, and note potential avenues for future research on other invasive species.

Comparisons between native and non-native populations of a species can provide help to clarify the biological and environmental factors that may contribute to range expansion and/or adaptation to climate change, and to reveal mechanisms by which organisms respond to novel ecological and environmental pressures. Moralez-Silva and Del Lama (2014) contribute to clarify the process of colonization and dispersal patterns of the cattle egret (*Bubulcus ibis*) across its native (Africa) and invaded range (Brazil). By means of genetic tools (Mitochondrial DNA analysis) the authors evaluate and describe the genetic diversity of the species in both areas, the genetic differentiation between populations in different regions of Brazil and Africa as well as the genetic signs of demographic expansion in both areas.

Lastly Abbas et al. (2014) worked on the effects of plant debris (wrack) burial on seed germination and seedling establishment of *Spartina densiflora* an invasive plant of saltmarshes in southern Europe, Northwest Africa and the West Coast of North America. Experimental results in accordance with field observations show an inverse relationship between germination and emergence of *S. densiflora* with wrack burial. With these findings authors provide useful information to predict invasion dynamics of the species and plan the management of invaded marshes.

Summarizing, the papers of this Special Issue illustrate the enormous complexity intrinsic to biological invasions and set out new challenges in order to improve our understanding of the issue and contribute to best knowledge management of invasive alien species.

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Supplementary material 1

Time to act! Biological invasions need a strong European legal framework urgently!

Authors: NEOBIOTA The European Group on Biological Invasions

Data type: image

Explanation note: Resolution adopted by the participants of the "7th European Conference on Biological Invasions" of the European Working Group on Biological Invasions - NEOBIOTA.

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REVIEW ARTICLE

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Advancing research on alien species and biological invasions

Quantifying the invasiveness of species

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Abstract

The success of invasive species has been explained by two contrasting but non-exclusive views: (i) intrinsic factors make some species inherently good invaders; (ii) species become invasive as a result of extrinsic ecological and genetic influences such as release from natural enemies, hybridization or other novel ecological and evolutionary interactions. These viewpoints are rarely distinguished but hinge on distinct mechanisms leading to different management scenarios. To improve tests of these hypotheses of invasion success we introduce a simple mathematical framework to quantify the invasiveness of species along two axes: (i) interspecific differences in performance among native and introduced species within a region, and (ii) intraspecific differences between populations of a species in its native and introduced ranges. Applying these equations to a sample dataset of occurrences of 1,416 plant species across Europe, Argentina, and South Africa, we found that many species are common in their native range but become rare following introduction; only a few introduced species become more common. Biogeographical factors limiting spread (e.g. biotic resistance, time of invasion) therefore appear more common than those promoting invasion (e.g. enemy release). Invasiveness, as measured by occurrence data, is better explained by inter-specific variation in invasion potential than biogeographical changes in performance. We discuss how applying these

comparisons to more detailed performance data would improve hypothesis testing in invasion biology and potentially lead to more efficient management strategies.

Keywords
Biogeographical comparisons, biological invasions, preadaptation, functional traits, increased vigour, invasion success, intrinsic vs extrinsic factors

Introduction

The economic and ecosystem impacts caused by species invasions are considerable (Gaertner et al. 2009; Pyšek et al. 2012b). However, the vast majority of species that are introduced remain rare, with only a fraction becoming widespread and dominating local communities (Williamson and Fitter 1996; Jeschke and Strayer 2005; Ricciardi and Kipp 2008; Stohlgren et al. 2011; Hulme 2012). Identifying the ecological and evolutionary factors that determine these disparate outcomes is the focus of a large body of published empirical work (van Kleunen et al. 2010a) including a growing number of hypotheses and synthetic frameworks (Catford et al. 2009; Blackburn et al. 2011; Gurevitch et al. 2011; Jeschke et al. 2012).

One reason for this expanding literature is a growing appreciation for the inherent complexity of ecological and evolutionary (eco-evolutionary) processes. But an additional factor may be a lack of appropriate data to rigorously evaluate multiple hypotheses for invasion success and the circumstances under which they are most applicable. To further explore this latter possibility, we review the hypotheses suggesting that some plant species are inherently good invaders, and those suggesting that invasiveness is acquired as a result of ecological and genetic differences between the native and introduced range. We introduce two simple metrics for quantifying the invasiveness of species on a relative scale and demonstrate their utility using occurrence data of native and introduced plant species in Argentina, South Africa, and Europe. We demonstrate how inter- and intraspecific comparisons using field surveys can improve testing of the major hypotheses of invasion success, and identify a significant data gap – namely, the lack of comprehensive field data measuring survival and reproductive rates in natural populations.

Hypotheses of invasion success

Hypotheses proposed to explain invasion success can generally be grouped into two categories based on whether they primarily attribute invasion success or failure to (i) extrinsic differences in ecological or evolutionary processes that differ between native and introduced ranges or (ii) intrinsic biological characteristic of particular species or higher-order taxonomic groups. Two key assumptions underlie these hypotheses. First, if invasiveness arises as a result of eco-evolutionary differences between the native and introduced ranges, then introduced populations in the introduced range should

exhibit enhanced performance relative to their conspecifics in the native range. Alternatively, if invasiveness is primarily an inherent characteristic, then invasive species should perform well in both ranges.

Perhaps the most common hypotheses in contemporary studies are those attributing the successful proliferation and spread of invasive species to altered ecological and evolutionary processes, an idea which dates back to the foundational literature of biological invasions (Elton 1958; Baker and Stebbins 1965). For example, introduced species experience an inhospitable abiotic environment (Mack 2000), or biotic resistance due to competition (Levine et al. 2004) or damage by native enemies (Parker et al. 2006), limiting the establishment, spread, and impact of the majority of introduced species. Additionally, establishment may fail because of insufficient propagule pressure (Lockwood et al. 2005) and Allee effects (Allee 1931), leading to stochastic extinction (Sax and Brown 2000). Alternatively, species may overcome these barriers given sufficient time (Pyšek and Jarošík 2005; Williamson et al. 2009), or by virtue of novel allelochemicals (Callaway and Ridenour 2004), altered soil microbial interactions (Reinhart and Callaway 2006), novel mutualisms (Richardson et al. 2000), and loss of natural enemies during the invasion process (Mitchell and Power 2003; Torchin et al. 2003). Introduction of historically isolated populations or species could also result in novel opportunities for interspecific hybridization leading to hybrid vigour in the introduced range (Ellstrand and Schierenbeck 2000), or intraspecific hybridization among historically isolated populations could occur, leading to novel adaptive gene combinations or simply increasing standing genetic variation available for adaptive evolution (Kolbe et al. 2004; Keller and Taylor 2010). Additionally, invaders are likely to be successful if they fill a novel role or function within an invaded ecosystem (Fetahi et al. 2011), if they are able to use resources not completely used by natives (Case 1990), or if they interact in novel ways with other non-native species (Simberloff and Von Holle 1999). Regardless of the specific mechanisms proposed, all of the hypotheses mentioned above assume that ecological or genetic differences between the native and introduced ranges (i.e. extrinsic factors) are responsible for making species invasive.

An alternative class of hypotheses regard invasiveness as an intrinsic quality of some species, implicitly assuming that ecological differences between ranges are minor relative to the identity and functional traits of the invader. This idea also dates back to early literature on invasive species, particularly Baker's (1965) characterization of a hypothetical 'ideal weed' possessing a particular suite of traits associated with invasiveness. Baker also noted that some invasive species exhibit a 'general purpose genotype' with high phenotypic plasticity and fixed heterozygosity, which he hypothesized made them capable of occupying a broad range of habitats in their native and introduced ranges (Baker 1965). These ideas have stimulated a large number of studies suggesting particular traits that promote invasion, but few generalities have emerged (Pyšek and Richardson 2007; van Kleunen et al. 2010b). However, the traits of invasive species are often similar to abundant or widespread native species (Lind and Parker 2010; van Kleunen et al. 2010b), suggesting that it may be possible to predict performance based on species' traits alone. The use of key traits or trait combinations to predict a spe-

cies' potential invasiveness has obvious management benefits, including the creation of 'blacklists' of potentially harmful species, 'whitelists' of species unlikely to pose a significant threat (Kolar and Lodge 2002; Hui et al. 2011), and formal risk assessment for particular applications (Kumschick and Richardson 2013). Interestingly, if invasions are the result of traits intrinsic to the invader *per se*, some species may simply be ecologically dominant both home and away - a quantitative prediction that has received surprisingly little attention in the literature despite the profound impacts it would have on our understanding and management of invasions (Firn et al. 2011).

Inter- vs. intra-specific comparisons

The contrasting hypotheses outlined above attribute the successful (or failed) spread and proliferation of introduced species to either (i) intrinsic differences in performance among species (or higher-level taxonomic groups) often manifested through functional traits, or (ii) extrinsic consequences of the invasion process (e.g., release from natural enemies, novel weapons, biotic resistance). Two types of data would be particularly helpful to explore these alternatives. First, field data are needed to quantify the performance of introduced species relative to other species within a particular community or assemblage (i.e. interspecific field comparisons). Second, field data from populations of individual species are needed to compare biogeographical differences in performance between the native and introduced ranges (i.e. intraspecific field comparisons).

Inter- and intra-specific field comparisons can be conceptualized as separate but non-independent axes along which to classify invasiveness in a purely ecological context (Fig. 1). The interspecific comparison axis (ω) quantifies the ecological performance or 'invasiveness' of a species in its introduced range without regard to the mechanisms responsible. Here we define invasiveness as a composite measure of performance of introduced species, particularly rates of survival and reproduction in natural populations that lead to high abundance and competitive exclusion of native species. The intraspecific comparison axis (δ) quantifies the degree to which performance changes from the native to the introduced range resulting from differences in ecological and evolutionary processes. Note that performance measurements may include abundance, survival, reproduction, or more complex population demographic parameters.

Comparing species along the axes in Fig. 1 could provide a simple but powerful characterization of whether a particular species is invasive because it performs well everywhere ($\omega >> 0$ and $\delta \sim 0$ in Fig. 1), or because it benefits from eco-evolutionary differences between ranges ($\omega >> 0$ and $\delta > 0$). This comparison can also distinguish non-invasive species ($\omega << 0$) that are successful natives that fail to become invasive as a result of eco-evolutionary differences between ranges ($\delta < 0$), from species that are simply rare species regardless of range ($\delta \sim 0$). Moreover, the literature tends to inconsistently categorize species as 'invasive' if they have large economic or ecological impacts (Daehler 2001, Richardson et al. 2011), treating invasive and non-invasive species as distinct categories, whereas our approach quantifies invasiveness along a continuum.

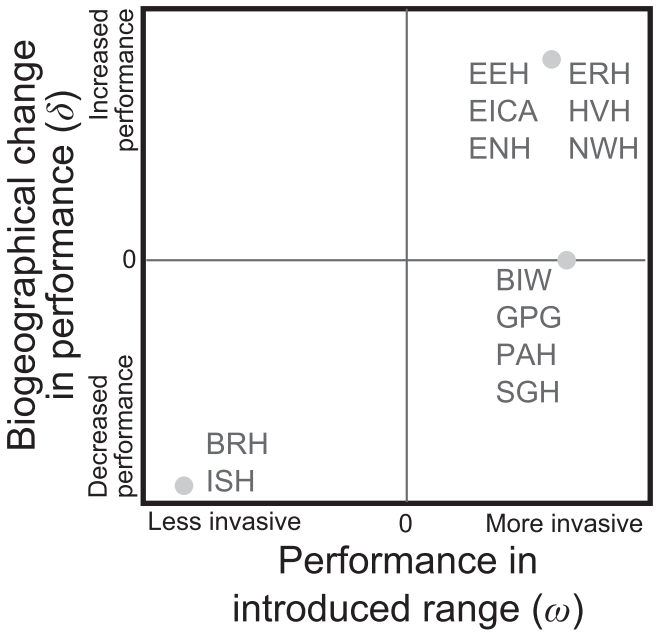

Figure 1. Testing hypotheses of invasion success could be improved by quantifying interspecific (ω) variation in performance among introduced species and intraspecific (δ) changes in performance between introduced and native populations. Dots show relative positions of a species predicted by the enemy of my enemy hypothesis (EEH), evolution of increased competitive ability (EICA), empty niche hypothesis (ENH), enemy release hypothesis (ERH), hybrid vigour hypothesis (HVH), novel weapons hypothesis (NWH), Baker's ideal weed (BIW), general purpose genotype (GPG), pre-adaptation hypothesis (PAH), specialist-generalist hypothesis (SGH), biotic resistance hypothesis (BRH), and the increased susceptibility hypothesis (ISH).

Quantifying the invasiveness of species could motivate appropriate study organisms for testing particular hypotheses of invasion success. For example, the enemy release hypothesis predicts that invasiveness results from native-introduced differences in the communities of natural enemies (Keane and Crawley 2002), which should yield an increase in performance ($\delta > 0$). Similarly, species that do not benefit from escaping natural enemies should have similar performance in the native and introduced ranges ($\delta \sim 0$), whereas those that gain more harmful enemies should have reduced performance in the introduced range ($\delta < 0$), on average. A more quantitative prediction of the enemy release hypothesis is therefore a positive relationship between the degree of escape from natural enemies and δ. Analogous predictions from other hypotheses of invasion success are approximated in Fig. 1.

In addition to simple statistical correlations, incorporating field measurements of ω and δ as continuous variables can lead to more rigorous statistical tests of invasion success. For example, simple least-squares models or more advanced statistical approaches, such as a path analysis (Wootton 1994), might be used to test one or more predictor variables on ω and δ, such as the degree of escape from natural enemies, or changes in allelopathic chemicals, or extent of genetic admixture. Other factors could

be incorporated in such an analysis to control for time of invasion, phylogenetic relatedness or to test the relative importance of different hypotheses. Importantly, a path analysis could test direct effects on invasiveness (ω), indirect effects on invasiveness via biogeographical differences in performance (δ), and the relative effect to ω and δ of the different predictor variables examined. Below we present a general mathematical approach to quantify inter- and intraspecific field measurements of performance, and then we demonstrate their heuristic and analytical value using occurrence data for plant species in Argentina, South Africa, and Europe.

Quantifying inter- vs. intra-specific difference in performance

A simple index that compares the relative performance (*W*) of a focal species (*j*) in a pool of *S* species is the following log ratio:

$$\omega_j = ln\left(S * W_j / \sum_{x=1}^{S} W_x\right) \quad \text{(Eq. 1)}$$

This equation is simply performance (*W*) measured for a focal species (*j*) divided by the average performance of all species (*S*) in the pool. It is designed to quantify interspecific variation in performance on a relative scale, which is necessary to compare the same species in different habitats or in different species assemblages. For example, performance could be measured as the relative abundance of an introduced species and compared across habitats with different species communities and productivity levels (e.g. Firn et al. 2011).

Quantifying performance on a relative scale provides a convenient method for comparing a species in its native and introduced ranges. For example, to quantify the biogeographical change in performance of an introduced species, consider the log-ratio of the relative performance of species *j* (from Eq. 1) calculated in its native (*n*) and introduced (*i*) ranges (see also Hufbauer and Torchin 2007), or:

$$\delta_j = ln\left(\frac{S_i * W_{j,i} / \sum_{x=1}^{S_i} W_{x,i}}{S_n * W_{j,n} / \sum_{y=1}^{S_n} W_{y,n}}\right)$$

which is mathematically equivalent to the difference in Eq. 1 between the native (ω_n) and introduced (ω_i) ranges:

$$\delta = \omega_i - \omega_n \quad \text{(Eq. 2)}$$

Using the intraspecific comparison given by Eq. 2, a positive δ represents an increase in the relative performance of species in the introduced range compared to the native range ($\omega_i > \omega_n$), whereas a negative value represents a decrease in relative performance ($\omega_i < \omega_n$).

One potential limitation of having non-independent axes is that an error in calculating ω_i will also increase δ_i, leading to spurious correlations if the same performance data are used. One solution to this problem would be to calculate these indices from different performance measurements. For example, one could calculate ω_i using range size, but measure intrinsic growth rates of native and introduced field populations to calculate δ_i. Incorporating these into the kind of statistical framework described above would be useful to test whether an extrinsic factor of interest (e.g. enemy release, heterosis) could explain differences in population vital rates between native and introduced populations (δ) and whether this could account for variation in range size (ω), after controlling for other factors like time since invasion and phylogenetic relatedness. The choice of performance measurements used to calculate ω and δ will ultimately depend on the hypotheses to be addressed.

In addition to testing scientific hypotheses, this approach could help to guide management decisions. For example, species found in the top left quadrant of Fig. 1 have increased performance in the introduced range, perhaps by escaping enemies or otherwise experiencing novel conditions, but have not (yet) become invasive. These species may become invasive if ecological conditions change (e.g., habitat alteration, global warming) or just given enough time (e.g., finding suitable habitats, evolutionary adaptation). These species may provide a high return on investment in control programs as they represent introduced species that are likely to become more invasive if proper measures are not taken. Additionally, species in the lower left quadrant are introduced species that are currently not invasive but could be if ecological conditions become more similar to those in the native range, for example with new disturbance regimes or a changing climate. Identifying several of these potential invaders within a particular region or habitat might help to motivate conservation efforts to limit anthropogenic influences that would cause these species to become more invasive.

