

Life history trait differentiation and local adaptation in invasive populations of *Ambrosia artemisiifolia* in China

Xiao-Meng Li · Deng-Ying She · Da-Yong Zhang ·
Wan-Jin Liao

Received: 10 January 2014 / Accepted: 18 October 2014 / Published online: 2 November 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract Local adaptation has been suggested to play an important role in range expansion, particularly among invasive species. However, the extent to which local adaptation affects the success of an invasive species and the factors that contribute to local adaptation are still unclear. This study aimed to investigate a case of population divergence that may have contributed to the local adaptation of invasive populations of *Ambrosia artemisiifolia* in China. Common garden experiments in seven populations indicated clinal variations along latitudinal gradients, with plants from higher latitudes exhibiting earlier flowering and smaller sizes at flowering. In reciprocal transplant experiments, plants of a northern Beijing origin produced more seeds at their home site than plants of a southern Wuhan origin, and the Wuhan-origin plants had grown taller at flowering than the Beijing-origin plants in Wuhan, which is believed to facilitate pollen dispersal. These results suggest that plants of Beijing origin may be locally adapted through female fitness and plants from Wuhan possibly locally adapted through male fitness. Selection and path analysis suggested that the phenological and growth traits of both populations have been influenced by natural selection and that flowering time has played an important role through its direct and

indirect effects on the relative fitness of each individual. This study evidences the life history trait differentiation and local adaptation during range expansion of invasive *A. artemisiifolia* in China.

Keywords Flowering time · Latitudinal cline · Reciprocal transplant experiments · Selection analysis · Range expansion

Introduction

Successful biological invasions are most often associated with large-scale geographical expansion from newly colonised areas, which usually exposes populations to strong selection pressures under novel environmental conditions (Colautti et al. 2010). Local adaptation is considered to be one of the most important mechanisms underlying the successful range expansion of invasive species (Eriksen et al. 2012; Sherrard and Maherali 2012; Colautti and Barrett 2013; Kilkenny and Galloway 2013). A recent study found clear evidence that the adaptation of invasive species to the variations in climate conditions encountered during range expansion has a greater impact on the species' fitness than its escape from competitive pressures (Colautti and Barrett 2013). However, local adaptation is not always observed; for example, common garden and reciprocal transplant experiments revealed no evidence for local adaptation in invasive populations of *Buddleja davidii* (Ebeling et al. 2011). Therefore, the extent to which local adaptation affects the success of an invader varies across different situations. The degree to which adaptation is a local phenomenon, which has been identified as one of the 100 fundamental ecological questions (Sutherland et al. 2013), is also an important concept in invasive biology and requires further study.

Communicated by Jennifer A. Lau.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-014-3127-z) contains supplementary material, which is available to authorized users.

X.-M. Li · D.-Y. She · D.-Y. Zhang · W.-J. Liao (✉)
State Key Laboratory of Earth Surface Processes and Resource Ecology and MOE Key Laboratory for Biodiversity Science and Ecological Engineering, Beijing Normal University, Beijing 100875, China
e-mail: liaowj@bnu.edu.cn

One powerful approach for understanding adaptation is to identify the agents and targets of natural selection (Conner and Hartl 2004). For species with large-scale distributions, natural selection for ecologically important traits may lead to phenotypic differentiation as a result of environmental variation along geographic gradients. According to a number of reports, sets of phenotypic traits can be genetically differentiated among populations of different origins in both invasive and non-invasive species (Leger and Rice 2007; Gonzalo-Turpin and Hazard 2009; Eriksen et al. 2012). Apart from the studies conducted by Colautti and Barrett (2010, 2013), the effects of natural selection on phenotypic traits in invasive species have rarely been measured. Therefore, the factors that contribute to local adaptation are still unclear, despite the importance of adaptation in evolutionary biology and invasion biology.

Reproductive timing is an important adaptation to variable conditions along a latitudinal gradient (Griffith and Watson 2005, 2006; Barrett et al. 2008; Colautti and Barrett 2010). Populations at higher latitudes or elevations might evolve to mature to seed-producing stages earlier to ensure successful reproduction in shorter growing seasons, resulting in a clinal pattern of plant size and flowering time (Santamaria et al. 2003; Griffith and Watson 2006; Haggerty and Galloway 2011). A recent study on the invasive species *Lythrum salicaria* demonstrated that the evolution of earlier flowering at its northern invasion front significantly increased the fitness of this species and facilitated its range expansion (Colautti and Barrett 2013). The clinal photoperiod along latitudinal gradients might also influence the initiation of flowering (Deen et al. 1998; Santamaria et al. 2003; Haggerty and Galloway 2011). In short-day species in particular, the plants' initial flowering occurs when the day length decreases to a critical photoperiod. Different threshold day lengths for flowering may evolve under different photoperiods at different latitudes, which may result in a clinal pattern of flowering time (Deen et al. 1998; Genton et al. 2005a).

Sex allocation, either through investment allocation or temporal allocation (dichogamy), may exhibit adaptive plasticity or differentiation in relation to plant size or environmental conditions (Friedman and Barrett 2011). For example, smaller plants may have larger male investment, because the energetic and temporal limitations of the smaller plants may impose severe limitations for female success, as the size-advantage hypothesis and time-commitment hypothesis predict (Paquin and Aarssen 2004). However, taller wind-pollinated plants may have greater male investment due to the more effective pollen dispersal (Paquin and Aarssen 2004; Friedman and Barrett 2011). Variable sex allocation under diverse environments has been reported in several species and is thought to be

adaptive (Freeman et al. 1981; Paquin and Aarssen 2004; Friedman and Barrett 2011).

