A multi-species experiment in their native range indicates pre-adaptation of invasive alien plant species

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Summary

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- To understand prerequisites of biological invasions, it is imperative to know whether species have traits that pre-adapt them to become invasive elsewhere. However, few experimental studies have explicitly tested this by comparing traits between invasive and noninvasive species in their native range instead of in the nonnative range.
- We used native plant material of 14 European congeneric pairs of herbaceous species that were all introduced to North America, and of which one species per pair is invasive.
- In our germination and common garden experiment with and without fertilizer addition, the invasive species germinated faster, produced more biomass and had a higher proportion of flowering plants than the noninvasive congeners.
- Our results indicate that species traits, which lead to a high plant performance in the native range, can confer pre-adaptation to become invasive. We suggest that such traits may be especially relevant for use in risk-assessment protocols before introduction elsewhere.

Key words: biological invasions, common garden experiment, exotic species, phylogenetically independent association, plant biomass, prediction, risk assessment.

Introduction

Biological invasions are among the major drivers of global environmental change (Vitousek *et al.*, 1997; Ricciardi, 2007), and have a huge financial impact (Pimentel *et al.*, 2005). Therefore, it is paramount to advance our mechanistic understanding of current biological invasions, and to gain power in predicting outcomes of future species introductions (Pyšek & Richardson, 2007). Despite increased scientific efforts in studying biological invasions, it is still not well known why some introduced species become invasive and others do not (van Kleunen & Richardson, 2007; Moles *et al.*, 2008). Such knowledge is, however, imperative to prevent further invasions.

Based on theoretical considerations, plant species with certain traits, such as fast and profuse germination, fast growth and high reproductive effort, are suggested to be more likely to become weedy and invasive (Baker, 1974). In addition, it has been suggested that species showing high environmental tolerance in such traits or that are better able to take advantage of high amounts of resources might become more invasive (Baker, 1974; Davis *et al.*, 2000; Richards *et al.*, 2006). Experimental comparisons of such traits between invasive and noninvasive species suggest that these traits are, at least sometimes, associated with invasiveness (reviewed in Daehler, 2003; Pyšek & Richardson, 2007). However, it is difficult to draw general conclusions because most studies were restricted to very few species, and because invasive species have been compared with different types of control groups. The majority of studies compared invasive alien species with native species either directly in the field or in common garden settings (Daehler, 2003; Pyšek & Richardson, 2007). Such studies tested which traits of invading species enhance their potential to increase over native species instead of investigating why some alien species become invasive and others do not (Hamilton *et al.*, 2005; Muth & Pigliucci, 2006). Clearly, the latter can only be tested by comparing invasive alien species with noninvasive alien species.

Of the few studies that experimentally compared invasive alien species with noninvasive alien species in common environments, almost all were performed in the introduced (i.e. nonnative) range of the species (but see van Kleunen &

Johnson, 2007). Such studies, however, cannot tell whether trait differences between invasive and noninvasive species were already present before the introduction – these are the ones most relevant for risk assessment – or whether they evolved (or further evolved) after introduction in the new range. Moreover, it could be that certain traits pre adapted species to establish in a new range, and were subsequently selected against once the species had become established (Dietz & Edwards, 2006). Consequently, these traits may no longer show differences between invasive and noninvasive alien species in the introduced range. Clearly, to determine whether invasive and noninvasive alien species had different traits before introduction, and whether such traits are likely to confer pre adaptation to become invasive, we need to study them in their native range.

Invasiveness is often linked to taxonomic group or phylogeny (Daehler, 1998; Pyšek, 1998; van Kleunen *et al.*, 2007), which implies that analyses testing for traits associated with invasiveness should correct for taxonomic or phylogenetic affinity. This can be done *a posteriori* by accounting for the variation explained by families and genera or by using phylogenetically independent contrasts (Felsenstein, 1985). However, if invasiveness is highly confounded with taxonomy or phylogeny, particularly when the total number of species is limited, taxonomically or phylogenetically independent tests are not possible. Therefore, a better approach is to select multiple related species pairs (e.g. congeneric pairs) *a priori*, each consisting of an invasive and a noninvasive species (Felsenstein, 1985).

To test whether invasive herbaceous plant species are already likely to be pre adapted to become invasive in their native range – that is, before they are actually introduced – we used a study system consisting of 14 congeneric pairs of herbaceous species representing 10 families. All 28 species are native to Europe and naturalized in North America, where one species of each pair is invasive while the other is not. We used seeds from the common native range (i.e. Europe) of all 28 study species in two common environment experiments. First, in a germination experiment in a glasshouse, we tested whether invasive and noninvasive species differed in time to germination and proportion of germinated seeds. Then, in a growth experiment in outdoor pots with and without added fertilizer, we tested whether the invasive and noninvasive species differed in root : shoot ratio, specific leaf area, leaf production rate, biomass production, mortality and likelihood to flower, or in a combination of these traits. This allowed us to address the following major question: do invasive and noninvasive alien species differ in germination characteristics, growth and performance related traits and their responses to soil fertilizer in their native range? In other words, we tested whether these traits likely pre adapt species to become invasive elsewhere. We additionally discuss the use of multi species comparisons for studying traits associated with invasiveness,

and the implications of our results for the development of risk assessment protocols.

Materials and Methods

Study species and seed collection

We used 14 congeneric pairs of invasive and noninvasive species, representing 10 families of terrestrial herbaceous plants. All 28 species are native to Europe and naturalized in North America. For each congeneric pair, we categorized the species that is most widely naturalized in North America (occurring in ≥ 31 US states + Canadian provinces, data retrieved from the USDA Plants Database, http://plants.usda.gov) as invasive and the less widely naturalized species (occurring in ≤ 18 US states and Canadian provinces) as noninvasive (Table 1). Most of the invasive species are also listed as 'invaders of natural areas' or as 'noxious weeds' in North America (Table 1). Moreover, all invasive species also have more entries in the Global Compendium of Weeds (Randall, 2002) than their congeneric noninvasive species (Table 1), indicating that our distinction between invasive and noninvasive species holds not only for North America but also on a global scale. Additionally, our results are shown to be robust after correcting for variation among species in time since first record in North American herbaria (Table 1, see the 'Discussion' section).