Despite the scientific and management value of this approach, even simple performance measurements such as annual survival and reproductive rates are not available for most species in most regions. Given this limitation, we instead apply occurrence data available from plant species surveys to demonstrate the potential utility of Eqs. 1 and 2.

Example: occurrence data

To demonstrate the value of the inter- and intra-specific comparisons described above, we analysed occurrence data that has previously been published (Stohlgren et al. 2011). These data are simply the occupancy rates of individual plant species in 10 × 10 km cells in Great Britain (Preston et al. 2002), 11 × 12 km cells in the Czech Republic (from the CzechFlor database), 0.25-degree cells in the Republic of South Africa (Henderson 1998; Germishuizen and Meyer 2003), across 23 countries in Europe (Winter et al. 2008; DAISIE 2009), and 24 bioregional sub-regions in Argentina (Zuloaga and Morrone 1996). In other words, each regional dataset includes an inventory list of all native and introduced species identified in each region, as well as the number of cells (or countries or bioregions) in which each species is known to occur.

Importantly these data are not sufficient to account for potential influence of phylogenetic non-independence and time of invasion. Residence time in particular can have a large impact on spread measured at a particular point in time (Pyšek and Jarošík 2005; Williamson et al. 2009). Moreover, species occurrence data represent only a rough approximation of numerical abundance and dominance (Royle and Dorazio 2008). Occurrence data will tend to over-estimate the invasiveness of species that are weak competitors but widespread, while under-estimating the invasiveness of recently established species that dominate where present but are not yet widely distributed. Our analysis is therefore intended as an introduction to the utility of the metrics described above, rather than to provide a definitive quantification of invasiveness.

We used Eq. 1 to quantify the performance of each species (ω_i) within each regional dataset as the number of occupied geographic cells relative to the number of cells averaged across all native and introduced species in a given region. The relative performance index of each species (ω_i) in its native and introduced ranges is shown in Fig. 2 for each of the eight pairwise comparisons between regional datasets. We include both pairwise transcontinental comparisons (Europe, Argentina and South Africa) and a within-continent contrast between the Czech Republic and Britain. This demonstrates the utility of Eq. 1 to compare performance between regions despite differences in species communities and census cell sizes (e.g. 23 countries in Europe vs. 0.25 degree cells in South Africa).

We found that in each region the majority of introduced species (66.6%) rated below-average on the relative performance axis (ω) in the introduced range (Fig. 2, y-axis < 0; $G = 112.3$, 1 *d. f.*, $P < 0.001$). This includes recent invaders that are still spreading, but also is consistent with the generally accepted view that only a minority of introduced species are able to establish and spread widely (Williamson and Fitter 1996; Jeschke and Strayer 2005; Ricciardi and Kipp 2008; Stohlgren et al. 2011; Hulme 2012). The majority of introduced species may simply be intrinsically weak invaders, or extrinsic environmental factors such as biotic resistance, genetic bottlenecks or simply time since introduction could prevent them from becoming invasive. We found that most species (73.7%) experienced a reduction in relative performance compared to the native range (i.e. below 1:1 line in Fig. 2; $G = 233.9$, 1 *d. f.*, $P < 0.001$), suggesting that time since invasion and environmental, rather than intrinsic factors, often prevent species from becoming more common than they are in their native range.

After calculating relative performance of species between each pair of regional datasets, we used Eq. 2 to calculate biogeographical changes in relative performance of each species (Fig. 3). This equation simply uses the x and y coordinates of each species in Fig. 2 to calculate delta values (δ) for each introduced species in each region. The distribution of δ can provide insight into environmental and biotic differences between ranges given that $\delta_i = 0$ represents a species performing similarly in the native and introduced range. For example, the majority of species introduced from Argentina to Europe have decreased in relative performance (δ < 0 in Fig. 3: AR-->EU), but species introduced from Argentina to South Africa have increased in relative performance, on average (δ < 0 in Fig. 3: AR-->ZA). A number of factors could be investigated to explain the weaker

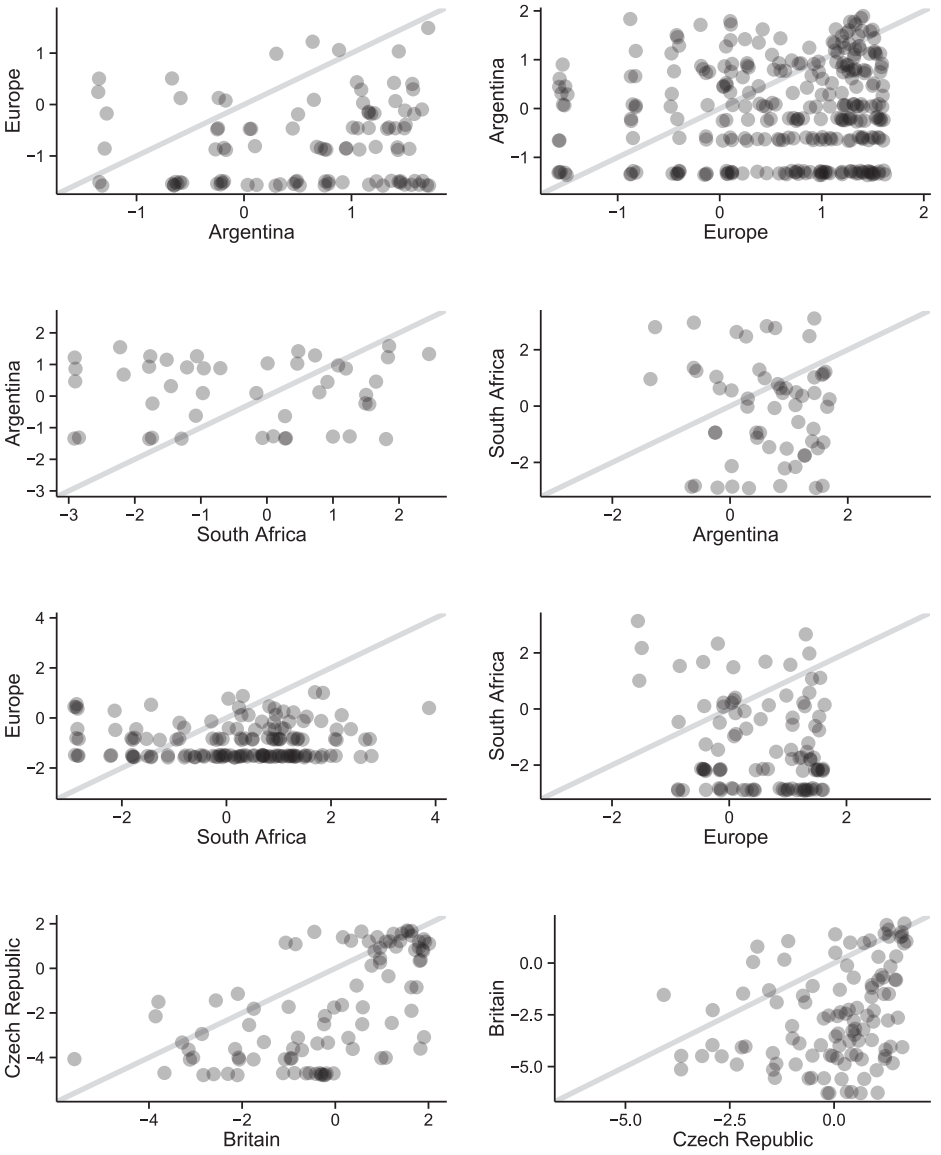

Figure 2. Bivariate plots comparing standardized performance measurements of species in their native (x-axis) and introduced ranges (y-axis). Each point is a species and the 1:1 line is shown in grey. Performance is measured as the number of occurrences, standardized for each region using Eq. 1 (see main text). Slight random noise was added to increase visibility of overlapping points**.**

performance of Argentinian native species in Europe relative to South Africa, such as stronger competition, or more aggressive generalist herbivores and diseases. Climate matching is also likely to be important given the reduced performance of European species introduced to South Africa (EU-->ZA) and Argentina (EU-->AR).

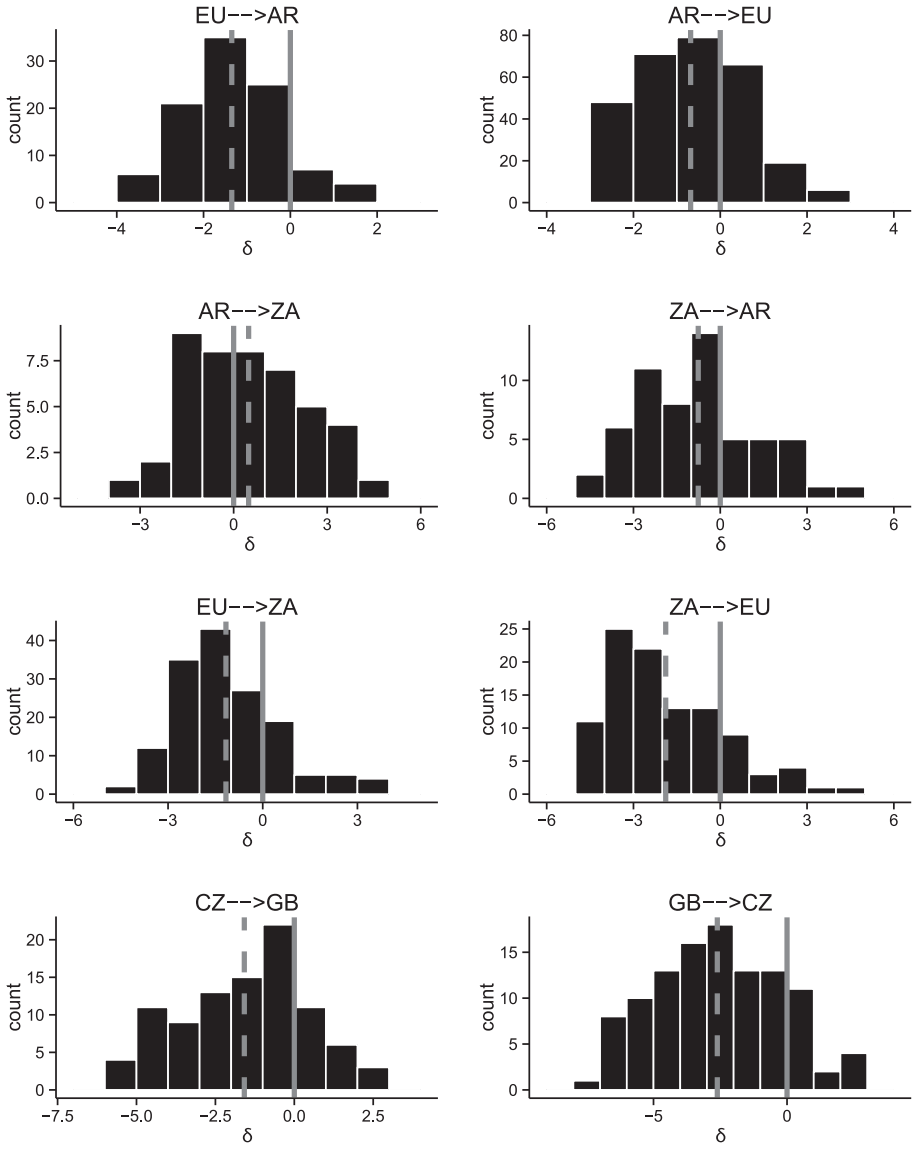

Figure 3. Frequency distribution histograms of biogeographical changes in performance (δ), for species native to one region and introduced to another, in the form of labels: "*native --> introduced*". Performance changes are based on the number of grid cells or regions of occurrence, standardized using Eq. 2 (see main text). Regions are abbreviated for Europe (EU), Argentina (AR), South Africa (ZA), the Czech Republic (CZ) and Britain (GB). Solid grey lines indicate equal performance in the native and introduced range, and the dotted lines shows the average δ.

Extrinsic ecological and genetic differences between the native and introduced range therefore appear to suppress most species from becoming common. But are the most common invaders more likely to belong to a subset of species that are common

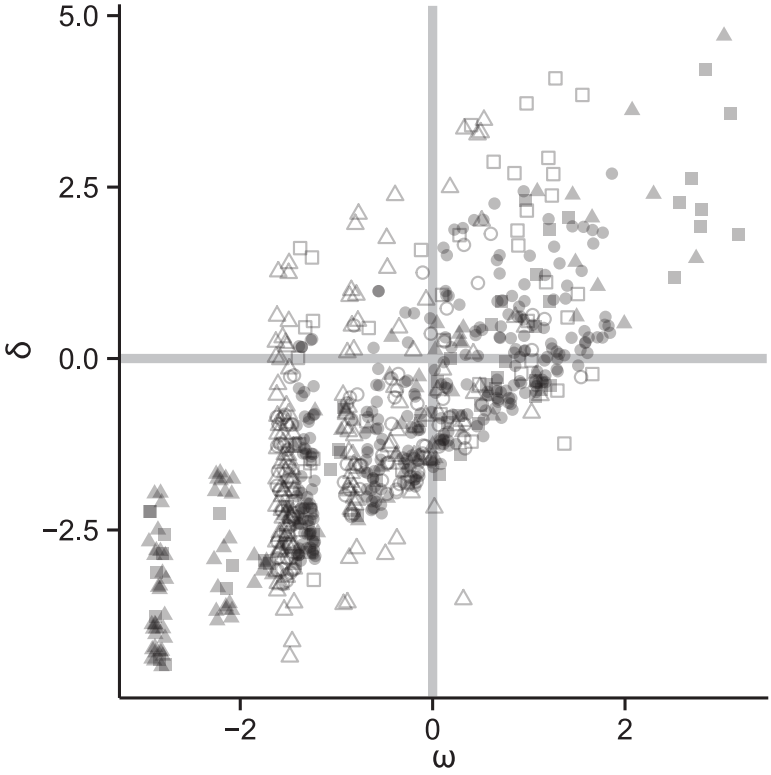

Figure 4. Bivariate plot of interspecific (x-axis) and intraspecific (y-axis) performance comparisons, using occurrence data. The x-axis shows performance of a species (ω) relative to the average performance of all species in its introduced range (ω = 0, vertical grey line). The *y*-axis shows the degree to which the biogeographical difference in performance (δ) deviates from equality in the introduced range relative to the native range (δ = 0, vertical grey line). Each point is an individual species from one of the following regional comparisons: species native to Europe and introduced to Argentina (open circles), Argentina to Europe (filled circles), Europe to South Africa (open triangles), South Africa to Europe (filled triangles), Argentina to South Africa (open squares), and South Africa to Argentina (filled squares). Slight random noise was added to increase visibility of overlapping points.

in their native range, or are they species that benefit most from eco-evolutionary processes (e.g. enemy release, novel weapons)? Following the heuristic approach in Fig. 1, we plotted ω and δ for each species in each pairwise comparison (Fig. 4) and found evidence for both scenarios. Of the 70 most common invaders (ω > 1.17), 85% (60 of 70) increased their performance relative to the native range (δ > 0), suggesting that extrinsic factors (e.g. enemy release, novel weapons, etc.) are important for explaining successful spread of the most common invaders. However, at any given point along the δ-axis in Fig. 4, species varied by up to an order of magnitude in ω, even though there is a strong correlation between these non-independent indices (R = 0.866). In other words, the extent of invasive spread (ω) varies significantly among species even after accounting for extrinsic environmental factors that cause differences in δ. Thus,

we find evidence that both intrinsic and extrinsic factors contribute to the relative invasiveness of species.

Simultaneously accounting for variability in both axes in Fig. 4 would improve statistical tests of invasion success, as measured by occurrence data. In particular, few characteristics of species successfully predict invasion success across a range of taxa (Pyšek and Richardson 2007; van Kleunen et al. 2010b). Without controlling for variation in δ, inherently good invaders are confounded with inherently poor invaders that become widespread due to extrinsic factors like enemy release or novel weapons. Similarly, inherently poor invaders are confounded with inherently good invaders that are prevented from becoming common by extrinsic factors like propagule pressure, a recent invasion history, or mismatched climate. In these cases, including δ in statistical tests for traits associated with ω would improve power to detect functional traits associated with inherently strong invaders.

In addition to examining intrinsic differences in invasion potential among species, extrinsic factors can also be better tested by accounting for variation in both axes in Fig. 4. Without accounting for variation in ω, introduced species that become common through extrinsic factors that increase performance are confounded with species that become common because they are inherently good invaders. Additionally, species that fail to become common because of reduced performance are confounded with species that are inherently poor invaders. Accounting for variation in ω would therefore improve statistical power to test for extrinsic genetic or environmental factors that influence the invasiveness of species. Testing enemy release, novel weapons, hybrid vigour, and other hypotheses for invasion success (or failure) based on extrinsic factors could be improved in this manner.

