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ORIGINAL PAPER

Distribution, growth performance and genetic variation of *Erigeron annuus* in the Swiss Alps

Miluse Trtikova · Sabine Güsewell · Matthias Baltisberger · Peter J. Edwards

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Abstract We investigated whether local adaptation has been important in enabling the invasive apomictic species *Erigeron annuus* to extend its altitudinal range in the Swiss Alps. We first conducted a field survey along several major roads crossing the Swiss Alps to study the distribution and growth performance of *E. annuus* along an altitudinal gradient. We then used amplified fragment length polymorphism to assess genetic variation within and among populations originating from different altitudes. To complement the molecular analyses, we compared the performance of genotypes with different distributions (i.e. local, occasional, widespread genotypes) in two common gardens at 400 m and 1,000 m a.s.l. Although *E. annuus* was seldom found above 1,000 m, plant performance in field populations did not decrease with increasing altitude. However, there was a significant decline in genotypic diversity within populations, and highland (711–1,100 m) populations were more differentiated (Gst = 0.55) than lowland (200–530 m) populations (Gst = 0.33). In the common garden experiment, local genotypes (i.e. those restricted to a single population) grew less vigorously than widespread genotypes, and were less likely to reproduce. We found no evidence for on-going adaptive changes and conclude that any selection acting on particular genotypes at the altitudinal limit is weak. This leads us to propose that the patterns in the distribution of genotypic diversity in *E. annuus* are governed by processes of occasional sexual reproduction, dispersal and extinction that are to a large extent independent of altitude.

Keywords AFLP · Altitudinal distribution · Apomixis · Genetic variation · Invasive plant · Local adaptation

M. Trtikova (✉) · S. Güsewell · M. Baltisberger · P. J. Edwards
Institute of Integrative Biology, ETH Zurich, 8092 Zurich, Switzerland
e-mail: miluse.trtikova@env.ethz.ch

Introduction

Many ecological factors influence whether or not an introduced species becomes invasive; apart from species traits, the susceptibility of the habitat to invasion (i.e. invasibility) and propagule pressure are important factors (Colautti et al. 2006; Dietz and Steinlein 2004). And there is increasing evidence that adaptive evolution also plays a role in enabling some plant species to become successful invaders (Lambrinos 2004; Lee 2002; Sakai et al. 2001). Indeed, rapid adaptive changes after introduction have been reported in several aggressive invaders, including *Solidago gigantea* in Europe (Weber and Schmid 1998), and *Tamarix ramosissima* in North America (Sexton et al. 2002).

Recently, it has been emphasised that the importance of different mechanisms promoting the spread of an introduced species may change during the

course of an invasion (Dietz and Edwards 2006; Theoharides and Dukes 2007). During the early stages, high phenotypic plasticity is likely to be important because it enables plants to tolerate a broad range of environmental conditions (Sexton et al. 2002); however, for a species to expand its range into habitats with more limiting conditions, for example at higher altitudes, local adaptation may be necessary (Dietz and Edwards 2006). But such adaptation may be hindered by a lack of genetic variation at the invasion front (Hoffmann and Blows 1994) or by maladaptive gene flow from lowland populations (Kirkpatrick and Barton 1997). Despite these hindrances, it seems that an initial reduction in genetic variation due to founder effects and genetic bottlenecks can be overcome over longer timescales (Dlugosch and Parker 2008), and some invasive species are able to maintain high genetic variation even in the peripheral populations (Lavergne and Molofsky 2007).

In this study we investigated whether adaptive evolution was important in permitting the spread of *Erigeron annuus* in the Swiss Alps. *E. annuus* is native to North America but was introduced in the 17th century to Europe, where it has become abundant on roadsides and in ruderal places (Edwards et al. 2006). In Switzerland, the species occurs mainly in the lowlands, though it has been reported up to 1,790 m a.s.l. (Becker et al. 2005). The species is an apomictic winter annual, producing large numbers of minute seeds that are genetically identical to the mother plant. Most populations, however, contain several genotypes, suggesting that sexual reproduction does occur occasionally, although this has never been directly demonstrated (Edwards et al. 2006).

In a previous study of genetic variation in *E. annuus*, it was shown that despite considerable genetic diversity within and among populations a few genotypes (strictly, RAPD phenotypes) were widely distributed in both the native range and in Europe. Furthermore, widely distributed genotypes accounted for a larger proportion of plants in the introduced range than in the native range (Edwards et al. 2006). Assuming that the high frequency of these genotypes reflected a more fixed form of apomixis in some clonal lineages, it was argued that asexual reproduction was probably advantageous during the early stages of invasion in Europe. However, in the longer term, genotypes capable of occasional sexual reproduction may be favoured because they would permit adaptation to local conditions (Edwards et al. 2006). We hypothesized that local adaptation would be important at higher altitudes because of more difficult growth conditions. Thus, we would expect genotypes with a restricted distribution (i.e. local genotypes) to replace the widely distributed genotypes (i.e. widespread genotypes) that are common in the lowlands, and we would also expect these local genotypes to perform better than widespread genotypes when grown at high altitudes.