For 20 of the 28 species, we collected seeds from four to 16 plants (i.e. seed families) in each of one to three populations in Switzerland or France (i.e. in the native range). Populations were defined as continuous stands of species, each covering an area between 4 and 40 000 m^2 that were separated by at least 10 km. For the eight remaining species and for six of the species with seeds from natural populations, we ordered native seeds from two commercial seed suppliers (UFA Samen, BH Gärten & Bio Suisse, Switzerland; Table 1).

Germination experiment

We sowed 25 seeds per seed family or per seed lot provided by a seed supplier (totalling 14 345 seeds) in *c.* 4 cm high 1.3 l trays filled with a sand : soil : peat mixture (2 : 2 : 1) in the second half of April 2008. The pots were randomly allocated to positions in an uncontrolled glasshouse (N 46°55′16.31″, E 7°30′08.53″, 550 m asl, Muri near Bern, Switzerland) and watered daily. We counted the number of germinated seeds in each seed family, three times a week for the first 5 wk, once a week for the next 4 wk and once every 2 wk for another 4 wk.

Growth experiment

We transplanted an average of 41 seedlings per species (range = 2–101 owing to variable germination success; total

Table 1 List of 14 congeneric pairs of invasive and non-invasive species, including for each species the family name, nonnative range sizes in North America, stings as noxious weeds and as natural-area invaders in North America, global invasiveness, life span, earliest record in the USA and sample sizes that were used in the growth experiment

Family	Species	Invasiveness status[b,c]	No. of US states occupied[c]	No. of Canadian provinces occupied[c]	Invader of natural areas[d]	Declared noxious weed[e]	No. of references in GCW[f]	Life span in native range[g]	Earliest herbarium voucher[i]	Sample size in experiment (suppliers + no. of populations, no. of plants)
Asteraceae	*Centaurea stoebe* L.[a]	Inv	46	7	24	18	40	bhp[h]	1918	3, 77
Asteraceae	*Centaurea montana* L.	Non	13	5	–	–	9	p	1914	3, 11
Asteraceae	*Cirsium vulgare* (Sav.) Ten.	Inv	51	11	24	11	70	b	1883	3, 90
Asteraceae	*Cirsium palustre* (L.) Scop.	Non	5	5	–	1	11	bh	1961	S + 3, 31
Campanulaceae	*Campanula rapunculoides* L.	Inv	40	10	–	1	23	p	1879	S + 2, 19
Campanulaceae	*Campanula patula* L.	Non	3	0	–	–	5	p	1990	S + 1, 41
Caryophyllaceae	*Dianthus armeria* L.	Inv	48	8	1	–	19	b	1892	3, 88
Caryophyllaceae	*Dianthus carthusianorum* L.	Non	4	0	–	–	3	p	1978	3, 101
Caryophyllaceae	*Silene vulgaris* (Moench) Garcke	Inv	41	12	3	1	30	p	1886	3, 65
Caryophyllaceae	*Silene nutans* L.	Non	5	0	–	–	6	p	1896	3, 57
Fabaceae	*Lotus corniculatus* L.	Inv	44	12	9	–	37	p	1899	3, 57
Fabaceae	*Lotus pedunculatus* Cav.	Non	7	6	–	–	13	p	1940	S + 2, 71
Fabaceae	*Trifolium repens* L.	Inv	51	13	7	–	49	p	1867	3, 21
Fabaceae	*Trifolium medium* L.	Non	6	5	–	–	11	p	1995	2, 2
Fabaceae	*Vicia cracca* L.	Inv	37	14	3	1	24	p	1890	3, 9
Fabaceae	*Vicia sepium* L.	Non	7	6	–	–	10	p	1970	S + 2, 20
Geraniaceae	*Geranium dissectum* L.	Inv	29	2	–	–	31	a	1862	S, 19
Geraniaceae	*Geranium pratense* L.	Non	8	10	–	–	9	p	1992	S, 5
Malvaceae	*Malva sylvestris* L.	Inv	42	7	–	–	30	p	1871	S, 20
Malvaceae	*Malva alcea* L.	Non	13	5	–	–	3	p	1907	S, 6
Plantaginaceae	*Plantago lanceolata* L.	Inv	51	10	9	10	70	p	1876	S, 20
Plantaginaceae	*Plantago media* L.	Non	11	5	–	–	14	p	1956	S, 20
Rosaceae	*Potentilla argentea* L.	Inv	35	10	–	–	13	p	1878	S + 3, 78
Rosaceae	*Potentilla erecta* (L.) Raeusch.	Non	1	2	–	–	4	p	1950[c]	1, 5
Rubiaceae	*Galium verum* L.	Inv	35	9	1	–	13	p	1912	S, 13
Rubiaceae	*Galium album* M.	Non	1	0	–	–	2	p	1979[c]	S, 16
Scrophulariaceae	*Verbascum thapsus* L.	Inv	51	10	20	2	65	b	1867	3, 91
Scrophulariaceae	*Verbascum nigrum* L.	Non	6	2	–	–	6	b	1933	3, 83

[a] *Centaurea maculosa* auct. non Lam. and *Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek treated as synonyms.

[b] Invasiveness status: invasive ≥ 31, non invasive ≤ 18 US states + Canadian provinces occupied.

[c] Data retrieved from The Plants Database (http://plants.usda.gov).

[d] No. of US states retrieved from Weed US – database of plants invading natural areas in the United States (http://www.invasive.org/weedus).

[e] No. of US states retrieved from INVADERS Database System: noxious weed list (http://invader.dbs.umt.edu).

[f] Global Compendium of Weeds (Randall, 2002).

[g] Life span: a, annual; b, biennial; h, hapaxanthic perennial; p, perennial; data retrieved from BioFlor (http://www.bioflor.de).

[h] Introduced geocytotypes are of the perennial type (Müller-Schärer *et al.*, 2004) which we also used for our experiments.

