

Multiple common garden experiments suggest lack of local adaptation in an invasive ornamental plant

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Abstract

Aims

Adaptive evolution along geographic gradients of climatic conditions is suggested to facilitate the spread of invasive plant species, leading to clinal variation among populations in the introduced range. We investigated whether adaptation to climate is also involved in the invasive spread of an ornamental shrub, *Buddleja davidii*, across western and central Europe.

Methods

We combined a common garden experiment, replicated in three climatically different central European regions, with reciprocal transplantation to quantify genetic differentiation in growth and reproductive traits of 20 invasive *B. davidii* populations. Additionally, we compared compensatory regrowth among populations after clipping of stems to simulate mechanical damage.

Important Findings

Our results do not provide evidence for clinal variation among invasive *B. davidii* populations: populations responded similarly to the

different environments, and trait values were not correlated to climatic conditions or geographic coordinates of their home sites. Moreover, we did not detect differences in the compensatory ability of populations.

We suppose that the invasive spread of *B. davidii* has been facilitated by phenotypic plasticity rather than by adaptation to climate and that continent-wide shuffling of cultivars due to horticultural trade may have limited local adaptation so far.

Keywords: *Buddleja davidii* • reciprocal transplantation • biological invasion • geographic clines • climatic conditions

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INTRODUCTION

Invasive, non-native species offer the opportunity to study evolutionary responses to a new environment in a contemporary time frame (Weber and Schmid 1998). To invade a new area, non-native species have to cope with novel environments they are usually not adapted to (Allendorf and Lundquist 2003). Rapid evolution and phenotypic plasticity

are two but not mutually exclusive mechanisms, which may explain successful invasion of these new environments (e.g. Bossdorf *et al.* 2005). Ecologically important evolutionary changes can happen rapidly (Bone and Farres 2001; Thompson 1998), and adaptation to habitats is a common feature of many native plant species (e.g. Becker *et al.* 2006; Joshi *et al.* 2001; Linhart and Grant 1996). There is increasing awareness that the ability for adaptive evolution in novel environments

may also be a key feature of successful plant invaders (Mooney and Cleland 2001; Sakai *et al.* 2001).

Adaptive evolution in invasive species can be important on different spatial scales: First, adaptive divergence may take place between the introduced range of a species and its native range, caused by the novel abiotic and biotic environment the species encounters after introduction (e.g. Bossdorf *et al.* 2005). Second, spread across large geographic gradients within the introduced range can be facilitated by adaptive differentiation among invasive populations ('regional adaptation': Ridley and Ellstrand 2010). At this spatial scale, climate is one of the main driving forces of natural selection and does often lead to geographic clines within plant species (Clausen *et al.* 1940). Clinal variation can be expressed in eco-physiological traits (Anderson *et al.* 1996; Ebeling, Welk, *et al.* 2008) and in life-history traits such as flowering phenology or plant size (Becker *et al.* 2006; Olsson and Agren 2002; Santamaria *et al.* 2003). While most studies on evolutionary changes in invasive plants have compared population differences between the native and introduced ranges, only few studies have investigated geographic clines within the new range (Colautti *et al.* 2010; Keller *et al.* 2009; Kollmann and Banuelos 2004; Leger and Rice 2003; Maron *et al.* 2004b; Montague *et al.* 2008; Ridley and Ellstrand 2010; Weber and Schmid 1998). Adaptive divergence of plant populations can also occur on smaller spatial scales leading to local adaptation to habitats within a region or even to microsites within a habitat (Kawecki and Ebert 2004; Linhart and Grant 1996). Whereas invasion of different environments on such small spatial scales may be facilitated by phenotypic plasticity (Parker *et al.* 2003; Ross *et al.* 2009), spread across large geographic gradients is often suggested to involve adaptive evolution (Montague *et al.* 2008). Hence, the question at which spatial scale plant species are adapted to their environments is not only of general interest to evolutionary biology (Becker *et al.* 2006; Bischoff *et al.* 2006) but also crucial for understanding the mechanisms behind biological invasions.

Local adaptation as result of divergent selection is characterized by higher fitness of resident genotypes compared to genotypes from other habitats ('local vs. foreign' criterion; Kawecki and Ebert 2004) and can be investigated using reciprocal transplant experiments. This approach can also be applied to study adaptive divergence at large-scale geographic gradients (Becker *et al.* 2006; Bischoff *et al.* 2006; Joshi *et al.* 2001). Alternatively, adaptation to particular conditions can be inferred from correlative evidence, if differentiation detected in common garden experiments can be related to the environmental conditions of the populations' home site (e.g. Anderson *et al.* 1996; Kollmann and Banuelos 2004; Weber and Schmid 1998). Common garden experiments have the advantage that a large number of populations can be compared, at the disadvantage that the 'local vs. foreign' effect cannot be rigorously tested. Since genetic and environmental factors often interactively affect the plant phenotype, using just one common garden can produce misleading results

(Williams *et al.* 2008). Nevertheless, studies investigating genetic differentiation among invasive plant populations in more than one environment are still rare (but see Rice and Mack 1991 and Ross *et al.* 2008 for transplantations within the introduced range, Maron *et al.* 2007 and Williams *et al.* 2008 for reciprocal common gardens between continents).

