

An explicit test for the contribution of environmental maternal effects to rapid clinal differentiation in an invasive plant

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Abstract: Population differentiation of alien invasive plants within their non-native range has received increasingly more attention. Common gardens are typically used to assess the levels of genotypic differentiation among populations. However, in such experiments, environmental maternal effects can influence phenotypic variation among individuals if seed sources are collected from field populations under variable environmental regimes. In the present study, we investigated the causes of an altitudinal cline in an invasive plant. Seeds were collected from *Senecio inaequidens* (Asteraceae) populations along an altitudinal gradient in southern France. In addition, seeds from the same populations were generated by intra-population crossings in a climatic chamber. The two seed lots were grown in a common garden in Central Belgium to identify any evidence of environmentally induced maternal effects and/or an altitudinal cline in a suite of life-history traits. Results failed to detect any environmental maternal effects. However, an altitudinal cline in plant height and above-ground biomass was found to be independent of the maternal environment.

Keywords: altitude; climatic gradient; clinal variation; invasive plant; maternal environment; seed mass.

Introduction

The way and the speed organisms will cope with, react or adapt to rapid environmental changes is a prime question for evolutionary ecology in the context of global change. Biological invasions are an outstanding opportunity to address fundamental questions concerning rapid species evolution in relation to environmental change (Sakai *et al.*, 2001; Lee, 2002; Callaway & Maron, 2006). As a result, the evolution of invasive species during range expansion is receiving increasingly more attention, and questions of population differentiation within introduction ranges of invasive species have been addressed in several common garden studies in the last decade (Weber & Schmid, 1998; Parker *et al.*, 2003; Kollmann & Banuelos, 2004; Maron *et al.*, 2004; Leger & Rice, 2007; Montague *et al.*, 2008). Such studies often indicated latitudinal (Weber & Schmid, 1998; Kollmann & Banuelos, 2004; Maron *et al.*, 2004; Montague *et al.*, 2008) or altitudinal (Monty & Mahy, 2009) clines, most often interpreted as the result of rapid adaptive processes. However, seeds used in those common garden studies were all obtained from natural populations (but see Weber & Schmid, 1998, where clones were used), and future studies are also likely to use field-collected propagules for practical facility. Therefore, environmental maternal effects (EMEs) cannot be ruled out, even if most results suggest *a posteriori*, a low maternal influence on measured traits. Yet, the role of EME on such clinal variation has never been explicitly tested. This is, however, important because if most studies use seeds collected in the field, an insufficient understanding of the influence of EME can lead to misinterpretation of the results and overestimation of the capacity of rapid adaptation to changing environment.

Alien species expansion into introduced ranges depends on the species capacity to cope with environmental heterogeneity. Population expansion over climatic gradients is possible due to the presence of phenotypic variability within a species. Phenotypic variability among populations can be the result of one or more of the following factors: phenotypic plasticity (Annapurna & Singh, 2003; Parker *et al.*, 2003), evolutionary differentiation (Cox, 2004) and/or maternal effects (Roach & Wulff, 1987). The latter are influences of the maternal parent on the phenotype of its offspring via mechanisms exclusive of the genetic information carried on chromosomes (Roach & Wulff, 1987). Maternal effects are determined by either genetic or environmental factors (Roach & Wulff, 1987; Platenkamp & Shaw, 1993).

Nongenetic or environmental maternal effects are elicited when the maternal plant environment influences the expression of traits in its offspring (Roach & Wulff, 1987). For example, the maternal tissue environment in which the zygote develops into a sporophyte can influence the phenotype of the offspring following germination

(Roach & Wulff, 1987; Kirkpatrick & Lande, 1989; Schmid & Dolt, 1994). Previous studies demonstrated that EME imposed a greater influence on offspring than genetic maternal effects (Platenkamp & Shaw, 1993; Schmid & Dolt, 1994; Byers *et al.*, 1997). EME on individual plant growth often appears to be transitory (Miao *et al.*, 1991; Wulff & Bazzaz, 1992; Schmid & Dolt, 1994), but may still play a role if the period of influence is important to plant fitness (Alexander & Wulff, 1985). EME are often mediated by seed size or seed mass, and several studies found a positive relationship between seed size, the probability and speed of germination, and subsequent seedling size (e.g. Weis, 1982; Hendrix, 1984; Schmid & Dolt, 1994). However, other studies did not detect these same properties (Dolan, 1984; Schmitt & Antonovics, 1986) and emphasized the possible role of other seed qualities (Hereford & Moriuchi, 2005). EME studies have generally considered one or two specific sources of environmental variation on maternal plants, which can influence the seeds and/or the offspring. Sources of variation in soil nutrient levels and water availability have been thoroughly addressed (Stratton, 1989; Aarssen & Burton, 1990; Philippi, 1993; Schmid & Dolt, 1994; Luzuriaga *et al.*, 2005), and other studies focused on the effects of photoperiod (Munir *et al.*, 2001), intra- or inter-specific competition (Platenkamp & Shaw, 1993; Donohue, 1999) or herbivory (Weiner *et al.*, 1997). However, few studies have questioned the effects of climatic conditions on seed and seedling characteristics (Alexander & Wulff, 1985; Blödner *et al.*, 2007). Most studies experimentally induced variations in the maternal plant environment and ascertained the consequences of phenotypic maternal variation on seeds and seedlings. Studies on EME in natural populations are scarce (but see Philippi, 1993; Galloway, 1995, 2001) and little is known about their contribution to phenotypic variation under field conditions.

