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No evidence for local adaptation in an invasive alien plant: field and greenhouse experiments tracing a colonization sequence

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- *Background and Aims* Local adaptation enables plant species to persist under different environmental conditions. Evolutionary change can occur rapidly in invasive annual species and has been shown to lead to local adaptation. However, the patterns and mechanisms of local adaptation in invasive species along colonization sequences are not yet understood. Thus, in this study the alien annual *Impatiens glandulifera* was used to investigate local adaptation to distinct habitats that have been consecutively invaded in central Europe.
- *Methods* A reciprocal transplant experiment was performed using 15 populations from alluvial deciduous forests, fallow meadows and coniferous upland forests, and a greenhouse experiment was performed in which plants from these habitats were grown under treatments reflecting the main habitat differentiators (shade, soil acidity, competition).
- *Key Results* Biomass production, specific leaf area, plant height and relative growth rate differed between habitats in the field experiment and between treatments in the greenhouse, but not between seed origins. Overall, there was no indication of local adaptation in either experiment.
- *Conclusions* Since *I. glandulifera* is a successful invader in many habitats without showing local adaptation, it is suggested that the species is coping with environmental variation by means of high phenotypic plasticity. The species seems to follow a 'jack-and-master' strategy, i.e. it is able to maintain high fitness under a wide range of environmental conditions, but performs particularly well in favourable habitats. Therefore, the proposed colonization sequence is likely to be based primarily on changes in propagule pressure. It is concluded that invasive alien plants can become dominant in distinct habitats without local adaptation.

Key words: Biological invasions, colonization history, general-purpose genotype, greenhouse experiment, home site advantage, invasive alien plant, *Impatiens glandulifera*, jack-and-master strategy, local adaptation, phenotypic plasticity, propagule pressure, reciprocal transplant experiment.

INTRODUCTION

Environmental variability causes opposing selection pressures and therefore favours genetic adaptation of plant species. Adaptation, in turn, enables plant species to persist in a set of different environmental conditions (Leimu and Fischer, 2008). If adaptation has taken place, resident genotypes will have a higher relative fitness than foreign ones ('local vs. foreign' criterion; Kawecki and Ebert, 2004). While at large scales climatic differences are important for adaptation (Macel *et al.*, 2007), at local scales distinct habitat characteristics might be more relevant (Hereford and Winn, 2008), e.g. soil conditions (Raabová *et al.*, 2011), shade (Godoy *et al.*, 2011) or biotic interactions (Grøndahl and Ehlers, 2008). Many studies have found evidence for adaptation in plant species (e.g. Becker *et al.*, 2008; Hufford *et al.*, 2008; see meta-analyses by Leimu and Fischer, 2008; Hereford, 2009), but others have not (e.g. Leiss and Müller-Schärer, 2001; Hereford and Winn, 2008; Ebeling *et al.*, 2011; Garrido *et al.*, 2012). Recently, progress has been made in explaining the mechanisms influencing local adaptation (e.g. Leimu and Fischer, 2008; Hereford, 2009; Lopez *et al.*, 2009), but it is still a challenge to understand the underlying patterns and drivers of adaptation in plants.

In invasive alien species, evolutionary change can occur rapidly (Maron *et al.*, 2004; Bossdorf *et al.*, 2005). Although there are several mechanisms that are believed to inhibit adaptation, e.g. low genetic variability (Taylor and Keller, 2007), local adaptation of invasive alien plants to distinct habitat types has been demonstrated repeatedly (e.g. Scott *et al.*, 2010; Godoy *et al.*, 2011). Sax *et al.* (2007) suggested that invasive alien species can be used as model organisms for studying ecological and evolutionary processes in real time. Therefore, invasive alien species are a suitable study system to investigate the evolution of local adaptation.

Local adaptation can broaden species' ecological niches. This is particularly important in secondary invasions (Dietz and Edwards, 2006). According to Dietz and Edwards (2006), plant invasions occur in two stages. During the primary invasion, alien species establish in habitats with the highest propagule pressure, e.g. along transport corridors, while in the secondary invasion additional habitats with distinct environmental conditions are colonized. These two stages do not have to be entered consecutively, but in many plant invasions the most accessible habitats are colonized first, before secondary invasion to new habitat conditions takes place. We expect local adaptation in the secondary invasion to be more pronounced for early-invaded habitats due to longer residence time. When different habitats have been colonized consecutively, a sequence of local adaptation can be studied along this colonization sequence. For example, Erfmeier *et al.* (2011) found a shift in life history strategy during secondary invasion of a deciduous tree, suggesting on-going adaptation to less favourable habitats. However, there are two possible alternative explanations for secondary invasion in invasive alien plants. First, the species' ecological niche can also be broadened by high phenotypic plasticity (Dietz and Edwards, 2006; Moloney *et al.*, 2009). Invasive plants can profit from high phenotypic plasticity in morphological and physiological traits by two main strategies (Richards *et al.*, 2006): the 'jack-of-all-trades' strategy is able to maintain high fitness in a set of distinct habitats (general-purpose genotype; Baker, 1965), while the 'master-of-some' strategy can increase fitness in especially favourable habitats (e.g. Sultan, 2001). Second, changing patterns of local propagule pressure may also contribute to secondary invasion. Propagule pressure depends mainly on the distance to (Rouget and Richardson, 2003) and the size of donor populations (Richardson and Pyšek, 2006), i.e. older, larger populations are more likely to donate propagules to other sites. Land use alteration can further change temporal patterns in propagule pressure through alterations of disturbance regimes and transport pathways.

A prominent invasive alien plant species that has colonized distinct habitats in Europe over a long time period is *Impatiens glandulifera*. In the invaded range, this species frequently occurs in near-natural habitats, primarily in riparian habitats, fenland, mesotrophic grassland and deciduous woodland (Andrews *et al.*, 2005). *Impatiens glandulifera* is a suitable species to study local adaptation to different habitats because it is an outcrossing annual with potentially fast evolution (Beerling and Perrins, 1993). Previous work showed that *I. glandulifera* exhibits latitudinal trends in growth which might reflect an adaptation to the length of the growing season (Kollmann and Bañuelos, 2004). In the congeneric *I. capensis*, potential to develop local adaptation was shown, especially with regard to shade (Dudley and Schmitt, 1995) and density (Donohue *et al.*, 2001). Additionally, Walker *et al.* (2009) found substantial genetic variation in *I. glandulifera* in northeast England using microsatellite analysis, and Zybartaite *et al.* (2011) revealed four major genetic groups of populations in Lithuania using randomly amplified polymorphic DNA.