To test these hypotheses, we examined patterns of genetic variation and genotype distribution within and among *E. annuus* populations in the Swiss Alps using the amplified fragment length polymorphism (AFLP) method. We sampled populations at different altitudes along several major roads crossing the Alps, and made measurements of the growth performance of *E. annuus* plants along the altitudinal gradient. In addition, we assessed performance of genotypes in two common gardens, representing the altitude at which the species is most abundant (400 m) and the usual altitudinal limit (1,000 m). With this approach we were able to address the following questions: (1) Does the growth performance of *E. annuus* plants change along the altitudinal gradient? (2) Does genetic variation within and among *E. annuus* populations change along the altitudinal gradient? (3) Do local and widespread genotypes have different altitudinal distributions? and (4) Do they differ in performance, especially near the altitudinal limit?

Materials and methods

Field survey

A total of 124 sites along major roads crossing the Swiss Alps were intensively searched for *E. annuus* in summer 2004 (May–July) and autumn 2005 (September). The roads included eleven mountain passes ranging in altitude from 1,008 to 2,478 m. The sites surveyed ranged from 243 m to 2,065 m a.s.l., and included car parks, roadsides, railway stations, industrial areas and dump-sites.

Morphological measurements and seed germination test

In August 2004, 26 sites (325–965 m) were revisited and the following morphological traits measured for

3–10 plants per site: height, number of stems, number of branches per stem, and stem diameters. All of these plants eventually produced seeds, though at the time of collection seeds were only available from 211 plants.

The field-collected seeds were germinated in the greenhouse in March 2005. Sixty seeds per plant were scattered evenly on a wet filter paper placed on a layer of fine sand in a Petri dish. After 2 weeks the numbers of germinating seeds in each Petri dish were counted and the seedlings were used for the common garden experiment.

Common garden experiment

The aim of the common garden experiment was to determine how different genotypes responded to climatic conditions at different altitudes. The common gardens were set up at the ETH research stations in Chamau (400 m) and Früebüel (1,000 m) in the canton of Zug. Seedlings from 197 mother plants were grown in trays for 1 month and then transplanted into 1-l plastic pots filled with standard potting soil (Universalerde Capito, Landi Schweiz) mixed with perlite (4:1). Three seedlings were planted per pot, with two pots per mother plant. After the seedlings had been hardened, one pot was placed in the common garden at 400 m (on 10 June 2005) and the other in the garden at 1,000 m (on 16 June 2005). Pots were arranged randomly into six plots (3.5 × 1.5 m). The plants were watered regularly using tap water from the same source and protected from slugs using molluscicide.

To assess growth, number of stems, rosette diameter and plant height were recorded when the plants were harvested (29 September–5 October 2005). The phenological stage of each plant was assessed as (1) bolting, (2) forming flower buds, (3) flowering, and (4) setting seed. The aboveground fresh biomass of all plants was determined by weighing, and the dry biomass was estimated from the dry matter content (dry/fresh biomass) of a subset of plants dried at 70°C. The 171 plants used in the experiment were assigned to 43 different genotypes using amplified fragment length polymorphisms (AFLPs; see below).

Sampling for AFLP analyses

Leaf material of *E. annuus* was collected during the field surveys in 2004 and in 2005. Where there were sufficient plants, ten randomly chosen plants at least 2 m apart were sampled per site. A total of 46 sites ranging from 243 to 1,762 m were sampled (Fig. 1). These sites, which are referred to here as populations, were at least 2 km apart and could be grouped into four distinct regions: (1) the northern region near the city of Zurich, (2) the western region near the city of Bern, (3) the southern region near the city of Bellinzona, and (4) the eastern region near the city of Chur. The leaves were collected in paper bags and kept dry with silica gel; in the laboratory, they were lyophilised and stored at −80°C until used for DNA extraction.