Despite the inherent limits of focusing our analysis on occurrence data, we have demonstrated above the potential value of using Eqs. 1 and 2 to better inform management decisions and to improve hypothesis testing. In the next section we consider alternative data sources for characterizing ω and δ that would significantly improve the approach we have advocated.

Improved performance measurements

Interspecific field comparisons

What sorts of data are available to quantify the performance of invasive species relative to other species within a particular community or assemblage? A number of studies have used an interspecific comparative approach to test hypotheses of invasion success. Many of these have been included in a recent meta-analysis (van Kleunen et al. 2010b), which reviewed data from 116 comparative studies involving 321 species. However, most of these (96%) did not distinguish invasive from non-invasive introduced species, but rather compared native and non-native species. In addition to the five remaining studies in the meta-analysis, we identified 27 studies that contrasted phenotypic traits or ecological aspects (e.g. herbivore load) of invasive and non-invasive introduced spe-

cies, several of which were published after the (van Kleunen et al. 2010b) meta-analysis (Table 1). We did not collect these studies using a methodical review of the literature but rather biased our search toward more recent studies demonstrating the different types of data currently available to quantify invasiveness for a large number of species (Table 1). Of these 32 studies, only four quantified invasiveness (Mihulka et al. 2003; Mitchell and Power 2003; Hamilton et al. 2005; Parker et al. 2006). The remaining 28 studies binned introduced species into two (24 of 28 studies) or more (4 studies in Table 1) categories of invasiveness.

These results show that comparative studies testing hypotheses of invasion success generally have used a categorical rather than a quantitative approach like the one we advocate in Figs 1–4. Moreover, invasiveness categories were determined primarily on expert opinion or presence/absence data in these studies, although occurrence data were occasionally combined with information on date of introduction to estimate rates of spread. This limited review therefore suggests that invasion biologists currently define species' invasiveness on a categorical scale, despite the obvious interspecific variation in performance that we demonstrate in Figs 2 and 4.

Intraspecific field comparisons

In addition to interspecific comparisons, testing hypotheses of invasion success also requires performance comparisons of natural field populations in the native and introduced range, yet these data are surprisingly rare (Firn et al. 2011). For example, a recent meta-analysis (Parker et al. 2013) compared size, reproductive traits, and abundance between native and introduced populations of the World's Worst Invaders (Lowe et al.

Table 1. Overview of interspecific comparative studies testing hypotheses of invasion success. The number of categories in a study is in parentheses for those using categorical classification. N is the number of species included in the study.

Citation	Invasiveness criteria	Classification	N
Burns 2004	Expert opinion	Categorical (2)	6
Burns and Winn 2006	Expert opinion	Categorical (2)	8
Cadotte et al. 2006	Occurrence data	Categorical (5)	1153
Cappuccino and Arnason 2006	Expert opinion	Categorical (2)	39
Cappuccino and Carpenter 2005	Expert opinion	Categorical (2)	18
Forcella et al. 1986	Expert opinion	Categorical (2)	3
Gerlach and Rice 2003	Expert opinion	Categorical (2)	3
Gioria et al. 2012	Occurrence and spread	Categorical (2)	321
Grotkopp and Rejmánek 2007	Spread rate	Categorical (2)	26
Hamilton et al. 2005	Occurrence data	Quantitative	152
Hejda et al. 2009	Occurrence and spread	Categorical (2)	282
van Kleunen et al. 2011	Occurrence data	Categorical (2)	28
Kubešová et al. 2010	Occurrence and spread	Categorical (2)	93

Citation	Invasiveness criteria	Classification	N
Lake and Leishman 2004	Expert opinion	Categorical (2)	57
Lloret et al. 2005	Expert opinion	Categorical (4)	354
Mihulka et al. 2003	Occurrence and spread	Quantitative	15
Mitchell and Power 2003	Expert opinion	Quantitative	473
Moravcová et al. 2010	Occurrence and spread	Categorical (2)	93
Murray and Phillips 2010	Expert opinion	Categorical (2)	468
Muth and Pigliucci 2006	Occurrence data	Categorical (2)	8
Nilsen and Muller 1980	Occurrence data	Categorical (2)	2
Parker et al. 2006	Expert opinion	Quantitative	51
Perrins et al. 1993	Spread rate	Categorical (4)	4
Phillips et al. 2010	Expert opinion	Categorical (2)	468
Pyšek and Jarošík 2005	Occurrence data	Categorical (3)	203
Pyšek et al. 2009a	Occurrence data	Categorical (2)	1218
Pyšek et al. 2009b	Occurrence and spread	Categorical (2)	17
Pyšek et al. 2011a	Occurrence and spread	Categorical (2)	1221
Pyšek et al. 2011b	Occurrence and spread	Categorical (2)	1007
Rejmánek and Richardson 1996	Spread rate	Categorical (2)	24
Richardson et al. 1987	Occurrence data	Categorical (2)	4
Skálová et al. 2011	Occurrence and spread	Categorical (2)	3

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2004). Despite an exhaustive search of the peer-reviewed and grey literature, performance data were not available for a majority of the investigated species (53 of 89), primarily because of a lack of published studies from the native range (Parker et al. 2013).

This paucity of performance data is surprising for plant taxa given the availability of large databases of presence/absence data (Zuloaga and Morrone 1996; Henderson 1998; Preston et al. 2002; Pyšek et al. 2002, 2012a; Germishuizen and Meyer 2003; Winter et al. 2008; DAISIE 2009; Stohlgren et al. 2011), and given that invasion biologists have repeatedly called for more studies from the native ranges of introduced species (Hierro et al. 2005; van Kleunen et al. 2010a). Impediments to progress on this front may include funding priorities, which tend to focus on problematic invaders and endangered native species. Increasingly the solution to data gaps might require the formation of international research networks and citizen-science efforts such as the "Nutrient Network" (http://nutnet.umn.edu), which is monitoring performance of hundreds of native and introduced plant species across 39 sites with similar habitat characteristics (Firn et al. 2011), or the "Global Garlic Mustard Field Survey" (http://www.garlicmustard.org), which measured performance of a single plant (*Alliaria petiolata*) across almost 400 sites in both its introduced and native ranges (Colautti et al 2014). More efforts like these could provide invaluable performance information to complement available distribution data for a variety of species.

In addition to these data limitations, it is often not clear which performance data are most appropriate for biogeographical comparisons. For example, one of the most common intraspecific comparison of invader performance is individual size (e.g. length or biomass), and sometimes time to maturity, ostensibly because fast-growing or long-lived individuals can become large and lead to increased population growth rates, population density, and abundance (Grime 1977). Individuals in introduced populations can indeed be larger in some species (Crawley 1987; Jakobs et al. 2004; Darling et al. 2011; Hinz et al. 2012, Parker et al. 2013), but other studies do not show a general increase in size between the native and introduced ranges (Thébaud and Simberloff 2001; Grosholz and Ruiz 2003). Moreover, many introduced plants form latitudinal clines in size and reproductive timing, suggesting that these traits are under divergent natural selection across large spatial scales (Colautti et al. 2009). Recent work on the invasive plant *Lythrum salicaria*, for example, shows that clines in size and reproductive timing arise because these traits trade off, resulting in an optimum phenotype that changes with latitude (Colautti and Barrett 2010; Colautti et al. 2010). Therefore, size and time to maturity in field populations may be poor measurements for contrasting native and introduced differences in performance. Population abundance and individual reproductive rates may be more informative performance metrics, but these have rarely been sampled in native and introduced populations even though these measurements are not difficult to obtain, particularly for plants (Vilà et al. 2005; Pergl et al. 2006; Ebeling et al. 2008). Measuring abundance, survival rates, and reproductive output of introduced species across their native and introduced ranges should be a priority for the field of invasion biology, as it would allow a quantitative comparison of different performance measures for assessing invasiveness.

Rates of survival and reproduction, as well as abundance data from natural field populations would be valuable for testing hypotheses of invasion success and setting management priorities. More to the point, the ideal dataset for testing the hypotheses of invasion success would include: (i) a census of all major life stages, and (ii) vital rates (i.e. survival and reproduction) at each life stage, perhaps with experimental manipulations and demographic modelling to better understand ecological dynamics of native and introduced populations (Williams et al. 2010; Roy et al. 2011). Additionally, time since invasion can be an important confounding factor if these vital rates change over time, increasing as introduced populations become locally adapted (Colautti et al. 2010) or decreasing as populations approach their carrying capacity (Lankau et al. 2009) and accumulate natural enemies (Hawkes 2007). Therefore, temporal replication of these measurements would also facilitate better models of invasive spread.

We recognize that although ideal, extensive spatial and temporal replication of demographic data and manipulative field experiments would be difficult to obtain for even a single species, let alone the dozens or hundreds needed to test the generality of invasion success hypotheses. We therefore wish to stress that even basic performance data would be a significant improvement over most currently available data. Given likely financial and time constraints, large-scale sampling efforts that quantify relatively simple performance measurements in a large number of populations across entire native and introduced distributions should be a priority. Measurements of abundance, survival and reproductive rates as a complement to large presence-absence datasets would significantly improve our ability to identify the biological basis of invasiveness.

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RESEARCH ARTICLE

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Advancing research on alien species and biological invasions

The Global Garlic Mustard Field Survey (GGMFS): challenges and opportunities of a unique, large-scale collaboration for invasion biology

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Abstract

To understand what makes some species successful invaders, it is critical to quantify performance differences between native and introduced regions, and among populations occupying a broad range of environmental conditions within each region. However, these data are not available even for the world's most notorious invasive species. Here we introduce the Global Garlic Mustard Field Survey, a coordinated distributed field survey to collect performance data and germplasm from a single invasive species: garlic mustard (*Alliaria petiolata*) across its entire distribution using minimal resources. We chose this species for its ecological impacts, prominence in ecological studies of invasion success, simple life history, and several genetic and life history attributes that make it amenable to experimental study. We developed a standardised field survey protocol to estimate population size (area) and density, age structure, plant size and

fecundity, as well as damage by herbivores and pathogens in each population, and to collect representative seed samples. Across four years and with contributions from 164 academic and non-academic participants from 16 countries in North America and Europe thus far, we have collected 45,788 measurements and counts of 137,811 plants from 383 populations and seeds from over 5,000 plants. All field data and seed resources will be curated for release to the scientific community. Our goal is to establish *A. petiolata* as a model species for plant invasion biology and to encourage large collaborative studies of other invasive species.

Keywords
Alliaria petiolata, citizen science, model system, plant invasion, research network

Introduction

How is it that invasive species, which are introduced to novel geographical regions where they lack an adaptive evolutionary history, are nonetheless able to establish and proliferate? Since investigation into this topic was launched over a half-century ago (Elton 1958; Baker and Stebbins 1965), research on this question has expanded rapidly, leading to a large and growing number of ecological and evolutionary hypotheses (Sakai et al. 2001; Facon et al. 2006; Catford et al. 2009; Gurevitch et al. 2011; Jeschke et al. 2012). Biological hypotheses of invasion success generally fall into one of two categories: (i) biogeographical differences between native and introduced ranges and (ii) functional traits that differ between species or higher-order phylogenetic groups (Colautti et al. 2014). Hypotheses in the first category attribute the success of invasive species to biological differences between native and introduced regions that are more favourable in the latter. These differences include escape from natural enemies (Mitchell and Power 2003; Torchin et al. 2003; Mitchell et al. 2010), novel effects of biochemical weapons (Callaway and Ridenour 2004), novel biotic interactions (Reinhart and Callaway 2006), or increased anthropogenic disturbance (Byers 2002). Hypotheses in the second category associate invasion success with functional traits that differ among species or higher-order phylogenetic groups. For example, invasive species may be the subset of species from native source pools that possess particular ecological or evolutionary characteristics that promote introduction, establishment, spread and competitive displacement of natives (Pyšek and Richardson 2007; van Kleunen et al. 2010). These hypotheses are not exhaustive or mutually exclusive and different species may be invasive for different reasons (Mack et al. 2000). A challenge for ecology is to evaluate these hypotheses for individual invasions, eliminate those unlikely to explain invasion success, quantify the relative importance of the remaining hypotheses and identify context dependencies that allow for a robust general theory of invasion success.

A key distinction between biogeographical and trait-based hypotheses is whether introduced populations have increased in performance relative to native populations, where performance may be measured as abundance, range size, demography, and individual survival, reproduction or competitive ability. Biogeographical hypotheses that seek to explain how introduced species escape the regulatory mechanisms present in

their native range make an inherent assumption that introduced populations benefit from an ecological or evolutionary increase in performance relative to native populations, on average. We call this the "increased vigour assumption". In contrast, trait-based hypotheses assume that native and introduced populations perform similarly, with invasion success owing to preadaptation and environmental similarities between ranges. Therefore it is possible two very different types of invaders may exist: (i) species that become abundant and widespread through niche expansion (e.g. escaping regulation or gaining access to new resources) and (ii) species that expand their range through human agency but ultimately perform the same in native and introduced regions. Moreover, the species that both perform well in their native range and expand their niche in invaded regions should be the most successful invaders. However, few studies have measured performance of natural populations to directly test the assumption of increased vigour and these have been limited in size and geographical scope (Bossdorf et al. 2005; Parker et al. 2013; Colautti et al. 2014). Indeed, even basic field performance comparisons between native and introduced populations are often not available for many of the world's most notorious invasive species, and where available, there is often little information about variation in performance among individuals or populations within each range (Parker et al. 2013). Comprehensive field data are therefore crucial for testing the assumption of increased vigour.

In addition to testing for increased vigour, direct field measurements help to assess the ecological relevance of factors proposed to explain invasion success. By "ecological relevance" we mean the extent to which factors affecting fitness components measured under experimental conditions directly translate to invasion success in natural populations and at larger biogeographical scales. Field measurements of natural populations are important because complex interactions among ecological and evolutionary processes can limit the predictive power of ecological and genetic factors identified in controlled experiments. For example, many plants and animals have lost specialist enemies following introduction to geographically distant locales (Mitchell and Power 2003; Torchin et al. 2003), but this does not often translate to increased performance relative to native competitors in natural field settings where multiple factors interact (Agrawal et al. 2005; Parker et al. 2006; Parker and Gilbert 2007). Without comprehensive field data from natural populations, it is unclear how often invasion success owes to escape from natural enemies. Performance measurements of individuals in natural populations provide an important link between experimental results and ecological relevance.

Testing the assumption of increased vigour, distinguishing biogeographical and phylogenetic effects, and measuring the ecological relevance of hypotheses of invasion success are difficult tasks. Ideally, a study of increased vigour in a single species would involve (i) extensive field surveys measuring performance of native and introduced populations, (ii) large-scale field manipulations to study population dynamics at nested spatial scales, (iii) development of genetic resources, and (iv) an integrated experimental approach involving experts in a variety of areas including chemical ecology, community dynamics, population ecology, population genetics, developmental biology, and genomics. To facilitate such an approach, our goal is to build a model

species for invasion biology, develop novel resources and encourage international and interdisciplinary collaboration to coordinate detailed and robust research on a single species: garlic mustard, *Alliaria petiolata*. Such an approach could then be applied to additional species in the future. Below we review our motivation for the project, introduce the sampling protocol, describe constraints on protocol design and implementation, summarize the extent of participation, outline our curation and quality control procedures, and note potential avenues for future research.

Rationale

The Global Garlic Mustard Field Survey (GGMFS) was conceived during a 2008 meeting of the Global Invasions Network, funded by a Research Collaboration Network grant from the U.S. National Science Foundation (see Acknowledgements). The meeting involved 35 invasion biologists representing a broad range of empirical and theoretical backgrounds in ecology, evolution and genetics. Discussions within this group identified the need for standardized field measurements of performance traits, and led to our choice of study species and experimental design.

Large-scale collaborations in ecology

A major challenge in ecology is generalizing from individual studies done under controlled conditions or in particular locations to broad-scale ecological patterns. This is particularly difficult in invasion biology where ranges often span continents and resources are split among studies of hundreds of different invasive species. Just as large genomic databases and bioinformatics techniques are advancing scientific understanding of genetics and evolution, large-scale ecological data collection networks that focus on a common goal can provide comprehensive data to improve understanding of ecological processes and interactions (Silvertown 2009; Cadotte et al. 2010; Firn et al. 2011; Moles et al. 2011; Silvertown et al. 2011). Large networks of professional scientists are ideally suited to collecting spatially extensive ecological data in a standardized format that is also scientifically rigorous (Craine et al. 2007). Such planned, coordinated endeavours provide much more consistent and reliable data than those which could be obtained through reviews and meta-analyses of smaller studies that typically differ in methodology and sample size, and in some cases were not designed to answer the scientific questions being addressed (Moles et al. 2011). Our general approach is similar but intermediate to two increasingly popular models of large-scale research networks: citizen science projects and "coordinated distributed experiments" or CDEs (Fraser et al. 2012). These studies are generally conducted on a global scale, but vary in the level of expertise, number of participants, sample size and data depth (Figure 2).