Environmental maternal effect can complicate the interpretation of common garden experiments. Phenotypic variation among plants in a common garden is generally attributed to genetic differentiation (Clausen *et al.*, 1940). However, environmental conditions in seed production can result in maternal carry-over effects. To minimize this source of error, plants should be grown for one or several generations in a common garden prior to the experiment (Roach & Wulff, 1987). However, many studies on plant differentiation fail to do so for practical reasons.

The invasion history of the African ragwort *Senecio inaequidens* DC. (Asteraceae) in southern France suggests that all the populations originated from a single introduction site and dispersal was largely spontaneous and/or aided by roads and railway traffic (Guillerm *et al.*, 1990; Ernst, 1998). Differences between French populations are therefore not attributable to multiple introduction events. Seeds from French *S. inaequidens* populations collected *in situ* and grown in a common garden environment showed elevational clinal variation in growth traits, i.e. plants from higher elevations showed lower height and above-ground biomass (Monty & Mahy, 2009). The nature of this cline could, however, not be ascertained, as both genetic differentiation and BME could have lead to the pattern observed. Therefore, the species represents a suitable model to assess the importance of BME in natural populations, and compare it to genetic differentiation along an altitudinal gradient during range expansion.

In this study, we conducted a common garden experiment with populations of *S. inaequidens* known to present a clinal phenotypic differentiation along an altitudinal gradient (Monty & Mahy, 2009) with the aim to examine the role of BME in phenotypic variation in plant traits. We specifically addressed the following questions: (i) to what extent does seed size explain phenotypic variation? (ii) exclusive of seed size, can phenotypic differences between plants from different maternal environments, i.e. natural and controlled, be detected? and (iii) is the clinal pattern dependent on the maternal environment?

Materials and methods

Senecio inaequidens natural history

Senecio inaequidens is an herbaceous perennial native to South Africa and Lesotho (Ernst, 1998). The species was accidentally introduced to Europe in the late 19th to early 20th century, where only tetraploids are reported. In its native range, the species occurs as two co-existing cytotypes (Lafuma *et al.*, 2003). The first records of the species are considered introduction events tied to the wool industry. For several decades, specimens were only documented in the vicinity of wool-processing centres in Europe (Ernst, 1998): Hannover (D) in 1889 and Bremen (D) in 1886; Edinburgh (UK) in 1928; Liège (B) in 1892 (Verloove, 2006); and Mazamet (F) in 1936 (Guillerm *et al.*, 1990). In southern France, Guillerm *et al.* (1990) documented the progressive colonization of the species, and found that for decades, it was only reported in the vicinity of the wool-processing centre of Mazamet. However, it was not until after 1950 that it steadily expanded throughout areas geographically removed from existing wool industrial centres. By the 1970s, the species had colonized areas of south-western France well within the first introduction region and was considered an agricultural weed. In the early 1980s, the

species reached the Mediterranean coast from Mazamet. A decade later, although the species was largely distributed along roads and railways, it was considered a threat to natural habitats (Michez, 1995). Today, *S. inaequidens* is still absent from several areas in central France, indicating that invasion fronts from Mazamet and other introduction sites have not yet converged (A. Monty, unpublished data). Historical data and the present distribution of the species strongly suggest seeds were introduced with the sheep wool trade and that several different independent colonization events occurred.

Senecio inaequidens reaches 1 m tall at its maximum height and possesses numerous stems bearing yellow capitula. Each plant can produce up to 1500 flowering heads during the reproductive season, each one bearing roughly 100 achenes (Lopez-Garcia & Maillet, 2005). Flowering initiates in late spring and continues through late autumn. The species is considered self-incompatible, but in France, some individuals were observed exhibiting partial self-fertility (Lopez-Garcia & Maillet, 2005). Entomophilous pollination with generalist pollinators is most common. The fruit is an achene bearing a pappus to aid in wind-dispersal (Monty *et al.*, 2008).