DNA extraction

Total genomic DNA was extracted from 16 to 20 mg of the lyophilised leaf samples using a modified version of the CTAB protocol of Doyle and Doyle (1987). The leaves were ground with a glass bead in a 2 ml tubes for 3 min at amplitude of 80 using a vibration mill (Retsch MM 2000). The ground material mixed with 300 µl of CTAB buffer with 5% 2-mercaptoethanol was incubated at 65°C for 30 min, extracted twice with 300 µl chloroform-isoamylalcohol (24:1), precipitated with 100 µl isopropanol, and washed with 250 µl 70% ethanol. Finally, DNA was suspended in 50 µl of double-distilled water. DNA quality was checked by electrophoresis on 1% agarose gel. The amount of DNA

Fig. 1 Spatial distribution of the 124 sites visited during the field surveys in 2004 and in 2005. The different symbols represent 59 sites where *E. annuus* was absent, 19 sites where *E. annuus* was present, but no samples were collected, 20 sites where leaf material for AFLP analyses was collected, and 26 sites where leaf material for AFLP analyses and seeds were collected

was quantified for a subset of samples with a Mini-Fluorometer TBS-380 (Turner Biosystems).

AFLP analyses

Amplified fragment length polymorphisms (AFLPs) were determined using a slightly modified version of the protocol of Vos et al. (1995). Total genomic DNA (150–300 ng) was digested with *Eco*RI and *Mse*I restriction enzymes (New England Biolabs—NEB). The subsequent ligation of *Eco*RI and *Mse*I adapters was performed at room temperature for 3 h. All restriction and ligation reactions were performed with NEB buffer no. 2. PCR amplifications were performed using GoTaq Flexi DNA polymerase and buffer (Promega). The amplification cycles followed the description in Bratteler et al. (2006). A total of 48 primer combinations were tested using between 4 and 16 of the DNA samples, and the following primer pairs were then chosen for the subsequent analyses: *Eco*RI + ACC/*Mse*I + CCC, *Eco*RI + ACC/*Mse*I + CCA, *Eco*RI + ACC/*Mse*I + CAT. The fragment analyses were performed with ABI PRISM 3130xl Avant Genetic Analyzer using an internal size standard GeneScan 500 (−250) LIZ (Applied Biosystems—ABI).

AFLP scoring

The fragments 75–500 base pairs (bp) in length were scored as present (1) or absent (0) in the software Genemapper 4.0 (ABI). For two of the primer pairs—*Eco*RI + ACC/*Mse*I + CCC and *Eco*RI + ACC/*Mse*I + CCA—the selection of markers for scoring involved three steps. First, all markers were identified for which at least one peak exceeded 200 relative fluorescent units (RFU); this yielded 121 and 160 markers, respectively, for the two primer pairs. Second, the AFLP profiles for these markers were checked for any shifts or ambiguities, and the number of the markers was reduced to 103 and 99, respectively. Third, only those markers were used for which fewer than 10% of peaks were below 100 RFU (68 and 64 markers, respectively). For the third primer pair (*Eco*RI + ACC/*Mse*I + CAT), the RFU values were generally lower, and the selection procedure involved only two steps. First, 71 markers were identified for which at least one peak exceeded 100 RFU. Then, these markers were checked for shifts and ambiguities, leaving 45 markers that were used in the final scoring.

Several precautions were taken to minimise genotyping and scoring errors, as recommended by Bonin et al. (2004, 2007), and Pompanon et al. (2005). First, we only used markers that could be scored clearly and consistently in most AFLP profiles. Second, we used blanks and reference DNA samples to check the reproducibility of the amplification process. Finally, for 96 markers we investigated the error rate per individual locus using 39–52 replicate samples per primer pair. This led to the exclusion of two markers for which the error rate per locus was higher than 0.1. For the remaining 94 markers, the mean genotyping error rate per locus was as low as 0.01.

Genotype identification and estimation of genetic variation

Because AFLPs are dominant characters, it is not possible to distinguish between homozygous and heterozygous individuals. In the strict sense, therefore, we recorded AFLP phenotypes, although we refer to them here as genotypes. Individuals were assigned to genotypes on the basis of 57 polymorphic markers using the software GenoType (Meirmans and Van Tienderen 2004). A small proportion of peaks could not be clearly scored as present or absent, and these data were therefore discarded (mean of 0.7% per marker). Subsequently, the clonal diversity indices were calculated with the software GenoDive (Meirmans and Van Tienderen 2004).

The genetic distance between all pairs of individuals was determined using squared Euclidean distance (e^2). Thus, the distance between a pair of individuals corresponded to the number of markers that differed between the two AFLP profiles. When the profiles differed in one or two markers, then these individuals were considered to belong to the same clonal lineage. This conservative approach was based on the numbers of differences observed when the same plant material was analysed twice (including DNA extraction and AFLP profiling). Comparing the results from 136 repeated analyses, we obtained identical AFLP profiles in 71% of cases, while profiles differed by one and two markers in 22 and 6% of cases, respectively; there was also one case in which the profiles differed by three markers.