Pure citizen-science projects like project bud-burst (http://budburst.org) and the Evolution MegaLab (http://www.evolutionmegalab.org) (Silvertown et al. 2011) gen-

erate large amounts of data at a low cost but rely heavily on private citizens of low expertise who may have no formal education in biology, and this has led to concerns about data accuracy (Delaney et al. 2008; Fitzpatrick et al. 2009; Dickinson et al. 2010). In contrast, large-scale coordinated distributed experiments typically involve a smaller group of professional scientists but require a higher levels of funding. For example, the International Tundra Experiment (ITEX) (http://www.geog.ubc.ca/itex), and the Nutrient Network (NutNet) (http://nutnet.science.oregonstate.edu) involve scientists using standardized protocols to collect ecological data at a series of sites around the world (Craine et al. 2007; Fraser et al. 2012). The GGMFS is intermediate to these two general approaches as it involves a larger number of highly-trained professional scientists, covering a larger number of sites, but with less intensity of research and low research cost per site (Figure 2).

Challenges and trade-offs

Large-scale collaborative research projects involving hundreds of scientists create a number of specific challenges, trade-offs and financial considerations. Development of a standardized protocol for large distributed studies requires decisions about the intensity of study and sophistication of participants (Figure 2). Given limited human resources and time, increased intensity of sampling at each site will trade off with the number of sample sites. Additionally, more sophisticated measurements require more expertise, reducing the number of qualified participants. In contrast to most citizen science projects, which usually include very simple measurements (e.g. presence of a species) with little or no equipment or training, we developed a protocol that requires only basic measuring supplies, but can be slightly challenging for participants with no formal science education, and can take several hours to sample a dense population. Nonetheless, we deliberately excluded more complicated but time-intensive field manipulations or measurements such as survival rates, seed production, edaphic measurements, and community composition to encourage an increased number of participants and to keep the protocol accessible to non-scientists. As a result, GGMFS participants are mostly professionally-trained, full-time scientists at academic and governmental organizations who are aware of the need for careful and unbiased data collection. A smaller number of populations have been sampled by non-scientists, and these will be analysed for potential data quality issues in future analyses.

Study system

Garlic mustard, *Alliaria petiolata* (M. Bieb.) Cavara & Grande, is native to most of Europe and western Asia below 68°N latitude (Cavers et al. 1979). Several factors make this species suitable as a model species for invasion biology.

A widespread and successful invader with demonstrated impacts

Herbarium records (Cavers et al. 1979) and neutral genetic markers (Durka et al. 2005) suggest introductions of *A. petiolata* to North America from multiple locations across its native Eurasian distribution beginning in the early 19th century, probably for food and medicinal uses. Like many invasive species, it remained inconspicuous for decades, after which it spread at an exponential rate. It is now present in at least 37 U.S. states and five Canadian provinces and has been declared a prohibited or noxious weed in eight states (USDA Plants Database). *Alliaria petiolata* invades nutrient-rich, semi-shaded habitats such as forest edges and moist woodlands. Dense invasive populations can reduce native plant diversity and limit recruitment of native trees (Carlson and Gorchov 2004; Stinson et al. 2007) by disrupting mycorrhizal communities that are important for economically valuable trees and native understory plants (Stinson et al. 2006; Burke 2008; Wolfe et al. 2008; Barto et al. 2011; Lankau 2011), and by altering litter decomposition as well as soil nitrogen and phosphorus cycles (Rodgers et al. 2008).

Implicated in several key hypotheses of invasion success

Invasive populations of *Alliaria petiolata* have been studied frequently. In North America, the species lacks its native specialist herbivores (Blossey et al. 2001), and suffers less herbivore damage (Lewis et al. 2006). Several previous studies tested whether this enemy release has led to evolution of decreased herbivore defences and increased competitive ability (EICA hypothesis). Some aspects of plant defence were consistently decreased in introduced populations (Bossdorf et al. 2004b; Hull-Sanders et al. 2007), but overall evidence was rather ambiguous and did not support the EICA hypothesis (Bossdorf et al. 2004a, b; Cipollini et al. 2005; Hull-Sanders et al. 2007; Cipollini and Lieurance 2012). *Alliaria petiolata* contains a broad array of secondary metabolites that putatively affect soil microbial communities (Cipollini 2002; Cipollini et al. 2005; Callaway et al. 2008; Bressan et al. 2009; Lankau 2011) and likely play a role in defence against herbivores and pathogens (Haribal and Renwick 1998, 2001; Haribal et al. 1999; Renwick et al. 2001; Kumarasamy et al. 2004; Cipollini and Gruner 2007). In connection with field surveys and further genetic studies, this body of research provides an excellent basis for characterizing individual-level variation in secondary chemicals, and potentially for linking this variation to ecosystem-level processes.

Simple lifetime fitness estimate

Alliaria petiolata is a biennial monocarpic species. Seeds germinate in spring, overwinter as rosettes, flower the following spring, and produce fruits in early summer (Cavers et al. 1979). Sampling populations in the summer therefore allows for simultaneous

Figure 1. Diagnostic characteristics of *Alliaria petiolata.* **A** populations **B** rosettes **C** bolting inflorescences and **D** individual flowers and developing siliques.

measurements of reproductive output of individual plants as well as population demographic structure (i.e. first-year- vs. second-year plants). Reproductive stems reach approximately 1 m in height and typically produce 10–25 siliques, each containing 10–20 seeds produced primarily through self-pollination. This relatively simple life cycle means that total lifetime reproductive success can be measured in the field and in greenhouse or growth chamber experiments.

Easily identified

Alliaria petiolata is the sole member of its genus found in Europe and North America (Cavers et al. 1979). Both juvenile and adult plants are very distinct from naturally co-occurring plants. Adult plants are easily recognized by their characteristic inflorescences in late spring (Figure 1A). First year rosettes vary in size (2–20 cm), and usually have 5–10 toothed leaves (Figure 1A) before developing inflorescences and siliques (Figure 1C). Leaves on mature plants vary from deltoid at the base to lanceolate toward the apex (Figure 1D). Flowers are 6–7 mm in diameter, each with four white petals (Figure 1D).

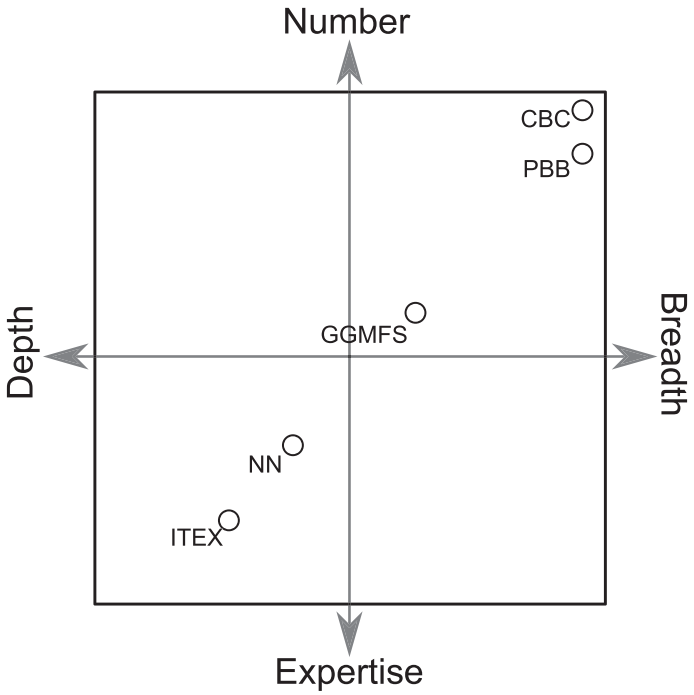

Figure 2. Schematic showing two hypothetical trade-off axes: **1.** (x-axis) Sampling intensity: depth of data collection (e.g. number of measurements per site) increases towards the left while the breadth of coverage (e.g. number of sites) increases towards the right; **2.** (y-axis) Participant sophistication: number of participants increases towards the top while the average level of expertise per participant increases toward the bottom. Approximate position of the Global Garlic Mustard Field Survey (GGMFS) is shown in relation to other large collaborations in ecology: Nutrient Network (NN); National Ecological Observatory Network (NEON); International Tundra Experiment (ITEX); and the Alpine Stress Gradient Project (ASG), and to citizen science projects: Christmas Bird Count (CBC) and Project Budburst (PBB).

Genetically tractable

The Brassicaceae is a genetically well-studied family of angiosperm. It includes many economically important horticultural and crops species and the model species *Arabidopsis thaliana,* which diverged from a common ancestor with *Alliaria petiolata* only about 20 million years ago (Koch et al. 2000). As a result of recent divergence, the two species share 98.3% of 1383 base pairs in the large subunit of ribulose-1,5-bisphosphate carboxylase (rbcL) (GENBANK accessions JQ933212.1 and AP000423.1). This genetic similarity is useful for annotating sequencing data and identifying candidate genes underlying phenotypic traits using common genomic tools. While metabolism and expression of defensive chemicals have not been well characterized in *A. petiolata*, related compounds have been extensively studied in many Brassicaceae species, and in particular the core biosynthetic pathway for glucosinolate production has been almost completely mapped in *A. thaliana* (Halkier and Gershenzon 2006). Identification of candidate genes and comparative genomics is quickly becoming eco-

nomically feasible for genetic studies of non-model organisms as sequencing costs have declined roughly 100,000-fold over the last decade (Lander 2011). This will be of particular value for genomic studies of *A. petiolata* because it is an autotetraploid with a relatively large genome (C-value 1.95) – about 14× that of *A. thaliana* (Bennett 1972).

Field survey

We established the GGMFS in 2009 to measure population size (i.e. area of coverage), density and age structure (i.e. proportion of first-year rosettes vs. second-year reproductive adults), as well as size, reproduction, herbivory and fungal damage of individual plants, plus some important environmental variables such as habitat type and canopy cover. The current full protocol and data collection sheet are available at http://www.garlicmustard.org, archived on FigShare (doi: 10.6084/m9.figshare.729274), and are described briefly below.

Setup and site choice

Contributors to the GGMFS are expected to sample populations in the late spring or early summer after >95% of plants have finished flowering. The specific date varies by climate because sampling is intended to standardize phenology across populations and to collect mature seeds for future experiments. We generally encourage participants to include one site with low light (e.g. a forest interior location), and one with high light (e.g. forest edge or roadside). However, to limit selection bias, any site containing 20 or more *A. petiolata* plants can be included in the study.

Data collection, curation and quality control

Measurements begin by pacing out the approximate area of the population of A. petiolata (length × width). The participants then lay out a 10 m transect to measure plants from one edge of the population moving toward the centre. In each of 10 adjacent 1 × 0.5 m plots, all juvenile rosettes and adult plants are counted. The size of the nearest juvenile and adult plant, as well as the height, leaf number and fecundity (number of fruits) of the adult are measured at five intervals, 20 cm apart, along each plot. On the adult plants, participants also count both the number of undamaged leaves and the number of leaves with >10% herbivore damage. In 2011, we added a simple measurement for pathogen damage, by noting both the total number of plants in each plot that exhibited signs of leaf pathogens as well as the number of damaged leaves. To estimate % canopy cover at each site, photos are taken of the forest canopy at three points across the sampled transect and a visual estimate of average cover is recorded. We use digital

camera photos for these estimates because they are common equipment whereas a fish-eye lens is a more accurate but specialized piece of equipment that many people may not have access to. The participants also note whether there is any information of past or ongoing disturbance or control efforts applied to the population they are sampling.

After measurements are recorded, participants harvest inflorescences from the first 20 adult plants in each population. These collections are dried for at least two weeks and then mailed along with the original data sheets and canopy photos. Seed collections and photos of sampled populations provide confirmation that the correct species was sampled, which is of concern mainly for private citizen contributions. Upon receiving these collections we visually inspect the online and hand-written data for potential typographical errors, and we clean and store all seed collections at 5°C. We use ImageJ (Abramoff et al. 2004) to estimate canopy cover from canopy photos, and we compile approximate bioclimatic variables for each sampled location from the WorldClim database (http://www.WorldClim.org) (Hijmans et al. 2005). We use Google Earth (Version 5.0) to confirm population locations and habitat information. With precise GPS locations, Google Earth images are of high enough resolution to verify key characteristics entered as site descriptions, such as altitude and habitat type. Finally, we run a series of statistical tests for typos and outliers and organize all text into a single data file using *R* (R Core Team 2012).

Participation and current extent of sampling

Across four field seasons (2009–2012), 164 participants in 16 countries across North America and Europe (Figure 3) collected data and sampled seeds from 383 field sites (Figure 4). These participants included many academic scientists – faculty, postdocs, and graduate students – but also weed managers, conservation groups, and citizen scientists. Sampling intensity was strongly skewed as most contributors sampled a small number of populations, but a few individuals sampled many sites (Figure 3A). Interestingly, participation at each site was generally all-or-nothing; only five entries were excluded due to incomplete data. Sampling began in 2009 but was most heavily promoted in 2010 and 2011 through direct invitation of individual scientists, as well as general announcements on listserv (e.g. ECOLOG-L, EvolDir), and citizen science channels. After the 2011 season, participants were no longer directly solicited, and participation dropped to 19 populations in 2012. The total field data contributed thus far includes 383 populations concentrated in the northeast U.S. and central Europe (Figure 4). These samples represent a fair proportion of North American and European distribution of *A. petiolata* (Welk et al. 2002). Over 5,000 seed families have been collected from these populations and are currently being evaluated for viability and subsequently grown for seed production. Collectively, participants, counted 137,811 plants and recorded 47,514 individual measurements.

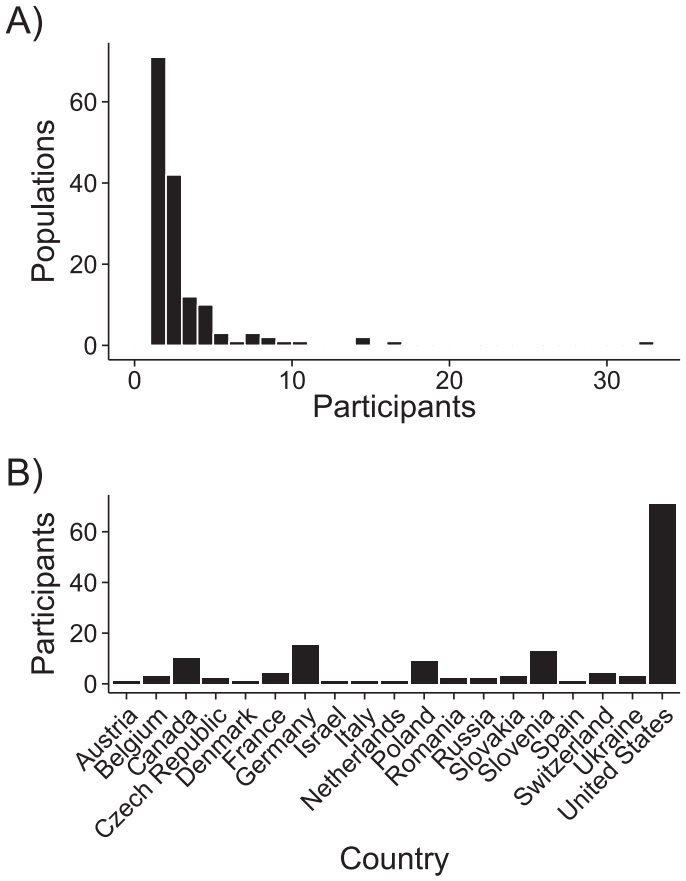

Figure 3. Frequency histograms of **A** sampling effort by each participant, and **B** number of participants per country. Note that "participant" in this case is either an individual or a group of people sampling together.

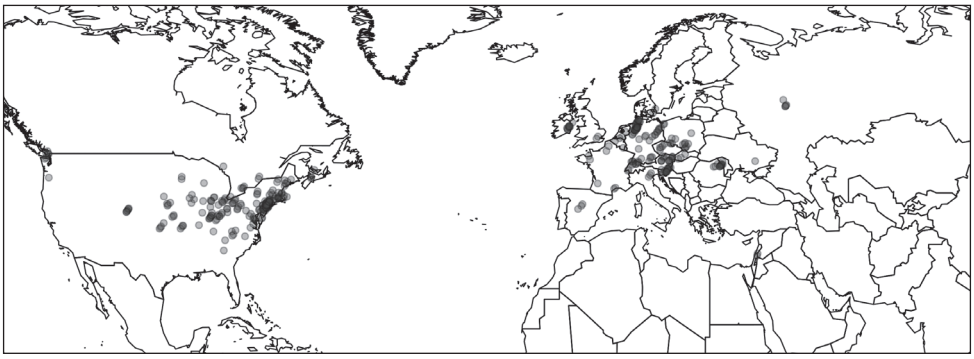

Figure 4. Map showing 383 sample locations from 2009–2012 inclusive, representing both the native (Europe) and introduced (North America) ranges. Dots are translucent resulting in darker areas that indicate regions with higher sampling intensities.

