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Pre-adaptation or genetic shift after introduction in the invasive species *Impatiens glandulifera*?

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Shared first authorship between EME and KPA. KPA and BJG designed the study. KPA, PAD and ZAR collected samples in the wild. KPA set up the experiment. EME did the measurements in the glasshouse and wrote the manuscript with help from KPA and IN. JT and EME did statistical analysis. All coauthors commented on the manuscript.

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ABSTRACT

Invasive exotic plants often grow fast, reproduce rapidly and display considerable phenotypic plasticity in their invasive range, which may be essential characteristics for successful invasion. However, it remains unclear whether these characteristics are already present in native populations (pre-adaptation hypothesis) or evolve after introduction (genetic shift hypothesis).

To test these hypotheses we compared means and phenotypic plasticity of vegetative and reproductive traits between populations of *Impatiens glandulifera* collected from either the invasive (Norway) or native range (India). Seeds were sown and the resulting plants were exposed to different experimental environments in a glasshouse. We also tested whether trait means and reaction norms harbored genetic variation, as this may promote fitness in the novel environment.

We did not find evidence that invasive populations of *I. glandulifera* grew more vigorously or produced more seeds than native populations. Phenotypic plasticity did not differ between the native and invasive range, except for the number of nodes which was more plastic in the invasive range. Genetic variation in the slope of reaction norms was absent, suggesting that the lack of change in phenotypic plasticity between native and invasive populations resulted from low genetic variation in phenotypic plasticity initially harbored by this species. Post-introduction evolution of traits thus probably did not boost the invasiveness of *I. glandulifera*. Instead, the species seems to be pre-adapted for invasion.

We suggest that differences in habitat between the native and invasive range, more specifically the higher nutrient availability observed in the new environment, are the main factor driving the invasion of this species. Indeed, plants in the more nutrient-rich invasive range had greater seed mass, likely conferring a competitive advantage, while seed mass also responded strongly to nutrients in the glasshouse. Interactions between habitat productivity and herbivore defense may explain the lack of more vigorous growth in the new range.

Keywords: biological invasion; genetic shift hypothesis; *Impatiens glandulifera*; pre-adaptation; phenotypic plasticity

1. INTRODUCTION

Biological invasion is a global phenomenon causing ecological, economic and social disruption (Davis et al. 2000). Alien invasive species often outcompete native species, affecting biodiversity and altering the structure and function of invaded ecosystems (Vitousek et al. 1996; Bossdorf et al. 2005). Identifying traits or mechanisms responsible for species invasiveness is crucial to predict the fate, spread and impact of invasive species and to take sound management decisions.

Numerous studies have identified traits associated with invasiveness in plants (e.g. Theoharides and Dukes 2007; Hayes and Barry 2008; Whitney and Gabler 2008; van Kleunen et al. 2010). These include – among others – vigorous growth, abundant reproduction and high phenotypic plasticity (i.e. the ability of an organism to change its phenotype in response to different environments) of morphological and physiological traits (Richards et al. 2006; Whitney and Gabler 2008; van Kleunen et al. 2010; Godoy et al. 2011). However, where in the invasion pathway such traits originate is still unclear. According to the pre-adaptation hypothesis, species become invasive if characteristics that promote invasiveness are already present in the native range (Lee and Gelembiuk 2008; Mason et al. 2008; Hejda et al. 2009; Schlaepfer et al. 2010). Alternatively, the genetic-shift hypothesis states that invasive phenotypes arise from rapid evolution after introduction (DeWalt et al. 2004; Caño et al. 2008; Lande 2009; Qing et al. 2011; Colautti and Barrett 2013). In the latter case, the small size of an introduced population typically first causes reduced genetic diversity (founder effect) which can be accompanied by inbreeding (Kaufmann and Smouse 2001; DeWalt et al. 2004; Maron et al. 2004; Bossdorf et al. 2005; Caño et al. 2008; Prentis et al. 2008; Qing et al. 2011). Post-introduction natural selection by – and local adaptation to – the abiotic and biotic conditions in the invasive range will further change the species genotype, giving rise to a novel micro-evolutionary trajectory (Kaufmann and Smouse 2001; Blair and Wolfe 2004; DeWalt et al. 2004; Maron et al. 2004; Bossdorf et al. 2005; Caño et al. 2008; Feng et al. 2008; Barney et al. 2009; Qing et al. 2011; Li et al. 2015).