We could clearly identify the most frequent genotypes, but the number of infrequent genotypes decreased as the number of pairwise differences within one clone was increased. Although we may have slightly underestimated genotypic diversity by attempting to eliminate scoring errors, the main conclusions were unaffected.

The calculation of the genotypic diversity was based on Nei's (1973) measures of gene diversity, but using genotype frequency instead of allele frequency. According to this approach, total genotypic diversity (Ht) represents the sum of the genotypic diversity within (Hs) and among populations (Dst). Genotypic diversity within populations was corrected for the sample size as follows: $Hs = n/(n-1)(1-\Sigma p^2)$, where n is the sample size and p is the genotype frequency. The coefficient of gene differentiation (Gst) was calculated as follows: $Gst = (Ht-Hs)/Ht$.

As a measure of genetic dissimilarity between the different genotypes within a single population, we used the simple mismatch coefficient (Kosman and Leonard 2005). This is identical to the normalized squared Euclidean distance (i.e. $m = e^2/n$), where n is the total number of markers used for the analysis.

Based on the number of the populations in which the genotypes occurred, the following three groups of genotypes were distinguished: (1) local genotypes found in only one population, (2) occasional genotypes found in two to four populations, and (3) widespread genotypes found in five or more populations.

Statistical analyses

The relationship between altitude and presence/absence of *E. annuus* was analysed with logistic regression. Since altitude was spatially autocorrelated, and since the presence of *E. annuus* at one site might be influenced by its presence at surrounding sites, an autologistic model was used which included, beside altitude, an autocovariate to account for neighbourhood effects. The autocovariate was calculated as the weighted average ($w = 1$/distance) of presences (1) and absences (0) at all sampling sites within a radius of 50 km.

Plant performance along the altitudinal gradient was analysed with polynomial regression. Due to non-normal distribution, data for number of stems, number of branches per stem and stem diameter had to be log transformed, while those for seed germination were square root transformed. Relationships between altitude and genotypic diversity within populations and population interclonal dissimilarity were analysed with linear regressions. The uppermost population was excluded from the analysis as an altitudinal outlier and two populations were excluded due to a small number of individuals. We used analyses of covariance to test the effects and interactions of genotype group (i.e. widespread, occasional and local genotypes) and altitude on number of genotypes, number of plants per genotype, genotype growth performance and seed germination.

Genotype performance at the two growth sites (400 and 1,000 m) in 2005 was analysed using two-way analysis of variance. To meet statistical requirements, data for dry mass and number of stems were log transformed. Means of dry mass were compared using Tukey's HSD test. Proportions of plants that reached different phenological transitions were compared between genotype groups using ordinal logistic regression and likelihood ratio test. The software R 2.9.2. (R Development Core Team 2009) was used for autologistic regression and tests of spatial autocorrelation; all other statistical analyses were performed with the software JMP 6.0 (SAS Institute Inc. 2006).

Results

E. annuus abundance and growth performance along the altitudinal gradient

E. annuus was present at 65 out of the 124 sites surveyed (243–2,065 m) (Fig. 1). The species was mainly confined to the lowlands and was found at only five sites above 1,000 m, the highest being at 1,762 m. Autologistic regression showed a significant negative relationship between altitude and probability of presence ($\chi^2 = 22.84$, $P < 0.0001$). In addition, the probability of presence was higher when many neighbouring sampling sites were occupied ($\chi^2 = 35.68$, $P < 0.0001$).

The growth performance of plants in the field was investigated for 26 populations between 325 and 965 m. A total of 218 plants were recorded, all of which flowered and produced seeds. The germination

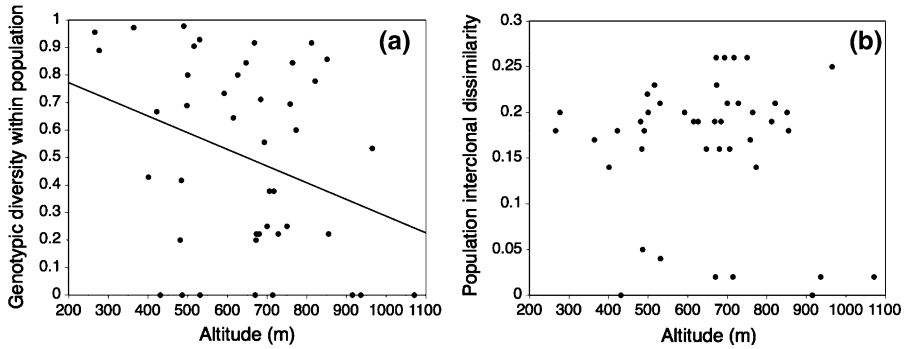

Fig. 2 Genetic variation of 43 populations along the altitudinal gradient: **a** genotypic diversity within each population ($R^2 = 0.10$, $P = 0.0378$), **b** population interclonal dissimilarity represented by simple mismatch coefficient ($R^2 = 0.01$, $P = 0.4884$)