[i] Earliest record in North America based on the collection date of the oldest vouchers (Rejmánek, 2000) found among on-line herbaria from the US states of California (Consortium of California Herbaria, http://ucjeps.berkeley.edu/consort/um), Florida (Atlas of Florida Vascular Plants, http://www.plantatlas.usf.edu), Idaho, Montana, Oregon, Washington and Wyoming (INVADERS Database System, http://invader.dbs.umt.edu), Minnesota (Herbarium of the University of Minnesota, http://www.wildflowers.umn.edu), New York (New York Flora Atlas, http://www.newyork.plantatlas.usf.edu) and Wisconsin (Wisconsin Herbarium Plant Specimens, http://www.botany.wisc.edu/herbarium). On-line databases were accessed February 12, 2009.

1136 seedlings) in June 2008. We pricked the seedlings to 1.2 l pots filled with the same soil mixture as used in the germination experiment. To avoid confounding effects of fertilizer treatments and genetic differences, we randomly assigned plants of each seed family and population or of each seed supplier to each fertilization treatment. The substrate contained 3.8 g N and 1.2 g P per pot (Kjeldahl extracts, $n = 5$; this mostly includes N and P in organic form that is not directly available for the plant). The fertilized plants received an additional 0.66 g N and 0.17 g P as a slow release fertilizer (Osmocote exact 5 6M; Scotts, Heerlen, the Netherlands).

Three weeks after transplantation, we moved the pots to an outdoor common garden close to the glasshouse, and randomly assigned them to one of 10 blocks. At this time, we determined the number of leaves and the length of the longest leaf blade (i.e. leaf length without petiole) on each plant. For plants with few branches, we counted all leaves, and for highly branched plants we counted the number of leaves on one randomly selected branch and multiplied this by the total number of branches per plant. We randomized the position of pots in each block every 4 wk throughout the experiment. About 4 wk after the first measurements, we again counted the number of leaves and measured the length of the longest leaf blade on each plant. We calculated total leaf length as the product of leaf number and leaf blade length. Then we calculated relative leaf production rate and relative leaf length production rate as follows: (log (leaf trait at second measurement) log (leaf trait at first measurement))/(number of days between measurements) (Grotkopp et al., 2002).

Between the end of August and the beginning of November 2008, we checked for mortality and flowering of plants, and harvested above and below ground biomass of each plant. We harvested each species at the moment of its maximum biomass production. For flowering species this was at their peak of flowering, and for nonflowering species this was when their first leaves started to senesce. We dried the plant samples at 70°C for at least 72 h, and weighed them to the nearest mg. We measured the combined area of ≥ 10 randomly selected leaves per plant with a leaf area meter (LI 3100C; Li Cor, Lincoln, NE, USA) to the nearest mm^2, and divided the area by the dry weight of these leaves to calculate specific leaf area (cm^2 g^{1}).

Statistical analyses

We analysed all data with linear mixed models, that is, models including both fixed and random terms. Because the final data set was unbalanced, particularly so for the growth experiment as a result of poor germination of some species, we present results of restricted maximum likelihood (REML) analysis of variance (Payne et al., 2008). However, we obtained very similar results when using ANOVA (Supporting Information, Table S1).

For the analysis of time to germination and proportion of germinated seeds, we included invasiveness status (invasive, noninvasive) as a fixed factor. To account for possible effects of seed mass on germination characteristics, we included 1000 seed mass of species (data from the 'Seed Information Database 7.1', http://www.kew.org/data/sid, accessed February 1, 2009) as a covariate in the random model. On average, the invasive and noninvasive species did not differ in seed mass (paired t test, $t_{13} = -1.27$, $P > 0.226$). To account for the hierarchical taxonomic structure of the experiment, we included plant family, genus nested within plant family, species nested within genus and population nested within species as random factors. We square root transformed time to germination and arcsine square root transformed proportion of germinated seeds before analysis.

The data collected in the growth experiment included outliers that were outside the biologically possible range. Possibly, this was because the leaf area meter was not sensitive enough to measure small and thin leaves accurately, and because some soil sticking to the roots had gone unnoticed. Therefore, we report results based on analyses with all statistical outliers excluded (77 146 of 1136 plants per trait), that is, we excluded data points beyond a distance of 1.5 times the interquartile range (computed as Tukey's hinges) for each combination of variable and main factor (Tukey, 1977). Nevertheless, even when we analysed the complete data set, including the outliers, all effects of the main factors of interest (i.e. the fixed factors) that were significant in the reduced data set remained significant (compare Table 4 with Table S2). For the analysis of root : shoot ratio, specific leaf area, relative leaf production rates and above and below ground biomass, we included fertilizer treatment (fertilizer, no fertilizer), invasiveness status (invasive, noninvasive) and their interaction as fixed terms. Because fixed terms are sequentially fitted, we ran two analyses with reciprocated order of both main factors, and report results for each factor from the analysis in which the other factor was fitted first.

We included blocks as a random factor, and accounted for possible effects of different starting dates of the experiment, duration of the experiment and whether the plant was flowering or not by including these variables as covariates in the random model (Payne et al., 2008). To account for the hierarchical taxonomic structure of the experiment, we included plant family, genus nested within plant family, species nested within genus and population nested within species, and the interactions of plant family, genus and species with fertilizer treatment as random terms. We additionally tested for a potential allometric effect on root : shoot ratio with a model that included total biomass as a covariate. Log transformation of specific leaf area and double log

transformation of root : shoot ratio, above and below ground biomass satisfied test assumptions.

In all REML analyses, variance components were con strained to positive values. The level of significance of each fixed factor was determined using F ratio tests and numeri cally estimated denominator degrees of freedom (Payne *et al.*, 2008). Differences in REML means of the invasive ness status × fertilizer treatment interaction were compared with Tukey Kramer honestly significant differences (HSD). The level of significance of each random term was deter mined by χ^2 tests for changes in deviance between the com plete model and a model with the term in question dropped (Littell *et al.*, 1996). For all analyses, we report the signifi cance of both fixed and random terms in Tables 2 4, but because only the fixed terms were of major biological inter est, we focus on these in the 'Results' section.

