

Diss. ETH No. 18015

Altitudinal limit of *Erigeron annuus* in the Swiss Alps

A dissertation submitted to

ETH ZURICH

for the degree of

DOCTOR OF SCIENCES

presented by

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2008

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Summary

Plant invasions mainly affect lowland regions. In the Swiss Alps, the number and abundance of introduced plant species strongly decrease above 1000 m. Thus, most introduced plants have so far been unable to establish or persist at higher altitudes, probably because they lack adaptations needed to maintain fitness above their current altitudinal limit. The evolution of such adaptations requires genetic variation in the relevant plant traits as well as selection for the appropriate traits at the current altitudinal limit. In introduced plant populations, low genetic variation (e.g. due to genetic bottlenecks) or insufficient selection (e.g. due to gene flow from the lowlands) might impede the adaptation process. Important questions in assessing the risk of plant invasions into mountain regions are therefore (i) which mechanisms reduce plant fitness at the altitudinal limit, (ii) whether there is sufficient genetic variation in fitness upon which selection could act and (iii) whether there is selection for adapted genotypes at the altitudinal limit.

In this thesis, the annual forb *Erigeron annuus* was used as a model species to evaluate the role of adaptive evolution in promoting the spread of introduced plants towards higher altitudes in the Swiss Alps. As a native of North America, *E. annuus* was introduced to Switzerland and is nowadays widespread in the lowlands and up to 1000 m in the mountains. The species is predominantly asexual, and most plants in Switzerland belong to a few genotypes, but additionally there are many genotypes with a restricted distribution. If there is selection for locally adapted genotypes at the altitudinal limit, locally restricted genotypes should be abundant in this zone.

To identify the mechanisms responsible for the altitudinal limit in the distribution of *E. annuus*, seeds were collected from *E. annuus* plants at different altitudes (325-965 m), and their offspring were grown in common gardens at 400 m and at 1000 m. Plant growth, phenology and seed production were compared between the two gardens and related to the altitude of seed collection. A second experiment investigated the effects of competition and mowing on growth and reproduction of *E. annuus* to test whether these factors exacerbate negative climatic impacts at the altitudinal limit. Finally, leaf samples collected from the plants along the altitudinal gradient were genotyped with the AFLP method to assess the altitudinal changes in genetic variation and to see whether better adapted genotypes occur at the altitudinal limit.

In the first common garden study, established *E. annuus* plants grew vigorously at both 400 and 1000 m; however, 27% of the seedlings planted in autumn at 1000 m died

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during the winter. Furthermore, plant phenology was significantly delayed at 1000 m, and the total number of capitula produced was 39% lower than at 400 m. Growth and reproduction of the plants were unrelated to their altitude of origin. These results indicate that a lack of adaptations to cool and short growing seasons limits the spread and establishment of *E. annuus* at higher altitudes in the Swiss Alps.

In the second common garden study, mowing and competition reduced the vegetative growth and reproduction of *E. annuus* more at 1000 m than at 400 m. Compared to control plants, competition reduced the reproductive output by 13% at 1000 m, whereas the reproductive output was not affected by competition at 400 m, suggesting that *E. annuus* can tolerate competition in the lowlands. Similarly, mown plants produced 28% more capitula than control plants at 400 m, but 28% fewer capitula at 1000 m, and seed production was delayed up to the very end of the growing season (September) at 1000 m. Thus, mowing does not prevent the spread of *E. annuus* in the lowlands but may hinder its establishment at higher altitudes.

Genetic analyses of 404 *E. annuus* plants from 46 populations revealed a significant decline in genetic variation with altitude: on average, samples of six to ten plants within a population contained 4.6 genotypes in the lowlands (200-530 m) but 2.7 genotypes in the highlands (711-1100 m). This reduction in genetic variation was not associated with decrease in growth performance. Distribution of genotypes did not indicate that better adapted genotypes occurred at the altitudinal limit, i.e. many genotypes found at 1000 m also appeared at lower altitudes, and genotypes restricted to a single location were represented by fewer individuals within locations than the more widespread genotypes. Consistently with these results, in the first common garden experiment, genotypes found only at single locations in the field survey generally performed less well than the more widespread genotypes, and did not show any sign of adaptation to growth at 1000 m. Thus, altitudinal patterns in the distribution of genetic variation in *E. annuus* seem to be governed by processes of genotype formation, dispersal and extinction, which act to a large extent independently of altitude, preventing local adaptation at the altitudinal limit.

A lack of adaptations to current climatic conditions thus limits the distribution of *E. annuus* above 1000 m in the Swiss Alps. However, climate warming might promote the upward range expansion of *E. annuus* by reducing winter mortality and by increasing the chance of producing seeds before the end of the growing season.

Zusammenfassung

Pflanzeninvasionen betreffen vor allem tiefgelegene Gebiete. In den Schweizer Alpen nimmt die Anzahl und Häufigkeit gebietsfremder Pflanzenarten oberhalb von 1000 m stark ab. Die meisten gebietsfremden Arten konnten sich also bisher oberhalb von 1000 m nicht dauerhaft ansiedeln, vermutlich mangels Anpassungen, mit denen sie dort eine genügende Fitness aufrecht erhalten könnten. Die Entwicklung solcher Anpassungen bedingt genetische Variation in relevanten Pflanzenmerkmalen sowie Selektion zugunsten der günstigen Merkmalen im Bereich der Höhengrenze. In eingeführten Pflanzenpopulationen könnte eine reduzierte genetische Vielfalt (z.B. aufgrund der Einfuhr weniger Genotypen) oder ungenügende Selektion (z.B. wegen starkem Genfluss aus tieferen Lagen) diesen Anpassungsprozess verhindern. Wichtige Fragen bei der Beurteilung des Invasionsrisikos in Berggebieten sind deshalb (i) welche Mechanismen die Fitness invasiver Pflanzen an ihrer Höhengrenze reduzieren, (ii) ob genügend genetische Variation in relevanten Merkmalen vorhanden ist, um eine Selektion je nach Höhenlage zu ermöglichen, und (iii) ob die Selektion besser angepasster Genotypen an der Höhengrenze tatsächlich stattfindet.

In dieser Arbeit wurde am Beispiel des einjährigen Berufkrautes, *Erigeron annuus*, untersucht, wie wichtig die Entwicklung von Anpassungen für die Ausbreitung gebietsfremder Pflanzenarten in höhere Lagen der Schweizer Alpen ist. Als Art nordamerikanischer Herkunft hat sich *E. annuus* seit seiner Einfuhr in den tiefen Lagen der Schweiz massiv ausgebreitet, kommt in Berggebieten jedoch nur bis ca. 1000 m häufig vor. Die Art vermehrt sich vor allem durch asexuell gebildete Samen, so dass einige Genotypen einen grossen Teil der Schweizer Bestände darstellen; daneben existieren zahlreiche weniger häufige, nur lokal vorkommende Genotypen. Falls im Bereich der Höhengrenze Selektion zugunsten neuer, besonders angepasster Genotypen stattfindet, sollten seltenere Genotypen dort vermehrt zu finden sein.