Table 1 Genotypic diversity within and among *E. annuus* populations in the four different regions in Switzerland

Region	Number of populations	Number of individuals	Number of genotypes	Total genotypic diversity	Within population diversity	Genetic differentiation (Gst)
Zurich (north)	13	120	24	0.93	0.40	0.57
Bern (west)	7	60	18	0.91	0.57	0.38
Bellinzona (south)	15	126	31	0.93	0.70	0.24
Chur (east)	11	98	26	0.92	0.44	0.52

test, performed using seeds from 211 plants, showed that 95% of plants produced viable seeds. All morphological traits and seed germination rates varied widely within populations. There was no relationship between altitude and the number of stems, but there were weak quadratic relationships for the other morphological traits and seed germination: plant height ($R^2 = 0.04$, $P = 0.0072$), number of branches per stem ($R^2 = 0.19$, $P < 0.0001$), stem diameter ($R^2 = 0.08$, $P < 0.0001$), and germination rates of the seeds collected in the field and germinated in the greenhouse ($R^2 = 0.05$, $P = 0.0036$).

Genetic variation along the altitudinal gradient

Fifty-seven of the 94 selected markers (61%) were polymorphic. On average, each polymorphic marker appeared in 68% of the populations, but there was a wide spread in their frequency; thus, 20 markers were found in all 46 populations, while one marker occurred in only a single population. The mean number of markers per population sample (3–10 individuals) was 38.8 (SD = 5.3). On average, 41% of the markers were variable within populations.

Allowing individuals of the same clone to differ by up to two markers, 64 different genotypes were identified among 404 individuals from 46 populations. The number of markers per genotype ranged from 21 to 38. Eight population samples were monoclonal, while the 38 multiclonal populations had a mean of 3.9 genotypes (SD = 2.0). Genotypic diversity within populations declined significantly with altitude (Fig. 2a). However, the dissimilarity among clones within a population was unrelated to altitude (Fig. 2b).

Nearly half of the total genotypic diversity occurred among populations (Gst = 0.46). However, lowland populations (200–530 m) were significantly less differentiated (Gst = 0.33) than those at higher altitudes (711–1,100 m; Gst = 0.55). The Gst values were also different for the four regions (Table 1), though only 3% of the total genotypic diversity was found among the regions (Gst = 0.03).

Genotype distribution and growth performance along the altitudinal gradient

There were 36 local genotypes (i.e. those occurring in only one population), 17 occasional genotypes (found

Table 2 F ratios and significance levels of ANOVA testing the effects of growth site (experimental garden at 400 or 1,000 m), genotype group (local, occasional or widespread), and their interaction on genotype performance in 2005

	Rosette diameter	Number of stems	Height	Biomass
Site	40.26***	51.37***	296.29***	163.40***
Genotype group	2.34	4.23*	1.93	5.55**
Site * Genotype group	1.57	0.30	2.59	0.97

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

on average in 3 of the 46 populations) and 11 widespread genotypes (occurring on average in 7 populations). The mean number of genotypes per population was significantly higher for widespread genotypes than for local and occasional genotypes ($F_{2,123} = 6.86$, $P = 0.0015$). However, the decline in numbers of genotypes with altitude ($F_{1,123} = 9.16$, $P = 0.0030$) was similar for the three genotype groups, and there was no tendency for either local or widespread genotypes to become relatively more abundant (i.e. there was no significant genotype by altitude interaction).

There was also significant variation among the genotype groups in the number of individuals per genotype in a population ($F_{2,73} = 4.55$, $P = 0.0138$). Closer analysis showed that this was because local genotypes tended to be represented by fewer individuals per population (mean = 1.6 individuals) than occasional and widespread genotypes (3.4 and 3.8 individuals, respectively). However, the number of individuals per genotype was unrelated to altitude, both across all genotypes (i.e. altitude effect not significant) and for each of the three groups of genotypes (i.e. genotype group by altitude interaction not significant).