Performance related traits coded as binary data (i.e. mor tality and flowering) were analysed with generalized linear mixed models (GLMM) assuming a binomial distribution and using the logit link function. We aggregated data per species × fertilizer treatment combination; otherwise, test assumptions were violated because of a large number of zeros. Binomial totals were set to the total number of plants for each species × fertilizer treatment combination. We included invasiveness status, fertilizer treatment and their interaction as fixed factors, and plant family, genus nested

Table 2 Summary of restricted maximum likelihood (REML) analyses of germination characteristics of 14 invasive and 14 noninvasive natural ized species measured in the germination experiment

	Proportion of germinated seeds			Time to germination		
Effect	df, ddf	F or χ^2	P	df, ddf	F or χ^2	P
Fixed term[a]						
Invasiveness status	1, 12.9	1.34	0.268	1, 15.3	7.84	**0.013**
Random terms[b]						
1000 seed mass (g)	1	1.82	0.178	1	2.31	0.128
Family	1	1.02	0.313	1	0.52	0.471
Genus (family)	1	0.15	0.694		Bound	
Species (genus)	1	8.88	**0.003**	1	21.76	**< 0.001**
Population (species)	1	98.86	**< 0.001**	1	1.66	0.197
Residuals	576			439		

[a]Fixed effects were tested with numerical approximations of the F statistic and denominator degrees of freedom (ddf).
[b]Random effects were tested with deviance differences as χ^2 statistic and one degree of freedom (df). See text for description of transforma tion of variables. 'Bound' refers to restricted variance components set to zero. Bold P values indicate significance levels smaller than 0.05 .

Table 3 Summary of generalized linear mixed models (GLMM) analyses of mortality and flowering of 14 invasive and 14 noninvasive natural ized species grown with and without fertilizer in the growth experiment

	Mortality			Flowering		
Effect	df, ddf	F or χ^2	P	df, ddf	F or χ^2	P
Fixed terms[a]						
Fertilizer treatment	1, 6.1	0.77	0.414	1, 7	0.81	0.397
Invasiveness status	1, 28.8	2.38	0.134	1, 18.4	277.36	**< 0.001**
Invasiveness status × fertilizer treatment	1, 30.2	4.72	**0.038**	1, 18.6	0.24	0.629
Random terms[b]						
Family	11	22.44	**0.021**		Bound	
Genus (family)		Bound		1	0.92	0.338
Family × fertilizer treatment	25	25.52	0.433	1	0.89	0.345
Genus (family) × fertilizer treatment	1	4.29	**0.038**	1	4.67	**0.031**
Residual	43			35		

[a]Fixed effects were tested with numerical approximations of the F statistic and denominator degrees of freedom (ddf).
[b]Random effects were tested with deviance differences as χ^2 statistic and one degree of freedom (df). See text for description of transforma tion of variables. 'Bound' refers to restricted variance components set to zero. Bold P values indicate significance levels smaller than 0.05.

Table 4 Summary of restricted maximum-likelihood (REML) analyses of traits related to biomass allocation, growth and biomass production of 14 invasive and 14 non invasive native naturalized species grown with and without fertilizer in the growth experiment

Effect	Root:shoot ratio (g g⁻¹)			Specific leaf area (cm² g⁻¹)			Relative total-leaf-length production rate			Relative leaf production rate			Above-ground biomass (g)			Below-ground biomass (g)		
	df, ddf	F or χ²	P	df, ddf	F or χ²	P	df, ddf	F or χ²	P	df, ddf	F or χ²	P	df, ddf	F or χ²	P	df, ddf	F or χ²	P
Fixed terms[a]																		
Fertilizer treatment	1, 85	5.97	**0.039**	1, 75	0.40	0.544	1, 9	19.99	**0.002**	1, 61	11.12	**0.015**	1, 88	72.04	**<0.001**	1, 109	41.8	**<0.001**
Invasiveness status	1, 114	1.72	0.216	1, 125	0.77	0.397	1, 127	0.03	0.876	1, 106	1.03	0.334	1, 121	41.87	**<0.001**	1, 163	4.46	0.051
Invasiveness status × fertilizer treatment	1, 122	0.09	0.770	1, 124	0.20	0.664	1, 8	4.39	0.070	1, 134	0.45	0.514	1, 77	12.95	**0.008**	1, 787.3	11.48	**<0.001**
Random terms[b]																		
Start of treatment (d)	1	0.31	0.581		Bound		1	7.03	**0.008**	1	4.75	**0.029**	1	0.76	0.382	1	Bound	
Duration of experiment (d)	1	6.53	**0.011**		Bound			Bound			Bound		1	0.00	0.958	1	7.813	**0.005**
Flowering	1	17.26	**<0.001**	1	4.74	**0.030**	1	5.29	**0.021**	1	15.30	**<0.001**	1	18.50	**<0.001**	1	Bound	
Block	1	32.26	**<0.001**	1	17.93	**<0.001**	1	12.05	**<0.001**	1	22.01	**<0.001**	1	8.54	**0.004**	1	6.028	**0.014**
Family		Bound		1	4.79	**0.029**		Bound		1	0.39	0.535	1	0.74	0.391	1	4.306	**0.038**
Genus (family)	1	0.52	0.470	1	1.15	0.284	1	3.12	0.078	1	0.72	0.396	1	4.37	**0.037**	1	Bound	
Species (genus)	1	21.49	**<0.001**	1	25.93	**<0.001**	1	7.06	**0.008**	1	6.26	**0.012**	1	9.41	**0.002**	1	22.025	**<0.001**
Population (species)	1	11.15	**<0.001**	1	18.28	**<0.001**	1	5.65	**0.018**	1	19.17	**<0.001**	1	19.57	**<0.001**	1	18.788	**<0.001**
Family × fertilizer treatment	1	2.25	0.134	1	1.57	0.211		Bound		1	0.01	0.921	1	5.14	**0.023**	1	Bound	
Genus (family) × fertilizer treatment		Bound			Bound		1	0.25	0.619		Bound		1	0.03	0.872	1	2.142	0.143
Species (genus) × fertilizer treatment	1	2.86	0.091	1	0.98	0.322	1	0.27	0.607	1	1.75	0.186	1	0.08	0.779	1	Bound	
Residuals	953			1011			1016			974			949			979		

[a] Fixed effects were tested with numerical approximations of the F-statistic and denominator degrees of freedom (ddf)