In this paper, we use a multiple common garden experiment to examine genetic differentiation among invasive populations of the Butterfly Bush (*Buddleja davidii*). The shrub species is native to China and was introduced to Europe and other continents for ornamental reasons. Descendants of the cultivated plants are invasive in Europe, Australia, New Zealand and parts of North America (Csurshes and Edwards 1998; Leeuwenberg 1979; Reichard and Hamilton 1997; Tutin 1972; Webb *et al.* 1988). Invasive European *B. davidii* populations perform better than native populations (Ebeling, Hensen, *et al.* 2008) and occur predominantly in oceanic to subcontinental regions from the northern temperate to the Mediterranean climate zone (Rothmaler 2002). We hypothesized that the successful spread of *B. davidii* across different climatic zones in Europe has been facilitated by adaptation to their novel climatic conditions. To test this hypothesis, we compared offspring from 20 invasive *B. davidii* populations sampled across western and central Europe in a common garden experiment. We replicated the common gardens in three central European regions, ranging from an oceanic to a subcontinental climate and incorporated a reciprocal transplantation experiment among the three populations that originate from the places hosting the common gardens. The combination of common garden and reciprocal transplantation experiments has rarely been employed so far (but see Maron *et al.* 2004b; Santamaria *et al.* 2003) and allowed us (i) to investigate genetic differentiation of a large number of populations, (ii) to explicitly consider genotype \times environment interactions among all populations and (iii) to test the 'local vs. foreign' effect among the reciprocally transplanted populations. Moreover, we included a stem clipping treatment to compare compensatory ability of populations because tolerance to damage is known to have a heritable basis (e.g. Agrawal *et al.* 2004; Strauss and Agrawal 1999), and because re-sprouting ability is important for *B. davidii* to recover from damage caused by frost or floods (Smale 1990).

In particular, we asked the following questions:

1. Is there evidence for clinal variation among invasive *B. davidii* populations in traits of growth and reproduction, associated with climatic conditions of the populations' home site?
2. Do the transplanted populations perform better at their local site than 'foreign' populations indicating adaptation to climatic conditions?
3. How does clipping of stems affect plant growth and reproduction, and does its effect vary between populations and among common gardens?

MATERIALS AND METHODS

Study species

The Butterfly Bush (*B. davidii* Franch., Scrophulariaceae) is a 3–5 m tall multi-stemmed shrub. Height and basal diameter growth follow an exponential pattern indicating rapid early growth over the first 15 years (Smale 1990). *B. davidii* is native to China and was introduced about 1890 for ornamental reasons to Europe (see CABI 2009). It flowers from July to September, usually 1 year after germination (Esler 1988) but sometimes already in the same year. The terminal inflorescences are thyrsoid panicles appearing at current-year stems or branches and are up to 30 cm in length (Leeuwenberg 1979; Wu and Raven 1996). *B. davidii* is butterfly pollinated and mainly outcrossing (Schreiter et al. 2011). Each panicle may produce 100 to >1700 capsules (Brown 1990; Kreh 1952) and the small seeds are dispersed by wind or water (Campbell 1984). *B. davidii* prefers naturally or anthropogeni-

cally disturbed areas in the native and invasive range, such as river banks, roadsides and railways (Kunick 1970; Randall and Marinelli 1996) and tolerates a wide range of climatic and soil conditions (Kreh 1952; Webb et al. 1988). A first spread of the species occurred after World War II in bombed and wasted areas of several European cities. The species escaped from gardens also in North America, Africa, Australia and New Zealand has become naturalized or invasive and can now be found on several plant watching lists (Leeuwenberg 1979; Tutin 1972; Webb et al. 1988; http://www.cps-skew.ch/english/eng_index.html, last accessed 23 February 2011).

Experimental design—common gardens and reciprocal transplantation

We carried out a common garden experiment replicated in three central European regions with distances of 316–540 km (Oldenburg and Halle in Germany and Basel in Switzerland; Fig. 1). We selected these three regions because they are

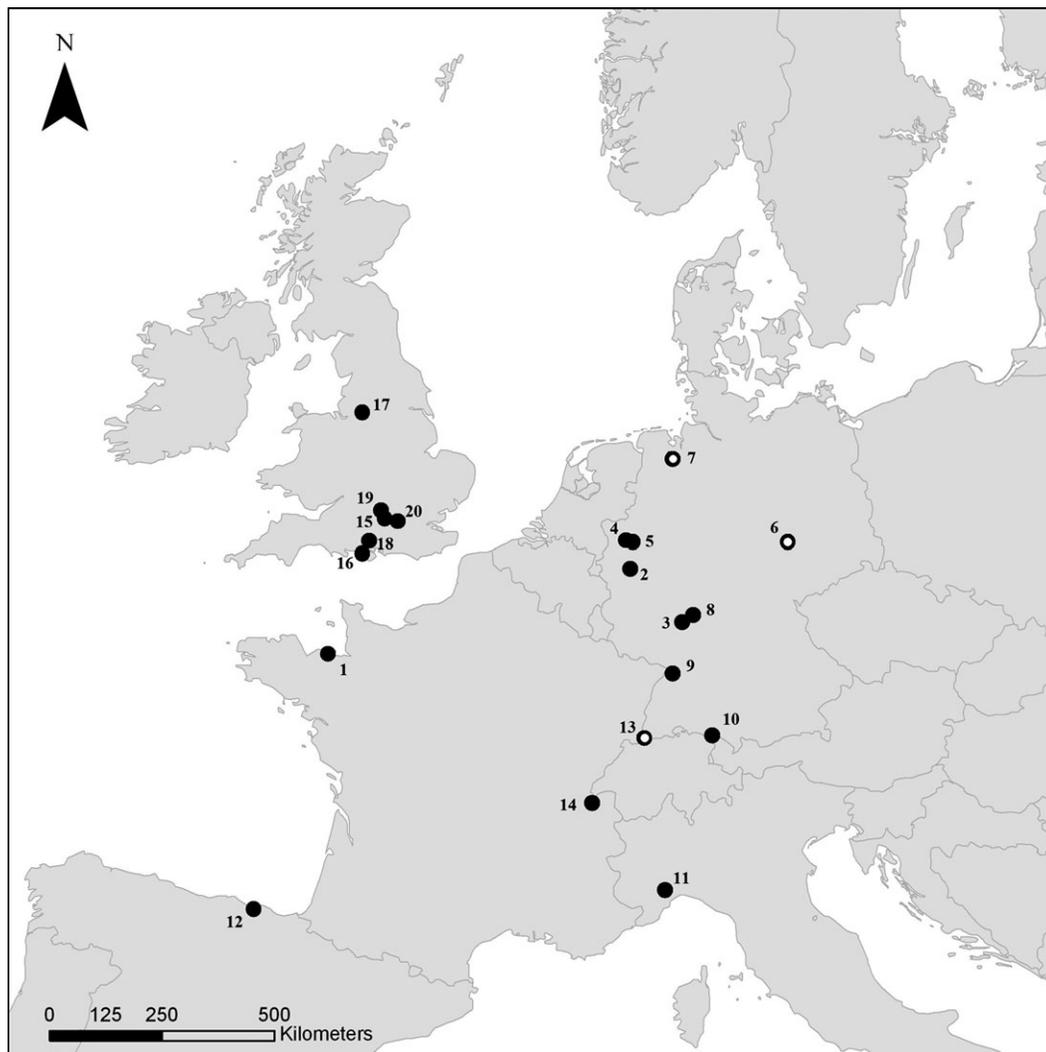


Figure 1: map of the location of the 20 sampled invasive populations of *B. davidii* and the three common gardens (open circles) in Europe.