Seed collection and production

We used the same populations as Monty & Mahy (2009), located along an altitudinal gradient in Southern France. The transect was located within the geographic area representing populations known to originate in Mazamet (Guillerm *et al.*, 1990). It encompassed the maximal altitudinal range of the species in its introduced range. Along the transect, we selected five reference altitudes: 0, 200, 400, 800 and 1600 m. Two seed populations were selected at each altitudinal reference (Table 1; Fig. 1). All populations chosen for the study were located on similar soil environment along roadsides on rocky, sandy and/or gravelly soils and were at least 2 km apart. The two populations occupying the central part of the transect (400 m) were located in the initial introduction area and, most probably, derived from parental plants involved in the initial introduction to Mazamet.

In November 2005, seeds were collected on 2-3 capitula from 10 randomly selected individuals per population and stored in paper bags at 4 °C. In November 2006, the seeds were sown in pots. Each of the 10 field sampled individuals in each of the 10 populations was represented by one offspring. The 100 plants were grown under controlled conditions in an insect-proof climatic chamber (18 °C constant temperature; 16 h photoperiod; 70-80% humidity). In March 2007, hand pollinations were performed when all capitulum florets were receptive (flowering is centripetal within a capitulum but stigmas remain receptive for several days if not pollinated). Manual crossings were made by gently rubbing two capitula together. Six individuals per population were randomly assigned as the maternal parent and received pollen from one (or two) other individual(s) in the population. The resulting seeds were sorted using a dissecting microscope to discard aborted seeds or those with obvious abnormalities. Ten seeds per maternal individual were randomly selected for a common garden experiment and collectively weighted to the nearest 0.1 mg. These seeds corresponded to the 'controlled' maternal environment.

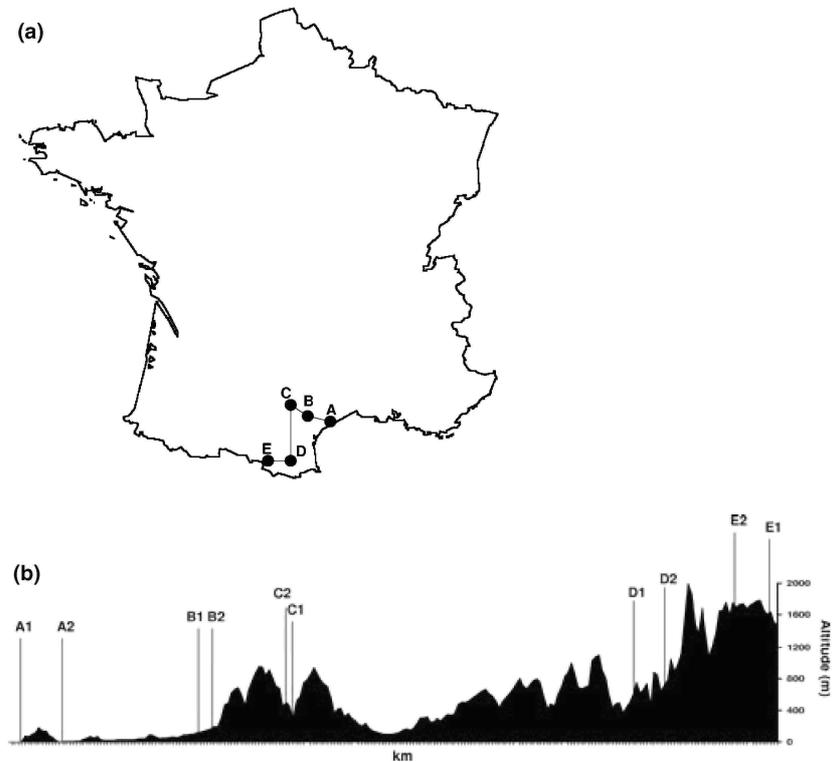
Table 1: Seed population coordinates and corresponding climatic stations, climatic data and seed mass data.