Historical reconstructions suggest that *I. glandulifera* colonized different near-natural habitats consecutively in the past 100 years, starting from settlements and riparian habitats (Pyšek and Prach, 1995). In the Czech Republic, for example, the species was first recorded in riparian habitats in 1900, in fallow meadows in 1934 and in forests in 1941 (Pyšek and Prach, 1995). Rivers act as dispersal corridors and it took about 20 years from the first occurrence of the species on main rivers until invasion proceeded upstream along tributaries and laterally away from the rivers (Malíková and Prach, 2010). Invasion in the Czech Republic is still in progress and expected to continue (Malíková and Prach, 2010). Accordingly, first records from southern Germany date to the first two decades of the 20th century (Hegi, 1925–1965) and it can be assumed that habitat colonization in Germany has progressed in a similar way as in the Czech Republic. Deciduous forests along rivers were most probably invaded first, whereas colonization of fallow meadows and coniferous upland forests, which are spatially separated from riverine habitats, started later. These three habitats differ mainly with regard to shade, soil acidity and competition. Forest habitats are characterized by moderate to high shade, while fallow meadows are mainly open. Soils in coniferous forests are usually more acid compared with alluvial deciduous forests and fallow meadows. Competition among herbs is more intense in fallow meadows and alluvial deciduous forests compared with coniferous forests with sparse herb layers. These contrasting habitat conditions should favour local adaptation.

We conducted a reciprocal transplant experiment in southern Germany to test for local adaptation in *I. glandulifera* to three habitat types along a colonization sequence consisting of alluvial deciduous forests, fallow meadows and coniferous upland forests. Additionally, we manipulated shade, soil acidity and competition as main habitat differentiators in a factorial greenhouse experiment to extract the ecological factors that are likely to lead to local adaptation. Our main aim was to test for local adaptation in *I. glandulifera*. More specifically we hypothesized (1) higher fitness of local origins when reciprocally sown in the three habitats in the field ('home habitat advantage'). Based on habitat characteristics, we expected that in the greenhouse (2a) under high shade, low soil acidity and high competition, plants originating from alluvial deciduous forests have higher fitness compared with other origins; (2b) under low shade, low soil acidity and high competition, plants from fallow meadows have highest fitness; and (2c) under high shade, high soil acidity and without competition, plants from coniferous upland forests have the highest fitness. We further predicted local adaptation to be most pronounced in origins from alluvial deciduous forests, followed by those from fallow meadows and those from coniferous upland forests.

MATERIALS AND METHODS

Study species

Impatiens glandulifera (Balsaminaceae) is a herbaceous annual species that was introduced from the Himalaya to Europe as an ornamental plant in the 19th century (Beerling and Perrins, 1993), and has become abundant with considerable impact in 19 European countries within latitudes 30–64 ° N (Kollmann and Bañuelos, 2004). It is common in open and shaded habitats in lowland and lower montane areas (<800 m a.s.l.), but occurs in the Alps up to 1550 m altitude (Kollmann and Bañuelos, 2004). *Impatiens glandulifera* grows up to 3 m tall, and the basal diameter of the stem can reach 5 cm (Beerling and Perrins, 1993). Germination takes place from February to April. The flowering period is from July to October, and the seeds disperse by dehiscent seed-capsules between August and October (Ammer *et al.*, 2011). They are transported over long distances through human activities and water dispersal (Hartmann *et al.*, 1995). The species has no clonal growth and a short-lived seed bank (Beerling and Perrins, 1993).

Study area and source populations

Seeds of *I. glandulifera* were collected in the region of Freising, southern Germany (study area: 48·39–48·45 °N, 11·65–11·88 °E, ~140 km^2, 366–506 m a.s.l.) in three different habitats, i.e. alluvial deciduous forests, fallow meadows and coniferous forests on nearby hills, each with five replicate populations to cover variability within habitats (hereafter 'source populations'). The alluvial deciduous forests were located in the floodplain of the River Isar. *Impatiens glandulifera* populations in this habitat were rather continuous and situated close to the main river channel as well as along forest roads. The tree layer was dominated by *Fraxinus excelsior* mixed with *Acer pseudoplatanus* and *Salix alba* (height 20–30 m), leading to deep shade (canopy cover 70–90 %). The understorey had 60–80 % cover of herbs and shrubs, mainly *Aegopodium podagraria* and *Rubus caesius*. The soil was moderately moist with neutral reaction. The fallow meadows occurred at plane to slightly inclined locations on loamy and moist soils with neutral reaction. *Impatiens glandulifera* populations in this habitat were rather separated by more intense land use around the patches. The vegetation was characterized by tall herbs and grasses, including *Arrhenatherum elatius*, *Galium mollugo* and *Phalaris arundinacea* (cover 80–100 %) with little shade from trees or shrubs. The coniferous upland forests were old-grown spruce plantations on sandy and less moist soils with slightly acidic reaction in the tertiary hills around Freising, with modest slopes under variable orientation. *Impatiens glandulifera* populations in this habitat were less dense than in the other two habitats, and patches were mostly continuous with small gaps in between. The canopy consisted of 20–30 m tall *Picea abies* leading to moderate shade (cover 60–80 %). The herb layer was sparser than in the other habitats (cover 30–50 %), including mosses, *Oxalis acetosella*, *Rubus fruticosus* agg. and young plants of *Quercus robur* and *Acer pseudoplatanus*. The three habitats were all relatively nutrient-rich, while there was a pronounced gradient in soil acidity (see Supplementary Data Table S1 for further information).