To distinguish whether a species is pre-adapted to invasion or has evolved after introduction, its traits need to be compared between native and invasive populations along a common environmental gradient (DeWalt et al. 2004; Maron et al. 2004; Caño et al. 2008; Qing et al. 2011; Moroney et al. 2013). Different trait means and different reaction norms to the environmental gradient indicate a genetic shift in respectively the traits themselves and their phenotypic plasticity (and indicate pre-adaptation otherwise). Such shifts can occur if genetic variation in trait means or trait plasticity exists in the introduced populations and if this variation yields a fitness advantage in the novel environment.

Some previous studies found evidence for pre-adaptation (e.g. DeWalt et al. 2004), while others support the genetic shift hypothesis (e.g. Qing et al. 2011). However, different studies used different approaches. While all studies compared trait means (e.g. Siemann and Rogers 2001; Hejda et al. 2009; Schlaepfer et al. 2010), fewer compared also the phenotypic plasticity of traits between native and invasive populations, using either plasticity indices (e.g. DeWalt et al. 2004) or the slope of the reaction norm to environmental treatments (e.g. Maron et al. 2004, Caño et al. 2008, Qing et al. 2011, Moroney et al. 2013). Plasticity indices provide quick estimations but are weaker for statistical comparison, whereas reaction norms are more reliable since they are the most direct way of exploring

phenotypic plasticity (Valladares et al. 2006). Studies testing pre-adaptation vs. genetic shift on new species should therefore use the best available methodology.

Here we investigate mechanisms underlying the invasion of a major invasive species in Europe, *Impatiens glandulifera*. We determine whether high competitiveness, abundant reproduction and high phenotypic plasticity were already present before invasion (pre-adaptation) or evolved after introduction (genetic shift) by comparing offspring from the native and the invasive range. Importantly, we also test for the presence of genetic variation in trait means and trait plasticity, which to our knowledge has not been done before in alien invasive species and could shed more light on the causes for possible changes in phenotypic plasticity during the invasion process.

2. MATERIALS AND METHODS

2.1 Study species and populations

The Himalayan balsam, *I. glandulifera* Royle (Balsaminaceae), is a widespread, often riparian forb native to the western Himalaya from Kashmir to Garhwal (India) (Hulme and Bremner 2006). After introduction in Great Britain in 1839 it spread through Europe (Beerling and Perrins 1993), where it increased dramatically in abundance and distribution during the past 30 to 40 years. The species is classified as one of the 100 most invasive species in Europe (DAISIE 2008). It occurs in many different habitats, but thrives best under high nutrient and water availability (Beerling and Perrins 1993; Pyšek and Prach 1995). *I. glandulifera* commonly reaches a height of up to 2.5 m and the plants in the invasive range are generally taller than in the native range (Beerling and Perrins 1993; Pyšek and Prach 1995; Clements et al. 2008; Tanner et al. 2014). The species is annual, does not reproduce vegetatively and has a limited seed bank; successful seed production and recruitment each year is therefore crucial for maintenance and spread of populations (Skálová et al. 2012). Seeds are dispersed by ballistochory (Willis and Hulme 2004), but long-distance dispersal often occurs by water currents, small rodents or man's deliberate or inadvertent spread (Beerling and Perrins 1993).

Table 1: Location, average soil nitrogen availability measured with four PRS probes and average soil temperature (measured with dataloggers) during six weeks of the growing season for the four study populations of *Impatiens glandulifera*.

Population	Coordinates	Altitude (m)	Nitrogen (μg m^2 per 6 weeks)	Soil temperature ($^{\circ}\text{C}$)
Invasive 1 (Malvik, Norway)	63.413 ° N/ 10.809 ° E	35	65.3	12.8
Invasive 2 (Stjørdal, Noway)	63.484 ° N/ 10.985 ° E	170	12.2	12.5
Native 1 (Gokhama, India)	34.077 ° N/ 74.488 ° E	1850	7.7	21.6
Native 2 (Paris Pora, India)	34.087 ° N/ 74.534 ° E	1700	1.9	21.5

The current study used the offspring from four populations of *I. glandulifera* (see Table 1 for population's coordinates and characteristics), two from the native range (Kashmir, India) and two from the invasive range

(Trondheim, Norway). The latter were chosen at the northern end of the invasive range in order to have maximal differences in environmental conditions relative to the native range (Table 1). This maximizes the selective pressure on the plants and thus the chance to detect any realized genetic shift (DeWalt et al. 2004; Maron et al. 2004; Bossdorf et al. 2005; Caño et al. 2008; Qing et al. 2011; Colautti and Barrett 2013). Moreover, the Species Map Service (Norwegian biodiversity information center and GBIF-Norway 2007-2014) indicates the presence of *I. glandulifera* around Trondheim since 1949, which is considered sufficiently long for genetic shifts to be expressed (Lee 2002).