Seed population		Climatic station							Mass of 10 seeds (10 ⁻⁴ g)					
Code	Location	Altitude	Latitude	Longitude	Location	Altitude	Latitude	Longitude	RR	TN	TM	TX	Natural	Controlled
		(m)	N	E		(m)	N	E						
A1	Narbonne-Plage	2	43° 10'	3°11'	Narbonne	5	43°11'	3°01'	41.4	15.3	19.2	23.1	30.67 ± 1.20	31.00 ± 1.83
A2	Narbonne	5	43°11'	3°02'									33.67 ± 2.94	33.83 ± 2.85
B1	Rieux-Minervois	95	43° 16'	2°37'	Laure-Minervois	80	43° 16'	2°31'	38.1	14.3	18.4	22.4	29.00 ± 1.57	32.50 ± 2.50
B2	Caunes-Minervois	175	43° 19'	2°32'									37.83 ± 1.11	35.00 ± 2.35
C1	Mazamet (Moulin de l'Oulne)	330	43°29'	2°22'	Rouairoux	345	43°28'	2°35'	57.7	12.2	16.6	20.9	30.17 ± 1.66	31.17 ± 1.28
C2	Mazamet (Castaunouze)	370	43°29'	2°23'									32.50 ± 2.17	40.17 ± 2.12
D1	Taurynia	760	42°35'	2°25'	Nohedes	1000	42°38'	2° 17'	37.4	9	13.1	17.2	27.17 ± 1.85	33.83 ± 1.60
D2	Nohèdes	785	42°37'	2°19'									28.83 ± 1.62	31.00 ± 1.39
E1	Egat	1635	42°30'	2°01'	Valcebollere	1420	42°23'	2°02'	46.7	5.5	11.9	18.4	31.83 ± 0.70	33.50 ± 2.58
E2	La Llagone	1695	42°32'	2°08'									38.50 ± 0.81	31.83 ± 0.91

Seed data are the population average mass (10⁻⁴ g) and standard error of the six lots of 10 seeds collectively weighted per parent population prior to the

common garden experiment, for both maternal environment (natural vs. controlled), separately. Climatic data are the cumulative rainfall (RR, mm), mean minimal temperature (TN, °C), mean maximal temperature (TX, °C) and mean temperature (TM, °C) during October 2006.

Fig. 1: (a) *Transect location in southern France, with the five altitudinal references (A: 0; B: 200; C: 400; D: 800 and E: 1600 m);* (b) *Seed population locations along the altitudinal transect. The transect in (b) correspond to linear sections between consecutive populations. Altitude was measured every km on the x-axis.*



In early November 2006, seeds on 2-3 capitula were field collected from 10 randomly selected individuals and stored in paper bags at 4 °C. The seeds were subsequently sorted and 10 seeds (without obvious anomalies, i.e. abortion or malformation) per parent individual were randomly selected and collectively weighted to the nearest 0.1 mg. These seeds represented the 'natural' maternal environment. The characteristics of the two sets of seeds are presented in Table 1. Monthly mean minimal (TN) and maximal (TX) temperatures during October 2006, as well as cumulative rainfall were obtained from climatic stations located within 20 km or less from seed populations at each altitude (Table 1). Monthly mean temperature was defined as $(TN + TX)/2$. These data represented the climatic conditions in the field for the maternal plants during flowering and fruit maturation ('natural' maternal environment).

Common garden experiment

In spring 2007, a common garden was established using a randomized block experimental design on an open area in Gembloux (Belgium; 50°33'N; 4°42'E; alt. 160 m). Six north-south-oriented blocks consisted of two rows of 10 pots each. Pots contained 2.5 L sand, 2.5 L compost and 0.5 L hydro-granulates (Argex®, Argex NV, Zwijndrecht, Belgium). The 20 pots per block were randomly assigned to the 20 combinations of the maternal environments and the populations (two maternal environments, i.e. natural and controlled x 10 seed populations). To prevent above-ground competition, rows were placed 80 cm apart and within each row, pots were separated by 50 cm. The common garden was surrounded by two additional rows of potted individuals to prevent edge effects.

On 4 June 2007, all 10 seeds per parent were sown together in each pot. Pots were covered with a protective light-permeable canvas for 10 days. Seedlings were counted every 2-3 days. The first seedling that emerged in each pot was marked with a short rod and retained in the pot for measurement. Remaining seedlings were

removed after counting. Germination was considered complete on 23 June, 10 days after no additional seedlings had emerged. The following data were recorded: time to germination (d) since sowing; time to flowering (d) since germination (based on daily observations); and height at maturity (cm), defined as the height of the plant at first flowering. Plant height was measured on 6 August (63 days after sowing; henceforth referred to as 'intermediate height') and on 19 November (168 days after sowing and close to winter senescence; 'final height'). On 23 November, plants were harvested at ground level and above-ground biomass (g) was measured after oven drying for 48 h at 60 °C.

Data analysis

A two-way analysis of variance (ANOVA) was performed on the seed mass data. Maternal environment was treated as a fixed factor with two modalities represented by the controlled and natural seed maternal environments. The second fixed factor was the source population (10 modalities), and the two fixed factors were crossed. In the case of an interaction between the two factors, a one-way ANOVA was performed with the factor population, for each maternal environment separately. The presence or absence of clinal variation was assessed by testing for a linear contrast on the population mean seed masses against population altitude (Dagnelie, 2003).