The first specimen from the greater study region stored in the two most important herbaria of the federal state is dated to 1909 (Munich) and originates from a riverine site ~85 km upstream of the study area, situated at River Isar, which runs through the study area. It is assumed that invasion in the study area first covered habitats along River Isar and the tributary River Amper before it proceeded to habitats outside the floodplains. Small tributaries as well as roads are most likely to have served as secondary colonization corridors. From Rivers Isar and Amper, colonization most probably first reached fallow wet meadow habitats, often situated close to tributaries, while colonization of upland coniferous forests began later and is still in progress. The distances between source populations were 5·7 ± 2·9 km (mean ± s.d., accordingly throughout the article) for deciduous forests, 5·3 ± 2·2 km for fallow meadows and 7·5 ± 4·1 km for coniferous forests, and did not differ within habitats (ANOVA, $F = 1{\cdot}19$, $P = 0{\cdot}32$; see Supplementary Data Table S1 for distances to the closest source population overall and within each habitat).

Annual average temperature in the study region is 7·5 °C and annual precipitation 788 mm (Weihenstephan 1961–1990; Bayerische Landesanstalt für Landwirtschaft, 2012). Monthly mean temperature during the experiment (March–August 2012) was 2 °C higher than the long-term average (1961–1990). Precipitation from March to May 2012 was 54 mm, i.e. 29 % less than normal, while from June to August 2012 it was 125 mm (42 % more than normal in 1961–1990).

Seed material

In each population in the three habitat types we took two to five ripe capsules from 75 randomly chosen plants in September 2011 and again in October 2011 to account for possible temporal differences in seed quality. Seeds were dried at room temperature for 3 weeks and stored at 5 °C for 2 months prior to seed mass determination and stratification. Average seed mass of the plant material used was 13·6 ± 0·9 mg for populations in the deciduous forest, 12·8 ± 0·9 mg in the fallow meadows and 11·8 ± 1·3 mg in coniferous forest ($n = 5 \times 500$ seeds per source population and $n = 5$ populations per habitat). Seeds were cold-wet stratified on filter paper in Petri dishes and stored at 3 °C. Seed germination rate after 3 weeks (5/15 °C, 12:12 h, without light) was 73 ± 11 % for populations from deciduous forests, 93 ± 4 % for fallow meadows and 79 ± 12 % for coniferous forests ($n = 5 \times 50$ seeds per source population and $n = 5$ populations per habitat).

Reciprocal seed transplant experiment

In mid-March 2012, we established one experimental plot (0·8 m × 1·6 m) in close proximity to each of the 15 source populations (see Supplementary Data Fig. S1A for the experimental design). The 15 plot sites were chosen to be similar to the source populations, but free of *I. glandulifera* with a buffer zone of >2 m. The distances between plot and source population ranged from 34 to 962 m, with values of 279 ± 358 m for deciduous forests, 69 ± 39 m for fallow meadows and 379 ± 226 m for coniferous forests. Distances were not different within habitats (Kruskal–Wallis test, $P = 0{\cdot}075$; Supplementary Data Table S2).

In one half of each plot (0·8 m × 0·8 m) soil remained untreated ('undisturbed soil'). In the other half all aboveground litter and vegetation were removed and the soil was disturbed with a rake (10 cm deep) 1 week before sowing ('disturbed soil'). This treatment was included to cover variability within habitats and meant to simulate disturbance by wild animals, e.g. boars. Each half of each plot was divided into 16 subplots. The subplot size (0·2 m × 0·2 m) was chosen based on observed plant densities in the source populations and experiences from a preliminary study in 2011. While one of them remained as a control, 20 seeds of each of the source populations were sown into the other subplots. Subplots were equipped with plastic rings (diameter 10 cm, height 3 cm) that were gently pushed into the soil to prevent seed losses. Nevertheless, we found germination in 7 % of the control subplots, which we consider to have been caused by accidental dispersal from the other subplots. After 7 weeks, seedlings were thinned to a maximum of five per subplot to avoid bias due to intraspecific competition. In deciduous forests, 4 ± 4 seedlings per subplot (across all subplots within the habitat, but excluding controls) were removed, 6 ± 4 in fallow meadows and 1 ± 2 in coniferous forests. The removed seedlings were used to determine aboveground dry biomass per plant (after drying for 3 days at 70 °C).

All plants were harvested after 20 weeks in August 2012. Biomass was used as a proxy for fitness since aboveground biomass and seed production of annual species are often correlated (e.g. Thompson *et al.*, 1991; Shipley and Dion, 1992). In addition, specific leaf area (SLA), plant height and relative growth rate (RGR) were measured to detect plastic plant responses to the main habitat differentiators, i.e. shade, soil acidity and competition. One individual per subplot was chosen randomly to measure height. Three fully developed leaves of the same plant were photographed to determine SLA with the software ImageJ 1·46 (Schneider *et al.*, 2012), and dried afterwards. SLA was calculated as $\mathrm{SLA} = A\,W_L^{-1}$, where A is area and W_L is dry mass of the three selected leaves (Cornelissen *et al.*, 2003). We harvested aboveground biomass of all *I. glandulifera* individuals and determined dry biomass per plant. Mean RGR per subplot was calculated as $\mathrm{RGR} = [\ln(W_2)n_2^{-1} - \ln(W_1)n_1^{-1}]\,(t_2 - t_1)^{-1}$, where W_1 and W_2 are the aboveground dry biomasses of n individuals harvested at times t_1 (week 7) and t_2 (week 20), respectively, in each subplot. As suggested by Hoffmann and Poorter (2002), biomass was natural logarithm-transformed before averaging.

Greenhouse experiment

In the same time period, a greenhouse experiment was conducted at the Dürnast Research Centre (located within the study area; www.wzw.tum.de/ghl/) to identify the environmental factors potentially leading to local adaptation. Treatments included shade, soil acidity and competition, giving a full factorial design with a total of eight treatments, including all 15 source populations.

For the shade treatment, plants were grown under a single or a double layer of green fabric, resulting in ~10 % and 5 %, respectively, photosynthetic active radiation (PAR) in relation to full sunlight, which corresponds to moderate to deep shade, for example in coniferous forests (8·7 ± 4·9 % PAR) and deciduous forests (2·5 ± 1·9 % PAR; Supplementary Data Table S2). As the shade treatment was expected to alter not only light availability but also air humidity and temperature, we performed two shade treatments rather than comparing shade with no shade.