Um Mechanismen zu identifizieren, welche die obere Verbreitungsgrenze von *E. annuus* verursachen, wurden Samen von wildlebenden Pflanzen auf verschiedenen Meereshöhen (325-965 m ü.M.) in den Alpen gesammelt, und deren Nachkommen wurden in zwei Versuchsgärten auf 400 m und 1000 m ü.M. kultiviert. Das Wachstum, die Phänologie und der Samenansatz dieser Pflanzen wurden zwischen den zwei Gärten verglichen und zur Herkunftshöhe der Samen in Bezug gesetzt. Ein zweites Experiment untersuchte die Auswirkung der Konkurrenz und des Schnitts auf das Wachstum und die Fortpflanzung von *E. annuus*, um zu testen, ob diese Faktoren die Wirkung ungünstiger klimatischer Bedingungen an der Höhengrenze noch verstärken.

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Schliesslich wurden Blattproben der Pflanzen entlang des Höhengradienten mittels der AFLP-Methode genetisch charakterisiert, um Änderungen der genetischen Variation mit der Meereshöhe zu festzustellen und um zu prüfen, ob besser angepasste Genotypen im Bereich der Höhengrenze vorkommen.

In den Versuchsgärten wuchsen etablierte Pflanzen von *E. annuus* sowohl auf 400 m als auch auf 1000 m kräftig; allerdings starben 27% der Keimlinge, welche im Herbst gepflanzt wurden, während dem Winter. Zudem war die jahreszeitliche Entwicklung der Pflanzen auf 1000 m deutlich verzögert, und es wurden 39% weniger Samenköpfe produziert als auf 400 m. Wachstum und Fortpflanzung hingen nicht mit der Herkunftshöhe der Samen zusammen. Diese Ergebnisse weisen darauf hin, dass fehlende Anpassungen an kühlere und kürzere Wachstumsperioden die Ausbreitung und dauerhafte Ansiedlung von *E. annuus* in höheren Lagen verhindern.

Im zweiten Gartenversuch reduzierten Mahd und Konkurrenz das Wachstum und die Fortpflanzung von *E. annuus* auf 1000 m mehr als auf 400 m. Im Vergleich zu den Kontrollpflanzen war die Samenbildung auf 1000 m mit Konkurrenz um 13% reduziert, während sie auf 400 m nicht reduziert war. Ebenfalls produzierten Pflanzen, die einmal gemäht wurden, auf 1000 m 28% weniger Samenköpfe, während sie auf 400 m 28% mehr Samenköpfe bildeten als die Kontrollpflanzen. Die Samenbildung fand auf 1000 m nach Mahd zudem erst ganz am Ende der Vegetationszeit (September) statt. Eine einmalige Sommermahd verhindert also die Ausbreitung der Pflanzen in tiefen Lagen nicht, während sie die Ansiedlung in höheren Lagen erschwert.

Genetische Untersuchungen von 404 *E. annuus*-Pflanzen aus 46 Populationen zeigten eine signifikante Abnahme der genetischen Vielfalt mit der Meereshöhe: Die Proben von sechs bis zehn Pflanzen pro Population enthielten in tiefen Lagen (200-530 m) durchschnittlich 4.6 verschiedene Genotypen, während diejenigen höherer Lagen (711–1100 m) durchschnittlich 2.7 Genotypen enthielten. Mit dieser Abnahme der genetischen Vielfalt war keine Abnahme der Wachstumsleistung verbunden. Die Verteilung der Genotypen lieferte keinen Hinweis dafür, dass im Bereich der Höhengrenze speziell angepasste Genotypen vorkommen: Erstens wurden viele Genotypen, welche auf 1000 m vorkamen, auch in tiefen Lagen gefunden, und zweitens waren die Genotypen, welche nur an einzelnen Orten vorkamen, auch an diesen Orten weniger zahlreich vertreten als die weiter verbreiteten Genotypen, also offenbar weniger erfolgreich. Entsprechend hatten im ersten Gartenversuch die Genotypen, welche nur an einzelnen Orten vorkamen, allgemein eine geringere Fitness als die weiter verbreiteten Genotypen, und zeigten keine bessere Leistung auf 1000

m. Es scheint daher, dass die Verteilungsmuster der Genotypen bei *E. annuus* durch die gelegentliche Bildung neuer Genotypen, deren Ausbreitung und deren lokales Aussterben geprägt werden. Diese Prozesse fördern zwar einige Genotypen gegenüber den anderen; dies erfolgt aber weitgehend unabhängig von der Meereshöhe, was eine lokale Anpassung im Bereich der Höhengrenze verhindert.

Eine ungenügende Anpassung an die gegenwärtigen klimatischen Bedingungen begrenzt also die Verbreitung von *E. annuus* oberhalb von 1000 m in den Schweizer Alpen. Die Klimaerwärmung können jedoch eine Ausbreitung in höhere Lagen ermöglichen, indem sie die Wintersterblichkeit reduziert und die Wahrscheinlichkeit einer erfolgreichen Samenbildung vor dem Ende der Vegetationszeit erhöht.

General introduction

DISTRIBUTION LIMITS

Plant spatial distribution is complex and dynamic, and may be determined by many of factors. Although there can be apparent physical and topographic barriers limiting the dispersal of plants, such as mountain ridges or large water bodies, more often the causes for the distribution limits are less obvious (Kirkpatrick and Barton 1997). Along geographical gradients climate is often the primary limiting abiotic factor (Bruehlheide and Heinemeyer 2002, Woodward and Jones 1984). At higher altitudes and latitudes, growth and reproduction of plants may be, in particular, constrained by low temperatures (Angert 2006, Woodward 1990) and short growing seasons (Griffith and Watson 2005), and current climate warming has been consistently reported to cause significant upward shifts in the altitudinal distribution of many plants (Lenoir et al. 2008, Parolo and Rossi 2008). Biotic factors, such as herbivory (Bruehlheide and Scheidel 1999) or competition (Case et al. 2005), may also set limits to plant distribution, although the interactions among plants seem to be less negative at high altitudes (Callaway et al. 2002).

However, all abiotic and biotic factors are only limiting because a species has not evolved the morphological, physiological, or ecological capacities to overcome them (Gaston 2003). Plants may adapt to lower temperatures and shorter growing seasons at higher altitudes and latitudes by having higher freezing tolerance (Zhen and Ungerer 2008), smaller size (Olsson and Agren 2002), smaller, often pubescent leaves (Halloy and Mark 1996) and/or by reproducing earlier (Griffith and Watson 2005, Olsson and Agren 2002). Seed weight has also been shown to increase with altitude in some plant species (Pluess et al. 2005), probably because larger seeds have higher survivorship during establishment under less favourable conditions (Moles and Westoby 2004). Intensity and spectral composition of solar radiation also change with altitude (Körner 2003), and plants may need to increase their production of phenolic compounds, which have a UV protective function (Weinig et al. 2004). Presumably, if a plant population acquires these or other necessary traits, it might expand beyond its altitudinal limit.