During August and September 2011, mature seeds from a large number of capsules were collected from 30 randomly selected maternal plants in each population. After measuring their height, these plants were harvested and oven dried for 72 hours at 60°C to determine the aboveground biomass. The average number of seeds per capsule was obtained by dividing the total number of collected seeds by the number of collected capsules. The average seed mass was obtained by dividing the mass of all collected seeds by the total number of seeds. In each population, four PRS probes (Plant Root Simulator, Western Ag Innovations Inc., Saskatoon, Canada) were inserted vertically into the soil at 5 cm depth to measure soil nitrogen availability during six weeks of the growing season (mid-June to end of July 2011). These probes consist of an ion exchange resin membrane that is charged. When buried, soil ions displace the counter-ions at a rate that depends on their diffusion rate in the soil. The quantity of soil ions absorbed during a burial period is a function of all soil properties controlling nutrient availability in soil. After six weeks the PRS probes were removed from the soil and the amount of nitrogen per surface area of membrane was measured. Average soil nitrogen availability per population was calculated from the amount of nitrogen per surface area of membrane measured on the four PRS probes in every population. During the same period, two data loggers (DS 1921G, NexSens Technology, Fondriest environment Inc., Beavercreek, Ohio, USA) per population measured the top soil temperature at 2 cm depth every hour. All populations were located along roads and were assumed to have experienced similar disturbance regimes.

2.2 Experimental design

Thirty seeds from each of 20 randomly selected maternal plants were kept dry at room temperature for two months and then cold stratified at 4°C on moist filter paper in Petri dishes for three months. After germination, the seedlings were cultivated in the glasshouse of the Department of Biology at NTNU (Trondheim, Norway). Mid-March 2012, five developed seedlings were randomly selected from each maternal plant (family) and transplanted into one pot (diameter 12 cm, 15 cm deep) filled with peat soil. Once the seedlings entered the true two-leaves stage, around mid-April 2012, three of those five seedlings per maternal plant were transplanted individually into separate pots of the same size, and each seedling was subjected to one of three different environments (see below). This represented 240 individuals (2 ranges × 2 populations × 20 families × 3 treatments).

The experiment was performed in three compartments of the glasshouse, where pots with offspring from the four populations were randomly spread on a large tray filled with 5 cm of water. Environmental variation was generated by temporarily replacing the water by a nutrient solution with a fixed concentration of nitrogen at different frequencies, i.e. one day per week (High treatment), one day every two weeks (Intermediate treatment) or one day

every three weeks (Low treatment). The added nutrients (YaraLiva™ CalciNit) contained 15.5% nitrogen and were supplied to achieve a concentration of 640 ppm nitrogen in the nutrient solution. This nutrient solution was freely available for the plants to take up during 24 hours, after which the trays were filled with 5 cm of water again the rest of the time. There was no temperature control system in the different compartments; therefore differences in temperature between the treatments cannot be excluded. Although this prevents us from assigning the effects to differences in nutrient availability only, we expect the nutrient gradient to be most influential. Moreover, possible other effects do not compromise the comparison of phenotypic plasticity between populations from the native and invasive range because all populations were exposed to the same environmental variation.

2.3 Measurements

In July and August 2012, when the plants were four to five months old and had been subjected to the nutrient treatment for three months, we determined four reproductive and three vegetative traits on each offspring plant. Reproductive traits included the number of flowers, the average number of seeds per capsule, the mean seed mass, and the total reproductive output (see below). To estimate the average number of seeds per capsule, we performed hand pollinations with randomly assigned donors and receivers within the same population, on several flowers per plant (mean=25, min=2, max=57, depending on available flowers per plant). Hand pollination was carried out by removing matured anthers from a flower of the pollen donor and applying the pollen to the receptive stigma of a newly opened flower of the maternal individual. This was done after checking the stigma for any visible deposited pollen and removing the corolla and nectar glands to prevent further pollination by insects visiting the rooms. To prevent self-pollination, the anther from the pollinated flower was also detached (Nienhuis and Stout 2009). Hand pollinated capsules that developed were covered with paper teabags and collected when most capsules had burst. Seeds per capsule were counted and dried at room temperature for one year. All seeds collected per individual were then weighted and the average seed mass was obtained by dividing the total mass by the total number of seeds. The total number of flowers per plant was determined by counting the receptacles at the end of the growing season. A proxy value for total reproductive output was calculated by multiplying the total number of flowers by the average number of seeds per capsule and the percentage of pollinated capsules that formed seeds. The vegetative traits were number of nodes, plant height and aboveground biomass. The number of nodes on the main stem was counted at first flowering. Early August, plant height was measured from the base of the stem to the upper most photosynthetic tissue, excluding the inflorescence. After these measurements were taken, the aboveground biomass was harvested per individual, oven dried at 60°C for 72 hours and weighted to the nearest 0.1 g.