[b] Random effects were tested with deviance differences as χ^2-statistic and one degree of freedom (df). See text for description of transformation of variables. 'Bound' refers to restricted variance components set to zero. Bold P-values indicate significance levels smaller than 0.05
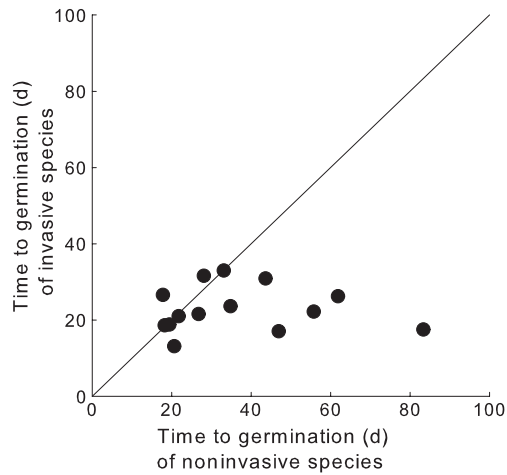
Fig. 1 Time to germination of 14 noninvasive (*x* axis) and 14 invasive (*y* axis) species measured in our glasshouse experiment. Significance of the effect of invasiveness status is given in Table 2.

within plant family and genus × fertilizer treatment interaction as random terms.

To assess the influence of trait combinations on the association with invasiveness, we performed a principal components analysis on the two germination and the eight plant performance related traits based on species means averaged over populations and fertilizer treatments. Traits were transformed as described earlier. Because traits were measured in different units, the correlation matrix was used as association matrix. We carried out all analyses using GenStat 11.1 (VSN International Ltd, Hemel Hempstead, UK).

Results

Germination of invasive and noninvasive species

The proportion of germinated seeds did not differ between invasive and noninvasive species (Table 2). However, time to germination was significantly shorter, by an average of 10 d, for invasive species than for noninvasive ones (Fig. 1; Table 2).

Growth and performance of invasive and noninvasive species and their responses to fertilizer

Twenty five of the 1136 plants died before harvest. Mortality was not significantly affected by the fertilizer treatment, and did not differ significantly between invasive (mean = 1.0%, SE range = 0.6 1.6%; back transformed predicted values) and noninvasive species (mean = 1.4%, SE range = 0.9 2.3%; Table 3) across the fertilization treatments. However, mortality was higher for noninvasive species than for invasive species in the high fertility treat

ment, in contrast to the low fertility treatment, where there was no such difference (indicated by the significant invasive ness × fertilizer treatment interaction in Table 3).

Relative leaf production rate and relative total leaf length production rate were higher with fertilizer than without fertilizer (Fig. 2a,b; Table 4), and did not differ significantly between invasive and noninvasive species (Fig. 2a,b; Table 4). Root : shoot ratios were significantly lower for fertilized plants than for unfertilized ones (Fig. 2c; Table 4) and did not differ significantly between invasive and noninvasive species (Fig. 2c; Table 4). However, when we additionally included total biomass as a covariate to account for potential allometric effects, the effect of invasiveness became marginally significant ($P = 0.076$), suggesting that at a given total biomass invasive species have lower root : shoot ratios than noninvasive species. Specific leaf area was not significantly affected by the fertilizer treatment, and did not differ significantly between invasive and noninvasive species (Fig. 2d; Table 4).

Invasive species produced significantly more above ground biomass than noninvasive species (Table 4), and this was consistently the case within each congeneric species pair (Fig. 2e). Invasive species also tended to have a higher below ground biomass than noninvasive species ($P = 0.051$; Fig. 2f; Table 4). In response to fertilizer, invasive species gained absolutely more above ground biomass (3.4 g) than noninvasive species (2.7 g), although the relative increase was larger for noninvasive species (159%) than for invasive species (85%; Fig. 2e; significant invasiveness × fertilizer treatment interactions in Table 4). In response to fertilizer, noninvasive species remained smaller in terms of below ground biomass than invasive ones, although the noninvasive species gained more biomass, both absolutely (2.3 g) and relatively (108%), than the invasive species (1.9 g and 49%, respectively) (Fig. 2f; significant invasiveness × fertilizer treatment interactions in Table 4). Overall, these results indicate that although the relative difference in biomass between invasive and noninvasive species was reduced in the treatment with fertilizer compared with the treatment without fertilizer, the absolute difference was maintained.

Of the 1111 plants that survived, 213 started flowering before the end of the season. Invasive species had, on average, a higher proportion of flowering plants (Fig. 3; Table 3), and this was independent of the fertilizer treatment (Table 3).