However, adaptive evolution is often limited by a lack of genetic variation in the traits responsible for the limit (Blows and Hoffmann 2005, Hoffmann and Blows 1994). For example, a low genetic variation for the reproductive timing in the marginal populations would constrain the evolution of early reproduction (Griffith and Watson

2006). Marginal populations may be small and scattered, and thus more prone to loss of genetic variation because of genetic drift and limited gene flow (Gaston 2003). On the other hand, a strong gene flow from the centre of the species range may lead to the continual immigration of maladapted genotypes, and may impede adaptation in the populations at the range margin (Kirkpatrick and Barton 1997). However, an increase in abundance or in genetic variation or a change in the environment, such as climate warming, might cause a range expansion (Kirkpatrick and Barton 1997).

BIOLOGICAL INVASIONS

Present distribution of many species is not solely determined by environmental conditions, but also by physical barriers to dispersal that prevent species spreading to otherwise suitable sites (Case et al. 2005). However, with recent increase in the transport, dispersal barriers have been regularly overcome, and many species have expanded their range with deliberate or accidental assistance of humans. In the novel environment, introduced species are often released from their antagonistic interactions with specialist herbivores and pathogens, which may result in the rapid increase in their distribution and abundance (Keane and Crawley 2002). Some plants may also become invasive because they bring novel mechanisms of interactions, such as allelopathy, and disrupt the coevolved interactions among native species (Callaway and Aschehoug 2000). However, introduced species not only lose enemies, mutualists and competitors; they may also gain new ones, and their success will depend on the outcome of these new biotic interactions, which can be directly influenced by abiotic conditions (Mitchell et al. 2006).

Some introduced plants spread successfully not because they are more competitive than native species, but rather because they benefit more from increased resource availability (Daehler 2003, Davis and Pelsor 2001). Indeed, many introduced plants are restricted to nutrient-rich and frequently disturbed environments, such as man-made or riverine habitats (Chytry et al. 2008, Rabitsch and Essl 2006). Only a few introduced plants occur in mountain regions, which are relatively undisturbed and also receive less propagules due to their remoteness (Becker et al. 2005, Pauchard and Alaback 2004). However, the decline in the number of introduced plants with altitude could also be explained by a lack of adaptation to harsher environmental conditions (Becker et al. 2005). While many introduced plants are preadapted to resource-rich and disturbed habitats, plastic or adaptive genetic changes in the introduced species may be necessary for their further spread into more natural habitats such as occur in mountain regions (Dietz and Edwards 2006).

Experimental approaches studying mechanisms that limit species range expansions are of crucial importance to control and prevent biological invasions. Although biological invasions are natural phenomena, and large-scale invasions and extinctions occurred in the geological past (Brown and Sax 2004, Vermeij 1991), the rates and geographical extents of present biological invasions exceed those in the past (Ricciardi 2007). At the same time, studying biological invasions may provide invaluable insights into the formation of geographic ranges, as all species ranges are the result of the successful past invasions (Keitt et al. 2001). Evolutionary insights emerging from research on invasive species challenge some of the long held views and theories (Sax et al. 2007). Genetic studies of invasive species revealed that adaptation to novel environments might occur rapidly, within 20 generations or less (Prentis et al. 2008), and rapid adaptations have been observed in the morphological and life-history characters of several invasive plant species (Leger and Rice 2007, Sexton et al. 2002, Weber and Schmid 1998). Most introduced populations have initially reduced genetic variation due to genetic bottlenecks (Dlugosch and Parker 2008), but even large losses of genetic variation do not seem to preclude rapid adaptations in the founding populations (Dlugosch and Parker 2008).

ERIGERON ANNUUS

The genus *Erigeron* encompasses over 400 species found nearly worldwide, but most typically in exposed montane habitats (Noyes 2000). Several species reproduce via apomixis, i.e. asexual seed production; these are generally hybrid and polyploid (Noyes 2000). Although less than 1% of all flowering plant species is estimated to be apomictic (Whitton et al. 2008), phylogenetic data indicate that apomixis has arisen at least three times in the genus *Erigeron* (Noyes 2000). In Switzerland there are seven *Erigeron* species native to the Alps (Lauber and Wagner 2007). These alpine species are diploid and so agamospermy is unlikely to be the mode of reproduction, however, low genetic variation within populations implies predominant selfing (Huber and Leuchtman 1992). The seven species are genetically and morphologically very similar, suggesting relatively recent geographical speciation (Huber and Leuchtman 1992). Two non-native *Erigeron* species are found in the lowlands: *E. karvinskianus*, which is restricted to the southern region of Tessin; and *E. annuus*, which is abundant in many parts of Switzerland (Lauber and Wagner 2007).

E. annuus is an annual forb native to eastern North America (Stratton 1992). It is a species of tall grass prairies (Edwards et al. 2006, Wilson and Hartnett 1998), which

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has probably spread as a weed and considerably expanded its range also in North America (Cronquist 1995). It was introduced to Europe in 17th century and has become abundant especially in ruderal habitats (Edwards et al. 2006). In Switzerland, it mainly occurs in the lowlands and up to 1000 m in the Alps, but was reported up to 1790 m (Becker et al. 2005). *E. annuus* is triploid ($2n=27$) and predominantly apomictic, reproducing by tiny (25 µg) wind-dispersed seeds with each plant being able to produce 10'000-50'000 genetically identical seeds in a season (Stratton 1989). It is usually a winter annual, but it may become short-lived perennial at higher altitudes or when the plants are mown. Seeds normally germinate in late summer (August), and overwintering rosettes bolt and flower (June) the following year.

Apomictic reproduction may have promoted the spread of *E. annuus*, because it allows it to found populations from a single propagule. Apomixis may also maintain so called 'general purpose' genotypes with broad environmental tolerance, which enables the species to colonise a wide range of habitats (Baker 1965). However, in the longer term, apomicts may suffer from the lower potential to maintain and increase genetic diversity, and so their ability to adapt to different environments might be limited (Baker 1965, Horandl 2006). This might probably explain why is *E. annuus* currently restricted to lowland regions. On the other hand, there is evidence that sexual reproduction does occur, albeit rarely, and that *E. annuus* is therefore a facultative apomict (Edwards et al. 2006, Noyes 2000). Indeed, most *E. annuus* populations contain several genotypes in both its native and its introduced range (Edwards et al. 2006), and so it might have a potential to evolve adaptations to conditions at higher altitudes. There is also evidence that it has advanced its altitudinal limit in the Swiss Alps (Becker et al. 2005), though whether this is due to adaptive changes or climate warming (or some other factor) is unknown.