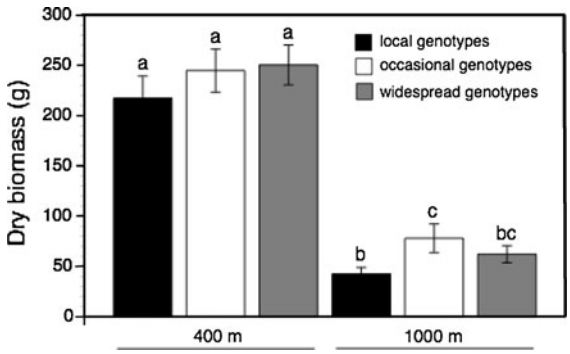

Fig. 3 Mean aboveground dry biomass produced by local, occasional and widespread genotypes in the common gardens at 400 and 1,000 m in 2005. Means (+SE) not connected by the same letters are significantly different

Regarding the individual growth performance and seed germination rates, the genotypes responded similarly to altitude (i.e. non-significant genotype group by altitude interactions for all plant traits and seed germination rates).

Genotype growth performance in the common gardens at two contrasting altitudes

All genotypes survived in both common gardens (400 and 1,000 m), but generally grew larger and produced more aboveground biomass at 400 than at 1,000 m (Table 2; Fig. 3). Furthermore, there were differences in vegetative growth between the three genotype groups, with local genotypes producing fewer stems and less aboveground biomass than occasional and widespread genotypes (Fig. 3). There were also significant differences between these genotype groups in the proportion of plants that reached the reproductive stage (likelihood ratio $\chi^2 = 30.28$, $P < 0.0001$), with local genotypes being less likely to reproduce than occasional and widespread genotypes (Fig. 4). However, local, occasional and widespread genotypes did not differ in their responses to the altitude of the common garden (i.e. no significant growth site by genotype group interactions in Table 2).

Discussion

E. annuus abundance in the Swiss Alps

In the field survey *E. annuus* declined rapidly in abundance above 1,000 m, although it did occur at sites as high as 1,762 m. In view of this predominantly lowland distribution, which is similar to that reported by Becker et al. (2005), we expected to observe signs of poorer growth at high altitudes. However, there was no decline in the performance of

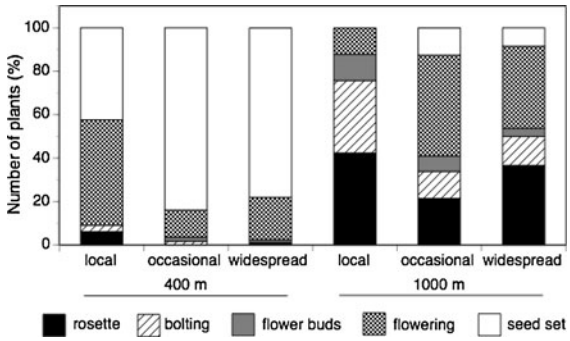

Fig. 4 The final phenological stage (rosette, bolting, flower buds, flowering, seed set) that the plants belonging to local, occasional and widespread genotypes reached at the end of the common garden experiments at 400 and 1,000 m in September/October 2005

E. annuus with altitude, and even plants growing at the altitudinal limit produced abundant viable seeds. A maintenance of vigorous growth across a broad altitudinal range has also been reported for another invasive species, *Rubus alceifolius* that occurs at altitudes between 50 and 1,200 m on the island of Réunion (Baret et al. 2004). However, in that case the authors observed a shift from predominantly sexual reproduction at low altitudes towards vegetative propagation at higher altitudes.

One possible reason for the increasing scarcity of *E. annuus* at higher altitudes is declining propagule pressure (Becker et al. 2005; Pauchard and Alaback 2004). However, this explanation seems improbable, since the study sites were located on major roads that presented no obstacle to the dispersal of tiny, wind-blown seeds. It seems more likely, therefore, that seedlings have a reduced chance of establishing at higher altitudes due to greater winter mortality (Trtikova et al. 2010). As a result, high altitude populations are less persistent and the proportion of potential sites that are occupied at any time declines with altitude.

Genetic structure of *E. annuus* populations along the altitudinal gradient

Previous studies of genetic diversity in *E. annuus* have used either isozymes (Hancock and Wilson 1976) or the RAPD (randomly amplified polymorphic DNA) method (Edwards et al. 2006). Our results obtained using the AFLP method show similar patterns in the distribution of genetic variation within and among populations to those obtained previously. Thus, we found that most populations (83%) were multiclonal and typically had high levels of genotypic diversity. Furthermore, while a few genotypes were frequent and widespread, most were represented by one or a few individuals.