Multivariate comparison of traits between invasive and noninvasive species

The first principal component based on the two germination characteristics, as measured in the germination experiment, and the eight traits related to biomass allocation and plant performance (averaged over the two fertilizer treat
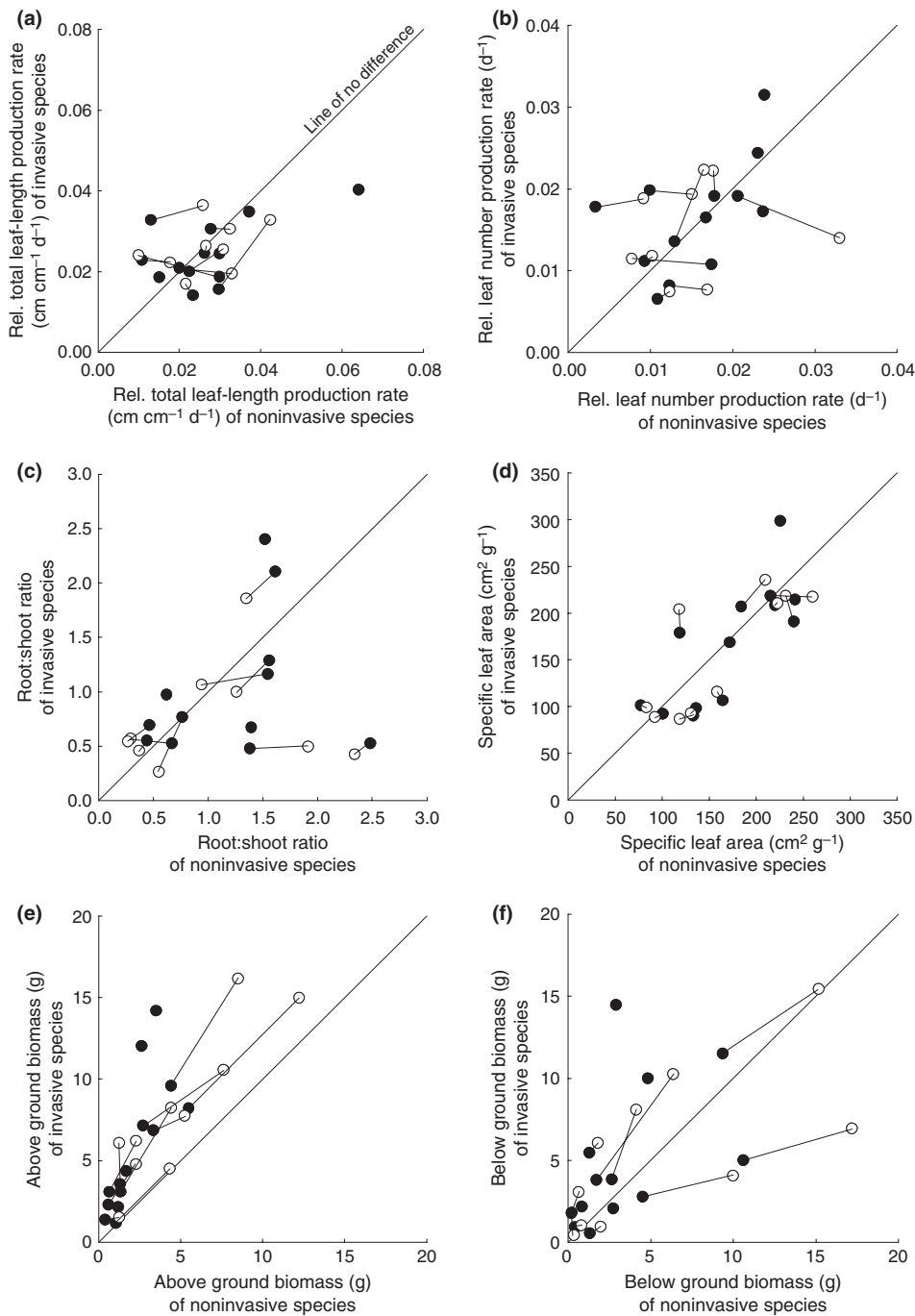
Fig. 2 Traits related to growth rate (a, b), biomass allocation (c, d) and biomass production (e, f) of 14 noninvasive (x axis) and 14 invasive (y axis) species measured under low (closed circles) and high (open circles) fertilizer treatments in our common garden experiment. Lines connect pairs of noninvasive and invasive species across fertilizer treatments. Root : shoot ratio and above and below ground biomass were double log transformed, and specific leaf area was log transformed before analysis. The graphs, however, show back transformed means. Signifi cances of the effects of fertilizer treatments, invasiveness status and their interaction are given in Table 4.

ments), as measured in the growth experiment, explained 31.0% of the total variation of these 10 traits, and the sec ond principal component explained a further 22.9%. The first principal component, which was positively associated with above ground biomass, below ground biomass and proportion of germinated seeds, and negatively associated with time to germination and mortality (Fig. 4b), signifi cantly separated invasive species from their respective non invasive congeners (Fig. 4a; paired t test, $t_{12} = 3.67$, $P < 0.003$).
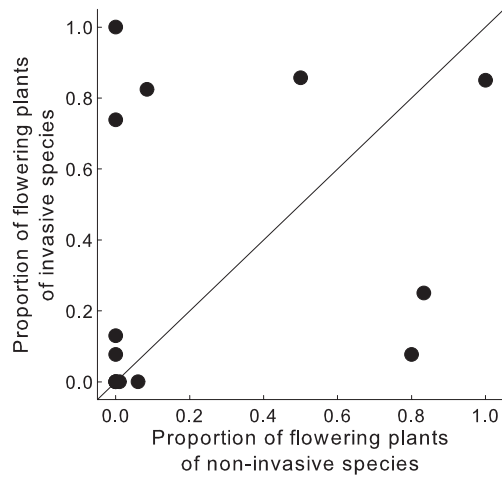
Fig. 3 Proportion of flowering plants of 14 noninvasive (*x* axis) and 14 invasive (*y* axis) species measured in our common garden experiment. Significance of the effect of invasiveness status is given in Table 3. Because the effect of fertilizer treatment was not significant, we averaged the proportion of flowering plants across the fertilizer treatments.

Discussion

Functional species traits in the native range and invasiveness elsewhere

Proportion of germinated seeds and timing of germination are major life history traits that likely play an important role in biological invasions (Baker, 1974). van Kleunen and Johnson (2007) showed that naturalization of South African Iridaceae elsewhere is positively associated with fast and profuse germination. Although our germination experiment revealed no association between proportion of germinated seeds and invasiveness, we found that rapid germination of seeds from the native range is positively associated with invasiveness of the species elsewhere. It is likely that rapid germination increases establishment success after recent disturbance, because it gives species a size advantage early in the season over species that germinate later (van Kleunen & Johnson, 2007), and subsequent asymmetric competition might prevent establishment or reduce growth and reproduction of the later germinating species (Weiner & Thomas, 1986).