2.4 Statistical analyses

Statistical analyses were done with R (R Development Core Team 2013). First we verified whether differences in means between the populations in the field could be explained by the native vs. invasive range. This was done by comparing models including population (four levels) or range (two levels) as the explanatory factor for every trait measured in the field (plant height, dry aboveground biomass, number of seeds per capsule and seed mass). Models were compared using a likelihood ratio test. A significant p-value indicates that the model with population (four

levels) explains the trait variation better than the model with range (two levels). Note that only the field data from the 20 mother plants used in the experiment were included in the analysis of the field populations.

To distinguish between genetic and environmental effects the offspring from these plants were grown in common garden in the glasshouse. Differences in reaction norms across populations in the glasshouse were tested with mixed-effects models where treatment and population were fixed factors and maternal family was a random factor (Appendix Table A 1). Treatments were categorized as -1, 0 and 1 for Low, Intermediate and High treatment, respectively, such that the intercept corresponded to the trait mean in the Intermediate treatment. As phenotypes were observed in three different environments, reaction norms could be non-linear. In environment x , the individual phenotypic value y is given by $y = A + Bx + cx^2 + E$, where A , B and c are the additive genotypic values for the intercept, the slope and the curvature of the reaction norm, respectively, and E is the environmental effect with a variance σ_E^2 . If a and b denote the population mean of A and B , respectively, and assuming no genetic variance in c , the observed phenotypes should be normally distributed with mean $E(Y) = a + bx + cx^2$ and a variance $\text{var}(Y) = \sigma_A^2 + \sigma_B^2 x^2 + 2\rho\sigma_A\sigma_B x + \sigma_E^2$, where the genetic variance and covariances of A and B are σ_A^2 , σ_B^2 , and $\rho\sigma_A\sigma_B$, respectively. In the mixed-effects models, the random (family) variance can be used to estimate the genetic variance in intercept (A) and slope (B). Indeed, considering two half-sibs in environments x_1 and x_2 , their phenotype will be determined by the parental genotypic values as

$$Y_1 = \frac{1}{2}(A_m + A_{f1}) + \epsilon_{A1} + \left[\frac{1}{2}(B\epsilon_m + B_{f1}) + \epsilon_{B1} \right] x_1 + cx_1^2 + E_1, \quad (1)$$

and

$$Y_2 = \frac{1}{2}(A_m + A_{f2}) + \epsilon_{A2} + \left[\frac{1}{2}(B\epsilon_m + B_{f2}) + \epsilon_{B2} \right] x_2 + cx_2^2 + E_2, \quad (2)$$

where A_m , A_f , B_m and B_f are the genotypic value for the intercept and the slope of the mother and father, and ϵ 's, representing Mendelian sampling and segregation, are normally distributed with zero means and variance $1/2 \sigma_A^2$ and $1/2 \sigma_B^2$ for the intercept and the slope, respectively. This can be fitted as a mixed effect regression model with random effect terms based on the mother identity on the intercept and slope, corresponding to the terms $1/2 A_{fi}$ and $1/2 B_{fi}$ in the above equations. From (1) and (2) it follows that, the variance of these random effect terms and their covariance can be seen to equal $1/4$ of the additive genetic variances in the reaction norm elevation and slope as well as $1/4$ of their covariance. Estimates of these variances are thus given by the estimated random effect variances multiplied by 4. Because random variance in the slope σ_B^2 was never observed, however, we did not have to consider the possible genetic covariance between the intercept and the slope. Assuming that the environmental variance equals the residual variance minus three times the family variance (because residual variance still contains the genetic variance due to the contribution of the male and Mendelian sampling variance equal to $\sigma_A^2/2$), heritability was estimated as: $h^2 = 4 \times \text{family variance} / (\text{residual variance} + \text{family variance})$. Note that if the different seeds from the same mother are sired by the same father, the estimate of the random variance will represent $1/2$ of the additive genetic variance.

In order to test the statistical significance of the random (family) variance we compared models fitted with restricted maximum likelihood (REML) with different random effect (random effect for the slope and intercept vs. random effect for the intercept only) using the likelihood ratio test. When statistically significant random variation in the

slope or intercept was found, we further tested whether populations differed in their random variation by fitting the model anew for each population separately. The total log-likelihood for the model with variances differing between each population was then given by the sum of the log-likelihood of each separately fitted model. We used the change in log-likelihood between the two models which is Chi-square distributed ($df = 3$) to test whether or not a model with different random variances was statistically better than a model with a single random variance common for the four populations. We performed model selection, based on Akaike information criterion (AIC) values, for the fixed effects using models fitted with maximum likelihood (ML). Parameter estimates were subsequently obtained for the best model by refitting the model with REML. Finally, we tested for differences in phenotypic plasticity between native and invasive populations by comparing the best model with a model where the factor population was replaced by a factor range of origin (invasive vs. native).