A. artemisiifolia is an aggressive annual weed native to North America that has invaded many ecosystems worldwide, and its wide distribution and disruptiveness to these ecosystems are of great concern. High levels of genetic diversity and an outcrossing mating system have been reported in both native and invasive populations of *A. artemisiifolia* (Genton et al. 2005b; Friedman and Barrett 2008; Chun et al. 2010; Gaudeul et al. 2011; Gladieux et al. 2011; Li et al. 2012). Phenotypic differentiation has been found in *A. artemisiifolia* populations from different latitudes in several studies through common garden experiments and reciprocal transplant experiments (Genton et al. 2005a; Hodgins and Rieseberg 2011), but the factors underlying the observed differentiation or local adaptation were not well assessed. In the present study, local adaptation over a latitudinal gradient was examined in invasive Chinese *A. artemisiifolia* populations through common garden experiments and reciprocal transplant experiments, coupled with a selection analysis and path analysis on southern and northern populations. Specifically, we aimed to address the following two questions:

1. Does genetic differentiation occur along latitudinal gradients for phenotypic traits?
2. Are populations locally adapted to their sites of origin in China, and if so, which traits are involved?

Materials and methods

Common ragweed, *A. artemisiifolia* (Asteraceae), is a monoecious annual weed in which female flowers are formed on the leaf and branch axils and male flowers form racemes on the primary stem and lateral branches (Payne 1963). The first invasive specimen of *A. artemisiifolia* collected in China was from Zhejiang Province in 1935, and this invasive species now exhibits a wide range, from Guangdong Province in the south to Heilongjiang Province in the north, covering latitudes ranging from 23°N to 46°N (Wan et al. 2009).

Common garden experiment

In 2008, we collected seeds from seven geographically separated populations along a latitudinal cline in China to perform common garden experiments (Online Resource 1, Table OR1). Seeds were collected from ten to 30 plants in each population of origin and stored separately for each maternal family. On 16 April 2009, three randomly selected seeds from each maternal family were sown per plastic pot

(350 × 330 mm) after 2 weeks of cold stratification for each population. The pots were placed at random in the Beijing Normal University garden (39.96°N, 116.36°E). Three weeks after planting, the surviving seedlings were thinned to one plant per pot. For each population, pots in which none of the three seeds germinated were randomly filled with a single seedling from another pot with more than one seedling. As a result, a total of 153 individuals from 131 families were investigated (Online Resource 1, Table OR1). The plants were watered as needed during the growing season. To investigate the variation of phenology across the seven populations, we recorded the day of cotyledon emergence and the first day of flowering for all 153 plants. The flowering time was defined as the number of days from sowing to the appearance of pollen release. When the first flower opened, we measured plant height.

Reciprocal transplant experiments

For field reciprocal transplant experiments, we collected seeds from 12 maternal plants from each of the following two *A. artemisiifolia* populations: one from Beijing (BJ) in the north and one from Wuhan (WH) in the south (Online Resource 1, Table OR1). Approximately 15 seeds from each maternal plant were subjected to cold stratification for 2 weeks and then planted in small, soil-filled pots in a greenhouse in Beijing in April 2011. The emergence of cotyledons was recorded as germination. In mid-May 2011, the seedlings with two fully opened cotyledons from each maternal plant were divided almost evenly into two groups, with 48 and 50 individuals being transplanted at the Wuhan and Beijing sites, respectively (Online Resource 1, Table OR1). At each transplant site, the seedlings from different origins were planted alternatively at a distance of 1.5 m, so that every seedling from one origin was surrounded by four seedlings from another origin. Extra seedlings were planted around the perimeter of the experimental plots to reduce edge effects. Ample water was supplied during the first month after transplantation.

For each plant, we recorded the dates on which the first male and female flowers began anthesis, which were defined based on the emergence of pollen release and forked stigmas, respectively, and quantified the flowering time as the number of days from sowing to the appearance of the first flower. Plant height was measured at time of first flowering, and the senescence time was estimated as the number of days from sowing to the date when all of the branches and leaves had perished. The lengths of the growth and reproductive periods were calculated as the number of days from germination to senescence and from the first flowering to senescence, respectively. We estimated the number of female and male flowers for each plant by

counting the female flowers and measuring the lengths of male inflorescences on a section of the branches, respectively, just before plant senescence. The branches to be investigated were selected evenly from different sections of the plants from the bottom up, and the number of branches investigated was one-fourth of the number of branches on that plant. We counted male flowers from a subset of 738 male inflorescences on 64 plants that were randomly selected from the two origins at the two transplant sites. The fit of a regression model relating the number of male flowers and raceme length was then assessed (for the BJ-origin plants at Beijing, $Y = 8.3 + 8.175X$, $r^2 = 0.873$, $P < 0.01$; for the WH-origin plants at Beijing, $Y = 14.365 + 5.407X$, $r^2 = 0.836$, $P < 0.01$; for the BJ-origin plants at Wuhan, $Y = 23.078 + 3.058X$, $r^2 = 0.656$, $P < 0.01$; and for the WH-origin plants at Wuhan, $Y = 16.146 + 4.248X$, $r^2 = 0.683$, $P < 0.01$), and the number of male flowers was estimated based on the raceme length recorded for each plant. The proportion of male flowers was used to estimate the sex allocation for each plant. When the plants senesced, we measured plant heights, harvested the aboveground parts and dried them at 80 °C for 48 h. We weighed the overall aboveground dry biomass and estimated 1,000-seed weight and seed number for every plant.

Statistical analyses

To determine whether life history traits varied among populations originating from different latitudes in the common garden experiment, the population means and SEs for flowering time and flowering plant height were derived from the estimated marginal means in a linear mixed model through restricted maximum likelihood analysis, with the population as a fixed effect and the maternal family nested within the population as a random effect (IBM SPSS Statistics 20). The correlations between the traits and latitudes were then assessed based on the population means.