Phenotypic variation among individuals has two possible sources under homogeneous conditions: genetic variation and maternal effects. Those two sources of variation can interact, as the influence of maternal conditions can differ according to genotype. A mixed model ANCOVA was used to distinguish the different sources of phenotypic variation observed among individuals in the common garden. Maternal environment (two modalities) and source population (10 modalities) were fixed factors. Block (six modalities) was treated as a random factor and all factors were crossed. The mass (mg) of the 10 seeds sown per pot served as the covariate. A significant covariate effect indicates that seed mass influences phenotypic variation, and consequently reveals EME mediated by seed mass, whereas a significant effect of the maternal environment indicates maternal effects not mediated by seed mass. For traits demonstrating a significant population effect, testing for a linear contrast between the population trait means and population altitude would provide evidence of an altitudinal cline. In addition, a significant maternal environment x population EME interaction would suggest that the cline was dependent on the maternal environment, and therefore indicate EME.

Analyses of variance and covariance were performed using the general linear model in MINITAB software version 14.20 (Minitab Inc., State College, PA, USA). Biomass data were log-transformed for distribution normality.

Results

Seed mass analysis

The two-way ANOVA performed on seed mass revealed no significant effect of maternal environment ($F_{1,100} = 2.68$, $P = 0.105$), but a significant maternal environment by population interaction ($F_{9,100} = 2.51$, $P = 0.012$). We therefore analysed separately the seed masses of the 'controlled' and the 'natural' maternal environment. Seeds from controlled conditions did not significantly differ in mass, according to population ($F_{9,50} = 1.84$, $P = 0.083$). In contrast, the population effect was highly significant for the 'natural' maternal environment ($F_{9,50} = 4.95$, $P < 0.001$). The altitudinal linear contrast tested for this maternal environment was not significant ($F_{1,50} = 1.78$, $P = 0.189$; Table 2).

Common garden experiment

Germination was successful in every pot in the experimental garden and all 120 selected plants survived until harvest. However, 18 plants, evenly distributed among the two maternal environments and the 10 populations (*data not shown*) failed to flower. Therefore, time to flowering and height at maturity were only recorded for 102 of 120 plants, ANCOVA (Table 3) revealed no significant covariate influence, indicating that seed mass failed to explain phenotypic variation in the measured traits. Furthermore, the maternal environment factor was never significant, i.e. no differences were detected between plants grown from *in situ* collected seeds and seeds produced under controlled conditions. The remaining two factors (source population and block) were not significant for time to germination, time to flowering and height at maturity. Significant variation among populations for intermediate height ($F_{9,94} = 2.28$, $P = 0.023$), final height ($F_{9,94} = 2.36$, $P = 0.019$) and above-ground biomass ($F_{9,94} = 2.26$, $P = 0.025$) was observed. A linear contrast for these traits was applied and revealed an altitudinal cline in final plant height ($F_{1,94} = 7.38$, $P = 0.008$) and above-ground biomass ($F_{1,94} = 8.81$, $P = 0.004$). The altitudinal cline in phenotypic traits was not dependent on maternal environment, as no significant maternal environment x population interaction was indicated (Table 3). The block factor was

significant for intermediate height ($F_{9,94} = 7.81$, $P < 0.001$), final height ($F_{9,94} = 6.01$, $P < 0.001$) and above-ground biomass ($F_{9,94} = 3.36$, $P = 0.008$). Table 4 presents means and standard deviations of the different plant traits, distributed by populations and maternal environments. Figure 2 illustrates the altitudinal pattern of variation in final plant height and above-ground biomass, for both maternal environments.

Table 2: Results of analyses of variance on seed mass: (a) two-way ANOVA depicting the effect of maternal environment and population on the whole data set. As the maternal environment x population interaction was significant, one-way ANOVA were separately performed for (b) the seeds produced under controlled conditions and (c) the seeds sampled in the field. As the latter showed a population effect, a linear contrast was tested against the altitude of the source population.

Source of variation	d.f.	MS	P
(a)			
Maternal environment	1	56.03	0.105
Population	9	77.61	< 0.001
Maternal environment x population interaction	9	52.63	0.012
Error	100	20.93	
(b)			
Population	9	45.67	0.083
Error	50	24.78	
(c)			
Population	9	84.57	< 0.001
Altitudinal linear contrast	1	30.32	0.189
Error	50	17.08	

Significant results are in bold.

Table 3: Results of ANCOVA analyses depicting the effect of seed mass, maternal environment, source population and block on the measured traits.