Many apomicts also retain a residual sexual function as pollen donors and they may increase their genetic diversity by crossing with their sexual relatives (Whitton et al. 2008). Indeed, triploid apomictic *E. annuus* produces pollen that functions in crosses with sexual diploids of its American relative, *E. strigosus* (Noyes 2000). Several introduced species hybridized with their previously isolated relatives (Prentis et al. 2008, Shibaike et al. 2002), and hybridization might even cause increased invasiveness in the resulting hybrid plants (Ellstrand and Schierenbeck 2000). Presumably, if *E. annuus* expands its current range, it could hybridize with its Swiss alpine relatives. However, the hybridization and possible replacement of the alpine *Erigeron* species via introgression would still be limited because of the niche differentiation, selfing and different ploidy levels (Horandl 2006).

OUTLINE OF THE THESIS

This thesis aims at a better understanding of the spatial patterns in the distribution of *Erigeron annuus* in the Swiss Alps. An extensive field survey along the roads crossing the Swiss Alps was combined with common garden experiments located at two altitudes. The lower garden (400 m) represented the core area of the current *E. annuus* distribution, whereas the upper garden (1000 m) was set up at the altitudinal limit, identified by the field survey.

In chapter 1 - *Is Erigeron annuus able to spread beyond its altitudinal limit in the Swiss Alps?* – climatic factors that might have limited the altitudinal distribution of *E. annuus* in the Swiss Alps are investigated. By comparing the performance of plants originating from different altitudes in the two common gardens, the role of local adaptation and phenotypic plasticity in the spread of the species is investigated.

In chapter 2 - *Effects of competition and mowing on growth and reproduction of the invasive plant Erigeron annuus* – responses of *E. annuus* to below-ground competition and simulated mowing are examined in order to test the hypothesis that disturbance facilitates the establishment and spread of the species.

In chapter 3 - *Distribution, growth performance and genetic variation of Erigeron annuus in the Swiss Alps* – genetic variation within and among *E. annuus* populations originating from different altitudes is assessed by using amplified fragment length polymorphism (AFLP). In order to understand the patterns in the genotypic distribution, the performance of differently distributed genotypes is compared in the common gardens.

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Chapter 1

Is *Erigeron annuus* able to spread beyond its altitudinal limit in the Swiss Alps?

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ABSTRACT

The altitudinal distribution of plants is restricted by various environmental factors, with climatic conditions being one of the primary constraints. Here, we investigate what limits the altitudinal range of the introduced species *Erigeron annuus* in the Swiss Alps. We planted offspring of *E. annuus* plants originating from different altitudes into two common gardens, one located at an altitude representing the main area of distribution (400 m) and the other close to the current altitudinal limit of *E. annuus* in Switzerland (1000 m). In both common gardens all established plants survived and grew vigorously during the growing season. However, there was high winter mortality of seedlings at 1000 m. Furthermore, plant phenology was delayed and reproductive output was reduced at 1000 m, although the seeds produced were larger. The general lack of adaptation to altitude and only moderate levels of plasticity suggest that there is little potential for *E. annuus* to spread beyond its current altitudinal limit in the Swiss Alps. However, climate warming might promote the upward range expansion of *E. annuus* by reducing winter mortality and the risk of not producing seeds within the growing season.

INTRODUCTION

The geographic distribution of plant species is determined by numerous environmental factors, such as climatic conditions and the distribution of competitors and herbivores (Hoffmann and Blows 1994). Temperature is one of the primary factors limiting species distributions along geographic gradients (Angert 2006). Low temperature, in particular, may prevent the establishment and persistence of a plant species beyond its range limit in various ways: plant individuals may be killed by low minimum temperatures in winter (Bruehlheide and Heinemeyer 2002, Woodward 1997) or by frost during the growing season (Taschler and Neuner 2004); plant growth (Angert 2006) or reproduction (Woodward 1990) may be inhibited by low temperatures during the growing season; and for annual plants, the growing season may be too short to complete the life cycle (Beerling 1993, Griffith and Watson 2005). Plant distribution may also be influenced by various aspects of the light climate, such as photoperiodic variation with latitude (Griffith and Watson 2005), and variation in the intensity and spectral composition of solar radiation with altitude (Körner 2003).

To colonize higher latitudes and altitudes, plants must therefore adapt to lower temperatures and different light conditions, and genetic differences between populations from differing altitudes or latitudes have indeed been observed in many species. A typical adaptive response to cooler and shorter growing seasons, for example, is reduced plant size and earlier reproduction (Clevering et al. 2001, Olsson and Agren 2002). Range limits arise where plants are no longer able to adapt to local environmental conditions (Angert and Schemske 2005, Kirkpatrick and Barton 1997). For example, the northern latitudinal limit of *Xanthium strumarium* in the USA appears to reflect constraints on the evolution of early reproduction (Griffith and Watson 2006). Accordingly, the range expansion of a species may be promoted by changes in environmental conditions (e.g. climate warming) or by genetic and demographic changes facilitating adaptive evolution, such as the introduction of new genotypes or a general increase in abundance (Bridle and Vines 2007, Kirkpatrick and Barton 1997).

Climatic limits and conditions for range expansion are important topics in invasion biology (Loebl et al. 2006, Richardson and Bond 1991, Walther et al. 2007, Willis and Hulme 2002). Many invasive alien species in Europe are presently limited by climatic conditions (Beerling 1993), so that both the number and abundance of invasive species decline with latitude and with altitude (Becker et al. 2005). However, such patterns may change over time. Numerous studies detected adaptive differentiation along geographic gradients in recently introduced plant populations (Kollmann and

Banuelos 2004, Leger and Rice 2007, Maron et al. 2004, Montague et al. 2008, Sexton et al. 2002, Weber and Schmid 1998), and further adaptation might eventually enable these species to invade new areas. In addition, although climatic factors often seem to limit the range of invasive alien species, some species may not yet have reached their range limits and their current distribution might simply reflect a low availability of propagules at the invasion front (Paiaro et al. 2007). Understanding the precise causes of the present distribution limits of invasive species is essential for predicting how they will change in the future, and for determining how future range expansion might be prevented.

In this study we investigate what limits the altitudinal distribution of the alien forb *Erigeron annuus* in the Swiss Alps. *E. annuus* is native to North America and was introduced to Europe in the 17th century (Edwards et al. 2006). In Switzerland, *E. annuus* is widespread and very abundant in the lowlands but seldom occurs above 1000 m (Becker et al. 2005). Given the fact that plants produce large numbers of tiny, wind-dispersed seeds (<50'000 seeds each weighing 25 µg; Stratton 1989), it is improbable that this altitudinal limit is due to low dispersal and seed availability at higher altitudes. Climatic factors are therefore more likely to cause the altitudinal limit, especially since *E. annuus* is sensitive to low winter temperatures in its native range (Stratton 1992). If so, an important question is whether the species is likely to become adapted to conditions beyond its current altitudinal limit through evolutionary change. This might potentially be the case if populations occurring at high-altitude sites exhibit local adaptation compared to populations from lower altitudes. Alternatively - or in addition - plants may adjust to higher altitudes through adaptive plasticity (e.g. morphological and physiological changes in response to high UV radiation), though such plastic responses may be insufficient to maintain fitness beyond a certain altitude (DeWitt et al. 1998).