Future directions

Undergraduate engagement

The GGMFS protocol has been incorporated into some undergraduate-level field courses, particularly at universities focused on undergraduate teaching and educating minorities. These faculty positions are typically associated with high teaching loads and low research budgets, which limit research output relative to faculty at major universities. At the same time, these schools tend to have smaller class sizes and more involved undergraduate students. Large collaborative networks like the GGMFS allow faculty with limited time and financial resources to collaborate within a global community of researchers and play a key role in collecting and analysing valuable scientific data (Bowne et al. 2011).

In addition to involving faculty in research, the GGMFS data and protocol introduce students to research and provide excellent opportunities for project-oriented undergraduate teaching. Active learning exercises, in which students engage directly in laboratory or field demonstrations, are more effective than class lectures and other passive modes of teaching (National Academies Press 2005; Michael 2006). We see two main avenues for teaching: (i) Students can use the formal GGMFS protocol to collect data in their area, and then analyse and discuss these data in comparison with other populations from the GGMFS. (ii) Students can incorporate climate data and satellite images of GGMFS sample sites to learn techniques and address fundamental questions in invasion biology, plant ecology and general biology. There are many more options for building elements of the GGMFS into future curricula, e.g. laboratory studies using seeds from the seed bank, which connect molecular or quantitative genetic information with the large amount of field and environmental information available for each of the seed origins.

Open science

One of the guiding principles of the GGMFS is the inherent value to society of completely open and accessible data, analyses, germplasm and any additional resources arising from this project. Creating these resources is also a crucial step for cultivating a diverse community of biologists with a variety of expertise but a common goal of understanding the biological mechanisms underlying the invasion success of an ecologically important invasive species. We plan to eventually release all data collected for the GGMFS, along with relevant analyses needed to replicate any results published in peer reviewed journals. These will be available to the scientific community with the expectation that future analyses using the data will also be made equally open-access. The release of the field data will likely occur in a series of stages following publication, with a sequester period of 1–2 years to facilitate novel analyses among project participants before releasing data to the scientific community.

Seed propagation and archiving

Seeds collected as part of the field survey will form the basis of inbred lines for laboratory demonstrations and for future research. Seed collections are stored under cool-dry conditions in three locations – University of Tübingen, Germany, Fordham University in New York, USA, and the University of British Columbia in Vancouver, Canada. To reduce the potential for maternal effects and to produce enough seeds for future experiments, we are currently propagating all viable seed families in an outdoor common garden in Tübingen.

Future projects

In addition to collecting valuable data and seed resources, the GGMFS has brought together a global network of scientists who posess a range of expertise and a unifying interest in understanding biological invasions. To build on the work described above, we propose to expand this network and to consider additional studies that complement the GGMFS data and address difficult but important questions about the ecology and evolution of invasive species. Anyone interested in participating should contact the lead authors of this paper or the project coordinators listed on the GGMFS website (http://www.garlicmustard.org). We have identified three projects in particular that build on the strengths of the GGMFS model:

(i) Temporal sampling: More detailed demographic measurements, and long-term sampling of the same populations across multiple years would help improve understanding of invasion dynamics at several nested spatial scales.

(ii) Additional invasive species: Replicating the GGMFS approach with other invasive species, including those with different life-history strategies and extent of invasiveness, would allow for a more general test of the increased vigour assumption and testing hypotheses of invasion success in other species. In addition to all of the benefits described above for *A. petiolata*, field surveys from multiple species will help to identify generalities in the relative importance of different ecological and evolutionary processes to invasion success.

(iii) Large-scale reciprocal transplant experiments: Reciprocal transplant experiments have a long history in plant biology but have rarely been used to study invasive species. Utilizing the GGMFS network and additional collaborators, a large transcontinental reciprocal transplant experiment across dozens of sites in North America and Europe and using a shared subset of GGFMS seed families would be particularly useful to test for genetic differences between, and local adaptation of, native and introduced populations.

Conclusions

Large collaborations are transforming many areas of science, but ecological and evolutionary studies of invasive species have spread limited resources across a broad range of

study systems and geographic locations. Focusing studies on a few model systems can help to comprehensively address the fundamental question of what determines invader success, and to evaluate different mechanisms of invasiveness. The Global Garlic Mustard Field Survey (GGMFS) is a step toward this more integrated approach to invasion biology; it provides much-needed comprehensive data on the performance of natural populations of an invasive species across its native and introduced ranges. Large field surveys can provide an important link from experimental results observed at local sites on a subset of populations to biogeographical patterns of invasion success occurring at continental and global scales.

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RESEARCH ARTICLE

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Colonization of Brazil by the cattle egret (*Bubulcus ibis*) revealed by mitochondrial DNA

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Abstract

The cattle egret (*Bubulcus ibis*) has recently colonized Brazil. This process offers an excellent opportunity for the study of colonization and dispersal patterns across extensive areas by non-native birds. The aims of the present investigation were a) to determine the genetic diversity of the cattle egret in Brazil and Africa, b) evaluate genetic differentiation between populations in different regions of Brazil and Africa, and c) detect genetic signs of demographic expansion in these two areas. Mitochondrial DNA (mtDNA) Control Region (CR) sequences were obtained from 112 cattle egrets in four Brazilian and four African (Kenya, Ghana and Nigeria) populations. Genetic diversity (H, *h*, θ_s) and population structure (AMOVA, F*st*) were assessed and the populations were tested for signs of recent demographic expansion. A total of 35 haplotypes were found: 22 exclusive to Africa, 10 exclusive to Brazil and three shared by both samples. The degree of genetic diversity, determined by mtDNA analysis, was similar between Brazil and Africa, demonstrating that the successful colonization of the non-native area occurred with no significant loss of diversity. The pairwise F*st* values among the Brazilian and African populations were all significantly different. The population in southern Brazilian exhibited the lowest degree of differentiation with respect to the African population, followed by the southeastern and northeastern populations of the country. The genetic differentiation data suggest that the colonization of Brazil by the cattle egret began in the southern region and expanded to the southeastern and northeastern regions of the country. This genetic differentiation pattern is in accordance with the higher number of cattle per grazing area in southern Brazil, which may have favored the onset of the successful establishment of the species. The findings indicate that mtDNA genetic diversity was retained during the colonization process and colonization began in the southern region of the country. Moreover, signs of demographic expansion were detected in the African sample.

Keywords
Cattle egret, colonization, control region, dispersal, diversity

Introduction

The cattle egret (*Bubulcus ibis* Linnaeus, 1758) primarily inhabits grassland habitats and forages in close association with grazing animals, such as cattle and other livestock, and it is classified in three subspecies. The subspecies *B. ibis* ssp. is native to tropical and subtropical Africa, southern Europe and western Asia (Brown et al. 1982). A number of bird species have been introduced to non-native areas through human intervention. However, the cattle egret is known to have established and expanded to the Americas without such intervention (Telfair 1983). This bird is considered an invasive species according to the International Union for Conservation of Nature's Invasive Species Specialist Group (ISSG) and is also listed among the invasive alien vertebrate species in the Galapagos Islands (Phillips et al. 2012). Although many details of its expansion are unknown, cattle egret populations from West Africa or southern Europe are thought to be the origin of populations in the Americas (Telfair 1983, Valverde 2003). Egrets that probably crossed the Atlantic Ocean were first recorded in coastal areas of northern South America (Palmer 1962, Wetmore 1963). The first sightings in the New World were reported for Suriname (Dutch Guiana, Palmer 1962) between 1877 and 1882, followed by sightings in British Guiana and Colombia (Wetmore 1963) and subsequent expansion throughout the Americas. In Brazil, the cattle egret was first recorded in the northern region of the country in 1964, feeding along with buffalos on Marajó Island in the state of Pará (Sick 1965). By 1973, its occurrence was reported in the state of Rio Grande do Sul (southern Brazil) (Belton 1974).

By approximately 1900, the cattle egret had also expanded its range in Africa (Telfair 1983). The species was first recorded in the Cape Peninsula of South Africa in 1867 (Skead 1952, Siegfried 1965). According to Chapin (1932), between 1920 and 1930, the breeding range of the cattle egret was restricted to the extreme north of Africa on the Mediterranean coast and a narrow sub-Saharan west-to-east corridor, descending through a broad area on the eastern coast, including Kenya, South Africa, Madagascar and the Comoro islands.

Novel colonizers can cause problems outside of their native range. While the cattle egret is not currently a threat to native fauna in Brazil throughout most of its geographic distribution, it has the potential to produce adverse effects, as evidenced by its occupation of island environments. For example, in the Fernando de Noronha archipelago, the cattle egret drives adult native seabirds from their nests in breeding colonies (Barbosa-Filho et al. 2009) and predates the Noronha skink (*Euprepis atlanticus*), which is endemic to the archipelago (Silva-Jr. et al. 2004). Similar behavior is seen in Hawaii, where predation by cattle egrets on the chicks of native waterbirds, such as the black-necked stilt (*Himantopus mexicanus*), has been reported (Stone and Anderson 1988).

Successful establishment in a new area by a novel colonizing species is determined by several factors, such as reproductive capacity, growth rate and suitable environmental features (Blackburn et al. 2009). When the invasive population grows exponentially in the non-native area and the landscape is uniform, the diffusion of individuals across the newly occupied area is random and the colonization front is expected to advance at a constant spread rate (Skellam 1951). This dispersion pattern is not expected for the cattle egret in Brazil due to the lack of uniformity in the landscape. During colonization, the habitat match is an important factor to consider because it can determine the selection of areas to be occupied by invasive species (Hayes and Barry 2008). The state of Rio Grande do Sul in southern Brazil is the only region of the country with extensive areas of natural grasslands within the "Pampas" biome (taken from MMA) and had the largest bovine breeding activities in the 1970s (census data; IBGE 1975), which are similar to conditions found for the cattle egret in its native range.

Genetic tools can provide data that assist in clarifying the process of colonization of non-native areas by alien species. Mitochondrial DNA (mtDNA) has been used in many studies on invasive species to identify historical introduction pathways or determine likely sources of introduced populations (e.g., Corin et al. 2007, Hoos et al. 2010). Rollins et al. (2011) clarified the invasion pathways of the common starling (*Sturnus vulgaris*) in Western Australia based on mtDNA data. According to the authors, mtDNA markers are useful for tracking dispersal in populations and can help predict future population expansions of an alien species. The evaluation of diversity patterns using neutral molecular markers is helpful to the investigation of species invasion (Yonekura et al. 2007). Genetic comparisons between populations in native and non-native areas may indicate different distribution patterns of genetic diversity that are interpreted as consequences of distinct phenomena (Allendorf and Lundquist 2003). A significant reduction in genetic variability in populations that have occupied non-native areas would be interpreted as a consequence of the founder effect, while a more diverse population in a non-native area than in the source population would be the result of an admixture of differentiated source populations (Kolbe et al. 2004, Lockwood et al. 2005, Colautti et al. 2006). Reductions in genetic variability in non-native vs. native ranges have been recorded for other bird species (Hawley et al. 2006, Rollins et al. 2011). The opposite pattern has also been described (Beneteau et al. 2012). Similar levels of genetic diversity in populations from non-native and native areas can be found when the entrance of a high number of founders occurred during a recent colonization or as the result of continuous migration between the source and colonized populations.

The aim of the present study was to investigate the colonization of Brazil by the cattle egret through the use of mtDNA Control Region (CR) sequences. Inferences are based on four Brazilian samples from different latitudes and four African samples in a more limited geographic area. The cattle egret has considerable dispersal potential and low fidelity to breeding sites (Telfair 1983), which are characteristics that favor a lack of structuring among populations. Based on historic records and background knowledge, three hypotheses are put forth: 1) supposing cattle egret populations are

not genetically differentiated in their native area, we hypothesize that genetic diversity in the four Brazilian populations will be lower or comparable to that found in the African population; 2) colonization of Brazil was directed by habitat match, with the cattle egret drawn to the southern region of the country due to the more frequent occurrence of cattle raising and extensive pasture areas, despite the fact that the entrance of the species in South America occurred in the northern region; and 3) genetic signs of demographic expansion could be detected in both Africa and Brazil populations, but genetic evidence of this process may not be detected due to the fact that range expansion occurred in recent times, especially in Brazil.

Methods

Sampling, DNA extraction and sequencing

Two types of biological samples were obtained for the study: blood samples were collected from nestlings in breeding colonies and molted feathers were collected beneath nests in breeding colonies. All sampling sites were reproductive colonies, except the Kankun National Park in Ghana, which is a roosting site. Blood samples (one per nest) were collected from each colony, pooled and each group was considered a single population, i.e., a group of individuals resulting from random interbreeding. The Brazilian samples (N = 51) consisted only of blood samples collected from breeding colonies and all African samples (N = 61) consisted of molted feathers (see Table S1 in Appendix for the complete list of locations sampled). Only feathers in good condition and with no signs of physical degradation were analyzed (Hogan et al. 2008). To avoid the collection of more than one feather from the same individual, only feathers separated by more than 3 m from each other were collected and no genotype was found twice among the feathers analyzed, except for Hap 14. DNA sequences obtained from blood and feathers had similar Phred quality scores. Total genomic DNA was extracted from blood samples following proteinase K digestion and using a phenol/chloroform/isoamylic alcohol protocol (Sambrook and Russell 2001). DNA was extracted from feather samples using the protocol described by Miño and Del Lama (2009), without the addition of SDS.

A fragment of 430 bp of the domain I of the CR mtDNA was amplified for 112 cattle egret samples using primers developed in the present study: Ardea L3 (5'-CAC CTA ACA CAA AAC ACA AAC-3') and BiDIH4 (5'-CTT CAG ATA CCG GTA CTT C-3'). Polymerase chain reactions were conducted in a total volume of 12.5 μL containing 10 ng of template DNA, PCR buffer containing 10 mM Tris-HCl, 50 mM KCl, 20 mM $((NH_4)_2SO_4$ and 2mM $MgCl_2$ (pH 9.0), 0.25 μM of each dNTP, 0.10 μM of each primer and 1 U of Taq DNA Polymerase (Biotools B&M Labs, S.A.). The cycling conditions were follows: denaturation for 5 min at 94°C; 5 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 1 min; 20 cycles of 94°C for 30 s, 58°C for 30 s, decreasing 0.1 °C per cycle; 10 cycles of 94°C for 30 s, 45°C for 30 s and 72°C for 1 min; and a final extension at 72°C for 10 min. The amplified fragments were sequenced in

an ABI Prism 3730 sequencer (Applied Biosystems Inc., California, USA). Sequences were initially aligned with CLUSTALW (Larkin et al. 2007). Alignments were verified by eye and trimmed using the BIOEDIT v7.0 (Hall 1999) software program.

Statistical analyses

Genetic diversity was examined by calculating the number of haplotypes (H), haplotype diversity (h) and molecular diversity (θ). Genetic diversity estimates were calculated using the ARLEQUIN program v3.5 (Excoffier and Lischer 2010). The molecular diversity estimator theta (θ) is equal to 2 Mμ, in which M is the population size of the haploid populations and μ is the overall mutation rate. The overall mutation rate can be estimated using different approaches that employ nucleotide diversity (θ_π), the number of polymorphic sites (θ_s) or the number of haplotypes (θ_k). Comparisons of the diversity among the African and Brazilian populations were based on θ_s, since the θ_π estimate reflects demographic changes in the more distant past and the interest of the present study was in changes over very recent time spans (approximately 100 generations), as proposed by Rollins et al. (2011). Both θ_s and θ_k are sensitive to recent demographic history and depend on assumptions of selective neutrality, constant population size and adherence to the appropriate mutational model ("infinite sites" model for θ_s and "infinite alleles" model for θ_k). The decision was made to use θ_s due to the fact that the "infinite sites" model of mutation is normally applied to DNA sequence data (Tajima 1996). The population structure was analyzed using analysis of molecular variance (AMOVA) and pairwise F*st* values were calculated using the ARLEQUIN program v3.5 (Excoffier and Lischer 2010).

Relationships among haplotypes were inferred through the construction of a statistical parsimony network by the TCS v 1.21 program (Clement et al. 2000). Ambiguous connections in the statistical parsimony network (loops) were solved using a hierarchical set of guidelines based on coalescent criteria (Crandall and Templeton 1993). The jMODELTEST v 0.1.1 (Posada 2008) program was used to select the best-fitting nucleotide substitution model from among three substitution-type models, using both the Akaike information criterion (AIC) (Akaike 1974) and the Bayesian information criterion (Schwarz 1978).

Deviation from selective neutrality was tested using Fu's Fs (Fu 1997) and Tajima's D (Tajima 1989) tests. Fu and Li's D^* and F^* statistics (Fu and Li 1993) and the *R2* statistic (Ramos-Onsins and Rozas 2002) were also computed to test for deviation from selective neutrality using the DNASP v5 program (Librado and Rozas 2009). The distribution of pairwise nucleotide differences (mismatch distribution) was calculated as an additional test for demographic expansion (Rogers and Harpending 1992), also using the DNASP program. To test whether the observed distributions significantly deviated from those expected under the population expansion model, the sum of square deviations (SSD) value was calculated using the ARLEQUIN v3.5 program (Excoffier and Lischer 2010).