Large size is likely to promote competitive ability of plants, and therefore is likely to promote invasiveness (Baker, 1974). Accordingly, invasive species in our experiment produced consistently more above ground biomass than related noninvasive species, and a similar trend was found for below ground biomass. Although invasive and noninvasive species differed significantly in their biomass response to fertilizer addition, invasive species produced more biomass under both fertilization conditions, indicating that the direction of the biomass difference between
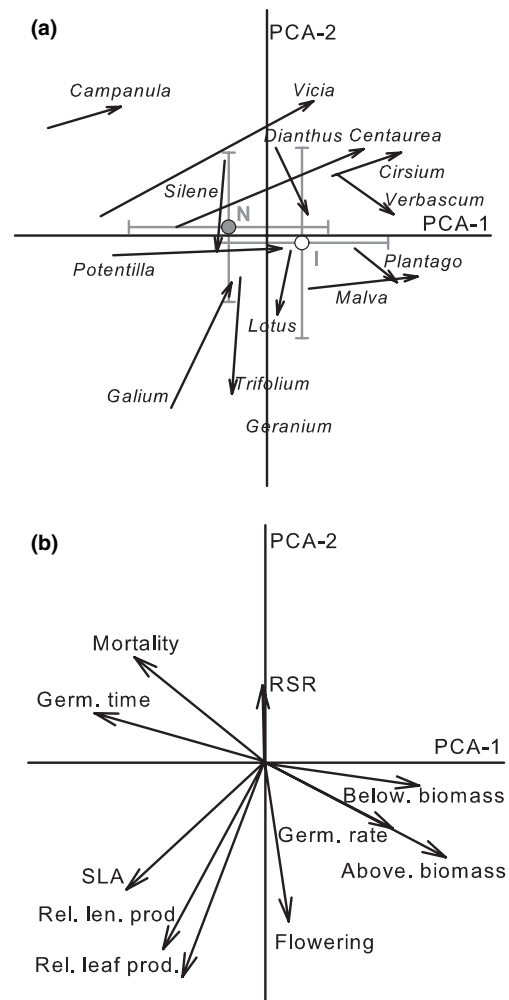

Fig. 4 Principal component analysis (PCA) based on two germination related traits and on eight traits related to biomass allocation and performance. (a) Invasive and noninvasive congeners were separated along the first PCA axis. Arrows denoting genera point from the noninvasive to the invasive congener. Data are based on means of each species across fertilizer treatments. Circles denote centroids ± 1SD (grey) of the invasive (I) and the noninvasive species (N). In the genus *Geranium*, after excluding outliers only the invasive species was included in the analysis; therefore, the label indicates the average of the invasive species of *Geranium*. Components 1 and 2 represent, respectively, 31.0 and 22.9% of total variation. (b) Vector loadings visualize trait associations with principal components as indicated by arrows.

invasive and noninvasive species is consistent across environments. A database study including > 1000 species of South African Iridaceae found that the ones that are naturalized elsewhere are taller in their native range than those that are not naturalized elsewhere (van Kleunen *et al.*, 2007). Similarly, a data base study of European species that are invasive in Canada showed that they are taller than non invasive congeners (Goodwin *et al.*, 1999), and a recent data base study showed that maximum height of North American trees is positively associated with their spread in

Europe (Bucharova & van Kleunen, 2009). Our study adds experimental evidence that species that grow tall and have high biomass production under different environmental conditions in their native range are likely to be pre adapted to become invasive elsewhere.

Traits related to resource acquisition, such as root : shoot ratio and specific leaf area, and leaf production rates are frequently associated with biomass production and plant performance. For instance, the capacity to capture soil resources increases with an increased root : shoot ratio (Hodge, 2004), and the ability to capture solar energy increases with specific leaf area (Wright & Westoby, 2001). Therefore, it is not surprising that, averaged over all species in our experiment, root : shoot ratio decreased, and that leaf production rates increased in response to fertilizer addition, while specific leaf area was not affected. It is remark able, however, that, although invasive species produced more biomass than congeneric noninvasive species, they did not differ significantly in root : shoot ratio, leaf production rates, specific leaf area and plastic responses of these traits in response to addition of fertilizer. This suggests that the higher biomass production of invasive species over noninva sive species is caused by other morphological or physiologi cal traits that we did not measure in our experiment.

It has been suggested that species with a short time to reproduction should be more likely to become weedy and invasive (Baker, 1974). Accordingly, we found that the invasive species, during the one season of our study, had a higher likelihood of flowering than their noninvasive cong eners. Similarly, Rejmánek & Richardson (1996) found in a database study on globally planted *Pinus* trees that a short time to reproduction was one of the major traits distin guishing invasive *Pinus* species from noninvasive ones. Our results on herbaceous species add to the idea that species that require little time to reproduction in their native range are pre adapted to become invasive elsewhere.

Overall, the results of our univariate analyses indicate that invasiveness of species is associated with rapid germination, high biomass production and a higher likelihood of flower ing during the first season. Differences between invasive species and noninvasive congeners were confirmed by their separation along the first principal component of the PCA based on the 10 traits under study (Fig. 4), that is, when taking the covariance among traits into account.

Nevertheless, there was still considerable trait variation among both invasive species and noninvasive species. This indicates that the power to predict invasive species based on traits in their native range is limited. Similarly, Pyšek *et al.* (2009) found in a recent database study that naturalization success of Czech plants elsewhere in the world was only indirectly determined by species traits, via their effects on size of the native range, and that only a small proportion of the variation in worldwide weediness of these plants was directly determined by species traits. Such low predictive power may either be a fundamental phenomenon in plant invasions or it may reflect that there are further biochemi cal, physiological or morphological traits of explanatory power, which have not been considered yet. At present, it is too early to draw final conclusions on this issue because ours is among the first experimental studies to use the native range approach. Therefore, more experimental studies involving native material of species naturalised elsewhere are needed, in particular, studies involving more species and assessing more species traits.

Multi-species comparisons

So far, only few multi species comparative database studies, which are usually restricted to simple traits that have not been assessed under common environmental conditions, have used the native range approach (Goodwin *et al.*, 1999; Prinzing *et al.*, 2002; van Kleunen *et al.*, 2007, 2008; Pyšek *et al.*, 2009). Only one previous multi species experiment compared invasive and noninvasive species in their native range under common environmental conditions, and showed that rapid and profuse germination of South Afri can Iridaceae is associated with their naturalization success elsewhere (van Kleunen & Johnson, 2007). To the best of our knowledge, our study is the first multi species com mon environment experiment to use invasive and noninva sive species from multiple families, and which, in addition to germination related traits, tested whether traits related to biomass allocation, growth and plant performance distin guish invasive from related noninvasive species in their native range (i.e. before introduction elsewhere).