To manipulate soil acidity, commercial peat (Floragard Floratorf; $\mathrm{pH_{CaCl_2}}$ 3·0–4·0; nitrogen, phosphate and potassium oxide <0·05 kg m^{-3} each) was mixed with fertilizer (Ferty® 2; nitrogen 0·15 kg m^{-3}, phosphate 0·05 kg m^{-3}, potassium oxide 0·25 kg m^{-3}, magnesium oxide 0·02 kg m^{-3}) and different concentrations of lime. To achieve a moderately low pH treatment, 6 kg m^{-3} pelleted lime (concentration 50 %) was applied, resulting in $\mathrm{pH_{CaCl_2}}$ 5·1. For neutral substrate, 14·5 kg m^{-3} pelleted lime (50 %) and 10 kg m^{-3} fine lime (95 %) were used. Additionally, $\mathrm{Ca(OH)_2}$ (0·3 %) was added with watering to achieve $\mathrm{pH_{CaCl_2}}$ 6·5; the $\mathrm{pH_{CaCl_2}}$ in the two treatments increased during the experiment to 6·4 and 7·2, respectively. The high pH treatment was comparable to the alluvial deciduous forests considered, where $\mathrm{pH_{H_2O}}$ was around 7·3 (Supplementary Data Table S1) which corresponds to a $\mathrm{pH_{CaCl_2}}$ of ~6·8 (after the Sillanpää equation: $\mathrm{pH_{CaCl_2}} = 1{\cdot}044\mathrm{pH_{H_2O}} - 0{\cdot}808$; Budoi *et al.*, 2003). The low pH treatment was comparable to fallow meadows with $\mathrm{pH_{H_2O}}$ 6·9, which corresponds to a $\mathrm{pH_{CaCl_2}}$ of 6·4. Plot sites had comparable soil reactions (Supplementary Data Table S2).

For the competition treatment, *I. glandulifera* seedlings were planted alone or together with five individuals of *Arrhenatherum elatius*. This grass species is known to be a good competitor and has been used in competition experiments for a long time (e.g. Mahmoud and Grime, 1976). Additionally, it grows in one of the habitats considered, i.e. fallow meadows. The grass seedlings were germinated from regional seed material (Rieger-Hofmann GmbH) and introduced 21 days before the target plants to create sufficient competition.

Seeds of *I. glandulifera* were pre-germinated on a standard growing substrate in multipots. In March 2012, cotyledon length of all seedlings was measured. Forty seedlings of each of the source populations were selected at random and potted individually (pot diameter 19 cm, volume 0·003 m^3). Remaining seedlings (minimum of 35 per habitat) were used to determine starting aboveground biomass. Plants were exposed to the eight treatments with five replicates, giving a total of 600 plants. The pots were arranged in five rows (see Supplementary Data Fig. S1B for the experimental design). Half of each row was covered with a double layer of green fabric (high shade), the other half with a single layer (low shade). Each row contained eight blocks. All blocks contained one plant of each source population. Within the two shade levels, the soil acidity and competition treatments were randomized, i.e. each row contained a randomized arrangement of one block per treatment with only the light levels being grouped together. To avoid edge effects, pots were randomized and rotated within the blocks, and blocks of the same shade treatment were rotated within rows once.

After 8 weeks (May 2012), three plants per source population were randomly selected from each treatment; the others were kept for further experiments. Aboveground biomass, plant height and SLA were determined as in the field experiment. To calculate RGR, plant dry mass W_1 was estimated based on the correlation between cotyledon length (x) and starting aboveground biomass of remaining seedlings at the beginning of the experiment (for deciduous forest populations, Pearson correlation, $W_1 = 0{\cdot}10x + 0{\cdot}04$, $r = 0{\cdot}41$, $P = 0{\cdot}004$; for fallow meadows, $W_1 = 0{\cdot}12x - 0{\cdot}06$, $r = 0{\cdot}70$, $P < 0{\cdot}001$; and for coniferous forest, $W_1 = 0{\cdot}24x - 0{\cdot}38$, $r = 0{\cdot}60$, $P < 0{\cdot}001$). Additionally, aboveground dry biomass of *A. elatius* per pot was determined in the competition treatment.

Statistical analysis

All statistical analyses were performed with R 2·15·1 (R Core Team, 2012), using the packages lme4 (Bates *et al.*, 2012) and multcomp (Hothorn *et al.*, 2009). For the reciprocal transplant experiment we fitted linear mixed effects models using maximum likelihood separately for the two treatments (undisturbed soil, disturbed soil) and the different response variables (biomass, SLA, plant height and RGR of *I. glandulifera*). Full models contained seed origin, habitat and their interaction. A significant interaction between seed origin and habitat would indicate an adaptation of *I. glandulifera* to the local environmental conditions (Van Groenendael, 1985; Leiss and Müller-Schärer, 2001). Seed mass and seedling emergence were included as covariates to partially control for maternal effects and varying intraspecific competition before the thinning to five seedlings per subplot after 7 weeks. We added source population nested in habitat and plot nested in habitat as crossed random factors to account for the spatial structure of seed sources and the experimental plot design.

To analyse the effects of seed origin and the eight treatments in the greenhouse on biomass, SLA, plant height and RGR of *I. glandulifera*, we also used linear mixed effects models fitted with maximum likelihood. We included seed origin, shade, soil acidity and competition with *A. elatius* and all possible two-way and three-way interactions as fixed factors. Seed mass and grass biomass (competition treatment) were included as covariates to partially control for maternal effects and variation in competition. Source population nested in habitat and block nested in rows within the shade treatment were included as crossed random factors to reflect the spatial component of seed origin and experimental design.

We simplified all models (field and greenhouse experiments) stepwise backwards based on likelihood ratio tests and removed non-significant fixed factors. Model checking plots were inspected to ensure that model assumptions were met. Biomass was natural logarithm-transformed to improve model fitting. No further transformations were necessary. Finally, we calculated *post hoc* Tukey contrasts for all significant factors with more than two levels in the minimum adequate models.

RESULTS

Plant performance in the field

We could not detect any influence of seed origin on aboveground biomass, SLA, plant height and RGR of transplanted *I. glandulifera* in the field experiment (Table 1). The response of all origins was very similar within each habitat (Fig. 1A–D and Supplementary Data Fig. S2A–D).