Other studies with clonal species have also shown high levels of genetic diversity, with sometimes similar patterns in the distribution of genotypes among populations. Ellstrand and Roose (1987) argued that because there is no known reproductive alternative to apomixis in *E. annuus*, the most important source of genetic variation is probably somatic mutations. However, Edwards et al. (2006) found clear evidence of recombination in *E. annuus*, suggesting that much of the genotypic diversity was generated by rare sexual reproduction. Indeed, only a small number of sexual individuals per generation could lead to a genotypically variable population (Bengtsson 2003). The distribution of genotypes within and among populations of *E. annuus*, thus, seems to reflect a variety of factors, including frequencies of both sexual reproduction and long distance dispersal. The fact that over half of all genotypes (56%) were restricted to single populations suggests that sexual reproduction occurs relatively frequently, while the high genetic differentiation among populations ($Gst = 0.46$) points to limited dispersal of seeds between populations. The most widespread genotype was recorded in just 12 out of 46 populations, and only two genotypes were found in all four regions.

Strong genetic differentiation among populations is common in mountainous regions, where high peaks and ridges present barriers to seed-mediated genetic exchange between populations (Ohsawa and Ide 2008). The lower genetic diversity in high altitude populations could be simply because these sites are further from large sources of propagules than those in the lowlands. However, the greater differentiation among high altitude populations and the fact that many potential sites are unoccupied support the idea that these populations are periodically destroyed during winter and the sites are then recolonised, mainly from lower altitudes. The rapid decline in the occurrence of *E. annuus* with altitude is also consistent with a decreasing persistence of local populations (Holt and Keitt 2000). Thus, the genetic structure of high altitude populations probably

reflects both recent founder effects and lower propagule pressure than lowland populations.

Is there evidence of adaptive evolution in *E. annuus*?

We found no significant genotype by environment (i.e. genotype by altitude) interactions that would suggest an adaptive explanation for the observed patterns of genetic variation. Similarly, Stratton (1994) showed that variation in the relative fitness of *E. annuus* genotypes did not correspond to patterns of environmental variation; thus, while the soil nutrients and the surrounding vegetation varied on a scale of 10–20 m, significant genotype by environment interactions only occurred at a scale of 10–20 cm. On the other hand, in our study we did not investigate the early stages of the life cycle, although another study suggests that the most important selection episode in *E. annuus* is winter survivorship (Stratton 1992). Therefore, we cannot exclude the possibility that selection on seedlings affected the distribution and abundance of genotypes.

If local adaptation of genotypes is important, then we might expect genotypes with a restricted distribution to perform better than more widespread genotypes under the relevant conditions. This is exactly the opposite of what we observed, since local genotypes (i.e. those restricted to a single population) tended to be represented by fewer individuals within populations than widespread genotypes. And in the common gardens local genotypes also performed worse than widespread genotypes, producing fewer stems and being less likely to produce viable seeds. Thus, the restricted distribution of some genotypes was a reflection not of local adaptation but of low fitness. Given that *E. annuus* is a triploid, predominantly apomictic species, this high proportion of local genotypes could reflect a rather irregular form of sexual reproduction that tends to produce progeny with a rather low fitness (Noyes 2000).

Other studies with introduced plants have shown that adaptation to local conditions is not always necessary to colonise a wide range of habitats, and that many successful invaders have what Baker (1965) described as a 'general purpose' genotype. For example, the invasive grass *Pennisetum setaceum* has a greater altitudinal range than any other grass on the island of Hawaii (Williams et al. 1995). However, populations of this species on Hawaii contain little genetic variation, and its invasive success is apparently due to high phenotypic plasticity (Poulin et al. 2005, 2007; Williams et al. 1995). A general purpose genotype rather than local adaptation also appears to have allowed *Verbascum thapsus* to spread to higher elevations in the Sierra Nevada (Parker et al. 2003). Similarly, our results indicate that *E. annuus* was able to extend its altitudinal range with generalist genotypes.

Conclusions

Our study has shown that growth performance of *E. annuus* does not decrease with increasing altitude in the Swiss Alps. Moreover, the ability of plants to maintain relatively high fitness up to the current altitudinal limit is probably due to phenotypic plasticity, since we found no evidence for local adaptation. These findings lead us to suggest that selection acting on the available genotypes at the altitudinal limit is weak, and that the observed patterns in the distribution of genotypic diversity in *E. annuus* are governed by processes of occasional sexual reproduction, dispersal and extinction that are to a large extent independent of altitude.