To investigate whether *E. annuus* is physiologically constrained by climatic conditions at its present altitudinal limit and whether it is likely to adapt to higher altitudes in the future, we planted offspring of *E. annuus* plants originating from different altitudes into two common gardens representing the main area of distribution (400 m) and the altitudinal limit of *E. annuus* in Switzerland (1000 m). We asked the following specific questions: (i) Is the survival, growth or reproduction of *E. annuus* reduced at 1000 m compared to 400 m? (ii) Does *E. annuus* exhibit adaptive plasticity in response to conditions at 1000 m? (iii) Is there evidence for local adaptation, i.e. is the performance of plants in the upper garden (but not in the lower garden) positively related to their altitude of origin?

MATERIAL AND METHODS

Plant material

Erigeron annuus L. (Asteraceae) is a triploid winter annual. Seeds normally germinate in late summer (August), and plants overwinter as rosettes from which flowering stems develop in the following spring (March-May). The species reproduces predominantly via apomixis, but rare sexual events also occur, maintaining a relatively high number of genotypes both in the native and in the introduced range (Edwards et al. 2006).

Seeds were collected during a field survey of the distribution of *E. annuus* along major roads crossing the Swiss Alps (Chapter 3). At a total of 108 sites spanning an altitudinal range from 243 m to 2065 m, the presence or absence of *E. annuus* was recorded in May-July 2004. If the species was present, sites were revisited in August 2004 to collect seeds. As a result, seeds from 197 plants in 26 populations (3-10 plants per population) growing at altitudes between 325 m and 965 m were available for the experiments. In the following, the “altitude of origin” of a plant refers to the altitude of the site from which its seeds were collected. Because the species is predominantly apomictic, seeds collected from the same mother plant are presumed to be genetically identical.

Common gardens

Common garden experiments were set up at the ETH research stations in Chamau (400 m) and Frübüel (1000 m) in canton Zug, Switzerland. At both sites an area of about 65 m² was ploughed and fenced in May 2005. Six plots (3.5 x 1.5 m) were established at each site. They were weeded regularly and protected from slugs with molluscicide pellets. *E. annuus* plants were grown in pots arranged in four rows per plot (20 cm apart) and set into the soil to 3/4 of their height. The plants at both sites were watered regularly using tap water taken from the same source. The monthly mean temperatures were on average 2.2°C lower at 1000 m than at 400 m (Table 1).

Plant cultivation

Seeds collected in August 2004 were stored at room temperature until used on 12 March 2005. They were left to germinate for two weeks on wet filter paper in Petri dishes in a heated greenhouse. The seedlings were transplanted into seedling trays and grown in standard potting soil (Universalerde Capito, Landi Schweiz) for one month, and then transplanted into 1-liter plastic pots filled with the standard potting

Table 1. Monthly mean temperatures during the common garden experiments in 2005 and 2006 (Zeeman unpubl.).

		May	June	July	August	September	October
2005	400 m	na	na	23.6*	16.1	15.3	11.4
	1000 m	na	na	22.7*	13.2	12.9	9.6
2006	400 m	13.3	17.5	21.7	15.0	16.7	12.4
	1000 m	10.4	14.9	19.4	11.8	14.9	11.1

na – non-available data, *data available only for the second half of July

soil mixed with perlite (4:1). Three seedlings were planted per pot with two pots per mother plant. The pots were kept in the heated greenhouse for two weeks, and then moved into an unheated greenhouse for hardening. After nine days (25 May 2005) the pots were moved outside into a garden in Zurich. They were transported to the 400 m site on 10 June and to the 1000 m site on 16 June. One pot per mother plant was placed at each of the two sites in a random arrangement.

This first experiment had three potential drawbacks. First, seeds were collected directly at the growth sites of the mother plants, so that any differences in performance or phenology related to the altitude of origin might reflect maternal effects rather than genetic differences (Stratton 1989). Second, seedlings were planted in the common gardens in spring rather than in autumn, and thus the typical phenology of a winter annual was not followed. And third, the late start of the experiment artificially delayed the onset of flowering and reduced the length of the growing season, which may have been crucial for plant performance at 1000 m (see below). To confirm the results, therefore, a second experiment designed to avoid these drawbacks was established in 2006.

Initially, the second experiment was set up in October 2005. However, most of the seedlings at 400 m died due to herbivory at the beginning of the experiment. Consequently, the winter survivorship of the seedlings could be assessed only at 1000 m. The experiment was repeated in 2006 with seeds collected from 35 plants (21 populations) in the common garden at 400 m in August 2005. Seeds were germinated over 10 days in December 2005 on wet filter paper in a climate chamber (day/night temperatures of 25/16°C). The seedlings were transplanted into seedling trays and grown in standard potting soil in a climate chamber for thirteen weeks, during which day/night temperatures were progressively reduced from 20/12°C to 12/4°C to simulate winter conditions. On 27 March 2006 the seedlings were moved outdoors to the institute terrace. Two-litre plastic pots were filled with standard potting soil

mixed with perlite (4:1) and slow release NPK fertilizer (Osmocote Exact Standard 8-9 months, Scotts) (0.5 kg per 100 l of soil and perlite mixture). Two seedlings were transplanted into each pot at the 400 m site on 3 May and at the 1000 m site on 5 May. After one month they were thinned to one plant per pot. At each site there were two replicates of each mother plant.

Measurements

Plant phenology was observed every second week in 2005 and every week in 2006. The times of the following transitions were recorded: (i) bolting (stem height ≥ 3 cm), (ii) flower buds formation, (iii) flowering (the white petals of the ray florets visible), and (iv) seed set (shedding of petals and appearance of the pappus).

In 2005, plant growth was assessed when the plants were harvested (between 29 September and 5 October 2005) by measuring rosette diameter, plant height, and number of stems. The fresh aboveground biomass of all plants was then weighed, and a subset of plants was dried at 70°C. Their dry matter content (dry/fresh mass) was used to estimate the shoot dry mass of all other plants. In 2006, the rosette diameter and the length of the longest leaf were recorded one month after transplant. The number of stems, the mean stem height and the number of leaves per stem were recorded when plants started to flower, and the final height of the whole plant was recorded when most plants had set seeds (10 August at 400 m, 4–7 September at 1000 m).