Source of variation	d.f.	Time to germination		Time to flowering		to Height maturity		at Intermediate height		Final height		Above-ground biomass	
		MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Seed mass	1	2.44	0.627	20.40	0.785	7.53	0.753	60.77	0.400	289.1	0.152	0.0064	0.807
Maternal environment	1	3.90	0.540	203.3	0.389	13.51	0.673	53.72	0.416	50.6	0.547	0.0204	0.662
Population	9	8.25	0.615	237.8	0.550	111.6	0.170	193.7	0.023	327.2	0.019	0.2394	0.025
Altitudinal contrast	linear 1	-	-	-	-	-	-	287.6	0.0688	1021.5	0.010	0.9338	0.004
Block	5	23.79	0.050	377.2	0.237	83.87	0.360	662.3	< 0.001	844.2	< 0.001	0.3564	0.008
Maternal environment x population interaction	9	12.30	0.306	342.2	0.272	68.92	0.517	49.9	0.804	70.7	0.864	0.0268	0.985
Error	94*	20.28		271.3		75.3		84.4		138.4		0.106	

In the case of a population effect, the statistics of the corresponding linear contrast against altitude are given. Significant results are in bold.

*For time to flowering and height at maturity, the degree of freedom of the error term was 76.

Discussion

Origin of the clinal phenotypic variation

Geographic variation in life-history traits associated with climate has been documented in plants within their native ranges, and clinal variation with latitude is common in wide-ranging species (e.g. Winn & Gross, 1993; Van Dijk *et al.*, 1997; Li *et al.*, 1998; Jonas & Geber, 1999; Clevering *et al.*, 2001; Olsson & Agren, 2002). Other studies have reported variation in life-history traits among native plants along altitudinal gradients, e.g.

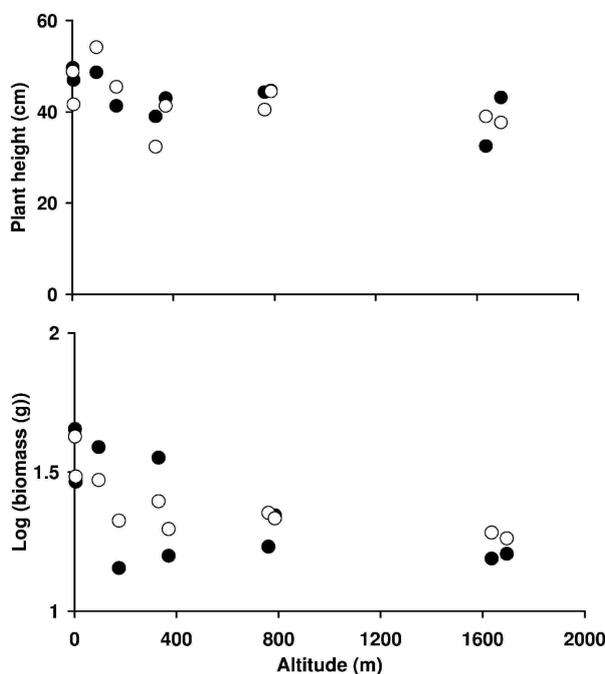
development time and leaf morphology (Galen *et al.*, 1991; Jonas & Geber, 1999). Furthermore, evidence of genetic differentiation in the form of geographic clines in growth traits has been shown in invasive species (Weber & Schmid, 1998; Del Pozo *et al.*, 2002; Sexton *et al.*, 2002; Kollmann & Banuelos, 2004; Maron *et al.*, 2004). Though, the importance of EME in such clines is unclear.

Table 4: Means \pm standard deviations of the plant traits measured in the common garden, distributed by populations and maternal environments.