To investigate genetic differentiation and environmental effects on phenological traits (flowering time and senescence time), growth traits (aboveground biomass, flowering plant height and final plant height), reproductive traits (the proportion of male flowers, 1,000-seed weight at harvest and seed number per plant) in the reciprocal transplant experiments, we performed a generalised linear mixed model analysis (IBM SPSS Statistics 20) using the transplant site, seed origin and their interaction as fixed effects and the maternal family nested within the original population as a random effect. The data were fit to a binomial distribution with a logit link function for the proportion of male flowers. Significant differences between the two original populations within the transplant sites were assessed by comparing the estimated marginal means in the models

through a least significant difference test (IBM SPSS Statistics 20). To identify the traits under direct selection that are likely to represent adaptations to different sites, we estimated selection gradients (β) for each site as the partial regression coefficients of the relative fitness on the standardised characters, including flowering time, senescence time, aboveground biomass and proportion of male flowers (Lande and Arnold 1983; Conner and Hartl 2004). Relative fitness was defined as the individual seed number relative to the mean seed number for the plants at each site. Phenotypic and genotypic β were estimated based on all individuals and family means, respectively. A likely path diagram was then constructed, and a path analysis was performed to elucidate the direct and indirect effects of the traits on the relative fitness. The path coefficients were calculated as regression coefficients in multiple regressions for the potentially causal traits on the relative fitness and the intermediate traits according to the path diagram for the two sites (SPSS version 20 Regression) (Mitchell 2001). The traits were standardized in the path analysis by subtracting the mean of the site from an individual value and then dividing the difference by the SD of the site.

Results

Latitudinal pattern of phenotypic traits

The traits measured in the common garden experiments in 2009, including flowering time and flowering plant height, displayed a significant clinal pattern along the latitudinal gradient (Fig. 1). Plants originating from northern populations (MD, SP and DD) flowered approximately 40 days earlier than plants from southern populations (NJ, WH, JD and NC). The flowering plant height from the northern populations was approximately half that of the southern populations.

Differentiation of phenological traits

In the reciprocal transplant experiment, the transplant site and plant origin significantly affected both flowering time and senescence time, and the interaction between the two factors significantly affected flowering time (Table 1). At the Beijing transplant site, the beginning of flowering varied from 1 to 24 August 2011 for the BJ-origin plants and from 12 August to 4 September 2011 for the WH-origin plants. At the Wuhan transplant site, the plants from BJ and WH began to flower from 29 June to 24 July 2011 and from 5 July to 22 August 2011, respectively. The plants from BJ flowered earlier (in Beijing, $F_{1,84} = 6.340$, $P < 0.05$; in Wuhan, $F_{1,84} = 27.200$, $P < 0.001$; Fig. 2a) and senesced earlier (in Beijing, $F_{1,84} = 6.340$, $P < 0.05$; in Wuhan,

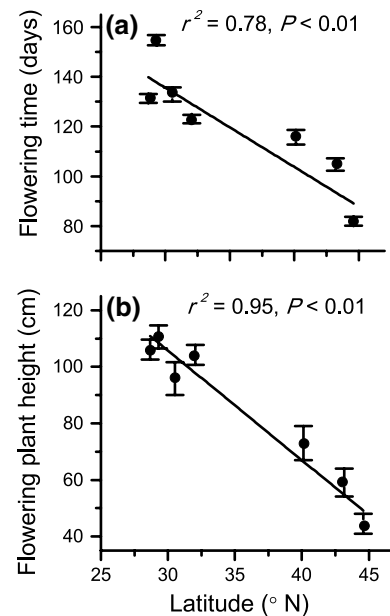


Fig. 1 Latitudinal variation in **a** flowering time and **b** flowering plant height among Chinese invasive populations of *Ambrosia artemisiifolia* in a common garden experiment. Data (mean \pm SE) were derived from the estimated marginal means in a linear mixed model through restricted maximum likelihood analysis, with population as a fixed effect and maternal family nested within the original population as a random effect. Fitted lines are regression analyses based on the mean values

$F_{1,84} = 27.200$, $P < 0.001$; Fig. 2b) than those from WH at both transplant sites. The plants from BJ exhibited shorter growth periods than those from WH at both sites (in Beijing, $F_{1,84} = 7.414$, $P < 0.01$; in Wuhan, $F_{1,84} = 4.614$, $P < 0.05$) but presented longer reproductive periods than the plants from WH at the Wuhan site ($F_{1,83} = 9.258$, $P < 0.01$).

Differentiation of growth traits

The three following growth traits were significantly affected by the transplant site: aboveground biomass, flowering plant height and final plant height. The origin of the plants significantly affected flowering plant height and final plant height, and the effect of the interaction between the transplant site and plant origin on aboveground biomass was significant (Table 1). At the Wuhan site, plants originating from the BJ population displayed shorter flowering plant height ($F_{1,84} = 11.345$, $P < 0.01$; Fig. 2c) and less aboveground biomass ($F_{1,81} = 7.785$, $P < 0.01$; Fig. 2e) than WH-origin plants, and plants from the BJ population were shorter at harvest than WH-origin plants at both transplant sites (in Beijing, $F_{1,66} = 6.350$, $P < 0.05$; in Wuhan, $F_{1,66} = 16.300$, $P < 0.001$; Fig. 2d). At the Beijing site, the plants of WH origin were slightly taller at flowering than

Table 1 Results of ANOVA for phenological, growth and reproductive traits of *Ambrosia artemisiifolia* in the reciprocal transplant experiments