Results

The number of haplotypes was higher in Africa (H = 25) than Brazil (H = 13). Table 1 displays the complete list of haplotypes and frequencies in the populations. Haplotype diversity (*h*) (mean ± SD) was 0.85 ± 0.04 in Africa and 0.90 ± 0.02 in Brazil. Molecular diversity based on the number of polymorphic sites (θ_s) was 3.33 ± 1.17 in Africa and 3.11 ± 1.17 in Brazil. The African and Brazilian populations exhibited 16 and 14 polymorphic sites, respectively.

Table 1. Number of domain I Control Region mtDNA haplotypes evidenced by distinct sequences in cattle egret (*Bubulcus ibis*) in two areas: A = Africa; B = Brazil.

S / H	47	69	74	114	117	148	215	242	278	288	290	294	296	316	325	373	375	378	403	413	B	A
Hap1	T	G	G	C	G	A	T	C	T	C	C	A	C	G	C	G	C	C	A	T	8	4
Hap2	.	.	.	.	.	.	C	T	C	.	.	.	T	.	.	.	T	T	.	.	11	-
Hap3	.	.	.	.	.	.	.	T	C	.	.	.	T	.	.	.	T	.	.	.	4	7
Hap4	.	.	.	.	.	.	.	T	.	.	T	.	T	.	.	.	T	.	.	.	4	-
Hap5	.	.	.	.	.	.	.	T	C	.	.	.	T	A	.	.	T	.	.	.	6	-
Hap6	.	A	.	.	.	G	.	.	C	.	.	.	T	.	.	.	T	.	.	.	3	-
Hap7	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	4	2
Hap8	.	.	.	.	.	.	.	.	.	T	T	.	.	.	.	.	T	.	.	.	2	-
Hap9	.	.	.	.	.	.	.	T	C	.	.	G	T	.	.	.	T	.	.	.	3	-
Hap10	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	.	C	.	1	-
Hap11	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	.	.	.	3	-
Hap12	.	.	.	.	.	.	.	.	C	.	.	G	T	.	.	.	T	.	.	.	1	-
Hap13	A	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.		.	.	.	1	-
Hap14	.	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	T	.	.	.	-	26
Hap15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	-	2
Hap16	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.		.	.	.	-	2
Hap17	.	.	.	.	.	.	.	T	C	T	.	.	T	.	.	.	T	.	.	.	-	1
Hap18	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	.	.	.	-	2
Hap19	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	A	T	.	.	.	-	1
Hap20	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	T	.	.	.	-	2
Hap21	.	.	.	.	A	.	.	.	C	.	.	.	T	.	.	.	T	.	.	.	-	2
Hap22	.	A	.	.	.	.	.	.	C	.	.	.	T	.	.	.	T	.	.	.	-	1
Hap23	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	.	T	.	.	.	-	1
Hap24	.	.	A	.	.	.	.	T	C	.	.	.	T	.	.	.	T	T	.	.	-	1
Hap25	.	.	.	.	.	.	.	.	C	.	T	.	T	.	.	.	T	.	.	.	-	2
Hap26	.	.	.	T	.	.	.	T	C	T	.	.	T	.	.	.	T	.	.	.	-	1
Hap27	.	.	.	.	.	.	.	.	C	.	.	.	T	.	T	.	T	T	.	.	-	1
Hap28	.	.	.	.	.	.	.	.	C	T	.	.	T	.	.	.	T	.	.	.	-	2
Hap29	.	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	T	T	.	.	-	2
Hap30	.	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	-	2
Hap31	.	.	A	.	.	.	.	.	C	.	.	.	T	A	.	.	T	T	.	.	-	1
Hap32	.	.	.	.	.	.	.	T	C	.	.	.	T	.	.	.	.	.	.	.	-	1
Hap33	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	T	.	.	-	1
Hap34	.	.	.	.	.	.	.	T	C	.	.	.	T	.	.	.	T	.	.	C	-	1
Hap35	.	.	.	.	.	.	.	T	C	.	.	G	T	.	.	.	T	.	.	.	-	1

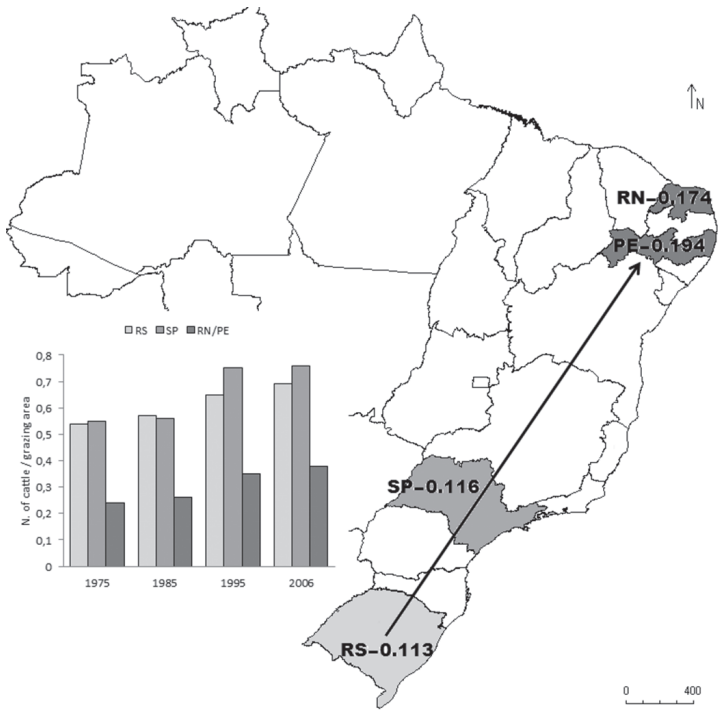

Figure 1. Map of Brazil showing states were cattle egret (*Bubulcus ibis*) populations were sampled. Levels of differentiation (F*st* values) between each Brazilian population and the total African population are shown. Arrow indicates probable path of colonization. Histogram shows the number of cattle per grazing area in four periods (10 years apart) in the states sampled.

AMOVA revealed that 8.73% of the detected variation was explained by differences between the two populations (African and Brazilian) and the remaining 91.27% was explained by individual differences within populations. The number of haplotypes found in the four Brazilian regions ranged from eight to nine, with no descending cline from the location of the first record of the cattle egret (northern Brazil) to the most distant population sampled (southern Brazil). The pairwise F*st* value between the Brazilian and African populations was 0.10. As this value was statistically significant ($P < 0.05$), pairwise F*st* values were calculated among all eight sampling sites (data not shown). Pairwise F*st* values involving only Brazilian pairs or African pairs of populations were non-significant, demonstrating no sign of population structuring within each region. The four Brazilian populations were also compared against the entire African population, considering Africa to be one of the probable sources of the Brazilian population, and possible differences in the degree of differentiation between the different Brazilian populations and the entire African population were tested. As expected, significant F*st* values were found for all paired comparisons ($P < 0.05$). The lowest degree of differentiation in relation to the entire African population was found in the southern region of Brazil (state of Rio Grande do Sul [RS]), followed by the southeastern region (state of São Paulo [SP]), and the most differentiated region was northeastern Brazil (states of Rio Grande do Norte [RN] and Pernambuco [PE]) (Fig. 1).

All private Brazilian haplotypes and some of the African haplotypes are located on branch tips in the haplotype network, which includes only three haplotypes shared by both continents (HAP-1, HAP-3 and HAP-7) (Fig. 2). HAP-1 is shared among all Brazilian populations and Ghana. HAP-3 is shared among all African populations and three Brazilian populations (RN, SP and RS). HAP-7 is shared among three Brazilian populations (RN, PE and RS) and Ghana.

Neutrality tests were performed assuming that the domain I sequence of the mtDNA is not under selection. Deviations from neutrality in these tests were considered to be indicative of demographic expansion (Table 2). Fu's *Fs* and *R2* tests revealed significant genetic signs of demographic expansion for the African population. The observed mismatch distribution curves displayed a unimodal shape for African and Brazilian populations and the SSD values were non-significant, indicating that the observed curves did not differ significantly from the population expansion model.

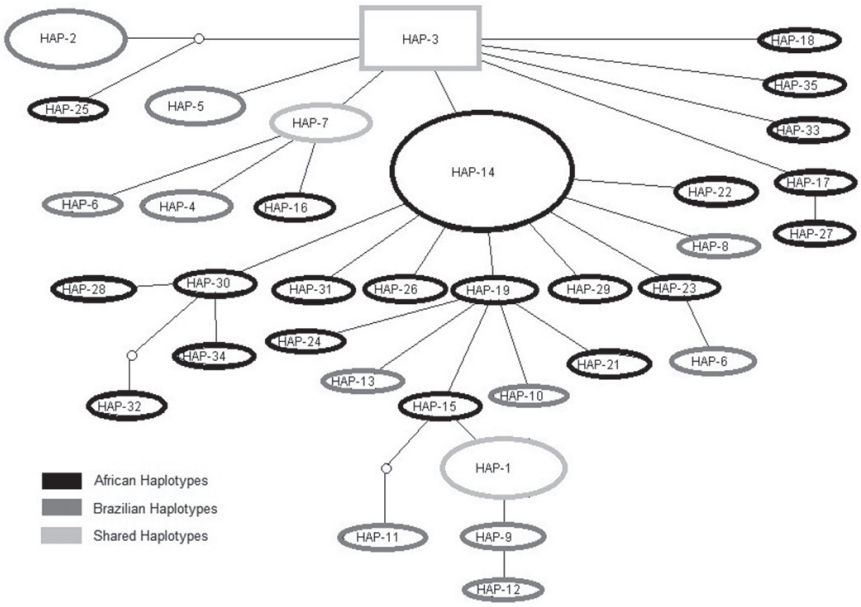

Figure 2. Haplotype network and relationships among 35 haplotypes of the domain I CR mtDNA region found in cattle egret (*Bubulcus ibis*) samples from Brazil and Africa. Areas of shapes are proportional to number of individuals sharing each haplotype.

Table 2. Results of neutrality tests performed for Brazilian and African populations of cattle egret (*Bubulcus ibis*).

Population	Tajima's D	Fu and Li's *D**	Fu and Li's *F**	Fu's Fs	*R2*
Brazil	0.45	0.57	0.63	-1.50	0.10
Africa	-1.28	-1.25	-1.50	-22.25[#]	0.10[#]

[#] significant values

Discussion

Understanding the colonization of the Americas by the cattle egret is a challenging task due to the lack of sufficient information and reports on entrance time, locality, number of events, and propagule pressure. Comparisons between native and non-native populations can provide a 'natural' experimental approach to clarify the biological and environmental factors that may contribute to range expansion and/or adaptation to climate change, and to reveal mechanisms by which organisms respond to novel ecological and environmental pressures (Schwartz et al. 2009, Neilson and Stepien 2011). The wide geographic distribution of the cattle egret requires exhaustive sampling of all occupied areas in the Americas for a complete study. However, since Brazil is located near the site of the first record of this species in South America and has huge dimensions, the present study involving Brazilian populations allows a first approach to this subject. For such, an analysis of domain I sequences was performed on mtDNA samples from both, Brazil and part of the native range in Africa, to determine the dispersal pattern of the cattle egret in Brazil. Previous studies on other organisms involving mtDNA data have been successful in providing evidence to support well-informed conclusions regarding invasion dynamics (Corin et al. 2007, Hoos et al. 2010, Rollins et al. 2011).

The present study is based solely on results from the analysis of mtDNA, which is maternally inherited, and included a limited sample from the African continent. This analysis revealed no decrease in the level of diversity in the Brazilian samples in relation to the African samples. Indeed, haplotype diversity (h) and molecular diversity (θ_s) were very similar between the African and Brazilian samples. According to Blackburn et al. (2009), there are more than 700 known non-native bird populations (representing more than 200 species) in non-native areas, but only 20 to 30 populations (representing seven species) have been surveyed for changes in genetic variability. The present results can be explained by multiple introductions or compared to reported cases in which no evident loss of variability was detected. For example, the saddleback (*Philesturnus carunculatus carunculatus*) has not exhibited a significant loss of variability after repeated population bottlenecks (Taylor and Jamieson 2008). According to Kekkonen et al. (2011), the house sparrow (*Passer domesticus*) has suffered a severe population decline in Finland over the last four decades, with no significant loss of genetic diversity. Since the details of initial colonization by the cattle egret are unknown and not all native areas were sampled, one can only affirm that the successful colonization of a non-native area occurred with no significant loss of genetic diversity through the evaluation of mtDNA.

The majority of mtDNA CR haplotypes were exclusive to either Brazil or Africa, with only three of the total 35 haplotypes shared between both areas. The admixture of Brazilian and African haplotypes could be due to retained ancestral polymorphism in these populations, which is expected for the recent colonization of a new area, such as in the case of the cattle egret in the Americas. The ancestral haplotype (HAP-3) was the fourth most frequent and it was found in both Brazil and Africa, as expected. The most common haplotypes in both the African and Brazilian populations were derived

by one or two mutational steps from this ancestral haplotype. It therefore seems that, among the haplotypes sampled, HAP-3 was the first-established lineage, giving rise to other haplotypes, which increased in number with the expansion of the species. All Brazilian haplotypes are on branch tips in the haplotype network. This pattern does not mean that they emerged in the history of Brazilian populations due to the lack of sufficient time for the appearance of new mutations, but suggests that these haplotypes emerged more recently in the evolutive history of these sequences. African haplotypes on branch tips reveal that the African populations sampled are in a geographic range where expansion on the native continent also occurred. The detection of shared haplotypes (among all Brazilian samples and mostly Ghana) and the fact that the ancestral haplotype is present in both regions support a common origin for the African and Brazilian populations sampled.

Genetic differentiation between populations from Brazil and Africa was evidenced by statistically significant pairwise F*st* values obtained for all paired comparisons in the two areas. Despite the fact that the African samples were not representative of all regions of the continent, the supposition was that the least differentiated population sampled in the non-native area in relation to the African samples would be more similar to the founder population. The results of the mtDNA analysis of genetic differentiation indicated that Brazil was first colonized in the southern region (RS population) (Figure 1). The first record of a breeding colony of cattle egrets in Brazil was on Marajó Island in the northern region of the country (Sick 1965), which was followed by several reports of the occurrence of the species in the southern region during the 1970s and a breeding colony in this same region in 1980 (Belton 1974). According to the supposition put forth here, although the species had arrived to the country through the north, it did not begin its expansion in this region of the country because the Brazilian landscape is not uniform. The cattle egret is capable of long-distance dispersal across unsuitable habitats and the amount of suitable habitats available in southern Brazil may have been the most important factor to determining its dispersal pattern. Thus, the reason for this order of occupation of non-native areas is the landscape similarity between native areas in Africa and non-native areas found in southern Brazil. Hayes and Barry (2008) showed that climate/habitat match and number of introduced organisms are consistently significant predictors of successful establishment across all of the biological groups in which they have been tested (birds, mammals and plants). The initial occupation of the state of Rio Grande do Sul (southern Brazil) is justified by the similarity between this region and the native African range of the cattle egret. As the cattle egret forages in close association with grazing animals, the large amount of cattle breeding activities and the presence of extensive areas of natural grasslands may also have influenced the establishment of this species in this state. Indeed, at the time of the establishment of the cattle egret in the southern region of Brazil, the states of Rio Grande do Sul and São Paulo (located in the southern and southeastern regions, respectively) had a higher number of cattle per grazing area (Fig. 1) (census data; IBGE 1975). Alternatively, one may suppose that entrance to the country also occurred in southern Brazil, which can be justified by the records

of occurrence (Belton 1974). After the occupation of this state, the species may have expanded its range in a northeasterly direction, occupying the southeastern and then northeastern regions of Brazil, where cattle numbers and the availability of pastures have grown over the past 40 years (Fig. 1).

All pairwise F*st* values between the Brazilian and African populations were statistically significant, as can be seen in Figure 1. This suggests a certain degree of constraint in gene flow. On the other hand, the partial admixture of Brazilian and African haplotypes detected in the haplotype network can be interpreted as a sign of gene flow between these two areas. The possibility of continuing migration to South America from different (non-sampled) regions of Africa cannot be ruled out, since a limited sample from Africa was included in this study. Valverde (2003) report a cattle egret banded in Spain and recovered in Central America in 1956, presumably following the proposed wind route that runs from southern Europe to the northern portion of South America. Cattle egrets have been also recorded on different South Atlantic islands, such as the St. Peter and St. Paul archipelago, Ascension, St. Helena and Tristan de Cunha (Telfair 1983). These facts and similar genetic diversity levels found in these two areas support continuous migration events from Africa to America favored by wind routes.