characterized by strong differences in frequency and abundance of invasive *B. davidii* populations (lowest in Halle and highest in Basel (personal observation), and because they range from an oceanic to a continental climate: According to the climatic stratification of Europe (Metzger *et al.* 2005), Oldenburg is located in the Atlantic-North, Basel in the Atlantic-Central and Halle in the Continental environmental zone. Although our common gardens did not cover the whole range of climatic conditions experienced by invasive *B. davidii* populations, they differed greatly in annual precipitation and growing degree days and to a lesser extent also in minimum and maximum temperatures (Table 1). We used plants derived from seeds sampled in 20 invasive populations across western and central Europe (Fig. 1; Table 1). The latitudinal gradient of sampling ranged from northern Spain (43°) to central England (53°) and the longitudinal gradient ranged from 3°W (northern Spain) to 12°E (eastern Germany), reflecting most of the European range of the species. In April 2005, seeds from five randomly selected individuals per population were germinated separately on potting soil in a greenhouse. In June 2005, when plants were ~10 cm in height, two progeny of each of the five seed families were planted randomly in each of five blocks established in each common garden. Individuals were separated by a distance of 50 cm. To simulate loss of biomass that can be caused by floods and frost, we applied a clipping treatment. In April 2006, just before first leaf flush, we clipped all stems 5

cm above ground of one of the two individuals per maternal plant in each garden using pruning shears. Since this treatment took place before plants started to grow, it should mimic mechanical damage as well as frost damage during winter. Within this common garden experiment, we implemented a reciprocal transplantation experiment in order to test the 'local vs. foreign' criterion (Kawecki and Ebert 2004). For this purpose, one population originating from each of the three places harboring the common gardens was included (Table 1).

In the first year (summer 2005), we quantified flowering phenology by monitoring the beginning of flowering of each plant every other day in the common garden in Halle. In January 2007, we measured several traits concerning plant size (number of stems, diameter of the thickest stem and length of five randomly selected stems per individual) and reproduction (number of inflorescences per individual, length of 20 randomly chosen inflorescences per individual) in all three common gardens. Because of the positive correlation between length of inflorescences and number of capsules (Brown 1990), we considered the number and length of inflorescences as surrogates of reproductive success. Additionally, we harvested all above-ground plant parts and determined reproductive biomass (biomass of inflorescences) and vegetative biomass separately by weighing it after being dried at 60°C. We calculated total above-ground biomass as the sum of reproductive and vegetative biomass and reproductive effort as the

Table 1: list of 20 sampled populations of *B. davidii* in Europe with climatic data according to Hijmans *et al.* (2005)

| No. | Population | Coordinates | | Altitude a.s.l. [m] | T_{Mean} [°C] | T_{Max} [°C] | T_{Min} [°C] | Mean annual precipitation [mm] | Growing degree days [°C] |
|-----|----------------------|-------------|-----------|------------------------|------------------------|-----------------------|-----------------------|-----------------------------------|-----------------------------|
| | | Latitude | Longitude | | | | | | |
| 1 | France/St. Malo | 48.6122 N | 2.0525 W | 40 | 11.23 | 21.0 | 2.8 | 737 | 1748.70 |
| 2 | Germany/Cologne | 50.9627 N | 6.9730 E | 46 | 10.13 | 23.6 | -0.8 | 770 | 1759.53 |
| 3 | Germany/Darmstadt | 49.8894 N | 8.6380 E | 127 | 9.73 | 24.5 | -2.4 | 642 | 1785.53 |
| 4 | Germany/Duisburg | 51.4827 N | 6.7856 E | 30 | 10.12 | 23.2 | -0.5 | 804 | 1701.57 |
| 5 | Germany/Essen | 51.4652 N | 7.0266 E | 37 | 9.91 | 22.7 | -0.6 | 834 | 1650.19 |
| 6 | Germany/Halle | 51.4869 N | 11.9683 E | 99 | 9.10 | 23.4 | -2.2 | 483 | 1643.17 |
| 7 | Germany/Oldenburg | 53.1438 N | 8.2138 E | 68 | 9.95 | 22.8 | -0.6 | 829 | 1514.61 |
| 8 | Germany/Seligenstadt | 50.0402 N | 8.9681 E | 114 | 9.99 | 24.7 | -2.0 | 639 | 1751.77 |
| 9 | Germany/Sulzbach | 48.8630 N | 8.3711 E | 470 | 8.76 | 23.1 | -3.1 | 867 | 1837.58 |
| 10 | Germany/Tett nang | 47.6300 N | 9.5847 E | 447 | 8.90 | 23.4 | -3.7 | 1063 | 1770.67 |
| 11 | Italy/Merano | 46.6667 N | 11.1666 E | 402 | 10.90 | 27.3 | -4.2 | 795 | 826.96 |
| 12 | Spain/Leioa | 43.3277 N | 2.9869 W | 37 | 14.03 | 22.9 | 6.2 | 1203 | 2166.24 |
| 13 | Switzerland/Basel | 47.5472 N | 7.5892 E | 280 | 10.04 | 24.6 | -0.8 | 782 | 1760.70 |
| 14 | Switzerland/Geneve | 46.2083 N | 6.1428 E | 387 | 10.29 | 25.9 | -1.8 | 934 | 1622.17 |
| 15 | UK/Eastleigh | 50.9666 N | 1.3506 W | 15 | 10.51 | 21.9 | 1.3 | 764 | 1590.50 |
| 16 | UK/Egham | 51.4305 N | 0.5467 W | 17 | 10.25 | 23.0 | 0.0 | 651 | 1539.74 |
| 17 | UK/Manchester | 53.4777 N | 2.2456 W | 50 | 10.50 | 21.8 | 1.6 | 932 | 1436.24 |
| 18 | UK/Reading | 51.4527 N | 0.9631 W | 42 | 9.93 | 21.9 | 0.0 | 701 | 1480.54 |
| 19 | UK/Wallingford | 51.6000 N | 1.1258 W | 48 | 9.96 | 21.4 | 0.5 | 657 | 1413.37 |
| 20 | UK/Yarmouth | 50.7027 N | 1.4967 W | 1 | 10.57 | 21.8 | 1.8 | 787 | 1547.22 |