	Transplant site	Origin	Transplant site × origin
Flowering time (days)	$F_{1,84} = 321.80^{***}$	$F_{1,84} = 321.80^{***}$	$F_{1,84} = 6.92^{**}$
Senescence time (days)	$F_{1,83} = 426.06^{***}$	$F_{1,83} = 8.01^{**}$	$F_{1,83} = 0.07$
Flowering plant height (cm)	$F_{1,84} = 32.99^{***}$	$F_{1,84} = 9.93^{**}$	$F_{1,84} = 1.75$
Final plant height (cm)	$F_{1,66} = 24.03^{***}$	$F_{1,66} = 20.64^{***}$	$F_{1,66} = 2.45$
Aboveground biomass (g)	$F_{1,81} = 52.21^{***}$	$F_{1,81} = 3.57^{\dagger}$	$F_{1,81} = 3.95^*$
Proportion of male flowers	$F_{1,79} = 73652.22^{***}$	$F_{1,79} = 2.44$	$F_{1,79} = 437.95^{***}$
Seed number per plant	$F_{1,80} = 89.15^{***}$	$F_{1,80} = 3.00^{\dagger}$	$F_{1,80} = 6.08^*$
1,000-Seed weight (g)	$F_{1,80} = 12.17^{***}$	$F_{1,80} = 7.14^{**}$	$F_{1,80} = 19.39^{***}$

A generalised linear mixed model was used, with the transplant site, plant origin and their interaction as fixed effects and maternal family nested within the origin as a random effect. Data were fitted to a binomial distribution, with a logit link function for the proportion of male flowers trait. Significance levels are indicated: $^{\dagger} 0.05 < P < 0.10$, $^* P < 0.05$, $^{**} P < 0.01$, $^{***} P < 0.001$

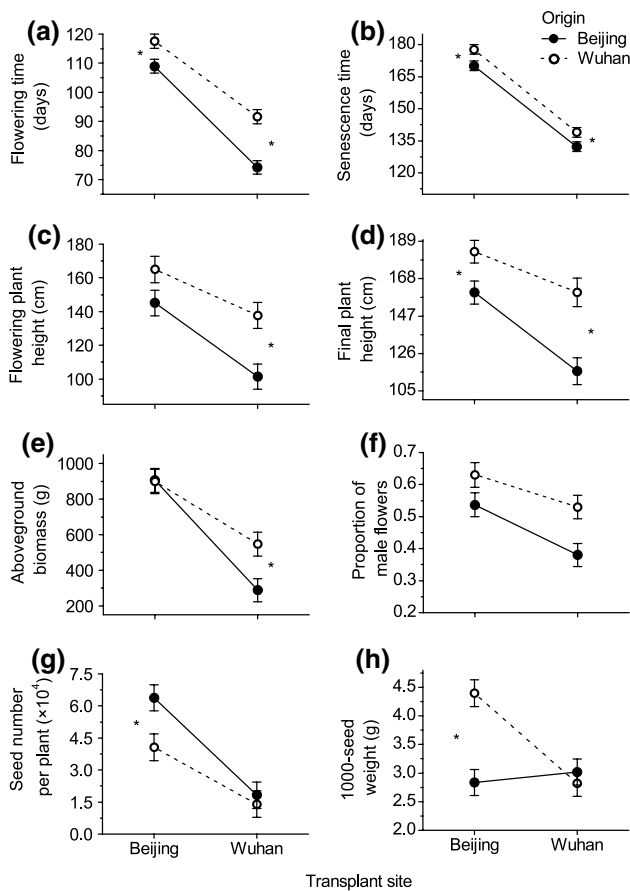


Fig. 2a–h Phenotypic traits (mean \pm SE) for plants from the two origins at each transplant site. Values were derived from the estimated marginal means in a linear mixed model, with transplant site, plant origin and their interaction as fixed effects and maternal family nested within the origin as a random effect. Asterisks indicate significant differences between the two origins ($P < 0.05$)

plants of BJ origin ($F_{1,84} = 3.317$, $P = 0.072$; Fig. 2c), and aboveground biomass was similar between the two original populations ($F_{1,81} = 0.005$, $P = 0.946$; Fig. 2e).

Differentiation of reproductive traits

The transplant site affected all the investigated reproductive traits, including proportion of male flowers, seed number and 1,000-seed weight. Only the 1,000-seed weight was influenced by plant origin. The proportions of male flowers, seed number and 1,000-seed weight were affected by the interaction between plant origin and the transplant site (Table 1). Comparisons between the two original populations indicated that the seed number and 1,000-seed weight were significantly differentiated. At the Beijing site, plants from BJ displayed more seeds per plant ($F_{1,80} = 6.884$, $P < 0.05$; Fig. 2g) and a smaller seed size ($F_{1,80} = 22.857$, $P < 0.001$; Fig. 2h) than those from WH. No difference was found for the proportion of male flowers ($F_{1,79} = 1.720$, $P = 0.193$; Fig. 2f) between the BJ and WH plants. At the Wuhan site, the plants from the two origins displayed similar seed numbers ($F_{1,80} = 0.233$, $P = 0.631$; Fig. 2g) and 1,000-seed weights ($F_{1,80} = 0.368$, $P = 0.546$; Fig. 2h). The WH plants had a larger proportion of male flowers than BJ plants ($F_{1,79} = 3.308$, $P = 0.073$; Fig. 2f).