Plasticity in leaf traits was measured by collecting two, fully developed but non-senescent rosette leaves from each plant in September 2005. Leaf area was measured using the leaf area meter (LI-COR Biosciences), and the leaves were then dried at 70°C, weighed and the specific leaf area calculated. The concentration of phenolics and UV absorbance of the epidermis were measured for a subset of 37 mother plants. Leaves were ground in a mortar, and 50 mg of leaf powder were extracted with 5 ml of 50% ethanol, followed by 4 min centrifugation at 10'000 rpm. The total concentration of soluble phenolics in the extracts was determined with the Folin-Ciocalteu method and expressed on a leaf area basis. To measure UV-A absorbance, 250 μ l of each leaf extract was mixed with 8 ml of distilled water, and absorption of the sample was measured in the UVI light photometer (Secomam). A wavelength of 330 nm was chosen because this was the absorption peak and yielded the best resolution; the results were expressed as absorption units per unit leaf area. Previous studies have shown that UV-A absorbance of leaf extracts usually correlates well with the UV-A and UV-B screening capacity of the epidermis (Markstadter et al. 2001).

Plant reproduction was assessed in 2006 by harvesting all inflorescences (stems with capitula but without leaves) on 15-18 August at 400 m and on 4-10 September at 1000 m. The number of capitula was counted on 20 inflorescences per site; these were then dried at 70°C and weighed. Based on the linear relationship between inflorescence dry biomass and the number of capitula, the number of capitula was estimated for the rest of the plants.

Seed size and germination rate were determined in January 2007 by counting and weighing 50 seeds per plant. These seeds were then germinated on wet filter paper in Petri dishes in a climate chamber (25/18 °C), and the number of germinated seeds was recorded after two weeks.

Statistical analyses

We used analysis of covariance to analyse the effects of growth site (400 m vs. 1000 m), altitude of origin (covariate) and their interaction on plant traits. To meet the statistical requirements, data for the number of stems (2005), dry mass (2005), the number of capitula per stem (2006) and the total number of capitula (2006) were square root transformed. Depending on the plant trait considered, a significant difference between the two sites may indicate reduced fitness at 1000 m (research question 1) or adaptive plasticity (question 2), whereas significant effects of altitude of origin or interactions between growth site and altitude of origin might indicate local adaptation (question 3).

Phenological records were analysed by fitting survival curves with a Weibull distribution separately for each phenological transition. The dependent variables were the number of days to bolting, formation of flower buds, flowering and seed set, respectively. If a phenological transition was not reached before the harvest, the observation was censored. Survival models were used to test the effects of growth site and altitude of origin on phenological rates and to estimate the time needed for 50% of the plants to reach each phenological transition in either garden. All analyses were performed in JMP 6.0 (SAS Institute Inc. 2006).

RESULTS

Plant performance at 400 m and at 1000 m

In both experiments (2005 and 2006), all established *E. annuus* plants survived and

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grew vigorously in both common gardens (400 m and 1000 m). Plants generally grew larger at 400 m than at 1000 m, with significantly bigger rosettes and more stems (Table 2). However, stem height in 2006 was larger at 1000 m than at 400 m (Table 2). Plant leaves showed significant plastic responses to altitude. At 1000 m, the specific leaf area was on average 19% lower than at 400 m, while the concentration of phenolics was 35% higher and the UV-A absorbance per unit leaf area 33% higher (Table 2).

Only some of the plants flowered and set seed in 2005, the proportion being much higher at 400 m (71%) than at 1000 m (9%). In 2006 all plants set seed but the reproductive output (number of capitula) was 64% higher at 400 m than at 1000 m (Table 2). However, the seeds produced at 400 m were 10% smaller and had 11% lower germination rates than those produced at 1000 m (Table 2). Plant

Table 2. Effects of growth site with two levels (400 m and 1000 m) and altitude of origin on plant performance in 2005 and 2006. All length measurements are in cm.

	Site (S)		400 m	1000 m	Altitude of origin (A)	S x A
Analysis of covariance	F ratio		mean	mean	F ratio	F ratio
<i>Vegetative growth</i>						
rosette diameter (05)	57.04 ***		40.2	33.0	0.37	1.60
nb stems (05)	279.90 ***		14	4	0.49	2.10
final height (05)	669.25 ***		152.2	82.0	2.51	0.17
dry mass (g) (05)	604.01 ***		243.3	63.5	0.00	0.30
rosette diameter (06)	120.02 ***		32.8	25.9	0.76	0.02
longest leaf (06)	156.26 ***		18.0	13.9	0.59	0.01
nb stems (06)	18.86 ***		10	8	0.16	0.13
nb leaves per stem (06)	56.82 ***		25	30	8.62 **	0.08
total nb leaves (06)	1.44		240	226	2.08	0.23
mean stem height (06)	36.53 ***		66.3	74.9	0.57	0.07
final height (06)	26.64 ***		107.9	117.8	1.07	1.08
<i>Rosette leaves traits (05)</i>						
SLA (cm ² .g ⁻¹)	70.96 ***		305.7	247.9	0.03	4.69 *
phenolics (mg.cm ⁻²)	17.14 ***		0.20	0.27	2.90	0.08
UVA absorbance (cm ⁻²)	17.20 ***		0.09	0.12	4.29 *	0.00
<i>Reproductive output (06)</i>						
capitula per stem	7.45 **		351	267	0.03	0.13
total nb capitula	47.26 ***		3189	1947	0.40	0.60
germination (%)	15.02 ***		71.2	79.7	2.56	0.00
seed mass (mg)	11.05 ***		0.037	0.041	0.42	0.08

*p<0.05, **p<0.01, ***p<0.001

phenology (presented as the estimated number of days for 50% of the plants to reach a phenological transition) differed conspicuously between the two growth sites and also between the two years (Fig. 1, Table 3). In 2005, plants started to bolt 43 days earlier at 400 m than at 1000 m, and this was followed by an earlier development of flower buds, flowers and seeds. In 2006, bolting at 400 m started only 4 days earlier than at 1000 m. Plants generally developed more slowly in 2005 than in 2006. In 2005 plants started to flower 31 days after bolting at 400m and 27 days after bolting at 1000 m, whereas in 2006 the equivalent periods were 17 and 26 days, respectively. Plants generally reproduced later in 2005 than in 2006 due to the later planting date in 2005 combined with a longer time to bolting.

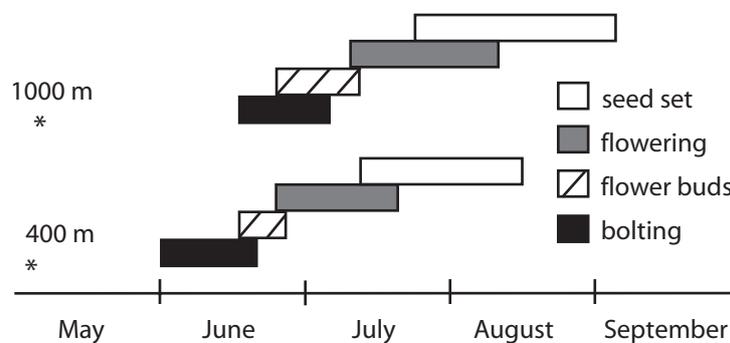


Fig. 1. Duration of each phenological transition in *E. annuus* plants in the common gardens at 400 m and at 1000 m in 2006. Asterisks indicate the dates of planting.