T_{Mean} : mean annual temperature, T_{Max} : mean maximum temperature of the warmest month, T_{Min} : mean minimum temperature of the coldest month, growing degree days: sum of (average day temperatures minus 5°C) from January to August.

ratio of reproductive biomass to total above-ground biomass. Seed mass was assessed by weighing a bulk sample of 100 seeds for each plant, which was divided by 100.

Experimental design—soil experiment

We are fully aware that we considered only climatic conditions using the reciprocal common gardens but ignored other abiotic and biotic conditions that may have influence on plant performance. Since we assume that the differences in soil conditions between the common gardens are also important for the performance of *B. davidii* plants, we carried out an additional greenhouse experiment. Therefore, we sampled the upper soil layer at five randomly chosen locations within each garden and mixed these samples for each garden. In April 2006, we sowed seeds from five individuals per population into 1-l plastic pots filled with soil of each garden. After germination, we reduced offspring to one seedling per maternal plant and kept the total of 300 pots in an unheated greenhouse. During winter, plants were held in a greenhouse at 10°C. In July 2007, we harvested the above-ground biomass and dried it at 60°C for 4 days. In addition, we determined chemical characteristics of the three soils: pH values in 0.1 N KCl solution using a Calimatic pH meter Typ 765 (Knick Elektronische Messgeräte GmbH & Co., Berlin, Germany), C and N concentrations by dry combustion and subsequent gas analysis using an Element Analyser Vario EL (Elementar Analysensysteme GmbH, Hanau, Germany) and conductivity (TetraCon 325 and WTW Cond 315i, Wissenschaftlich-Technische Werkstätten GmbH & Co. KG, Weilheim, Germany).

Statistical analyses

We compared the probability of survival and the probability of flowering between gardens and populations using a generalized linear model with binomial error distribution and logit link function (procedure GENMOD, SAS version 9.1). All other data from the common garden experiments were analyzed with general mixed effect models (procedure MIXED, REML method), with garden and clipping as fixed effects and block nested within garden, population as well as the population \times garden and population \times clipping interactions as random effects. In addition, length of the longest leaf (as a proxy for seedling size) at the start of the experiment had a strong effect on various plant traits at final harvest. We therefore included initial leaf length as a covariate in our analyses in order to account for maternal effects. We, thus, feel confident that our final results were only marginally influenced by the maternal environment of the experimental plants.

Random effects were assessed by comparing the full model with the reduced model using a likelihood ratio test (Littell *et al.* 1996) The same model was applied to the data set including only the three reciprocally transplanted populations from Basel, Halle and Oldenburg. Since the data were unbalanced due to different mortality between gardens, we used type III sum of squares (Shaw and Mitchell-Olds 1993). In case of the length of stem and the length of inflorescence, we used

the mean value of each plant for statistical analysis to avoid pseudo-replication. While reproductive effort was arcsine square-root transformed, the other dependent variables and the covariate were log-transformed to normalize their distribution prior to analysis. *P* values from the model were corrected by Benjamini and Hochberg FDR procedure (Verhoeven *et al.* 2005) to account for multiple comparisons without inflating the likelihood of type II errors (Cabin and Mitchell 2000).

Since flowering phenology was only measured in the common garden in Halle and before the clipping treatment took place, we used the procedure MIXED as described above but without garden and clipping as fixed factors. To compare the effects of the different soils in the additional greenhouse experiment, we used the general linear model procedure with origin of soil as fixed factor. The same procedure was applied to compare soil chemical properties between the three common gardens.

To investigate whether there is a relationship between plant traits and climatic conditions of the populations' home sites, we calculated the population means of each plant trait across the three common gardens and correlated these values with climatic variables, geographic coordinates and altitude of sampling locations using Pearson's product-moment correlation. As climatic variables, we extracted mean annual temperature, mean maximum temperature of the warmest month, mean minimum temperature of the coldest month and mean annual precipitation from <http://www.worldclim.org> (Hijmans *et al.* 2005). Accumulated growing degree days with a base temperature of 5° (January to August; mean values for the period 1971–2000) were obtained at a 10' grid resolution (Mitchell *et al.* 2004; New *et al.* 2000). We suggest these climatic variables to be important because size of *B. davidii* individuals is positively correlated with mean maximum temperature (Ebeling, Hensen, *et al.* 2008), and because precipitation and minimum temperatures vary with continentality of climate. To quantify phenotypic distances among populations, we calculated all pairwise Q_{ST} values of growth and reproductive traits (excluding seed mass) according to Merila and Crnokrak (2001):