Selection analysis

The selection analyses based on individual phenotypes or family means produced similar results for β (Table 2). At the Beijing site, there was weak selection to decrease flowering time, both across individual plants ($P = 0.031$), and marginally across families ($P = 0.099$) (Table 2). Additionally, the direct selection on aboveground biomass and the proportion of male flowers were significant, with a higher aboveground biomass and lower proportion of male flowers resulting in increased seed production. At the Wuhan site, flowering time and senescence time were significantly selected. Plants showing earlier flowering and later senescence were favoured in Wuhan. The selection favoured a lower proportion of male flowers based on

Table 2 Selection gradients (β) for phenotypic selection based on individual phenotypes and genotypic selection based on family means for *A. artemisiifolia* at each site in the reciprocal transplant experiments

	R^2	β			
		Flowering time (days)	Senescence time (days)	Aboveground biomass (g)	Proportion of male flowers
Beijing site					
Phenotypes	0.849 ($P < 0.001$)	-0.098 ($P = 0.031$)	0.046 ($P = 0.328$)	0.412 ($P < 0.001$)	-0.282 ($P < 0.001$)
Family means	0.876 ($P < 0.001$)	-0.136 ($P = 0.099$)	0.062 ($P = 0.365$)	0.341 ($P < 0.001$)	-0.315 ($P < 0.001$)
Wuhan site					
Phenotypes	0.500 ($P < 0.001$)	-0.409 ($P = 0.002$)	0.297 ($P = 0.013$)	0.178 ($P = 0.097$)	-0.219 ($P = 0.047$)
Family means	0.472 ($P = 0.004$)	-0.367 ($P = 0.029$)	0.401 ($P = 0.018$)	0.069 ($P = 0.544$)	-0.261 ($P = 0.108$)

β Were estimated from the partial regression coefficients of a linear multiple regression of relative fitness (seed number) on the four standardised characters: flowering time, senescence time, aboveground biomass and the proportion of male flowers. The overall and determinate coefficients for each trait (R^2) of the models and significance levels are reported

phenotypes ($P = 0.047$) and on family means at the Wuhan site ($P = 0.108$).

Path analysis indicated that the traits under direct selection at each site were correlated with flowering time either directly or indirectly (Fig. 3; Tables OR2, 3, Online Resource 1). At the Beijing site, aboveground biomass was strongly selected, with larger plant sizes leading to greater seed production. Aboveground biomass could be increased by later flowering through increased plant size at flowering (Fig. 3a; flowering time \rightarrow flowering plant height \rightarrow aboveground biomass). However, flowering time had a direct negative effect on aboveground biomass in our experiment (Fig. 3a; flowering time \rightarrow aboveground biomass). The proportion of male flowers displayed a strong negative correlation with relative fitness. At the Wuhan site, two phenological traits, flowering time and senescence time, were strongly directly selected, with earlier flowering and later senescence leading to increased seed production. However, senescence time was significantly correlated with flowering time, with later flowering leading to later senescence and greater seed production (Fig. 3b; flowering time \rightarrow senescence time \rightarrow relative fitness). On the other hand, the proportion of male flowers, which was negatively correlated with relative fitness, was influenced by the final plant height and senescence time. Specifically, plants flowering later presented greater final plant heights and larger proportion of male flowers (Fig. 3b; flowering time \rightarrow final plant height \rightarrow proportion of male flowers); on the other hand, plants flowering later led to later senescence and less proportion of male flowers (Fig. 3b; flowering time \rightarrow senescence time \rightarrow proportion of male flowers).

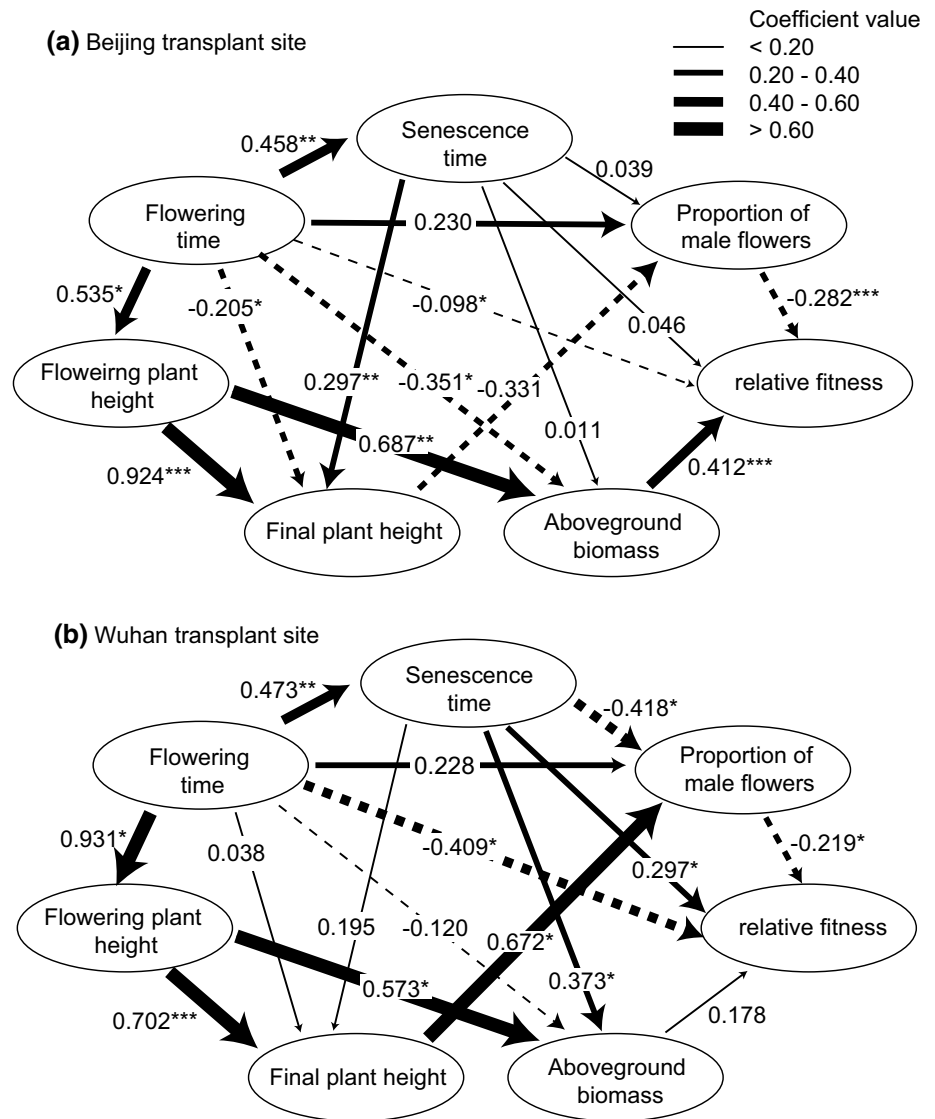
Discussion

Several studies have provided evidence of genetic differentiation in the life history traits of invasive plants in both