The present findings revealed no signs of demographic expansion in Brazil, as expected for a recent colonization process. However, *Fs* and *R2* values and the unimodal mismatch curves revealed significant genetic signs of demographic expansion in the African samples. According to Ramos-Onsins and Rozas (2002), *R*2 and Fu's *Fs* are the most powerful statistics for the detection of demographic expansion, while Tajima's *D* and Fu and Li's *F** and *D** have comparatively less power. Recent demographic expansion in Africa has been documented in the literature (Chapin 1932, Browder 1973, Telfair 1983). The cattle egret was originally restricted to tropical and subtropical regions of the African continent (Crosby 1972, Brown et al. 1982), but expanded to other regions. Between 1920 and 1930, cattle egret occurred without reproduction in the most of the Atlantic coast areas (including Ghana and Nigeria, which were sampled in the present study) and in the coastal portion part of central Africa, which was interpreted to mean that populations were temporarily occupying these areas. Currently, the species occurs and breeds throughout nearly the entire continent, except in the Sahara and Namib deserts (Browder 1973).

Conclusions

The cattle egret has retained most of the mtDNA genetic diversity during the colonization process in Brazil. Genetic signs of demographic expansion were detected in the African sample. The genetic differentiation analysis shows that this species began colonization in the southern region of the country and expanded in a northeasterly direction to the southeastern and northeastern regions. This dispersal pattern is supported by the environmental characteristics and larger amount of cattle raising and pasture areas in the south region in comparison to the two other regions sampled. Fu-

ture studies involving a greater number of samples in its native range and the inclusion of an analysis of nuclear genes will provide a more complete scenario of the proposed colonization process of the cattle egret in Brazil.

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Appendix

Table S1. Number of samples and locations in Brazil and Africa.

	Location name	Number of samples	Geographic coordinates
BRAZIL	Pernambuco state - PE	13	8°52'S, 36°28'W
	Rio Grande do Norte state - RN	12	5°37'S, 36°52'W
	São Paulo state - SP	13	22°30'S, 47°35'W
	Rio Grande do Sul state – RS	13	30°01'S, 51°31'W
AFRICA	Nigeria	11	9°58'N, 8°53'E
	Kenya	20	1°19'S, 36°51'E
	Kakun National Park - Ghana	15	05°32' N, 01°13W
	Korle Lagoon, Accra city - Ghana	15	09°05'N, 01°49W

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RESEARCH ARTICLE

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Wrack burial reduces germination and establishment of the invasive cordgrass *Spartina densiflora*

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Abstract

Germination and emergence of halophytes may decrease significantly by seed burial in dead plant material, or wrack, which is common and abundant in tidal marshes. The effects of plant debris (wrack) burial on seed germination and seedling establishment of *Spartina densiflora*, an invasive cordgrass, were studied under greenhouse conditions and compared with field observations. Five wrack burial depths were applied: control without wrack, 1 cm (1235 ± 92 g DW wrack m^{-2}), 2 cm (3266 ± 13 g DW m^{-2}), 4 cm (4213 ± 277 g DW m^{-2}), and 8 cm (6138 ± 227 g DW m^{-2}). Sediment pH, electrical conductivity, redox potential and temperature were recorded. Quiescence increased with wrack load up to ~20% at 8 cm deep. Germination decreased with wrack load from 96% to 14%, which could be related with anoxic conditions under the debris since sediment redox potential was as low as -83 ± 7 mV at 8 cm. Germination percentage increased and quiescent and dormant percentages decreased at higher daily sediment temperatures and with higher daily temperature fluctuations, conditions that were recorded without or under low loads of wrack. *Spartina densiflora* did not show primary dormancy, but its seeds entered into a non-deep physiological dormancy below 1 cm deep in plant debris. The establishment of *S. densiflora* seedlings was also greatly reduced by wrack burial since only 6 seedlings (11 ± 5 % of germinated seeds) emerged above plant debris from 1 cm and all seedlings died from deeper than 1 cm. *S. densiflora* seedling development was also reduced by wrack burial. The inverse relationship between germination and emergence of *S. densiflora* with wrack burial recorded in our study is useful to predict its invasion dynamics and to plan the management of invaded marshes.

Keywords
Coastal marshes, physiological dormancy, plant debris, seedling, *Spartina densiflora*

Introduction

Most salt marsh environments are unpredictable for plants, because of the occurrence of both salinity and flooding stress (Cantero et al. 1998). When conditions for seed germination are not favorable, ungerminated seeds of halophytes often remain under enforced dormancy in the soil and serve as a transient or persistent seed bank (Ungar 1995). Thus, seed banks are particularly important in maintaining populations of halophytes in saline marshes (Coteff and Van Auken 2006). Salt marshes are among the most heavily invaded systems in the world (Grosholz 2002). The ability of introduced species to bank seeds can contribute to invasion success, since seeds can persist while waiting for favorable conditions. This ability is especially useful in environments where opportunities for seed germination are infrequent or unpredictable such as salt marshes (Parker et al. 1989). Management of invasive species in salt marshes can be challenging because monitoring and control must continue for at least as long as their seeds persist in the seed bank (Panetta and Timmins 2004).

Rafts of dead plant material, or wrack, are common and abundant in tidal marshes. Due to high primary productivity rates and low consumption rates by herbivorous, halophytes, such as eelgrasses and cordgrasses, produce high quantities of wrack, especially in dye-back areas. This wrack is added up to that transported by rivers to salt marshes in estuaries. Then, tidal creeks provide corridors for wrack transport into salt marshes where it can disturb plant communities (Reidenbaugh and Banta 1980; Bertness and Ellison 1987; Valiela and Rietsma 1995; Tolley and Christian 1999; Brewer et al. 1998; Minchinton 2002). As part of the natural disturbance regime, deposited wrack mats often create bare spots by killing not only the surface vegetation, but also the below-ground biomass (Hartman 1988). Seeds of different salt marsh plants can germinate in the spaces opened by wrack accumulation, playing a key role in plant distribution (Hartman et al. 1983; Pennings and Richards 1998), but germination and emergence may decrease significantly by seed burial in plant debris. Buried seeds are recruited only when they are brought back to the surface by disturbances (Facelli and Pickett 1991). However, these effects are poorly understood since there is a general lack of research that examines the effects of wrack burial in salt marshes.

The austral cordgrass, *Spartina densiflora* Brongn. (Poaceae) is invading salt marshes in southern Europe, Northwest Africa and the West Coast of North America (Bortolus 2006). In invaded marshes, *S. densiflora* develops very dense populations where large amounts of dead matter are deposited (Nieva et al. 2001; Castillo et al. 2008). Moreover, *S. densiflora* seeds float so they are dispersed by currents and tides together with plant debris (Howard and Sytsma 2013). One of the keys to the success of *S. densiflora* invasion is its ability to produce large quantities of viable seeds (Nieva et al. 2001a,b).

Spartina densiflora produces many seedlings in intertidal mudflats but its recruitment is very low in marsh zones where wrack is accumulated.

While cordgrasses (*Spartina* genus) are one of the most abundant and frequent halophytes in salt marshes, there have not been any studies that examined how their seeds and seedlings respond to wrack burial. We examined under controlled greenhouse conditions the impact of five wrack burial depths (0, 1, 2, 4 and 8 cm) on germination, seed viability and seedling establishment of *S. densiflora*. Main abiotic factors conditioning seed germination and seedling establishment in salt marshes (sediment pH, electrical conductivity, redox potential and temperature) were also recorded for every wrack treatment. We hypothesized that *S. densiflora* germination and establishment would be reduced by wrack burial due to anoxia and/or low temperature fluctuations.

Methods

Seed and wrack collection

Spartina densiflora seeds were collected in August 2009 from multiple mature individuals chosen at random from a population growing at the periphery of an accreting, well-drained intertidal lagoon at Odiel Marshes (Southwest Iberian Peninsula; 37°08' - 37°20'N, 6°45' - 7°02'W; Fig. 1); see Castellanos et al. (1994) for a description of the site. Seeds were stored at 5 °C, under dry conditions and in darkness after harvest until the beginning of the experiment. Wrack was collected from the mean higher high water (MHHW) from the same marsh and it consisted of dead culms and leaves of different plant species (~50% of the debris was dead *S. densiflora*) together with pieces of shells (diameter < 1.0 cm). Collected wrack was checked for the presence of *S. densiflora* seeds and none was found, probably because seeds are dispersed before dead spiked shoots are transported by tides and currents.

Wrack treatments

A greenhouse experiment was conducted from December 2009 to February 2010, since most *S. densiflora* seeds germinate during winter, to test the effects of wrack burial on seed germination, and emergence and growth of seedlings of *S. densiflora*. The mean disseminule size of *S. densiflora* was 9.67 ± 0.15 mm by 1.47 ± 0.02 mm (n = 50) and its mean weight was 3.0 ± 0.1 mg (range: 1.3-4.2 mg). Mean *Spartina* caryopsis size was 4.68 ± 0.08 mm by 0.98 ± 0.02 mm (n = 50) and its weight was 2.0 ± 0.1 mg (range: 1.3–3.0 mg). Four replicates of 25 seeds were sown at 1 cm depth in clean sand in plastic containers measuring 18 cm width, 22 cm length and 11 cm height (containing ~1.6 kg of clean sand). The sand was collected from the same marsh where the seeds were obtained and it was sieved through 0.5-mm mesh size filling to eliminate pre-existing seeds and other plant material. A control treatment was set up without any

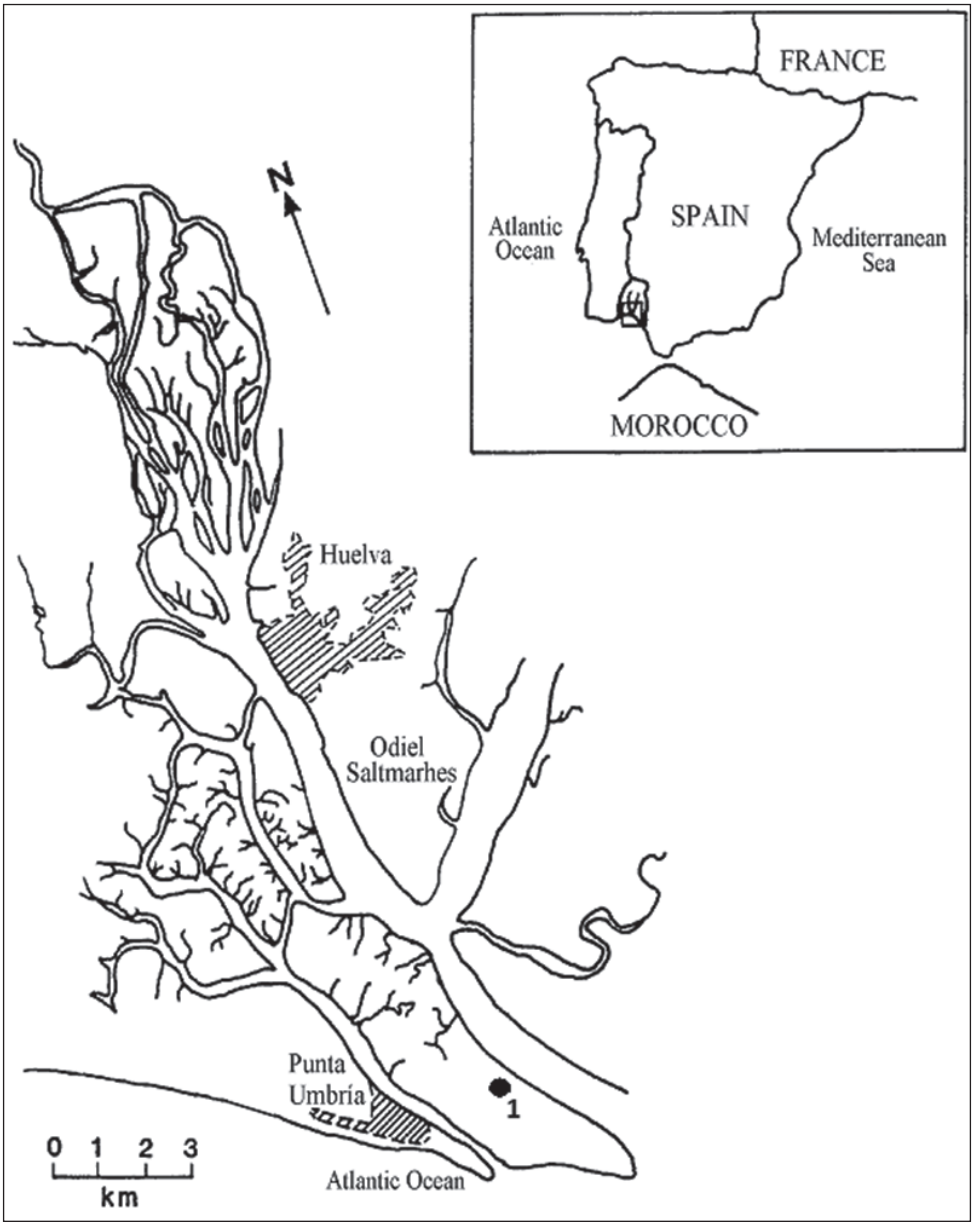

Figure 1. Location of the Odiel Marshes on the Atlantic coast of Southwest Iberian Peninsula (37°08'-37°20'N, 6°45'-7°02'W), and the sampling point where *Spartina densiflora* seeds were collected (1).

seeds added in order to test whether the sand we used contained seeds. Five wrack burial treatments were conducted: control (no wrack was added above the sand surface), 1 cm (1235 ± 92 g DW wrack m^{-2}), 2 cm (3266 ± 13 g DW m^{-2}), 4 cm (4213 ± 277 g DW m^{-2}), and 8 cm (6138 ± 227 g DW m^{-2}) of wrack burial depth. These treatments were decided following our field observations in Odiel Marshes (Southwest Iberian

Peninsula) where *S. densiflora* is very abundant (Nieva et al. 2001a). Each container had 25 seeds at one depth, so there were five treatments with four containers (replicates) per depth (n = 4 per treatment; n = 20, including all treatments together). Containers were irrigated gently once a day with water to ensure the moisture of the soil remained within 70% of its water-holding capacity. Fresh water (< 0.5 psu) was used to avoid salinity effects on germination since we wanted just to record seed responses to burial and avoid high salinity effects. *Spartina densiflora* is a facultative halophyte that can germinate, establish and develop in freshwater conditions (Nieva et al. 2001b; Castillo et al. 2005). The containers had small holes on the bottom to allow drainage, but these were covered with strips of cloth to prevent the loss of sand.

Sediment redox potential, electrical conductivity and pH were recorded at the end of the experiment in February 2010 at 1 cm depth in the sand. pH was recorded in the laboratory after adding distilled water to the soil (1:1, soil: distilled water, v/v) (pH/redox Crison with the electrode M-506). Soil salinity was measured as electrical conductivity (conductivity meter, Crison-522) after pH (1:2, soil: distilled water, v/v)). Redox potential was determined with a portable meter and electrode system in the greenhouse (Crison pH/mV p-506). Mean, maximum and minimum daily sediment temperature were recorded at 0, 1, 2, 4 and 8 cm depths using an electronic thermometer (SA880SSX, Germany) recording from 8 a.m. to 8 p.m. every 4 hours for 3 days (n = 3). Average daily air temperature during the experiment was 18.5 ± 0.5 °C, varying between a mean low temperatures of 10.4 ± 1.6 °C and a mean maximum temperature of 34 ± 3.5 °C. Mean daily air relative humidity was 74.5 ± 2.6%, varying between 32.5 ± 5.5% and 92.5 ± 1.1%.

Emerged seedlings from beneath the wrack were counted every 24 h (Cui et al. 2007). The experiment continued until no additional emergence was observed during 10 days. At the end of the experiment, the wrack and the sand were carefully removed and those seedlings that died before raising the wrack surface were counted and ungerminated seeds were collected. At the end of the experiment, shoot and root length were measured on all seedlings and the number of roots counted. Germination percentage was calculated as the number of germinated seeds by the total number of seeds per treatment. Seedling emergence percentage was calculated as the number of seedlings raising above the wrack surface by the number of germinated seeds per treatment. Seedling aerial growth rate was recorded as the difference in emerged seedling height between two consecutive measures (in two days period) divided by the number of days.

Ungerminated seeds were transferred to 0.5-cm depth in sand and germination and emergence percentages were recorded until no more seeds germinated for 10 days. Germinated seeds were considered to be in a quiescent state, which is different than true seed dormancy and occurs when a seed fails to germinate because external environmental conditions are not appropriate (Baskin and Baskin 1985). Then, ungerminated seeds were soaked in water at 30 °C for 24 h. Seed coats were cut and the embryo was soaked in 1% tetrazolium chloride (Panreac Quimica S.A., Barcelona, Spain) for 24 h at 30 °C. Pink embryos were scored as alive and considered to be in dormancy (Baskin and Baskin 2004). Non-coloured seeds were considered to be dead. Quiescence, dormancy and mortality percentages were calculated in relation to the total number of seeds per treatment.