Experimental multi species comparisons have more power to find traits that are generally associated with inva siveness than single species studies, but are still rarely per formed. Nevertheless, when testing for associations between traits and invasiveness among multiple species, as we did, there are several factors that could limit this power and therefore require careful consideration. First, we treated invasiveness as a dichotomous species characteristic, although in reality it is a continuous one. However, when we used a more continuous measure of spread, the number of US states and Canadian provinces in which a species occurs as a measure of invasiveness, all effects of the fixed factors that were significant in the analyses using a dichoto mous invasiveness criterion remained significant (compare Tables 2 4 with Table S3). Second, although all our 28 study species have been introduced to North America and also to other parts of the world, we cannot preclude the pos sibility that species differ in current invasiveness status because of differences in time since introduction rather than in other characteristics (Pyšek & Jarosik, 2005). Indeed, our invasive species have been recorded, on average, 70 yr ear lier in North America according to herbaria data than the noninvasive species (t_{13} = 6.64, P < 0.001; Table 1). How

ever, even when species are introduced at the same time, the ones that spread faster are more likely to be encountered by botanists collecting specimens for herbaria. Consequently, although the year of the earliest herbarium record is fre quently used as a proxy for year of introduction of a species, it might simply reflect invasiveness rather than the true year of introduction (Bucharova & van Kleunen, 2009). Never theless, even when we corrected for time since first record in North America (by adding it as first term in the fixed model of the REML analysis), only the significant difference in time to germination between invasive and noninvasive spe cies disappeared, while the differences in biomass produc tion and flowering remained significant (compare Tables 2 4 with Table S4). Third, we did not control in our study for potential differences in propagule pressure among our study species, which may be one of the impor tant drivers of invasions (Catford *et al.*, 2009; Pyšek *et al.*, 2009). However, because propagule pressure typically increases with time since introduction and our additional analysis showed robust results after correcting for time since first record, we assume that potential differences in propa gule pressure would not invalidate our results. Fourth, in our study, three of the congeneric pairs of invasive and non invasive species differed in life span (Table 1). However, when we excluded these three pairs from the analyses, the differences between invasive and noninvasive species remained significant (compare Tables 2 4 with Table S5). Overall, these additional analyses show that the observed differences in time to germination, biomass production and flowering between invasive and noninvasive congeners are robust with regard to potentially confounding factors. This confirms that experimental multi species studies allow one to determine traits associated with invasiveness.

Native-range approach vs introduced-range approach

The traits measured in the native range that we found asso ciated with invasiveness elsewhere most likely pre adapt spe cies for invading new regions. Other experimental studies compared invasive species to native species or, less fre quently, to noninvasive alien species in the introduced range (reviewed in Pyšek & Richardson, 2007). Such comparisons address partly different but complementary questions. Although we still do not have enough studies to generalize, comparisons between the different approaches might pro vide more insight into the causes of invasiveness than each approach provides separately. For example, differences between outcomes of studies comparing invasive and non invasive alien species using plant material from the intro duced range and outcomes of those using plant material from the native range would indicate the importance of evo lutionary processes for invasions.

The fact that invasive species germinate faster or more profusely is not only found in the native range, but is also repeatedly reported for the introduced range (reviewed in Pyšek & Richardson, 2007). Similarly, high and early reproduction is frequently associated with invasiveness in the introduced range (reviewed in Pyšek & Richardson, 2007). Furthermore, although narrative reviews concluded that biomass is not consistently associated with invasiveness in the introduced range (Daehler, 2003; Pyšek & Richard son, 2007), a recent, more powerful meta analysis showed that invasive species frequently grow larger than native spe cies as well as noninvasive alien species in the introduced range (van Kleunen *et al.*, in press). This indicates that these traits not only pre adapt species to become invasive else where, but also are important for later stages of invasiveness after introduction, and provide an advantage over native species.

We found no associations between invasiveness and traits related to growth rate in the native range, whereas in the introduced range, comparisons of invasive species to native species and to noninvasive alien species revealed such associ ations. Invasive species in the introduced range are fre quently associated with a lower root : shoot ratio (Ehrenfeld, 2003; Wilsey & Polley, 2006), a higher specific leaf area (Grotkopp *et al.*, 2002; Hamilton *et al.*, 2005; Grotkopp & Rejmánek, 2007) and a higher relative growth rate (Grotkopp *et al.*, 2002; Grotkopp & Rejmánek, 2007; Pyšek & Richardson, 2007). Therefore, our results would suggest that these differences in traits related to growth rate in the introduced range are not the result of pre adaptation in the native range but might evolve after introduction.

The patterns emerging from comparisons between the different approaches are still tentative because of the low number of experimental studies comparing invasive and noninvasive species. Moreover, the studies using different approaches may not be directly comparable because they use different species in different regions. Therefore, opti mally, future experimental and database work should com bine native and introduced range approaches.

Conclusions and implications

Traits measured in the native range that pre adapt species to become invasive elsewhere provide valuable knowledge in the context of predicting outcomes of new introductions. Such knowledge is imperative for quarantine authorities that decide whether an alien species can safely be intro duced, and for conservation authorities that allocate funds for control, containment or eradication of recently intro duced species that might become invasive.

Because we compared invasive and noninvasive congen ers, the associations of traits with invasiveness certainly hold when comparing species of the same genus, but not neces sarily when comparing species across genera. For quarantine authorities, this implies that they should always compare traits of species considered for introduction with traits of

already introduced congeneric species and their degree of invasiveness. Interestingly, in our study, even when we removed the taxonomic corrections from our analyses, the shorter time to germination, higher biomass production and higher likelihood of flowering of invasive compared with noninvasive species remained statistically significant (Table S6). This suggests that these traits may be useful for risk assessment even when not explicitly considering a taxo nomic framework.

Acknowledgements

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Supporting Information

The following Supporting Information is available for this article:

Table S1 Analyses of the experiments using generalized maximum likelihood (GML) instead of restricted maximum likelihood (REML)

Table S2 Analyses of the experiments with all data (i.e. including statistical outliers)

Table S3 Analyses of the experiments with invasiveness being estimated by distribution

Table S4 Analyses of the experiments accounting for minimum residence time

Table S5 Analyses of the experiments on a subset of congeners with the same life span

Table S6 Analyses of the experiments omitting taxonomic affinity

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