In the undisturbed soil treatment, SLA was the only measured trait that was affected significantly by habitat (Table 1): transplants in deciduous forests revealed the highest SLA (Tukey contrasts against both fallow meadows and coniferous forests $P < 0·001$), followed by those in coniferous forests (against fallow meadows $P = 0·002$) and fallow meadows (Fig. 1C). In the disturbed soil treatment, plant performance varied considerably across habitats and the influence of habitat was significant for all measured traits (Table 1). Plants transplanted to fallow meadows produced significantly more aboveground biomass than those in the two forest habitats (Tukey contrasts against deciduous forest $P = 0·004$, against coniferous forests $P > 0·001$) and did not differ between deciduous and coniferous forests ($P = 0·107$; Fig. 1B). SLA was similarly high in deciduous and coniferous forests ($P = 0·864$) and significantly lower in fallow meadows (Tukey contrast against both forest habitats $P < 0·001$; Fig. 1D). Height of plants in coniferous forests was significantly lower (Tukey contrasts against deciduous forests $P = 0·006$, against fallow meadows $P < 0·001$), but comparable between deciduous forests and fallow meadows ($P = 0·614$; Supplementary Data Fig. S2B). RGR was significantly higher in fallow meadows than in deciduous forests (Tukey contrast $P < 0·001$; Supplementary Data Fig. S2D).

Seedling emergence in the plots was 44 ± 29 % in deciduous forests (for all subplots together, but excluding the controls), 54 ± 26 % in fallow meadows and 14 ± 21 % in coniferous forests. The response of all five source populations of the same origin was similar for each habitat and treatment, and in all models the population factor within origin explained less than 5 % of the variance of the random factors. The plot site within a habitat explained some of the variance in most models (0–47 %), but most variance of the random factors remained unexplained.

Plant performance in the greenhouse

In the greenhouse experiment, seed origin had a significant influence on biomass production (Table 2). Nevertheless, there was no clear pattern and no better performance of each origin in the treatment reflecting its original habitat conditions (Fig. 2A, B). Including all treatments, biomass was highest for plants from coniferous forests (6·5 ± 4·0 g) and lowest for those from fallow meadows (5·6 ± 3·9 g; Tukey contrast $P = 0·001$). However, the maximum value for biomass was achieved by plants from fallow meadows (17·4 g). Biomass of plants from deciduous forests was in an intermediate range (6·0 ± 3·3 g) and was not significantly different from either fallow meadows ($P = 0·086$) or coniferous forests ($P = 0·385$). Biomass was additionally affected by soil acidity and the interaction of shade and competition (Table 2). Plants produced little biomass under high shade also in the absence of competitors,

TABLE 1. *Influence of seed origin, habitat and their interaction on aboveground biomass, specific leaf area (SLA), plant height and relative growth rate (RGR) of the invasive alien* Impatiens glandulifera *in a reciprocal transplant experiment in the invaded range in central Europe*

	Biomass		SLA		Height		RGR	
	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Undisturbed soil								
Origin	3·73	0·155	2·04	0·362	1·56	0·459	0·60	0·741
Habitat	1·41	0·493	**9·58**	**0·008**	3·34	0·188	2·82	0·245
Origin × habitat	2·77	0·596	7·17	0·127	2·97	0·563	7·58	0·108
Disturbed soil								
Origin	0·63	0·729	1·29	0·525	1·59	0·452	3·70	0·157
Habitat	**9·30**	**0·010**	**8·95**	**0·011**	**8·35**	**0·015**	**4·47**	**0·035**
Origin × habitat	1·95	0·745	7·36	0·118	1·14	0·887	0·97	0·617

χ^2- and *P*-values are based on maximum likelihood ratio tests for linear mixed effects models. χ^2- and *P*-values of non-significant factors refer to the respective step of the model simplification procedure. Significant terms were tested against the minimum adequate model. See Materials and methods section for information on random factors and covariates. For sample size see Fig. 1 and Supplementary Data Fig. S2.

Significant values ($P < 0·05$) are shown in bold type.