Because we were interested in comparing relative differences among populations and traits, we performed all analyses on log-transformed data. These models with log-transformed data had also a slightly better fit than the models performed on raw data (not shown).

3. RESULTS

3.1 Field measurements

In the field, models including only populations were better than those including only range to explain differences in plant height and dry aboveground biomass ($p < 0.001$). Therefore, for these two traits, possible differences between the native and invasive range were masked by the among-population differences within range. For the number of seeds per capsule and the seed mass, on the other hand, differences between ranges were statistically significant and larger than those among populations. Overall, plants in the invasive populations produced 62% less seeds per capsule than those in the native populations, while their seeds were 43% heavier (Table 2).

Table 2: Mean $\pm$ 1 se per population of *Impatiens glandulifera* for the four plant traits measured in the field. I=Invasive, N=Native.

Population	Number of seeds per capsule	Seed mass (mg)	Plant height (cm)	Aboveground biomass (g)
I1	4.64 $\pm$ 1.40	10.25 $\pm$ 0.56	154.38 $\pm$ 7.81	8.96 $\pm$ 1.05
I2	4.12 $\pm$ 0.63	10.92 $\pm$ 0.46	142.36 $\pm$ 5.97	9.07 $\pm$ 1.00
N1	11.67 $\pm$ 0.72	7.03 $\pm$ 0.22	109.20 $\pm$ 5.82	10.15 $\pm$ 1.33
N2	11.68 $\pm$ 0.65	7.80 $\pm$ 0.26	143.25 $\pm$ 7.39	27.29 $\pm$ 3.70

3.2 Glasshouse measurements

In general, trait values increased from the Low to the High treatment, except for the average number of seeds per capsule which was not affected (Fig. 1; Table 2 and 4). For the other traits, the increase from Intermediate to High was generally stronger than the increase from Low to Intermediate, generating non-linear reaction norms (Fig. 1; Table 3). In four out of the six traits affected by the treatment, neither the slope nor the curvature of the reaction norm differed among populations or ranges (Table 3, Fig. 1). Hence, for these traits, phenotypic plasticity was similar in native and invasive populations. For total reproductive output, the slope of the reaction norm differed among the populations. One invasive population showed a modestly increasing reaction norm between the Low and Intermediate treatment, while the other three populations showed a slight decrease (Fig. 1). For the number of nodes, the curvature of the reaction norm was more pronounced in plants from the invasive range (Fig. 1; Table 3). This indicates greater phenotypic plasticity for this trait in the invasive (33% and 23% increase from Low to High in invasive population 1 and 2, respectively) than in the native populations (4% and 6% increase from Low to High in native population 1 and 2, respectively).

Plants originating from the invasive range produced less seeds per capsule and also had lower total reproductive output than plants from the native range (Fig. 1, Table 2). For these two traits, the model with range of origin as explanatory variable was better than the model with the separate populations. For the other traits, the best model was the one including populations as explanatory variable, indicating that possible differences among ranges were overshadowed by the among-population differences within range ($p < 0.001$).

None of the traits showed statistically significant random variation in slope, and only one trait, the number of nodes, showed significant random variation in the intercept (Table 3), suggesting that only this trait harbored genetic variation. Furthermore, the amount of family variance for this trait differed among populations ($\chi^2 = 12.11$, $df = 3$, $p = 0.007$) and was statistically significant only in one of the native populations, while it was marginally significant in one native and one invasive population (Table 4). Heritability estimates for the number of nodes based on the assumption that individuals from the same maternal plant were half-siblings, were particularly high for the three populations harboring family variation (0.79 to 1.31). This suggests that most of the offspring from the same maternal plant, grown in our glasshouse-experiment, were full-sibs (Table 4).

287 Fig. 1: Mean $\pm$ 1 se on log scale of the trait values of *Impatiens glandulifera* for each population in each treatment for (a) Number of flowers, (b) Number of seeds
 288 per capsule, (c) Seed mass, (d) Total reproductive output, (e) Plant height, (f) Aboveground biomass and (g) Number of nodes.

Table 3: Parameter estimates ± 1 se on log scale for the effects of treatment and population (I: invasive, N: native) and statistical significance level of the random effects for the slope and intercept on seven traits in *Impatiens glandulifera* in the glasshouse. Parameters were obtained from the best model fitted with REML without global intercept. Consequently, the estimates for each population correspond to the mean value for each population in the Intermediate treatment. Models included population and treatment as fixed factors and maternal family as a random factor. The p-values for the random variance in the intercept were calculated using the exactRLRT function in R. Note: I=Invasive, N=Native.