their native and invasive ranges (Leger and Rice 2007; Colautti and Barrett 2010; Eriksen et al. 2012). The present study clearly demonstrated genetic differentiation in several life history traits among *A. artemisiifolia* populations along a latitudinal gradient within the invasive range of this species in China (Figs. 1, 2; Online Resource 2). These results are consistent with those from common garden experiments performed on *A. artemisiifolia* that indicated a negative correlation between the flowering time and latitude of origin (Genton et al. 2005a; Hodgins and Rieseberg 2011).

Furthermore, meta-analyses of local adaptation in plants revealed that the local plants generally performed better than introduced plants at their site of origin, but at a lower frequency than is commonly assumed (Leimu and Fischer 2008; Hereford 2009), and the results of our reciprocal transplant experiments suggest that the BJ and WH populations may be locally adapted to their habitats according to the 'local vs. foreign' criterion (Kawecki and Ebert 2004). In this study, the greater seed production observed in BJ-origin plants at their home site indicated adaptation of the BJ population to their site of origin through female function (Fig. 2g), since seed production has been thought as a good estimate of female fitness. On the other hand, although we lacked direct quantification of male fitness, the greater flowering plant height recorded in WH-origin plants may indicate local adaptation to the Wuhan site through male fitness, given that increased height can increase male fitness returns through more efficient pollen dispersal (Klinkhamer et al. 1997; Friedman and Barrett 2011). The slightly higher proportion of male flowers of WH-origin plants also implied that they may exhibit higher male fitness than BJ plants at the Wuhan site. Furthermore, the interaction between plant origin and transplant site was significant for the seed number (Table 1), which also suggested local adaptation because an interaction between genotype and environment for fitness is thought to be a

Fig. 3 Results of path analysis for significant determinants of relative fitness in population of *A. artemisiifolia* grown in common gardens at **a** Beijing and **b** Wuhan. Relative fitness was estimated based on seed number. *Line thickness* indicates the magnitude of path coefficients. *Solid lines* indicate positive correlations and *dotted lines* negative correlations. *Values above each line* are partial correlation coefficients; *asterisks* indicate significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$



prerequisite for local adaptation (Kawecki and Ebert 2004). A previous study by our group comparing the genetic variation between introduced and native populations of *A. artemisiifolia* demonstrated that the Chinese populations presented a similar genetic diversity to and twice the genetic differentiation of the native populations (Li et al. 2012). These results suggested that there is sufficient genetic variation on which selection can act and that more restricted gene flow exists in Chinese invasive populations than in native populations in North America. Taken together, the results of these studies may support the occurrence of local adaptation in Chinese invasive populations. Local adaptation has also been suggested in European invasive populations of this species (Genton et al. 2005a; Hodgins and Rieseberg 2011). Although it was previously reported that maternal effects were not strong in a common garden experiment on *A. artemisiifolia* (Hodgins and Rieseberg

2011), measurement of the maternal effects involved would make the findings of this study more convincing.

Remarkably, through selection analysis and path analysis, the present study showed that the flowering and senescence time, aboveground biomass and proportion of male flowers may contribute to local adaptation in Chinese invasive populations (Table 2). In Beijing, where the growing season is shorter with cooler temperatures, early flowering plants could increase the length of the reproductive period and consequently produce more seeds (Griffith and Watson 2005). Furthermore, flowering time may have indirect effects on female fitness through aboveground biomass. On one hand, earlier flowering plants may evolve to grow faster (Online Resource 2; Fig. OR1) and hence lead to more biomass (Fig. 3a); on the other hand, earlier flowering means a shorter period of vegetative growth and thus may show a smaller plant size, which tends to decrease biomass

(Fig. 3a). In our experiment, the negative effect of flowering time on fitness (-0.449 , calculated from the sum of the direct effect of flowering time on relative fitness and its indirect effect through aboveground biomass) was slightly larger than the positive effect (0.367 , calculated from the indirect effect of flowering time on relative fitness through the flowering plant height and aboveground biomass), suggesting that earlier flowering may be favoured in Beijing at higher latitudes (Fig. 3a).

In the selection analysis, we found that earlier flowering and later senescence were directly selected in Wuhan (Table 2). Because of the longer and warmer growing season of Wuhan, earlier flowering and later senescence may lead to a longer reproductive period and, hence, increased seed production, consistent with the time-commitment hypothesis (Paquin and Aarssen 2004). However, the selection for earlier flowering in Wuhan was estimated by using seed number as an index of fitness, which is an estimate of female fitness. The results of our reciprocal transplant experiments indicated that plants originating from WH may adapt through male function (Fig. 2). We found that final plant height (which was indirectly but positively influenced by flowering time) had a significant effect on the proportion of male flowers at the Wuhan site, which conformed to the ‘size-dependent’ hypothesis of sex allocation (Paquin and Aarssen 2004; Friedman and Barrett 2011). Meanwhile, senescence time, which was correlated with flowering time, was negatively correlated with the proportion of male flowers (Fig. 3b). This result was also consistent with the time-commitment hypothesis which presumes a shorter time commitment for male function than female function (Paquin and Aarssen 2004) (Fig. 3b). Overall, the indirect effects of flowering time on the proportion of male flowers through flowering plant height and final plant height (0.439) were stronger than the effects via senescence time (-0.198), suggesting that later flowering was favoured in the WH population, which may have adapted to its habitat through male function.