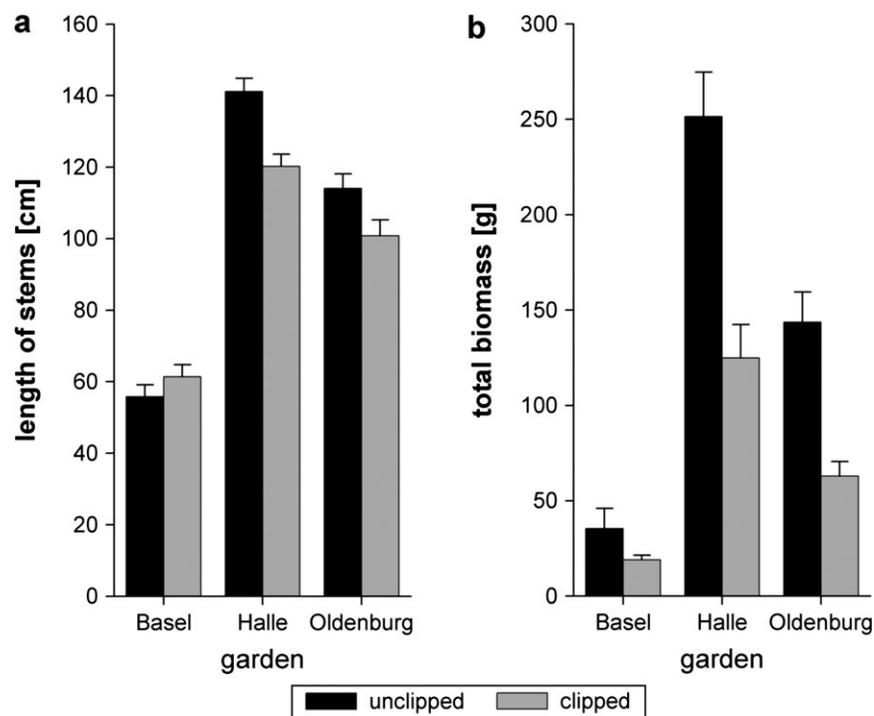
$$Q_{ST} = \frac{\sigma_b^2}{2\sigma_w^2 + \sigma_b^2} \text{ (where } b \text{ means between and } w \text{ means within populations).}$$

Variance components among and within populations were extracted from a separate mixed models on unclipped plants only and without population \times garden interactions. To analyze whether differentiation among populations is due to geographical distance, we correlated Q_{ST} values and geographic distances among populations and used Mantel's *t*-test (R Software) based on Pearson's product-moment correlation to test for statistical significance. It should be noted that any correlation of Q_{ST} with geographic distance does not allow disentangling neutral processes (isolation by distance) from local adaptation, since geographic distance is in our case confounded with environmental gradients.

Table 2: summary of mixed model analysis of covariance for 20 invasive *B. davidii* populations, planted in three common gardens in Oldenburg, Halle and Basel

| | d.f./error d.f. | <i>F</i> value (for fixed effects) or log likelihood ratio (for random effects) | | | | | | | | |
|--------------------------------|-----------------|---|--------------|-----------------|-------------------|----------------------|---------------------|-----------------------|--------------------------|-----------|
| | | Growth-related traits | | | | Reproductive traits | | | | |
| | | Total biomass | No. of stems | Length of stems | Diameter of stems | Reproductive biomass | Reproductive effort | No. of inflorescences | Length of inflorescences | Seed mass |
| Fixed effects | | | | | | | | | | |
| Garden | 2/12 | 52.95*** | 15.28*** | 92.21*** | 60.92*** | 37.01*** | 15.84*** | 15.85*** | 25.91*** | 8.37** |
| Clipping | 1/19 | 38.61*** | 4.00 | 4.34 | 60.11*** | 19.24** | 0.04 | 4.02 | 0.03 | 1.53 |
| Garden × clipping | 2/38 (35) | 4.12* | 1.29 | 4.36* | 11.45*** | 6.28 | 1.10 | 1.13 | 2.28 | 0.06 |
| Random effects | | | | | | | | | | |
| Covariate | 1 | 31.60*** | 3.70 | 2.80 | 0.90 | 4.60*** | 0 | 6.40* | 0 | 0 |
| Block (garden) | 1 | 34.20*** | 0 | 0 | 0 | 7.70*** | 0 | 0 | 0 | 4.10 |
| Population | 1 | 3.80 | 4.00*** | 0.30 | 0 | 2.10 | 10.60*** | 3.60 | 3.60 | 3.50 |
| Garden × population | 1 | 0 | 0 | 0 | 0 | 0.30 | 0 | 0.60 | 0.40 | 0 |
| Clipping × population | 1 | 0 | 0.20 | 0 | 0 | 0.50 | 1.70 | 0.60 | 0.60 | 0 |
| Garden × clipping × population | 1 | 0 | 0 | 0 | 0 | 0 | 0.60 | 0 | 0 | 0 |

Length of longest leaf at the beginning of the experiment was used as covariate. Numbers in brackets indicate error degrees of freedom for reproductive traits. *F* values are given for fixed effects, while log likelihood ratios are given for random effects (levels of significance: * $\alpha < 0.05$, ** $\alpha < 0.01$, *** $\alpha < 0.001$, after applying the Benjamini–Hochberg procedure to growth-related and reproductive traits in order to account for multiple comparisons).

**Figure 2:** comparison of (a) length of stems and (b) total biomass (mean + SE) of *B. davidii* between clipped and unclipped gardens at three different sites.

RESULTS

Differences among common gardens

Mortality of *B. davidii* differed significantly between the three common gardens (log likelihood ratio = 34.19, $P < 0.001$, d.f. = 2). After 19 months, mortality was highest in the common garden in Basel (28%), lowest in Halle (6.5%) and intermediate in Oldenburg (16%). Across the three common gardens, 20.7% of all plants flowered already in 2005, i.e. within the first year after germination. The probability of flowering in the first year was significantly different between the gardens (log likelihood ratio = 183.56, $P < 0.001$, d.f. = 2) and was lowest in Basel (0.05%), intermediate in Oldenburg (7%) and largest in Halle (52.5%). In the second year after germination, 83% of all surviving plants flowered. Flowering probability was lowest in Basel (71%), intermediate in Oldenburg (78%) and highest in Halle (96%; log likelihood ratio = 48.13, $P < 0.0001$, d.f. = 2).