	Number of flowers	Number of seeds per capsule	Seed mass (mg)	Reproductive rate	Plant height (cm)	Aboveground biomass (g)	Number of nodes
Fixed effects							
Population I1	4.54 $\pm$ 0.08	1.09 $\pm$ 0.05	1.28 $\pm$ 0.08	3.67 $\pm$ 0.13	4.89 $\pm$ 0.04	2.10 $\pm$ 0.07	1.91 $\pm$ 0.04
Population I2	4.45 $\pm$ 0.08	1.03 $\pm$ 0.05	0.80 $\pm$ 0.08	3.76 $\pm$ 0.13	4.74 $\pm$ 0.04	1.86 $\pm$ 0.07	1.91 $\pm$ 0.04
Population N1	4.40 $\pm$ 0.08	1.82 $\pm$ 0.05	0.86 $\pm$ 0.08	5.15 $\pm$ 0.13	4.67 $\pm$ 0.04	2.31 $\pm$ 0.07	2.05 $\pm$ 0.04
Population N2	4.63 $\pm$ 0.08	1.73 $\pm$ 0.05	0.74 $\pm$ 0.08	5.12 $\pm$ 0.13	4.57 $\pm$ 0.04	2.06 $\pm$ 0.07	1.95 $\pm$ 0.04
Treatment	0.35 $\pm$ 0.04		0.30 $\pm$ 0.04		0.14 $\pm$ 0.02	0.12 $\pm$ 0.04	
Treatment ²	0.36 $\pm$ 0.07		0.28 $\pm$ 0.07	0.75 $\pm$ 0.11	0.05 $\pm$ 0.03	0.23 $\pm$ 0.06	
Treatment:Population I1				0.93 $\pm$ 0.13			0.14 $\pm$ 0.02
Treatment:Population I2				0.45 $\pm$ 0.13			0.11 $\pm$ 0.02
Treatment:Population N1				0.53 $\pm$ 0.13			0.02 $\pm$ 0.02
Treatment:Population N2				0.61 $\pm$ 0.13			0.03 $\pm$ 0.02
Population I1:Treatment ²							0.15 $\pm$ 0.04
Population I2:Treatment ²							0.07 $\pm$ 0.04
Population N1:Treatment ²							0.03 $\pm$ 0.04
Population N2:Treatment ²							0.00 $\pm$ 0.04
Random effects							
Slope: <i>Chisquare</i>	1.56	1.05	1.6e-08	0.39	0	1.7e-08	0.94
<i>df</i>	2	2	2	2	2	2	2
<i>p-value</i>	0.46	0.59	1	0.82	1	1	0.62
Intercept: <i>RLRT</i>	0	0	0	0	0	0	7.00
<i>p-value</i>	0.49	0.49	1	1	0.49	1	<0.01

Table 4: Heritability (h^2) estimated for the number of nodes of *Impatiens glandulifera* from the different populations assuming that offspring were half- or full-sibs. The p-values and likelihood ratio test for the statistical significance of the random variance in the intercept for each population are also reported.

Population	h^2 full sib	h^2 half sib	p-value random effect
Invasive 1	0.00	0.00	0.477 (RLRT=0.00)
Invasive 2	0.43	0.86	0.057 (RLRT=2.39)
Native 1	0.39	0.79	0.072 (RLRT=2.03)
Native 2	0.65	1.31	0.011 (RLRT=5.48)

4. DISCUSSION

Overall we did not find evidence for invasive populations of *I. glandulifera* having greater vegetative growth, more abundant reproductive output or higher phenotypic plasticity than native populations, suggesting that invasiveness was not enhanced by changes in these traits after introduction.

The lack of differences in plant height and aboveground biomass between the native and invasive range in the field as well as in the glasshouse contradicts the expectation of more vigorous growth in the new region as predicted by the genetic shift hypothesis (Beerling and Perrins 1993; Pyšek and Prach 1995; DeWalt et al. 2004; Jakobs et al. 2004; Caño et al. 2008; Qing et al. 2011; Colautti and Barrett 2013; Moroney et al. 2013). Moreover, in the glasshouse, also the number of nodes and the total number of flowers produced per plant was similar in the offspring from both ranges. Consequently, these four traits were pre-adapted for invasion (Lee and Gelembiuk 2008; Mason et al. 2008; Hejda et al. 2009; Schlaepfer et al. 2010), at least if they effectively contribute to the species' invasiveness in the first place. Alternatively the lack of difference in plant height and aboveground biomass between the native and the invasive range could arise from the interaction between habitat productivity and herbivore defense (Zhang and Jiang, 2006). Coley et al. (1985) state that habitat productivity and defense against natural enemies may interact and as such shape the outcome of evolutionary changes in invasive plants (Zhang and Jiang, 2006). If species originate from a low productivity environment and invade a high productivity environment, as is the case for *I. glandulifera*, herbivore resistance can be reduced owing to re-allocation of resources from defense to growth (Zhang and Jiang, 2006). This may counteract the expected vigorous growth of the invasive species (Zhang and Jiang, 2006).