Statistical analysis

Analyses were carried out using SPSS 12.0 (SPSS Inc, USA). Data were tested for homogeneity of variance and normality with the Brown-Forsythe test and the Kolmogorov-Smirnov test, respectively ($P < 0.05$). Plant traits were compared between treatments by one-way analysis of variance (ANOVA, *F*-test). Tukey Honest Significant Difference (HSD) test between means was calculated only if the *F*-test was significant ($P < 0.05$). The effect of wrack load on seedling growth rate between two treatments was analyzed using a Student *t*-test. Pearson correlation coefficient and linear regressions were calculated between abiotic factors, germination and seedling traits, and the wrack load. When a biotic characteristic was correlated with two or more abiotic environmental factors, multiple regression analysis was carried out to explore relative weights (β). Deviations were calculated as standard errors of the mean (SEM).

Results

Abiotic environment

Sediment pH increased with the wrack burial load from 6.2 ± 0.5 in the control treatment to 7.7 ± 0.1 under 8 cm ($r = 0.96$, $P < 0.01$, $n = 20$). Electrical conductivity changed from 0.11 ± 0.00 to 0.23 ± 0.00 mS cm^{-1}. Sediment redox potential varied between -83 ± 7 mV under 8 cm of wrack and +255 ± 5 mV without wrack burial (Table 1). Mean daily sediment temperature decreased at higher depths ($r = -0.61$, $P < 0.05$, $n = 20$), but without showing significant differences between treatments, varying between 12.3 ± 1.0 °C for the control treatment and 10.1 ± 0.8 °C at 4 and 8 cm (ANOVA, $F = 0.39$, $P > 0.05$). Maximum daily sediment temperature changed between +25.0 ± 0.8 °C for the control treatment and +21.7 ± 0.3 °C at 1 cm (ANOVA, $F = 2.02$, $P > 0.05$), decreasing also when wrack burial depth increased ($r = -0.36$, $P > 0.05$, $n = 20$). Minimum daily temperature varied between +10.4 ± 0.4 °C at 8 cm and +7.9 ± 1.0 °C for the control treatment (ANOVA, $F = 0.86$, $P > 0.05$), increasing with depth ($r = 0.81$, $P < 0.0001$, $n = 20$). Daily variation between maximum and minimum temperatures decreased at higher depths ($r = -0.62$, $P < 0.05$, $n = 20$), varying between 17.7 ± 1.4 °C for the control treatment and 12.4 ± 0.3 °C at 8 cm (ANOVA, $F = 2.46$, $P < 0.05$) (Table 1).

Germination and establishment

The time to first emergence of *S. densiflora* was 23 days after the start of the experiment at control treatment and 37 days at 1 cm wrack depth. Germination decreased when wrack load increased ($r = -0.84$, $P < 0.0001$, $n = 20$), showing the highest value without wrack (96 ± 4%) and decreasing significantly under 8 cm deep (ANOVA, $P < 0.05$) (Fig. 2).

Table 1. Wrack load (g m^{-2}), sediment pH, redox potential (mV), electrical conductivity (mS cm^{-1}), daily mean, maximum and minimum sediment temperature (°C), and the difference between maximum and minimum temperature (°) (n = 3) for five wrack burial depths. Different letters indicate significant differences between treatments (ANOVA, $P < 0.05$) (n = 5).

Wrack depth (cm)	Wrack load (g m^{-2})	pH	Redox potential (mV)	Conductivity (mS cm^{-1})	Daily sediment temperature (°C)			
					Mean	Maximum	Minimum	Max-Min
0	0 ± 0^{a}	6.2 ± 0.5^{a}	+255 ± 5^{a}	0.23 ± 0.00^{a}	12.3 ± 1.0^{a}	25.0 ± 0.8^{a}	7.9 ± 1.0^{a}	17.7 ± 0.2^{a}
1	1235 ± 92^{b}	6.9 ± 0.3^{b}	+247 ± 2^{a}	0.20 ± 0.02^{a}	10.4 ± 0.9^{a}	21.7 ± 0.3^{a}	9.4 ± 0.5^{a}	13.0 ± 0.3^{b}
2	3266 ± 13^{c}	7.1 ± 0.5^{b}	+229 ± 3^{b}	0.16 ± 0.00^{b}	10.2 ± 0.9^{a}	22.5 ± 0.1^{a}	9.7 ± 0.5^{a}	13.1 ± 0.0^{b}
4	4213 ± 277^{d}	7.3 ± 0.1^{c}	+157 ± 11^{c}	0.13 ± 0.01^{c}	10.1 ± 0.8^{a}	22.7 ± 0.8^{a}	10.1 ± 0.4^{a}	12.7 ± 0.1^{b}
8	6138 ± 227^{e}	7.7 ± 0.1^{d}	-83 ± 7^{d}	0.11 ± 0.00^{d}	10.1 ± 0.8^{a}	22.5 ± 0.0^{a}	10.4 ± 0.4^{a}	12.4 ± 0.3^{b}

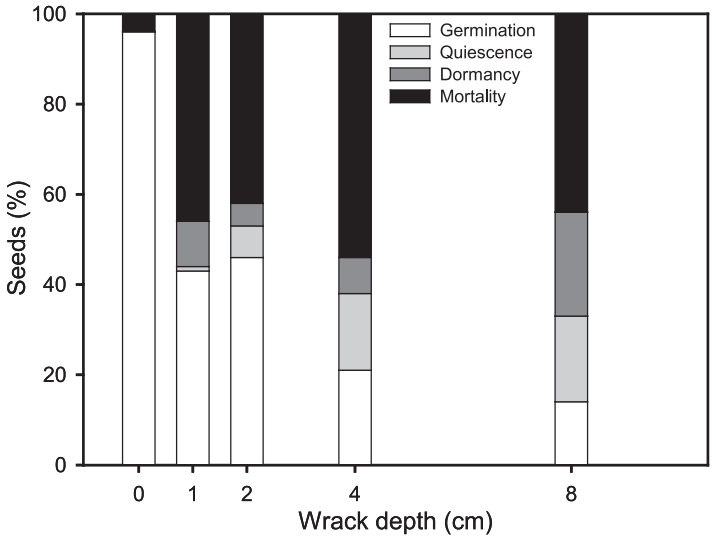

Figure 2. Germination, quiescence, dormancy and mortality of *Spartina densiflora* seeds for five wrack burial depths.

Germination percentage decreased at lower redox potentials (r = 0.65, P < 0.005, n = 20; β = -0.599), varying from 96 ± 4% at the control treatment with sediment redox potential +255 ± 5 mV to less than 15% under 8 cm deep with negative redox potential -83 ± 7 mV. In addition, germination percentage increased at lower minimum daily sediment temperature (r = -0.95, P < 0.0001, n = 20; β = -3.669) (Fig. 2).

Quiescence and dormancy percentages increased with wrack load (r = 0.67, P < 0.05, n = 20; r = 0.70, P < 0.001, n = 20, respectively). Quiescent percentage increased at higher minimum daily sediment temperature (r = 0.57, P < 0.05, n = 20; β = 3.265) and at lower redox potentials (r = -0.56, P < 0.01, n = 20; β = 0.495). No dormant seeds were recorded for the control treatment where seed mortality was the lowest (4%; 4 seeds). Dormancy and mortality increased to ~50% of the ungerminated seeds under wrack (Fig. 2). Dormant seed percentage and seed mortality increased mainly at higher daily minimum sediment temperature (r = 0.70, P < 0.001, n = 20; r = 0.81, P < 0.0001, n = 20, respectively).

No seedling emerged from deeper than 1 cm depth since every seedling died before emerging above the wrack surface. Minimum seedling mortality was recorded for the control treatment (7 ± 3% of germinated seeds) (ANOVA, P < 0.05). The highest seedling emergence percentage occurred without wrack (93 ± 3%) and only 6 seedlings emerged from under 1 cm of wrack (11 ± 5%) (Table 2).

Seedlings in the control treatment were much taller and had longer and more roots than those growing from under 1 cm of wrack and also than those seedlings dying under the wrack at 2, 4 and 8 cm (Fig. 3). These results also showed a higher aerial growth rate for seedlings at the control treatment (0.11 ± 0.01 cm day^{-1}) than those growing from under 1 cm of wrack (0.02 ± 0.01 cm day^{-1}) (t-test; P < 0.05).

Table 2. Emergence and mortality rates of germinated seeds for five wrack burial depths. Different letters indicate significant differences between wrack burial depths for the same trait (ANOVA, $P < 0.05$).

Wrack depth (cm)	Emergence (%)	Mortality (%)
0	93 ± 3[a]	7 ± 3[a]
1	11 ± 5[b]	89 ± 5[b]
2	0 ± 0[c]	100 ± 0[c]
4	0 ± 0[c]	100 ± 0[c]
8	0 ± 0[c]	100 ± 0[c]

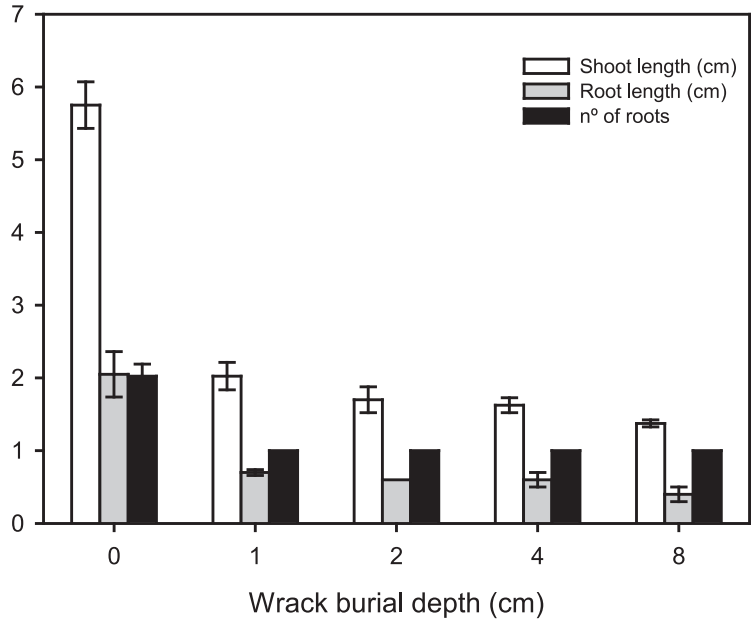

Figure 3. Shoot and root lengths (cm) and root number of *Spartina densiflora* seedlings for five wrack burial depths (seedlings from deeper than 1 cm were found dead and did not emerge above the wrack surface). Different letters indicate significant differences between wrack depths for the same trait (ANOVA, $P < 0.05$).

Discussion

This study shows that germination and establishment of *S. densiflora*, an invasive cordgrass in Europe, North America and North Africa, is greatly limited by wrack burial.

Sediment pH increased with wrack burial load, which may be related to the presence of shells in the debris that would add carbonate to the sediment. However, *S. densiflora* germination would not be altered within the narrow recorded pH range (6.2–7.7) (Curado et al. 2010). Similarly, electrical conductivity varied within a range (0.11–0.23 mS cm^{-1}) that would not influence *S. densiflora* germination significantly (Castillo et al. 2005). In contrast, sediment redox potential under the debris may have affected germination under 8 cm of wrack since it was as low as -83 ± 7 mV, and germination decreased and quiescence increased at low redox potentials as it has been described previously in anaerobic environ-

ments under organic material in lakes (Rich and Wetzel 1978) and in accordance with our hypothesis. Negative values of redox potential can decrease *S. densiflora* germination in the field (Mateos-Naranjo et al. 2008). Sediment anoxia affects seeds by consuming oxygen resulting from degradation of organic matter (Wu et al. 2009). Thus, poor soil aeration may induce quiescence (Vleeshouwers et al. 1995).

The highest germination was recorded without debris (96%), decreasing markedly (to values between 21–43%) between 1 and 4 cm deep with positive redox potentials (> +150 mV). Therefore, other environmental factors in addition to anoxia seemed to be limiting *S. densiflora* germination under the debris. The effects of burial on germination can be mediated by changes in the light regime as it has been described for some halophytes (Pons 1992; Khan and Gul 2002), which is not the case for *S. densiflora* germination that is similar in light and darkness under fresh water conditions (Nieva et al. 2001b). However, synergistic effects between light and other abiotic factors cannot be excluded. Germination of *S. densiflora* increased at lower sediment daily minimum temperatures, conditions that were recorded without or under low loads of wrack, indicating that burial probably caused an unsuitable temperature environment for germination (Benvenuti et al. 2000). Thus, quiescence and dormant seed percentages increased at higher sediment minimum temperatures. In temperate regions, many grass species require exposure to low winter temperatures to come out of dormancy (Baskin and Baskin 1998). Sensitivity to temperature fluctuation functions as a depth- or gap-detecting mechanism; in this way, germination is activated when temperature fluctuation increases at unvegetated areas exposed directly to solar radiation (Thompson and Grime 1983). Furthermore, allelopathic effects from the plant debris inhibiting the germination of *S. densiflora* seeds cannot be excluded (Li et al. 2010; Sieg and Kubanek 2013).

Spartina densiflora did not show primary dormancy since all its seeds germinated or died (only 4%) without wrack in optimal conditions. Instead, *S. densiflora* seeds entered a non-deep physiological dormancy (Baskin and Baskin 1988) under the plant debris. Dormancy percentage increased with higher daily minimum temperature determining a lower daily temperature variation under the wrack. Secondary dormancy may be induced by environmental factors such as high CO_2 levels produced by debris decomposition (Harper and Obeid 1967), poor aeration (Simpson et al. 1989) and low temperature fluctuations (Baskin and Baskin 1998). Longer seed dormancy at greater depths within the debris would be ecologically advantageous because seeds would survive in the dormant state in the seed bank until the upper layer of wrack would be removed. Nevertheless, wrack burial increased seed mortality.

The establishment of *S. densiflora* seedlings was also greatly influenced by wrack. Only six seedlings emerged above the plant debris from a burial depth of 1 cm, while no seedling emerged from deeper than 1 cm (wrack load > 3 kg DW m^{-2}). The seedling of *S. densiflora* has just one thin and sharp cotyledon that grows easily along an axis, being able to emerge straight away from 4 cm depth in water (Abbas et al. 2012) and in sand (A.M. Abbas, personal observation). In contrast, when a seedling grew within wrack it had to find the few hollows left open in the wrack as is reflected in its cotyledon growing in curves. Every *S. densiflora* seedling dying within the debris was shorter

than 2 cm, which seemed to be the longest length they were able to grow under these conditions. The dense structure of the debris would prevent *Spartina* from emerging from deeper than 1 cm due to the exhaustion of food reserves before getting to the surface (Martínez et al. 1992; Bond et al. 1999). It has been previously described how deeply sown seedlings in sediment died before emerging in wetlands (Hartleb et al. 1993; Jurik et al. 1994; Wang et al. 1994; Dittamar and Nelly 1999; Spencer and Ksander 2002; Ke and Li 2006). Live and dead standing biomass of *Spartina patens* (Aiton) Muhl prevented seedling emergence of subordinate annuals and perennials in coastal marshes (Brewer and Grace 1990; Baldwin et al. 1996). *Spartina densiflora* seed germination and quiescence presented a gradual response to wrack burial but seed dormancy, seed and seedling mortality and seedling emergence showed a threshold dynamic in response to wrack burial, increasing markedly even under just 1 cm depth. These results characterized *S. densiflora* as a very sensitive species to wrack burial during the establishment period, which may limit its invasion in those marshes accumulating high debris loads.

Spartina densiflora seedling development was also significantly reduced by wrack burial. Seedlings growing without wrack were ~5.8 cm tall at the end of the experiment while seedlings emerging from 1 cm deep under the wrack were ~2 cm tall (including their buried part), coinciding with lower growth rates. In adittion, seedlings in the control treatment had longer and more roots. In this sense, it has been reported that phytotoxins generated in anaerobic decomposition can inhibit the growth of freshwater plants (Barko and Smart 1986; Maun and Lapierre 1986).

Our experimental results are in accordance with our field observations. In tidal salt marshes, wrack is accumulated mainly coinciding with the mean higher high water. We have observed in the field (Odiel Marshes) that the wrack depth in these areas ranges from 2 to 14 cm. In these marshes, we have seen no *S. densiflora* seedling growing from within the wrack. Just very few *Spartina* adult clumps were observed within the debris areas, which seemed to have established before wrack accumulation or within open patches in the wrack. *Spartina densiflora* tussocks accumulate high densities of dead tillers in middle and high marshes (Nieva et al. 2001a) and when this necromasa is detached from the tussocks is accumulated as wrack. In view of our results, as *S. densiflora* invades a location it would decelerate its own invasion rate through the accumulation of wrack that may limit its establishment.

Conclusions

Data gathered during this study confirmed an inverse relationship between germination and emergence with wrack burial for the invasive cordgrass *S. densiflora*. Germination decreased from 96% without wrack to 14% at 8 cm deep in debris (ca. 6 kg DW m^{-2}). No seedling emerged above the wrack surface for seeds germinated at wrack burial depths greater than 1 cm (a wrack load of ca. 1 kg DW m^{-2}). The results from this study improved our understanding of *S. densiflora* invasion and they are useful

to predict invasion dynamics and to plan the management of invaded marshes. Thus, wrack may be used to limit *S. densiflora* colonization and should not be removed from those areas sensible to the invasion of this cordgrass.

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