Plant size at the beginning of our experiment (measured as length of the longest leaf) significantly influenced biomass and number of inflorescences at final harvest (Table 2). Our study populations differed significantly in all growth and reproductive traits among the gardens, as growth conditions were apparently best in the common garden in Halle and worst in Basel. For example, plants in Halle and Oldenburg produced on average 85% and 73% more biomass than plants growing in Basel (Fig. 2b). Similar differences were detected in reproductive traits, i.e. plants in Halle and Oldenburg developed more (78% and 58%), larger (31% and 20%) and heavier (89% and 78%) inflorescences than plants in Basel.

Response to clipping

Clipping of stems in spring was not completely compensated during the following growing period, as total biomass, stem diameter and reproductive biomass were significantly smaller in clipped plants compared to untreated plants. The difference between unclipped and clipped plants in total biomass and stem diameter was smallest in Basel (46% and 10% reduction, respectively), intermediate in Halle (50% and 30% reduction) and largest in Oldenburg (59% and 39% reduction). In contrast, stem length was reduced by 17.4% and 13.2% in Halle and Oldenburg, respectively, whereas clipped plants overcompensated slightly in terms of stem length in Basel (+8.9%; Fig. 2a). The significant garden \times clipping interactions in these growth-related traits indicate that compensatory ability of plants was strongly dependent on growing conditions of each site.

Similarly, there was a significant garden \times clipping interaction with respect to probability of flowering in the second year (log likelihood ratio = 10.11, $P = 0.006$, d.f. = 2): On average, probability of flowering among unclipped plants was lower than among clipped plants in Basel (66.7% vs. 75.8%), higher than among clipped plants in Oldenburg (87.8% vs. 68.3%) and nearly equal to them in Halle (97.9% vs. 94.6%).

Among-population variation and relationship to climatic gradients

Plant mortality did not vary among populations (log likelihood ratio = 27.38, $P = 0.096$, d.f. = 19), but populations differed significantly in their probability of flowering in the first year after germination (log likelihood ratio = 59.05, $P < 0.001$, d.f. = 19). For plants growing in Halle, flowering started on average 133.8 ± 2.2 days (mean \pm SE) after germination and differed significantly between populations ($F_{19,83} = 1.90$, $P = 0.025$). In the second year, flowering probability did no longer vary among populations. Populations did not differ in their response to clipping or to garden environments, neither in mortality nor in the probability of flowering.

At final harvest, populations differed significantly in the number of stems and reproductive effort, whereas no difference among populations in all other traits could be measured (Fig. 3, Table 2). In general, variation among populations as measured by the coefficient variation in plant traits (8.5% $<$ CV $<$ 49.0%) was much smaller than phenotypic plasticity in the same traits as measured by the coefficient of variation among the three gardens (25.2% $<$ CV $<$ 80.8%), except in seed mass, where variation was larger among populations than among gardens (11.7% vs. 6.0%). There was also no difference in the response of the 20 populations to the different common gardens, as indicated by the non-significant garden \times population interaction. We did not find significant correlations between any traits of growth or reproduction and climatic variables or geographic coordinates of the populations' home sites ($-0.41 < r < 0.43$, $n = 20$). Q_{ST} values among all 20 populations ranged from 0.0222 (in reproductive biomass) to 0.100 (length of inflorescences). Again, there was no significant relationship between Q_{ST} value of any trait and geographic distance among populations ($-0.0015 < r < 0.174$).

Considering the three populations reciprocally transplanted between the three places, we did not detect significant garden \times population interactions in any trait ($0.75 < P < 1.0$), which would be expected in case of local adaptation of these three populations (i.e. if the 'local vs. foreign' effect would be significant).

Response to soil conditions

In the greenhouse experiment, soil conditions significantly affected plant performance, especially the biomass ($F_{2,3} = 20.18$, $P < 0.001$). Plant biomass was, on average, lowest in the soil from Oldenburg (5.18 ± 0.42 g, mean \pm SE), intermediate in the soil from Halle (8.04 ± 0.35 g) and highest in the soil from Basel (8.48 ± 0.42 g). This contrasts the results of the common garden, as non-clipped plants growing in Halle and Oldenburg produced much more biomass (251.52 ± 23.27 g and 143.68 ± 15.76 g) than plants in Basel (35.38 ± 10.61 g). Our soil analysis showed that there are significant differences between sites in terms of pH values, C_{total} and N_{total} (Table 3). All three soils show a small C/N ratio indicating a high