Table 3. Effects of growth site and altitude of origin on plant phenology in 2005 and 2006. Numbers of days are estimates of time needed for 50% of the plants to reach each phenological transition.

	Site (S)		400 m	1000 m	Altitude of origin (A)		S x A
Survival analysis	χ^2		50% plants		χ^2		χ^2
<i>Phenology 2005</i>							
days to bolting	213.64	***	46	89	3.47	0.06	0.42
days to flower buds	199.65	***	64	107	6.67	**	0.25
days to flowering	176.47	***	77	116	8.90	**	0.16
<i>Phenology 2006</i>							
days to bolting	52.55	***	38	42	25.20	***	31.04 ***
days to flower buds	176.74	***	47	59	28.86	***	4.55 *
days to flowering	248.20	***	55	68	31.58	***	12.27 ***
days to seed set	231.44	***	73	92	22.11	***	5.27 *

*p<0.05, **p<0.01, ***p<0.001

Plant performance versus altitude of origin

Most of the variables describing vegetative growth, rosette leaves and reproductive output were not significantly related to altitude of origin, nor was there a significant growth site by altitude of origin interaction (Fig. 2, Table 2). The three significant effects detected in the analyses (Table 2) were not more than would be expected by chance alone when carrying out multiple tests at the 5% significance level. Thus, plants from different altitudes responded similarly to the altitude of growth site in terms of vegetative growth and reproductive output.

In contrast, plant phenology was significantly related to the altitude of origin in both years (Table 3). Plants originating from low altitudes developed flower buds and flowers earlier than plants originating from high altitudes. Furthermore, plants from low altitudes tended to be phenologically more plastic, i.e. to experience a greater delay in development at 1000 m compared to 400 m than plants from high altitudes (significant growth site by altitude of origin interaction in 2006; Table 3). Based on the regression of bolting time against altitude of origin, phenology was delayed by an average of 13 days for plants from the lowest altitude (325 m) and 8 days for plants from the highest altitude (965 m), and there was a similar trend for the other phenological variables in 2006.

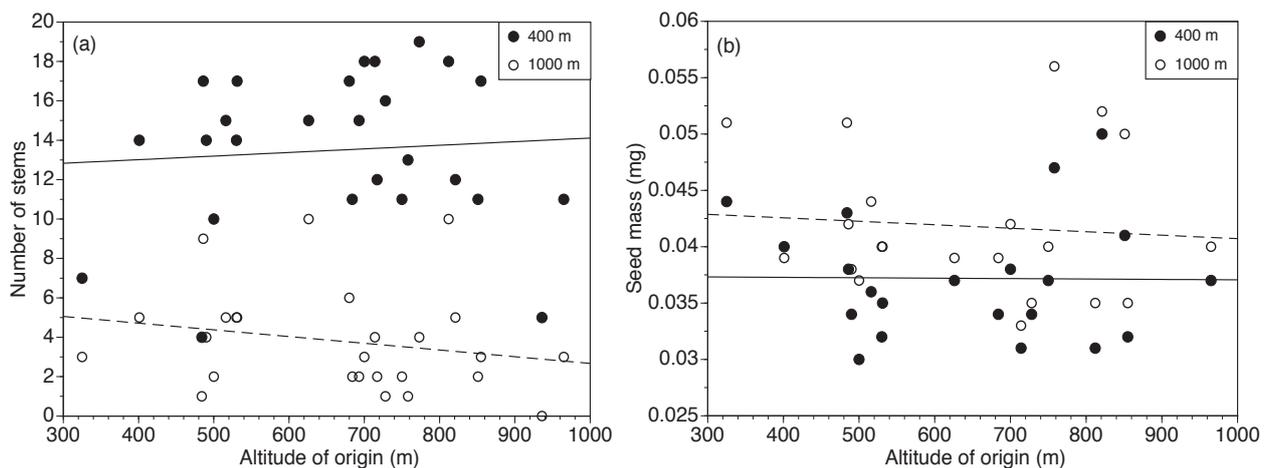


Fig. 2. Performance of *E. annuus* plants originating at different altitudes in the common gardens at 400 m and 1000 m: (a) mean number of stems in 2005, (b) mean seed mass in 2006. Regression lines only serve to highlight the differences in plant performance between the two growth sites; none of the relationships was significant.

DISCUSSION

Is the performance of *E. annuus* reduced at its altitudinal limit?

The two common gardens were located at altitudes representing the main area of distribution (400 m) and the current altitudinal limit of *E. annuus* in Switzerland (1000 m). If the altitudinal limit was caused by physiological limitations, the performance of *E. annuus* should have been severely reduced at 1000 m because of increased mortality, or reduced growth and fecundity, or both (Angert and Schemske 2005, Bruelheide and Heinemeyer 2002).

We did not observe any mortality during the growth experiments, which indicates that climatic conditions during the growing season are not critical for the survival of established *E. annuus* plants even at 1000 m. However, 27% of the seedlings planted in autumn at 1000 m died during the winter, suggesting that low winter temperatures might be critical for seedling survival. Stratton (1992) also observed considerable seedling mortality in *E. annuus* during winter due to frost heaving. Similarly, Regehr and Bazzaz (1979) found high winter mortality (16-86%) due to frost heaving in the winter annual *Conyza canadensis*. Woodward (1997) observed the total extinction of overwintering rosettes of young *Verbena officinalis* plants at -10°C . In another winter annual, *Digitalis purpurea*, frost damage to leaves occurred at or below -12°C (Bruelheide and Heinemeyer 2002). In the winter 2005-06 the minimum air temperature at 2 m above ground was -10°C at 400 m and -11°C at 1000 m (Zeeman unpubl.). Assuming that the frost tolerance of *E. annuus* is similar to that of these species, the minimal winter temperature may have been the critical factor for its survivorship at 1000 m. If so, seedlings of *E. annuus* may survive at higher altitudes in relatively mild winters but die in cold winters, with the risk of winter mortality increasing steeply above 1000 m. This risk may be further exacerbated by the presence of competitors. In a parallel experiment, we observed 81% winter mortality in *E. annuus* seedlings grown at 1000 m in competition with grasses (Trtikova unpubl.). Woodward and Jones (1984) also found the winter survival of *D. purpurea* to be negatively affected by increased plant density.

Winter survival therefore seems to be critical for *E. annuus*, especially under competitive conditions, and this factor alone might account for its current altitudinal limit in the Swiss Alps. However, the species seems to have advanced its altitudinal limit over the past few decades (Becker et al. 2005), possibly due to climate warming. In Switzerland mean winter temperatures (October – March) have increased by about 2°C since 1864 (Bader and Bantle 2004), and these warmer, shorter winters are believed to have

promoted the rapid spread of the invasive palm *Trachycarpus fortunei* in southern Switzerland (Walther et al. 2007). By increasing seedling survival, a further increase in winter temperatures might permit populations of *E. annuus* to establish at higher altitudes, especially in open disturbed sites.