Invasive populations of *I. glandulifera* produced less seeds per capsule than native populations, both in the field and in the glasshouse. This points to a genetic shift rather than pre-adaptation, probably induced by a genetic bottleneck after introduction (Kaufmann and Smouse 2001; DeWalt et al. 2004; Maron et al. 2004; Bossdorf et al. 2005; Caño et al. 2008; Qing et al. 2011; Colautti and Barrett 2013) and subsequent local adaptation to the low temperatures and short growing season in the invasive Norwegian populations (Kaufmann and Smouse 2001; Blair and Wolfe 2004; DeWalt et al. 2004; Maron et al. 2004; Bossdorf et al. 2005; Caño et al. 2008; Feng et al. 2008; Barney et al. 2009; Qing et al. 2011; Li et al. 2015). Plants in cold climates typically produce smaller amounts of seeds, though these seeds are generally larger than in warm regions (Landolt, 1967; Schütz & Stöckin, 2001; Baskin & Baskin, 2014). The latter may give rise to increased germination, better seedling survival, and greater vigour and competitive ability

(Schmitt and Ehrhard 1990; Skálová et al. 2011, Willis and Hulme 2004). In the current experiment, we indeed also observed greater seed mass in the invasive than in the native populations, which had an environmental rather than a genetic origin because it was no longer present in the offspring in the glasshouse. Nutrients are known to be important for seed mass (Baskin & Baskin, 2014), and seed mass in the glasshouse increased strongly when more nutrients were supplied. Moreover, in the field, we measured more available nutrients in the soil beneath the invasive populations than the native ones. Part of the invasiveness of this species may thus be generated by the production of larger seeds with higher seed quality under high nutrient conditions typical of the invasive range. In most of the traits we detected no difference in phenotypic plasticity between the ranges in the glasshouse. Therefore, we did not find evidence for an overall genetic shift in the plasticity of *I. glandulifera* in the invasive range. If phenotypic plasticity plays a role in the invasiveness of this species, then it may already be present at a similar level in the native populations, which is consistent with the pre-adaptation hypothesis (Lee and Gelembiuk 2008; Mason et al. 2008; Hejda et al. 2009; Schlaepfer et al. 2010). The additional random terms in the statistical model allowed us to ascertain whether differences in means and phenotypic plasticity could be explained by genetic variation (mean: intercept, phenotypic plasticity: slope). Sufficient additive genetic variation is essential for evolutionary adaptation to new environments (Lee 2002). The absence of genetic variation in the slope of the reaction norm suggests that the lack of change in phenotypic plasticity between native and invasive populations resulted from low genetic variation in phenotypic plasticity initially harbored by this species. One trait, the number of nodes, was more plastic in the invasive than in the native range (steeper slope and stronger curvature). However, for this trait as well, genetic variation in plasticity was absent, as only the mean value of this trait had genetic variation. Among the studies on other species that generally support the pre-adaptation hypothesis (e.g. DeWalt et al. 2004; Hejda et al. 2009; Schlaepfer et al. 2010), only DeWalt et al. (2004) studied phenotypic plasticity and they found no evidence for a shift in phenotypic plasticity of morphological and photosynthetic traits in *Climedia hirta*. On the other hand, studies lending support to the genetic shift hypothesis (e.g. Siemann and Rogers 2001; Maron et al. 2004; Caño et al. 2008; Qing et al. 2011; Colautti and Barrett 2013; Moroney et al. 2013) found increased phenotypic plasticity after introduction in *Senecio pterophorus* (Caño et al. 2008), *Spartina alterniflora* (Qing et al. 2011) and *Centaurea melitensis* (Moroney et al. 2013). The capacity to realize a genetic shift may thus be species-specific.

In conclusion, our study indicates that *I. glandulifera* is pre-adapted for invasion with respect to most of its traits, both for their mean value and phenotypic plasticity. Only the number of nodes was more plastic in the invasive than in the native range, pointing to a genetic shift in this trait. The number of seeds per capsule also showed a shift, but not toward more abundant reproduction as predicted by the genetic shift hypothesis. Invasive populations in the field did produce heavier seeds, probably owing to higher nutrient availability in the invasive range. From these findings we conclude that differences in habitat between ranges most likely drive the invasion of this species, as plants in the invasive range mainly occupy disturbed and nutrient rich sites. These conditions may therefore be the key determinant of the invasion process.