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References

Baker HG (1965) Characteristics and modes of origin of weeds. In: Baker HG, Stebbins GL (eds) The genetics of colonizing species. Academic Press, New York, pp 147–168

Baret S, Maurice S, Le Bourgeois T, Strasberg D (2004) Altitudinal variation in fertility and vegetative growth in the invasive plant *Rubus alceifolius* Poiret (Rosaceae), on Réunion island. Plant Ecol 172:265–273

Becker T, Dietz H, Billeter R, Buschmann H, Edwards PJ (2005) Altitudinal distribution of alien plant species in the Swiss Alps. Perspect Plant Ecol Evol Syst 7:173–183

Bengtsson BO (2003) Genetic variation in organisms with sexual and asexual reproduction. J Evol Biol 16:189–199
Bonin A, Bellemain E, Eidesen PB, Pompanon F, Brochmann C, Taberlet P (2004) How to track and assess genotyping errors in population genetics studies. Mol Ecol 13:3261–3273
Bonin A, Ehrich D, Manel S (2007) Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. Mol Ecol 16:3737–3758
Bratteler M, Lexer C, Widmer A (2006) A genetic linkage map of *Silene vulgaris* based on AFLP markers. Genome 49:320–327
Colautti RI, Grigorovich IA, MacIsaac HJ (2006) Propagule pressure: a null model for biological invasions. Biol Invasions 8:1023–1037
Dietz H, Edwards PJ (2006) Recognition that causal processes change during plant invasion helps explain conflicts in evidence. Ecology 87:1359–1367
Dietz H, Steinlein T (2004) Recent advances in understanding plant invasions. In: Esser K, Luttge U, Beyschlag W, Murata J (eds) Progress in botany, vol 65. Springer, New York, pp 539–573
Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449
Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15
Edwards PJ, Frey D, Bailer H, Baltisberger M (2006) Genetic variation in native and invasive populations of *Erigeron annuus* as assessed by RAPD markers. Int J Plant Sci 167:93–101
Ellstrand NC, Roose ML (1987) Patterns of genotypic diversity in clonal plant species. Am J Bot 74:123–131
Hancock JF, Wilson RE (1976) Biotype selection in *Erigeron annuus* during old field succession. Bull Torrey Bot Club 103:122–125
Hoffmann AA, Blows MW (1994) Species borders: ecological and evolutionary perspectives. Trends Ecol Evol 9:223–227
Holt RD, Keitt TH (2000) Alternative causes for range limits: a metapopulation perspective. Ecol Lett 3:41–47
Kirkpatrick M, Barton NH (1997) Evolution of a species' range. Am Nat 150:1–23
Kosman E, Leonard KJ (2005) Similarity coefficients for molecular markers in studies of genetic relationships between individuals for haploid, diploid, and polyploid species. Mol Ecol 14:415–424
Lambrinos JG (2004) How interactions between ecology and evolution influence contemporary invasion dynamics. Ecology 85:2061–2070
Lavergne S, Molofsky J (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proc Natl Acad Sci USA 104:3883–3888
Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17:386–391
Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol Ecol Notes 4:792–794
Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323
Noyes RD (2000) Diplospory and parthenogenesis in sexual × agamospermous (apomictic) Erigeron (Asteraceae) hybrids. Int J Plant Sci 161:1–12
Ohsawa T, Ide Y (2008) Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. Global Ecol Biogeogr 17:152–163
Parker IM, Rodriguez J, Loik ME (2003) An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. Conserv Biol 17:59–72
Pauchard A, Alaback PB (2004) Influence of elevation, land use, and landscape context on patterns of alien plant invasions along roadsides in protected areas of south-central Chile. Conserv Biol 18:238–248
Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: causes, consequences and solutions. Nat Rev Genet 6:847–859
Poulin J, Weller SG, Sakai AK (2005) Genetic diversity does not affect the invasiveness of fountain grass (*Pennisetum setaceum*) in Arizona, California and Hawaii. Divers Distrib 11:241–247
Poulin J, Sakai AK, Weller SG, Nguyen T (2007) Phenotypic plasticity, precipitation, and invasiveness in the fire-promoting grass *Pennisetum setaceum* (Poaceae). Am J Bot 94:533–541
Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Ann Rev Ecol Syst 32:305–332
Sexton JP, McKay JK, Sala A (2002) Plasticity and genetic diversity may allow saltcedar to invade cold climates in North America. Ecol Appl 12:1652–1660
Stratton DA (1992) Life-cycle components of selection in *Erigeron annuus*: I. phenotypic selection. Evolution 46:92–106
Stratton DA (1994) Genotype-by-environment interactions for fitness of *Erigeron annuus* show fine-scale selective heterogeneity. Evolution 48:1607–1618
Theoharides KA, Dukes JS (2007) Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. New Phytol 176:256–273
Trtikova M, Edwards PJ, Güsewell S (2010) No adaptation to altitude in the invasive plant *Erigeron annuus* in the Swiss Alps. Ecography 33:556–564
Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414
Weber E, Schmid B (1998) Latitudinal population differentiation in two species of *Solidago* (Asteraceae) introduced into Europe. Am J Bot 85:1110–1121
Williams DG, Mack RN, Black RA (1995) Ecophysiology of introduced *Pennisetum setaceum* on Hawaii: the role of phenotypic plasticity. Ecology 76:1569–1580