Population code	Altitude (m)	Time to germination (days)	Time to flowering (days)	Height at maturity (cm)	Height at Intermediate height (cm)	Final height (cm)	Above-ground biomass (g)
Natural							
A1	2	7.2 \pm 0.41	95.2 \pm 23.1	41.0 \pm 12.0	30.8 \pm 15.2	49.7 \pm 14.9	41.2 \pm 32.5
A2	5	7.0 \pm 0.00	103.6 \pm 22.5	45.0 \pm 10.8	27.8 \pm 11.3	47.0 \pm 14.3	51.9 \pm 36.9
B1	95	7.0 \pm 0.00	110.2 \pm 15.3	51.2 \pm 7.5	25.2 \pm 12.7	48.7 \pm 16.1	45.4 \pm 50.4
B2	175	7.0 \pm 0.00	114.0 \pm 15.1	39.5 \pm 6.9	23.7 \pm 7.3	41.3 \pm 8.2	21.8 \pm 5.6
C1	330	7.0 \pm 0.00	115.0 \pm 1.4	44.8 \pm 9.4	17.2 \pm 8.1	39.0 \pm 17.2	26.3 \pm 20.0
C2	370	7.0 \pm 0.00	95.0 \pm 9.3	43.6 \pm 9.2	29.7 \pm 9.7	43.0 \pm 14.9	30.1 \pm 22.0
D1	760	7.0 \pm 0.00	102.4 \pm 19.1	43.2 \pm 15.2	24.0 \pm 15.4	44.3 \pm 18.4	28.4 \pm 19.6
D2	785	7.0 \pm 0.00	111.7 \pm 13.5	42.7 \pm 7.3	21.7 \pm 6.9	44.7 \pm 7.2	26.3 \pm 17.9
E1	1635	10.8 \pm 8.91	89.3 \pm 13.0	41.5 \pm 5.7	23.7 \pm 12.6	32.5 \pm 17.3	23.9 \pm 15.6
E2	1695	7.0 \pm 0.00	102.0 \pm 7.6	41.8 \pm 5.0	28.3 \pm 7.7	43.2 \pm 5.1	20.3 \pm 7.4
Controlled							
A1	2	7.0 \pm 0.00	103.6 \pm 10.7	46.8 \pm 3.3	30.3 \pm 6.6	48.8 \pm 9.9	33.9 \pm 22.1
A2	5	7.3 \pm 0.82	111.3 \pm 10.8	49.3 \pm 5.3	25.3 \pm 10.7	41.7 \pm 18.6	49.4 \pm 21.6
B1	95	7.2 \pm 0.41	97.3 \pm 27.9	45.0 \pm 9.0	32.5 \pm 10.7	54.2 \pm 8.0	52.3 \pm 33.5
B2	175	7.2 \pm 0.41	120.3 \pm 16.5	43.3 \pm 11.2	23.8 \pm 9.0	45.5 \pm 10.9	17.2 \pm 8.1
C1	330	11.7 \pm 11.43	95.0 \pm 26.5	36.3 \pm 9.8	15.7 \pm 11.2	32.3 \pm 17.9	25.5 \pm 24.1
C2	370	7.8 \pm 2.04	104.0 \pm 9.1	46.8 \pm 8.6	26.5 \pm 12.6	41.3 \pm 16.2	54.4 \pm 53.7
D1	760	7.0 \pm 0.00	109.3 \pm 12.9	46.5 \pm 11.0	20.8 \pm 11.7	40.5 \pm 14.3	34.7 \pm 30.0
D2	785	7.5 \pm 1.22	109.2 \pm 12.5	40.0 \pm 6.0	24.8 \pm 13.6	44.5 \pm 8.1	20.4 \pm 14.0
E1	1635	7.0 \pm 0.00	114.8 \pm 16.0	39.2 \pm 5.5	21.3 \pm 8.5	39.0 \pm 5.0	16.6 \pm 4.5
E2	1695	7.5 \pm 1.22	105.2 \pm 20.6	34.2 \pm 6.8	19.3 \pm 5.4	37.7 \pm 8.7	17.4 \pm 8.8

'Natural' refers to plants grown from seeds collected in the field, 'controlled' refers to plants grown from seeds produced under controlled conditions.

Fig. 2: *Senecio inaequidens* populations and maternal environment in the common garden: population mean final height and above-ground biomass against altitude of source populations for the two maternal environments. Open symbols: plants grown from seeds collected from natural populations; closed symbols: plants from seeds produced under controlled conditions.



This study is one of the first to evaluate EME by growing an invasive species in a common garden from (a) seeds collected *in situ* and (b) seeds produced under controlled conditions. The cline observed by Monty & Mahy (2009) in plant size was used as a basis to investigate the importance of EME, when compared with genetic differentiation, on clinal variations observed in invasive plants. The experiment we report here clearly suggested that *S. inaequidens* experienced population genotypic differentiation during range expansion from the site of introduction towards higher and lower altitudinal areas, whereas no evidence for EME was detected. When grown under controlled conditions in the common gardens, plants from lower elevations were taller and larger than those from higher elevation, and varied gradually, independently on the maternal seed production environment. Surprisingly, however, plants grown from seeds produced under controlled conditions were more variable in some traits, e.g. above-ground biomass (Fig. 2). This pointed out the different behaviour of populations B2 and C1 (located at altitudes of 175 and 330 m, Table 4).

Seed mass analysis

Seed mass did not statistically differ between the two maternal environments. However, the pattern of variation between populations was different in the 'controlled' and the 'natural' maternal environment, as revealed by the maternal environment x population interaction. When environmental variability was controlled, seed mass did not significantly differ between populations. But in the field, the population influence was highly significant. This suggests a plastic response in seed mass to environmental conditions, at least in some populations. As the pattern of variation was found not to be linear with altitude, the plastic response in seed mass may arise from other environmental conditions than climate, e.g. soil nutrient levels. Our results are in line with those of Hereford & Moriuchi (2005), who found that maternal environment significantly affected seed weight in field populations of *Dioda teres* (Rubiaceae). It must be noted, however, that we collectively weighed each set of 10 seeds instead of recording individual seed mass. This lowers the precision of the analysis.