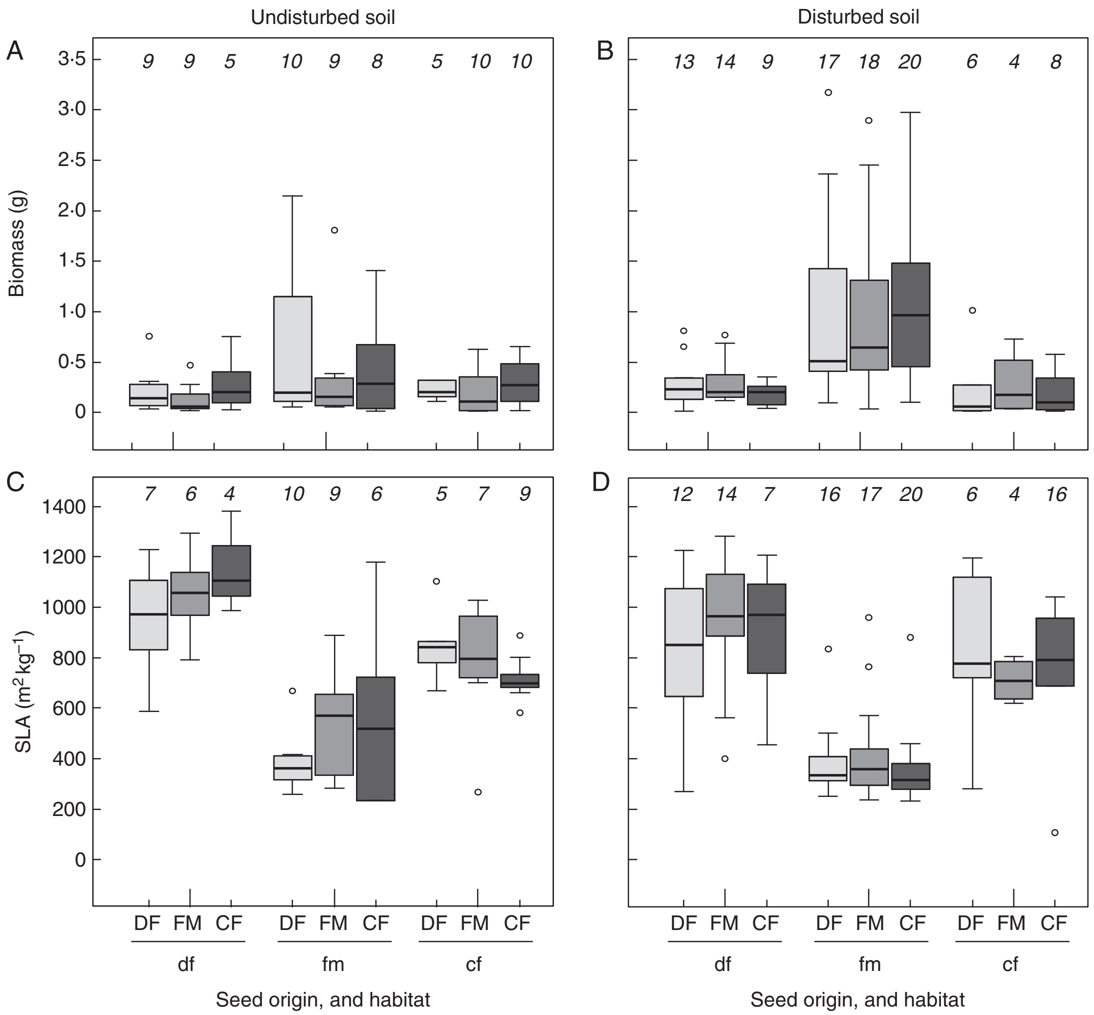

FIG. 1. Above-ground biomass (A, B) and specific leaf area (C, D) of the invasive alien *Impatiens glandulifera* when reciprocally transplanted between alluvial deciduous forests (df/DF), fallow meadows (fm/FM) and coniferous forests (cf/CF) in the invaded range. Seed origins are indicated with capital letters, plot habitats with lower-case letters. Plots remained either untreated (A, C) or were experimentally disturbed before planting (B, D). The number of plant individuals in each group is given in small italic numbers above the boxplots.

TABLE 2. *Effects of seed origin, shade, soil acidity, competition and their pairwise interactions on aboveground biomass, specific leaf area (SLA), plant height and relative growth rate (RGR) of the invasive* Impatiens glandulifera *in a greenhouse experiment*

	Biomass		SLA		Height		RGR	
	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Origin	**6·74**	**0·034**	3·46	0·177	n.a.	n.a.	**7·84**	**0·020**
Shade	n.a.	n.a.	**14·11**	**< 0·001**	n.a.	n.a.	**37·56**	**< 0·001**
Soil acidity	**4·04**	**0·045**	1·24	0·265	n.a.	n.a.	**6·43**	**0·011**
Competition	n.a.	n.a.	0·30	0·585	**9·80**	**0·002**	1·03	0·310
Origin × shade	4·70	0·096	4·79	0·091	**6·03**	**0·049**	4·69	0·096
Origin × soil acidity	1·17	0·558	0·86	0·650	**8·42**	**0·015**	1·60	0·450
Origin × competition	2·19	0·335	1·15	0·562	0·38	0·829	0·58	0·750
Shade × soil acidity	3·07	0·080	0·07	0·796	1·30	0·255	0·17	0·300
Shade × competition	**5·73**	**0·017**	1·09	0·296	0·34	0·560	0·93	0·334
Soil acidity × competition	0·01	0·912	0·20	0·652	2·25	0·134	0·02	0·891

χ^2- and *P*-values are based on maximum likelihood ratio tests for linear mixed effects models. χ^2- and *P*-values of non-significant factors refer to the respective step of the model simplification procedure. Significant terms were tested against the minimum adequate model. See Materials and methods section for information on model simplification, random factors and covariates. $N = 15$, except five cases where only 14 replicates were available. Main factors included in a significant interaction were not further explored (n.a., not assessed). All three-way interactions were not significant (not shown).

Significant values ($P < 0·05$) are shown in bold type.