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6. APPENDICES

Table A 1: Model selection for the different traits of *Impatiens glandulifera*. Models A and B are used to compare if the random effect for slope is significant. Models 1 to 8 are used to determine which fixed effects are needed in the model. We first present the various models that have been fitted and then their AIC values per trait.

- Model A** $\log Y \sim \text{population} + \text{treatment} : \text{population} + \text{treatment}^2 + \text{treatment}^2 : \text{population} + (1 | \text{mother})$
Model B $\log Y \sim \text{population} + \text{treatment} : \text{population} + \text{treatment}^2 + \text{treatment}^2 : \text{Population} + (\text{treatment} | \text{mother})$
Model 1 $\log Y \sim \text{treatment} + \text{population} + \text{treatment} : \text{population} + \text{treatment}^2 + \text{treatment}^2 : \text{population} + (1 | \text{mother})$
Model 2 $\log Y \sim \text{treatment} + \text{population} + \text{treatment} : \text{population} + \text{treatment}^2 + (1 | \text{mother})$
Model 3 $\log Y \sim \text{treatment} + \text{population} + \text{treatment} : \text{population} + (1 | \text{mother})$
Model 4 $\log Y \sim \text{treatment} + \text{population} + \text{treatment}^2 + (1 | \text{mother})$
Model 5 $\log Y \sim \text{treatment} + \text{population} + (1 | \text{mother})$
Model 6 $\log Y \sim \text{population} + (1 | \text{mother})$
Model 7 $\log Y \sim \text{treatment} + (1 | \text{mother})$
Model 8 $\log Y \sim 1 + (1 | \text{mother})$

Trait	Model	K	AICc	ΔAICc	AICcWt	AIC
Plant Height	A					-11.1884
	B					-7.1884
	1	14	-9.32	0.2	0.35	
	2	11	-4.28	5.25	0.03	
	3	10	-3.16	6.37	0.02	
	4	8	-9.53	0	0.39	
	5	7	-8.37	1.16	0.22	
	6	6	39.29	48.82	0	
	7	4	31.47	10.99	0	
	8	3	78.47	87.99	0	
Aboveground biomass	A					333.14
	B					337.14
	1	14	335.02	8.67	0.01	
	2	11	328.98	2.33	0.23	
	3	10	339.88	13.54	0	
	4	8	326.35	0	0.75	
	5	7	337.37	11.02	0	
	6	6	345.96	19.62	0	
	7	4	356.07	29.72	0	
	8	3	363.46	37.12	0	
Number of nodes	A					-201.79
	B					-198.73
	1	14	-199.92	0	0.73	
	2	11	-197.9	2.02	0.27	
	3	10	-189.64	10.29	0	

	4	8	-181.42	18.5	0	
	5	7	-175.28	24.65	0	
	6	6	-145.87	54.06	0	
	7	4	-168.56	31.37	0	
	8	3	-135.33	64.59	0	
Number of flowers	A					368.98
	B					371.42
	1	14	370.85	9.73	0.01	
	2	11	364.9	3.78	0.13	
	3	10	389.49	28.37	0	
	4	8	361.12	0	0.86	
	5	7	385.47	24.35	0	
	6	6	446.77	85.65	0	
	7	4	385.89	24.77	0	
	8	3	445.7	84.58	0	
Average number of seeds per capsule	A					189.50
	B					192.45
	1	14	191.42	6.65	0.02	
	2	11	190.3	5.5	0.03	
	3	10	190.26	5.49	0.03	
	4	8	185.82	1.05	0.25	
	5	7	185.77	1	0.26	
	6	6	184.77	0	0.42	
	7	4	286.01	101.24	0	
	8	3	284.7	99.93	0	
Average seed mass	A					359.22
	B					363.22
	1	14	361.14	3.85	0.1	
	2	11	359.21	1.91	0.25	
	3	10	372.85	15.56	0	
	4	8	357.29	0	0.65	
	5	7	370.44	13.15	0	
	6	6	415.13	57.84	0	
	7	4	401.72	4.43	0	
	8	3	440.07	82.78	0	
Total reproductive output	A					579.63
	B					583.25
	1	14	581.55	5.99	0.03	
	2	11	575.56	0	0.68	
	3	10	616.17	40.61	0	
	4	8	577.25	1.68	0.29	
	5	7	616.55	40.99	0	
	6	6	685.13	109.57	0	
	7	4	704.71	129.15	0	
	8	3	764.86	189.3	0	