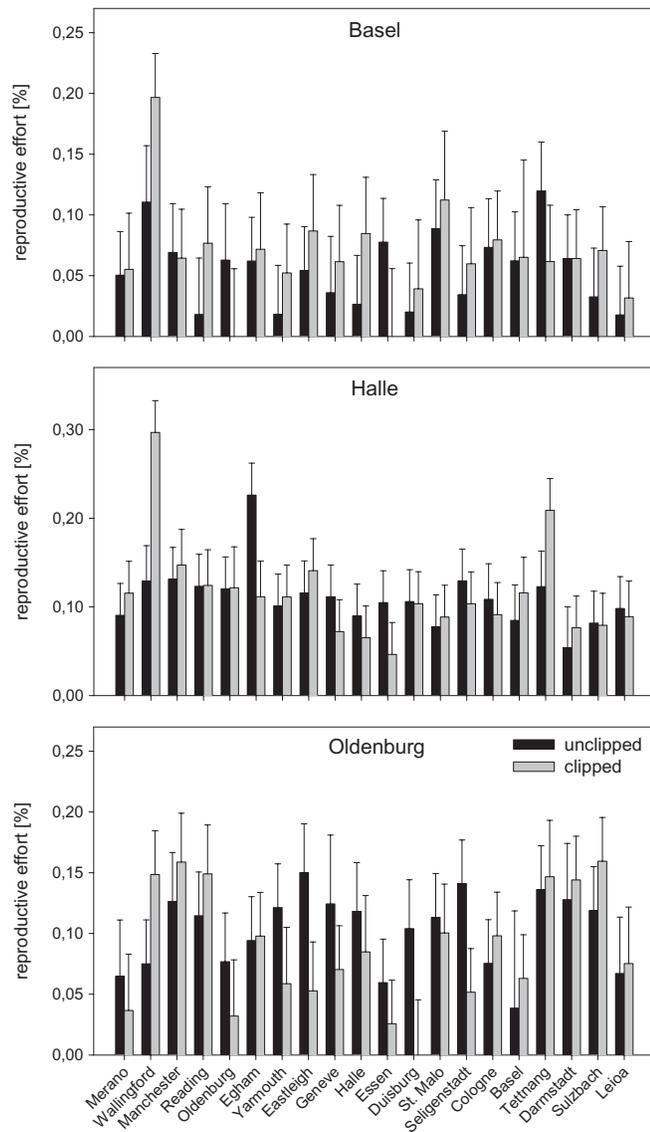


Figure 3: reproductive effort of *B. davidii* populations of 20 different origins in the three common gardens. Clipping and non-clipping treatments are shown for each population (mean \pm SD). The x -axis is in descending order of growing degree days [sum of (average day temperatures minus 5°C) from January to August].

Table 3: comparison of soil chemical properties of the three common gardens (mean \pm SE; degrees of freedom for F -tests: 2, 3; levels of significance: ** α < 0.01, *** α < 0.001)

| Garden | pH (in KCl) | C_{total} | N_{total} | Conductivity [μS] |
|-----------|-----------------|--------------------|--------------------|--------------------------------|
| Basel | 8.16 \pm 0.08 | 1.24 \pm 0.06 | 0.08 \pm 0.00 | 323 \pm 47 |
| Halle | 7.84 \pm 0.13 | 1.99 \pm 0.04 | 0.16 \pm 0.02 | 359 \pm 34 |
| Oldenburg | 5.92 \pm 0.60 | 3.64 \pm 0.16 | 0.27 \pm 0.01 | 241 \pm 38 |
| F value | 107.06*** | 58.38 *** | 86.22*** | 6.83** |

availability of nitrogen. The conductivity varied to some extent between gardens, but the differences were not significant.

DISCUSSION

The results of our study provide some evidence for genetic differentiation in phenotypic traits among invasive populations of *B. davidii* of central and west Europe. Environmental conditions of the three common gardens, however, had a much stronger impact on growth and reproduction. In contrast to our expectation, all populations responded in a similar way to the different physical environments of the common gardens suggesting a lack of adaptation to climate in *B. davidii* populations from different parts of Europe. Moreover, we found no evidence for clinal variation among the invasive *B. davidii* populations because none of the plant traits measured was correlated with the location or the climatic conditions of the populations' home sites. Even the reciprocal transplantation experiment, which was incorporated in the common gardens, revealed no evidence for the 'local vs. foreign' criterion. Clipping of stems strongly affected plant growth and reproduction, but there was again no difference between populations in their compensatory ability. Altogether, these findings do not support our hypothesis that adaptation to climatic conditions has facilitated the spread of invasive *B. davidii* populations in Europe, which is consistent with previous data on frost hardiness of these populations (Ebeling, Welk, *et al.* 2008).

One aim of our common garden experiments was to discover genetic differentiation among populations by reducing the environmental component of variation. We used open-pollinated seeds sampled at different field sites rather than seeds produced under identical conditions (Falconer and MacKay 1996). Phenotypic variation among populations may therefore overestimate genetic differentiation because it involves a genetic and a maternal environmental component. Maternal environment becomes often manifest in seed characters which, in turn, may influence germination and seedling growth (Roach and Wulff 1987; Rossiter 1996). Seed mass of maternal plants used for our experiments indeed varied considerably among the 20 populations (mean \pm SD 0.039 \pm 0.007 mg, range 0.030–0.057 mg, CV = 17%), as the variance among populations contributed 37.5% to the total variance among individuals ($z = 2.41$, $P = 0.008$). In contrast, offspring seed mass in the common gardens did not differ significantly between populations, suggesting that variation detected in the field-sampled seeds was primarily due to environmental effects. We are therefore confident that variation in initial seedling size did also reflect maternal environmental effects, and that the use of seedling size as covariate in statistical analyses did not mask genetic variation among populations.

The fact that we did not find any evidence for local adaptation is in a remarkable contrast to results of some previous studies, which used a similar sample size of populations and seed families and found strong evidence for the evolution of geographic clines in invasive plant species, e.g. in *Solidago altissima* and *Solidago gigantea* (Weber and Schmid 1998), *Hypericum perforatum* (Maron *et al.* 2004a), *Impatiens glandulifera* (Kollmann and Banuelos 2004), *Eschscholzia californica*

(Leger and Rice 2007), *Lythrum salicaria* (Montague et al. 2008), *Hypericum canariense* (Dlugosch and Parker 2008), *Senecio inaequidens* (Monty and Mahy 2009), *Silene vulgaris* and *Silene latifolia* (Keller et al. 2009), and *Raphanus sativus* (Ridley and Ellstrand 2010). Furthermore, a recent meta-analysis on common garden experiments revealed latitudinal clines for 14 out of 34 invasive plant species (Colautti et al. 2009), suggesting that adaptive evolution in the introduced range may be an important feature of successful plant invaders.

The contrasting results on invasive *B. davidii* populations may be attributed to the short time period since the first invasive spread of the species in Europe. *B. davidii* was introduced about 1890, while its first spread started after World War II. Several studies have demonstrated that plants can evolve in response to recent environmental conditions within few generations (Bone and Farres 2001; Davison and Reiling 1995; Thompson 1998). Perhaps, the selection regime for invasive populations of *B. davidii* imposed by climate is not as strong as in other well-known studies of local adaptation, e.g. in response to heavy metals or herbicide application (Bone and Farres 2001). However, this would not explain why adaptation along climatic gradients has been found in other native and invasive plant species (Becker et al. 2006; Kollmann and Banuelos 2004; Weber and Schmid 1998).