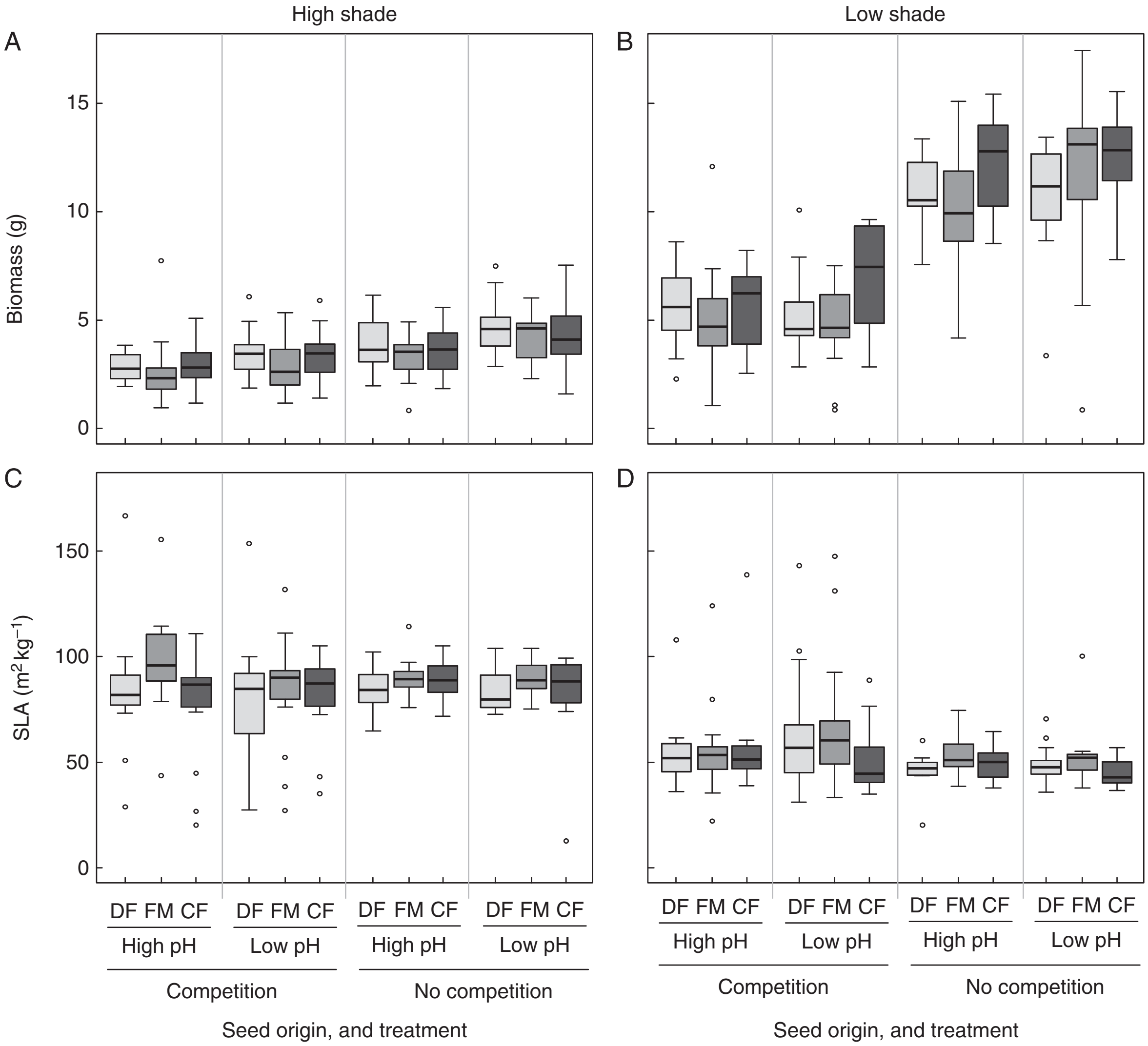

FIG. 2. Above-ground biomass (A, B) and specific leaf area (C, D) of invasive populations of *Impatiens glandulifera* in a greenhouse experiment. Plants were exposed to eight treatments in a full factorial design, including high and low shade, competition by a common grass species (*Arrhenatherum elatius*) and no competition, as well as low and high soil acidity. Plant material originated from three habitat types, i.e. alluvial deciduous forests (DF), fallow meadows (FM) und coniferous forests (CF). Most groups represent 15 replicates, except five cases where only 14 replicates were available.

while plants grown under low shade produced remarkably more biomass when released from competition (Fig. 2A, B).

For all origins, SLA was significantly higher in the high-shade treatment irrespective of additional treatments and origin (Table 2; Fig. 2C, D). Plants grown without competitors were generally taller than those grown under competition (Table 2; Supplementary Data Fig. S3A, B). Additionally, height was significantly increased under low shade, especially for plants from coniferous forests (significant origin × shade interaction, Table 2). Plants from coniferous forests and fallow meadows grew taller under low compared with high pH, while plants from alluvial deciduous forests were taller under high pH (Fig. 3; significant origin × soil acidity interaction, Table 2). In all treatments, RGR of plants originating from fallow meadows and coniferous forests did not differ ($P = 0{\cdot}503$), but RGR was smaller for deciduous forest origins compared with fallow meadows ($P < 0{\cdot}001$) and coniferous forests ($P = 0{\cdot}027$; Supplementary Data Fig. S3C, D). RGR was significantly higher for plants grown under low shade or high soil acidity (Table 2; Supplementary Data Fig. S3C, D).

All five source populations of the same origin responded similar to the experimental treatments in the greenhouse, and in all models the population factor explained <5 % of the variance of the random factors. The experimental block within row within shade treatment explained some of the variance in all models (0–14 %), but most variance of the random factors remained unexplained.

DISCUSSION

Explaining the lack of local adaptation

We could not find any indication of local adaptation of the invasive alien *I. glandulifera* to three distinct habitats, i.e. alluvial deciduous forests, fallow meadows and coniferous forests. Neither an interaction between origin and habitat nor higher fitness of

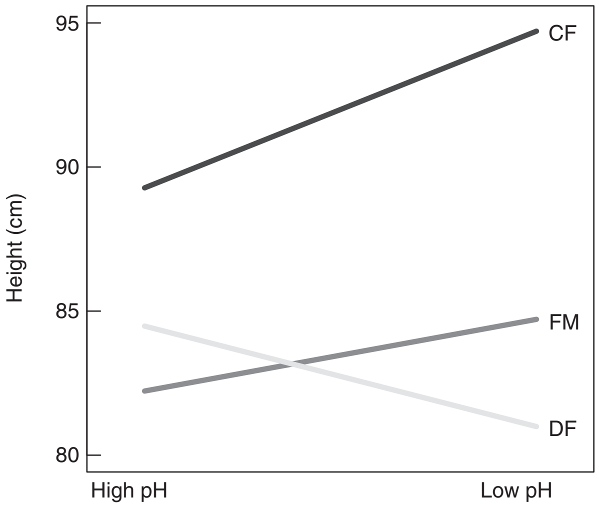

FIG. 3. Plant height of invasive populations of *Impatiens glandulifera* in a greenhouse experiment affected by a significant interaction ($\chi^2 = 8{\cdot}42$, $P = 0{\cdot}015$, for methods see text) between pH and seed origin, i.e. alluvial deciduous forests (DF), fallow meadows (FM) und coniferous forests (CF). Graphs were computed pooling all treatments.

local origins emerged when reciprocally sown to the three habitats in the field (Hypothesis 1), nor did the experimental treatments reflecting the three habitat types in the greenhouse favour the respective provenances (Hypothesis 2a–c). Thus, we could not explain the proposed colonization sequence by different degrees of local adaptation (Hypothesis 3). The lack of local adaptation found in adult plants seems to be consistent for other phases of the study species' life cycle. Our results support the observations by Skálová *et al.* (2012), who found least local differentiation in seedling traits of *I. glandulifera* when compared with congeneric *I. parviflora, I. capensis* and *I. noli-tangere* under controlled climate chamber conditions.