These results demonstrate the importance of flowering time in both male and female functions. A previous study detected significant selection on the time to first flower and vegetative size at flowering based on direct measurements of natural selection on phenotypic traits in the invasive plant *L. salicaria* (Colautti and Barrett 2010). A further study on *L. salicaria* showed that the rapid evolution of earlier flowering at the northern invasion front of this species increased the fitness of northern versus southern populations by as much as 37-fold (Colautti and Barrett 2013). Thus, flowering time is a trait that is likely to be under selection and may exhibit greater variation under novel conditions (Kollmann and Banuelos 2004; Samis et al. 2012; Novy et al. 2013), which is consistent with the results of the present study (Fig. 3). Our findings suggest

the existence of significant selection on flowering time and a latitudinal cline of phenological traits, providing evidence of the important role of the rapid evolution of flowering time as a local adaptation of invasive *A. artemisiifolia* during the spread of this species in China.

Acknowledgments We thank Ming-Xun Ren and Xue Gu for their assistance in the seed collection and field work in Wuhan and Professor Spencer Barrett for providing helpful comments on the manuscript. This work was supported by the National Natural Science Foundation of China (31121003) and the State Key Laboratory of Earth Surface Processes and Resource Ecology. The experiments comply with the current laws of China in which the experiments were performed.

References

- Barrett SCH, Colautti RI, Eckert CG (2008) Plant reproductive systems and evolution during biological invasion. *Mol Ecol* 17(1):373–383. doi:10.1111/j.1365-294X.2007.03503.x
- Chun YJ, Fumanal B, Laitung B, Bretagnolle F (2010) Gene flow and population admixture as the primary post-invasion processes in common ragweed (*Ambrosia artemisiifolia*) populations in France. *New Phytol* 185(4):1100–1107. doi:10.1111/j.1469-8137.2009.03129.x
- Colautti RI, Barrett SCH (2010) Natural selection and genetic constraints on flowering phenology in an invasive plant. *Int J Plant Sci* 171(9):960–971. doi:10.1086/656444
- Colautti RI, Barrett SC (2013) Rapid adaptation to climate facilitates range expansion of an invasive plant. *Science* 342(6156):364–366
- Colautti RI, Eckert CG, Barrett SCH (2010) Evolutionary constraints on adaptive evolution during range expansion in an invasive plant. *Proc R Soc B Biol Sci* 277(1689):1799–1806. doi:10.1098/rspb.2009.2231
- Conner JK, Hartl DL (2004) A primer of ecological genetics. Sinauer, Sunderland
- Deen W, Hunt T, Swanton CJ (1998) Influence of temperature, photoperiod, and irradiance on the phenological development of common ragweed (*Ambrosia artemisiifolia*). *Weed Sci* 46(5):555–560
- Ebeling SK, Stocklin J, Hensen I, Auge H (2011) Multiple common garden experiments suggest lack of local adaptation in an invasive ornamental plant. *J Plant Ecol* 4(4):209–220. doi:10.1093/jpe/rtr007
- Eriksen RL, Desronvil T, Hierro JL, Kesseli R (2012) Morphological differentiation in a common garden experiment among native and non-native specimens of the invasive weed yellow starthistle (*Centaurea solstitialis*). *Biol Invasions* 14(7):1459–1467. doi:10.1007/s10530-012-0172-6
- Freeman DC, McArthur ED, Harper KT, Blauer AC (1981) Influence of environment on the floral sex-ratio of monoecious plants. *Evolution* 35(1):194–197. doi:10.2307/2407956
- Friedman J, Barrett SCH (2008) High outcrossing in the annual colonizing species *Ambrosia artemisiifolia* (Asteraceae). *Ann Bot* 101(9):1303–1309. doi:10.1093/aob/mcn039
- Friedman J, Barrett SCH (2011) Genetic and environmental control of temporal and size-dependent sex allocation in a wind-pollinated plant. *Evolution* 65(7):2061–2074. doi:10.1111/j.1558-5646.2011.01284.x
- Gaudeul M, Giraud T, Kiss L, Shykoff JA (2011) Nuclear and chloroplast microsatellites show multiple introductions in the worldwide invasion history of common ragweed, *Ambrosia artemisiifolia*. *PLoS One* 6(3):e17658. doi:10.1371/journal.pone.0017658