Another cause why local adaptation is apparently absent in *B. davidii* populations could be that response to selection requires sufficient genetic variation in relevant traits. Since invasive plant species were often introduced in small numbers, genetic bottlenecks and drift may reduce their potential for adaptive evolution (Allendorf and Lundquist 2003; Colautti et al. 2010; Novak and Mack 1993). However, we found substantial genetic variation in some plant traits, at least among populations. Genetic diversity in introduced species may increase due to multiple introductions, hybridization as well as artificial selection and random processes (Allendorf and Lundquist 2003; Ellstrand and Schierenbeck 2000; Mooney and Cleland 2001; Ross et al. 2009). Plant breeding probably has an important impact on invasion success of non-native species by changing ecologically important traits and by promoting genetic differentiation among populations (Kitajima et al. 2006). *B. davidii* was introduced as an ornamental plant, and breeders focused on leaf and flower color, inflorescence morphology, growth and frost hardiness (Albrecht 2004; Wilson et al. 2004). The more than 90 cultivars which are currently available (Stuart 2006) may thus provide sufficient genetic variation for an evolutionary response to natural selection.

We suggest that large-scale horticultural trade with these cultivars, combined with frequent plantings in gardens and parks, may have impaired local adaptation in invasive *B. davidii* populations. Due to the popularity of *B. davidii*, it is a reasonable assumption that the cultivars are permanently shuffled by horticultural trade among the different European regions. This may have important consequences for the genetics of the invasive populations. First, it is likely that invasive populations of

B. davidii consist of progeny of multiple cultivars. Depending on frequency and residence time of cultivars provided on the market, the probability of an escape from cultivation rises (Dehnen-Schmutz et al. 2007). Thus, variation among invasive *B. davidii* populations may simply reflect genetic differences between cultivars haphazardly planted at each location and that gave rise to the invasion. Second, gene flow into a population can impose a limit to local adaptation of that population (Kawecki and Ebert 2004; Lenormand 2002). In particular, if populations at range margins experience novel environmental conditions, then genetic swamping from central populations—or in this case from planted cultivars—would lead to maladapted populations and might prevent local adaptation (Bridle and Vines 2007). We suggest that these causes are also responsible for the lack of correlation between Q_{ST} values and geographic distance among invasive *B. davidii* populations. The absence of such correlation indicates that gene flow and selection or drift are not at equilibrium (as elaborated for the geographic pattern of neutral genetic variation: Hutchison and Templeton 1999), which may be due to the short history of introduction of *B. davidii* together with horticultural trade and founder effects. Remarkable are the differences in the compensatory ability among populations to recover from clipping. The artificial clipping was designed to mimic heavy damage by wind, water or frost, which *B. davidii* is apparently able to tolerate by regrowth of new stems (Smale 1990). Moreover, gardeners recommend severe pruning of the bush in spring and removal of old wood to enhance growth of new basal stems and to produce denser and larger inflorescences (Armitage and Dirr 1995; Miller 1984; Ream 2006). Our results demonstrated that clipped plants nearly fully compensate the length of stems. This is neither mirrored by reproductive biomass nor by total biomass, which is nearly reduced by the half. Nevertheless, this compensatory ability is probably an important characteristic explaining the success of the species, even after natural or anthropogenic damage.

Apart from climatic effects, the significant differences in plant growth which we have revealed between the three common gardens might also be produced by soil differences. The three gardens showed differences in soil characteristics, which may have been mirrored by the performance of *B. davidii*. However, our additional greenhouse experiment showed that the plant growth in the common gardens was not correlated with the growth in the different soils in the greenhouse; moreover, the rank order of the three sites or soils, respectively, was reversed. Thus, we feel confident that the differences among the common gardens are not simply the result of the different soils but reflect differences between the whole set of abiotic and biotic factors. In accordance with Maron et al. (2004b) and Williams et al. (2008), we again emphasize that further studies on the importance of adaptive evolution for invasion success should incorporate multiple common garden experiments.

In conclusion, our study revealed no hints for adaptation to climatic conditions among invasive European *B. davidii*

populations. Based on the similar and strong response of all 20 populations to the different environments and to the clipping treatment, we speculate that high phenotypic plasticity of these populations allows them to grow across a wide range of climatic and soil conditions, thus contributing to the successful spread of the species on a geographic scale. Phenotypic plasticity itself has a genetic basis (Schlichting 1986) and is considered to be a key characteristic of colonizing or invasive species (Baker 1974; Fraser *et al.* 1965; Richards *et al.* 2006). Indeed, phenotypic plasticity has been found to contribute to the successful spread of several invasive plant species but usually on smaller spatial scales (Parker *et al.* 2003; Ross *et al.* 2009). Although our experimental data do not allow conclusions about fitness consequences of plasticity in *B. davidii*, our suggestion is supported by the large coefficient of variation among gardens (as measure of phenotypic plasticity) compared to the relatively small variation among populations (as a measure of genetic differentiation).

A more general implication of our study is that future research on evolutionary changes in invasive species should pay more attention to ornamental plants. First, they constitute a major part of the invasive flora worldwide: for instance, 52% of the naturalized alien plant species in Europe had been introduced for ornamental or horticultural purpose (Lambdon *et al.* 2008) and 82% of invasive woody plants in the United States had been used in landscaping (Reichard and White 2001). Second, besides their numerical contribution to invasive floras, ornamental plants have usually been strongly affected by artificial selection. Plant breeding often changes ecological traits that are also known for successful invaders, e.g. seed production (Kitajima *et al.* 2006) or resistance to frost, pathogens and insects (Auer 2008). We therefore suggest that cultivars of introduced plant species rather than non-manipulated genotypes from the native range play a role in the evolution of invasiveness. Third, as already outlined, horticultural trade may lead to a colonization history of ornamentals, which is different from that of spontaneously spreading species. However, only very few studies have been published so far on the colonization history and genetic structure, and the roles of plant breeding and adaptive evolution in invasive ornamental plants (Clarke *et al.* 2006; Culley and Hardiman 2007; Dlugosch and Parker 2008; Kitajima *et al.* 2006; Ross *et al.* 2008, 2009). Together with our results, these studies suggest that artificial selection and horticultural trade have to be taken into account in order to understand the mechanisms behind successful invasion of many introduced plant species.

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