Performance of *I. glandulifera* in the reciprocal field experiment was overall rather poor. Aboveground biomass in fallow meadow plots with disturbed soil treatment reached values comparable to those of a field study conducted in the Czech Republic (Skálová and Pyšek, 2009), while most other values were actually lower. SLA was comparable to values observed in a field study in England (~370–1000 $\text{cm}^2\,\text{g}^{-1}$; Andrews *et al.*, 2009), slightly exceeding them in deciduous forests and slightly falling below them in fallow meadows. Plant height was at the lower margin of values reported from England (Andrews *et al.*, 2005). In the greenhouse experiment, values of biomass and plant height were smaller than in a previous common garden experiment (Kollmann and Bañuelos, 2004).

There are several reasons why an invasive alien species may lack local adaptation. Based on the results of our study, three lines of arguments seem to be relevant. First, residence time in the new range might have been too short (Ross *et al.*, 2009; Haider *et al.*, 2010; Ebeling *et al.*, 2011). *Impatiens glandulifera* was introduced to England as early as 1839 (Beerling and Perrins, 1993), and the first herbarium specimen from a river ~85 km upstream from the study area dates back to the beginning of the 20th century. Other studies, however, found adaptation in annual invasive species over comparable time scales, e.g. in *Eschscholzia californica*, with a residence time in the invaded range of 110–150 years (Leger and Rice, 2007). Still, we cannot exclude that residence time may have been too short until now and local adaptation might evolve in future. Second, it is commonly assumed that high gene flow prevents the evolution of locally adapted genotypes (Haider *et al.*, 2011; 2012). *Impatiens glandulifera* is self-compatible but protandrous and thus is frequently cross-pollinated by several species of bumblebees, honeybees and wasps (Bartomeus *et al.*, 2010). Pollinators are capable of transferring pollen over several kilometres (Walker *et al.*, 2009), thus enabling long-distance gene flow. Beside pollination, effective seed dispersal can increase gene flow. At the local scale, seeds of *I. glandulifera* are dispersed up to 6 m by exploding fruits (Chapman and Gray, 2012), but long-distance dispersal via waterways (maximum 20 km; Wadsworth *et al.*, 2000), vehicles and contaminated soil is also common (Hartmann *et al.*, 1995). Long-distance pollen transfer and seed dispersal suggest effective gene flow in *I. glandulifera*, which probably counteracts local adaptation. Third, strong spatial and temporal fluctuations in populations can act against local adaptation. Although we have no data on the persistence of *I. glandulifera* populations in our study area, this idea is supported by molecular studies in northeast England that suggest frequent local extinction, re-colonization and repeated anthropogenic dispersal in populations of *I. glandulifera* (Walker *et al.*, 2009).

Reasons for the success of I. glandulifera in distinct habitats

Despite the observed lack of local adaptation, *I. glandulifera* was performing well in all studied habitats (see Supplementary Data Table S1 for plant height in the source populations). The most likely reason why the species is able to cope with distinct habitats without showing local adaptation is high phenotypic plasticity (Pigliucci, 2001), which might enable the species to expand its ecological niche (Richards *et al.*, 2006). It has been shown recently that *I. glandulifera* exhibits higher plasticity in seedling biomass, height and root–shoot ratio than the less invasive congeners *I. parviflora* and *I. capensis* (Skálová *et al.*, 2012). We found plasticity in the morphological traits SLA and height, which are particularly plastic (e.g. Flory *et al.*, 2011; Godoy *et al.*, 2011). SLA was larger in shaded habitats (i.e. deciduous and coniferous forests) compared with fallow meadows. Similarly, SLA increased under high shade compared with low shade in the greenhouse. Higher SLA allows plant species to better capture light under shaded conditions and thereby increases fitness (Grotkopp and Rejmánek, 2007). Plant height was comparable in the deciduous forests and fallow meadows, but lower in coniferous forests in the undisturbed soil treatment in the field experiment. In the greenhouse experiment, plants were taller under low shade and in the absence of the competing grass. Plant height is known to be linked to competitive ability, with larger species generally being able to suppress the growth of smaller species (Wang *et al.*, 2010), which in turn is a fitness advantage. As a result, plasticity in SLA and height can generally affect fitness.

From our study we have some indication for both the jack-of-all-trades and master-of-some strategies (Richards *et al.*, 2006). On the one hand, there are no significant fitness differences (measured as biomass) in transplanted *I. glandulifera* in the undisturbed soil treatment between habitats, suggesting a jack-of-all-trades strategy with high fitness in a set of distinct habitats. On the other hand, some of our results suggest a master-of-some strategy, with increased fitness under favourable conditions. In the disturbed soil treatment, fitness was higher in fallow meadow habitats compared with both forest habitats. In the greenhouse, low shade similarly led to increased biomass, particularly in the absence of the competing grass. Thus, there are indications for both good fitness in all considered habitats and increased fitness under especially favourable conditions. This suggests that *I. glandulifera* may follow a jack-and-master strategy (Richards *et al.*, 2006).

Alternative factors determining the colonization sequence

As we could not detect local adaptation in *I. glandulifera*, this mechanism cannot explain the consecutive colonization of different habitats in the invaded range. Therefore, there must be other reasons for the colonization sequence. If *I. glandulifera* is capable of colonizing distinct habitats due to high phenotypic plasticity, as suggested above, the species will be able to cope with a broad range of environmental conditions. Then, the colonization sequence must be related to landscape and land use characteristics that govern propagule pressure (e.g. Lockwood *et al.*, 2005; Colautti *et al.*, 2006). If propagule pressure instead of adaptation to environmental conditions is the main driver of the proposed colonization sequence, then *I. glandulifera* will colonize further habitats in the coming decades when propagule pressure continues to increase.

We conclude that invasive alien plants can become dominant in a set of distinct habitat types in the same region without local adaptation. These species may show high degrees of phenotypic plasticity following a jack-and-master strategy. Additionally, in these species the significance of propagule pressure and land use patterns will be high.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: main characteristics of the source populations. Table S2: main characteristics of the plot sites in the transplant experiment. Fig. S1: experimental design of the transplant and greenhouse experiments. Fig. S2: height and relative growth rate of *I. glandulifera* in the reciprocal transplant experiment. Fig. S3: height and relative growth rate of *I. glandulifera* in the greenhouse experiment.

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