- Genton BJ, Kotanen PM, Cheptou PO, Adolphe C, Shykoff JA (2005a) Enemy release but no evolutionary loss of defence in a plant invasion: an inter-continental reciprocal transplant experiment. *Oecologia* 146(3):404–414. doi:[10.1007/s00442-005-0234-x](https://doi.org/10.1007/s00442-005-0234-x)
- Genton BJ, Shykoff JA, Giraud T (2005b) High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple sources of introduction. *Mol Ecol* 14(14):4275–4285. doi:[10.1111/j.1365-294X.2005.02750.x](https://doi.org/10.1111/j.1365-294X.2005.02750.x)
- Gladieux P, Giraud T, Kiss L, Genton BJ, Jonot O, Shykoff JA (2011) Distinct invasion sources of common ragweed (*Ambrosia artemisiifolia*) in Eastern and Western Europe. *Biol Invasions* 13(4):933–944. doi:[10.1007/s10530-010-9880-y](https://doi.org/10.1007/s10530-010-9880-y)
- Gonzalo-Turpin H, Hazard L (2009) Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *J Ecol* 97(4):742–751. doi:[10.1111/j.1365-2745.2009.01509.x](https://doi.org/10.1111/j.1365-2745.2009.01509.x)
- Griffith TM, Watson MA (2005) Stress avoidance in a common annual: reproductive timing is important for local adaptation and geographic distribution. *J Evol Biol* 18(6):1601–1612. doi:[10.1111/j.1420-9101.2005.01021.x](https://doi.org/10.1111/j.1420-9101.2005.01021.x)
- Griffith TM, Watson MA (2006) Is evolution necessary for range expansion? Manipulating reproductive timing of a weedy annual transplanted beyond its range. *Am Nat* 167(2):153–164. doi:[10.1086/498945](https://doi.org/10.1086/498945)
- Haggerty BP, Galloway LF (2011) Response of individual components of reproductive phenology to growing season length in a monocarpic herb. *J Ecol* 99(1):242–253. doi:[10.1111/j.1365-2745.2010.01744.x](https://doi.org/10.1111/j.1365-2745.2010.01744.x)
- Hereford J (2009) A quantitative survey of local adaptation and fitness trade-offs. *Am Nat* 173(5):579–588. doi:[10.1086/597611](https://doi.org/10.1086/597611)
- Hodgins KA, Rieseberg L (2011) Genetic differentiation in life-history traits of introduced and native common ragweed (*Ambrosia artemisiifolia*) populations. *J Evol Biol* 24(12):2731–2749. doi:[10.1111/j.1420-9101.2011.02404.x](https://doi.org/10.1111/j.1420-9101.2011.02404.x)
- Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecol Lett* 7(12):1225–1241. doi:[10.1111/j.1461-0248.2004.00684.x](https://doi.org/10.1111/j.1461-0248.2004.00684.x)
- Kilkenny FF, Galloway LF (2013) Adaptive divergence at the margin of an invaded range. *Evolution* 67(3):722–731. doi:[10.1111/j.1558-5646.2012.01829.x](https://doi.org/10.1111/j.1558-5646.2012.01829.x)
- Klinkhamer PGL, deJong TJ, Metz H (1997) Sex and size in cosexual plants. *Trends Ecol Evol* 12(7):260–265. doi:[10.1016/s0169-5347\(97\)01078-1](https://doi.org/10.1016/s0169-5347(97)01078-1)
- Kollmann J, Banuelos MJ (2004) Latitudinal trends in growth and phenology of the invasive alien plant *Impatiens glandulifera* (Balsaminaceae). *Divers Distrib* 10(5–6):377–385. doi:[10.1111/j.1366-9516.2004.00126.x](https://doi.org/10.1111/j.1366-9516.2004.00126.x)
- Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* 37(6):1210–1226. doi:[10.2307/2408842](https://doi.org/10.2307/2408842)
- Leger EA, Rice KJ (2007) Assessing the speed and predictability of local adaptation in invasive California poppies (*Eschscholzia californica*). *J Evol Biol* 20(3):1090–1103. doi:[10.1111/j.1420-9101.2006.01292.x](https://doi.org/10.1111/j.1420-9101.2006.01292.x)
- Leimu R, Fischer M (2008) A meta-analysis of local adaptation in plants. *PLoS One* 3(12):e4010. doi:[10.1371/journal.pone.0004010](https://doi.org/10.1371/journal.pone.0004010)
- Li XM, Liao WJ, Wolfe LM, Zhang DY (2012) No evolutionary shift in the mating system of North American *Ambrosia artemisiifolia* (Asteraceae) following its introduction to China. *PLoS One* 7(2):e31935. doi:[10.1371/journal.pone.0031935](https://doi.org/10.1371/journal.pone.0031935)
- Mitchell RJ (2001) Path analysis: pollination. In: Scheiner SM, Gurevitch J (eds) Design and analysis of ecological experiments. Oxford University Press, New York
- Novy A, Flory SL, Hartman JM (2013) Evidence for rapid evolution of phenology in an invasive grass. *J Evol Biol* 26(2):443–450. doi:[10.1111/jeb.12047](https://doi.org/10.1111/jeb.12047)
- Paquin V, Aarssen LW (2004) Allometric gender allocation in *Ambrosia artemisiifolia* (Asteraceae) has adaptive plasticity. *Am J Bot* 91(3):430–438. doi:[10.3732/ajb.91.3.430](https://doi.org/10.3732/ajb.91.3.430)
- Payne WW (1963) Morphology of inflorescence of ragweeds (*Ambrosia-Franseria*: Compositae). *Am J Bot* 50(9):872–880. doi:[10.2307/2439774](https://doi.org/10.2307/2439774)
- Samis KE, Murren CJ, Bosssdorf O, Donohue K, Fenster CB, Malmberg RL, Purugganan MD, Stinchcombe JR (2012) Longitudinal trends in climate drive flowering time clines in North American *Arabidopsis thaliana*. *Ecol Evol* 2(6):1162–1180. doi:[10.1002/ece3.262](https://doi.org/10.1002/ece3.262)
- Santamaria L, Figuerola J, Pilon JJ, Mjelde M, Green AJ, De Boer T, King RA, Gornall RJ (2003) Plant performance across latitude: the role of plasticity and local adaptation in an aquatic plant. *Ecology* 84(9):2454–2461. doi:[10.1890/02-0431](https://doi.org/10.1890/02-0431)
- Sherrard ME, Maherali H (2012) Local adaptation across a fertility gradient is influenced by soil biota in the invasive grass, *Bromus inermis*. *Evol Ecol* 26(3):529–544. doi:[10.1007/s10682-011-9518-2](https://doi.org/10.1007/s10682-011-9518-2)
- Sutherland WJ, Freckleton RP, Godfray HCJ, Beissinger SR, Benton T, Cameron DD, Carmel Y, Coomes DA, Coulson T, Emmerson MC (2013) Identification of 100 fundamental ecological questions. *J Ecol* 101(1):58–67
- Wan FH, Guo JY, Zhang F (2009) Research on biological invasions in China. Science Press, Beijing