Lack of evidence for EME

In this study, we found no evidence for EME on phenotypic variation. Seed mass influence on the measured traits was not statistically significant. The effect of the maternal environment of seed production was neither on plant traits nor on the clinal variation observed in plant height and biomass. The lack of detectable maternal effects is not consistent with most published research on EME: when they are experimentally induced,

differences in environmental conditions of seed production generally influence germination, due to seed size (Alexander & Wulff, 1985; Stratton, 1989; Aarssen & Burton, 1990; Platenkamp & Shaw, 1993; Schmid & Dolt, 1994) or irrespective of seed size (Munir *et al.*, 2001). Even under natural (e.g. field) conditions, Galloway (2001) found the maternal environment influenced germination percentage in *Campanula americana* (Campanulaceae). In our study, we did not experimentally induce phenotypic variation among maternal plants to examine how the maternal environment influences progeny phenotype. Instead, we tested for maternal effects as expressed under natural conditions, due to different environmental (e.g. climatic) parameters encountered during seed formation.

Seed collection during November ensured that temperature conditions strongly varied along the transect. During October 2006, minimal and maximal mean temperatures were 15.3 and 23.1 °C at the lowest elevations, and decreased to 5.5 and 18.4 °C at the highest altitudes respectively (Table 1). Collecting seeds on a temporal basis would be an interesting approach to assess seasonal variation in seed characteristics. However, as no significant differences were found between seeds produced under varied field conditions, and seeds produced at a constant temperature (18 °C), our results merely suggested a very minor influence of maternal climatic conditions on the offspring phenotype. Despite our samplings cover the whole altitudinal range of the species in France, the absence of maternal effect along the transect might be ascribed to an environmental gradient not sufficiently strong to induce EME.

Our analysis of intermediate and final plant height was congruent with low EME. The longer the plants remained in the common garden, the more evident a linear trend in plant height. The linear contrast in plant height was only marginally significant (Table 3) in early August 2007 and corresponded to a reduction in mean population height by 15.4% (from 26.6 to 22.5 cm) from the lowest to the highest populations. In contrast, it was highly significant in November 2007 and corresponded to a reduction by 19.4% (from 44.3 to 35.8 cm). As maternal effects are known to be more pronounced in early life-history stages (Roach & Wulff, 1987), this supports the altitudinal cline as one of genetic origin, and not the result of maternal effects.

The absence of maternal environment effects on offspring phenotype is congruent with most interpretations from studies on invasive species differentiation within their non-native ranges. Environmental maternal influence appears to be negligible compared with the evolutionary differentiation along an environmental gradient. Previous studies have addressed the importance of EME on phenotypic differentiation in native plant species across environmental gradients. Olsson & Agren (2002) reported that EME were barely detectable compared with genetic differentiation over a latitudinal cline in *Lythrum salicaria* across Sweden. Other studies on EME in native plants generally concluded that EME were discernable, especially on seed and seedling characters, but had a relatively weak influence on offspring phenotypes compared with other factors influencing offspring fitness (Weiner *et al.*, 1997). The magnitude of EME appears to be so low that they play an insignificant role on species differentiation in response to climate.

Evolutionary interpretation of the clinal variation

Variation in life-history traits among introduced populations of *S. inaequidens* closely matched the theoretical expectations for selection on growth traits across an altitudinal and climatic gradient. Small plant size is expected to reflect adaptation to harsher conditions and shorter growing seasons, and is a common and expected feature of native species in mountain ecosystems (e.g. Galen *et al.*, 1991; Blanckenhorn, 1997; Körner, 2003). However, the clinal variation observed in the present study can also originate from demographic processes induced by colonization constraints. If repeated founder effects occurred during range expansion, genetic drift could be responsible for the genotypic differentiation of *S. inaequidens*. Reciprocal transplants is the next step to test the adaptive significance of the observed cline (Lacey, 1988; Rice & Mack, 1991). Furthermore, genetic structure analyses within the invaded range of *S. inaequidens* could be used to further elucidate the relative importance of genetic drift and adaptation.

Conclusions

A common garden experiment was used to study the influence of EME on a clinal variation in plant traits, among invasive populations of *S. inaequidens* selected along an altitudinal gradient in southern France. Plants were grown from two maternal seed environments, represented by (i) *in situ* collected seeds and (ii) seeds produced under controlled conditions. Seed mass did not differ according to the maternal environment, but the pattern of variation among population in the field was different from that under controlled conditions, indicating a plastic response of seed mass to environmental conditions. However, seed mass did not influence phenotypic variation of the offspring. As no difference were found between plants grown from the two maternal

environments, this study brought to light that EMEs played a negligible role on the observed cline.

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