Vegetative growth was significantly reduced at 1000 m compared to 400 m. The difference was very pronounced in 2005, when stem number was reduced by 71% and final aboveground biomass by 74%, but much less in 2006, with only 20% reduction in stem number and 6% decline in leaf number. The strong reduction in growth at 1000 m in 2005 may have been caused by photoinhibition. Just one week after the beginning of the experiment, in June 2005, the leaves had turned reddish, probably due to exposure to strong irradiation at relatively low temperature. Under both clear sky (reduced atmospheric turbidity) and overcast (thinner cloud layer) conditions solar radiation increases with altitude (Körner 2003). In a comparative experiment, the lowland plant *Pisum sativum* proved to be less tolerant of cold and light stress than alpine species, as indicated by higher sensitivity to photosynthesis damage, reduced catalase activity and bleaching of chlorophyll (Streb et al. 2003).

Acclimation to suboptimal temperature increases the capacity of plants to resist photoinhibition induced by chilling (Venema et al. 2000), which could explain why we found no evidence of photoinhibition in 2006, when plants were pre-grown at relatively low temperatures. Since the 2006 plants were able to grow vigorously even at the altitudinal limit, we conclude that photoinhibition does not significantly affect the growth of *E. annuus* in the field, and suggest that the effects observed in 2005 may have been an experimental artefact.

Shoot development (from bolting to seed set) took 15 days longer at 1000 m than at 400 m, but plants grew taller at the higher site. The tiny seeds of *E. annuus* are wind-dispersed, and plant height might be critical for effective dispersal. For another wind-dispersed annual, *Conyza canadensis*, maximum plant height was thought to be more important for effective dispersal than maximum energy allocation to seed production (Regehr and Bazzaz 1979).

The total number of capitula produced was 39% lower at 1000 m than at 400 m, suggesting that total reproductive output was also considerably lower. However, the seeds produced at 1000 m tended to be larger, and the mean germination rate under laboratory conditions was 12% higher. Seed size might have a greater influence on survivorship during establishment under less favourable field conditions (Moles

and Westoby 2004). The ability to produce larger seeds under severe environmental conditions also seems to facilitate the spread of the strongly invasive plant *Impatiens glandulifera* (Willis and Hulme 2004). Larger seed mass might, therefore, promote the establishment of *E. annuus* at higher altitudes more than was apparent in our experiment, but it remains open whether this would be sufficient to compensate for the lower seed production.

Does *E. annuus* exhibit adaptive plasticity in response to altitude?

While some of the observed differences in plant traits between the two gardens indicate a reduced fitness at 1000 m, others can be interpreted as adaptive plastic responses that may have contributed to limit the reduction in fitness. The latter clearly applies for the higher seed mass and germination rate at 1000 m. Alexander (2007) also showed that the seed mass of *E. annuus* increased along altitudinal gradients both in its native and introduced ranges. However, his results were based on field observations and did not reveal whether the increase in seed mass resulted from local adaptation or from phenotypic plasticity. In our common garden experiment, seed mass was not related to the altitude of origin, suggesting that the observed increase in *E. annuus* seed mass along the altitudinal gradient in the Swiss Alps is a purely plastic response.

E. annuus also showed significant adaptive plasticity in rosette leaf traits. Plants had smaller leaves and a reduced specific leaf area at 1000 m and the leaves contained more UV-absorbing phenolic compounds. Smaller leaf size may help to reduce frost damage due to radiative cooling during clear nights (Jordan and Smith 1995). A smaller specific leaf area may also be associated with better structural protection against drought stress as induced by frost and wind. Flavonoids and related phenolic compounds are among the most important protective substances responsible for UV-B absorbance (Körner 2003). Phenotypic plasticity in these leaf traits may therefore enable *E. annuus* to tolerate harsher climatic conditions at higher altitudes. However, the levels of plasticity in these traits displayed by *E. annuus* are similar to those of other species grown under contrasting temperature regimes (Atkin et al. 2006) and light conditions (Weinig et al. 2004), and they are probably insufficient to permit the spread of *E. annuus* at higher altitudes.

The plastic changes in phenology are more difficult to interpret. Bolting, flowering and seed set were delayed by up to three weeks at 1000 m compared to 400 m. The question remains whether the plastic response is adaptive or maladaptive. The delay in plant phenology might cause seed set or seed maturation to fail due to low

temperatures in an early and cold autumn (Griffith and Watson 2005). Furthermore, seedlings germinating in autumn may not have enough time to form sufficiently large rosettes before the end of the growing season to become resistant to frost heaving. In another winter annual *Conyza canadensis*, winter survival of seedlings was closely correlated with the size of their rosettes, with 100% survival of seedlings with rosettes larger than 5 cm, because they had larger roots and were better anchored (Regehr and Bazzaz 1979). However, a phenological delay of several weeks may contribute to avoid precisely this problem by postponing seed germination to the spring, when seedlings have a full growing season to reach a sufficient rosette size before the next winter. Poll (2007) observed that *E. annuus* changed from winter annual to biennial in a garden experiment at 1650 m. We observed the same pattern in another garden experiment, where seeds were sown in pots placed at three different altitudes in September 2004 (Trtikova unpubl.). Some of the seeds germinated immediately, others only in the next spring. In the uppermost garden, at 1700 m, none of the seedlings that emerged in September survived the winter. In contrast, seedlings that germinated in the following spring formed rosettes during the first growing season, and many of them survived the next winter to flower in the second growing season.

A prolonged life cycle thus seems to be necessary for successful survival and reproduction of *E. annuus* at higher altitudes. The observed phenological delay at 1000 m may contribute to this adaptive response provided that the delay does not prevent seed formation altogether. The balance between the risk of later reproduction and the advantage of postponed seed germination is likely to vary from year to year according to climatic conditions in autumn and winter.

Is there evidence for local adaptation to altitude in *E. annuus*?

Vegetative growth, leaf traits and reproductive output of *E. annuus* plants were unrelated to their altitude of origin. Plants originating from different altitudes also responded similarly to the altitudinal difference between the two common gardens. Thus, for these traits there was no evidence for adaptation to altitude.

For plant phenology, we found trends related to the altitude of origin, but little evidence for local adaptation to conditions at the altitudinal limit. Plants from high altitudes bolted and flowered slightly later than plants from low altitudes. This might be interpreted as a sign of local adaptation if we assume that at higher altitudes plants benefit from reproducing later, so that seed germination is postponed to the next spring. However, because plants from low altitudes were phenologically more plastic

than those from high altitudes, they reproduced at a similar time when grown at 1000 m, while reproducing earlier when grown at 400 m. Thus, even if we assume that there is selection for late reproduction at 1000 m (which would still need experimental confirmation), plants from high altitudes would not perform better in this respect than those from low altitudes.

The selective advantage of later reproduction in *E. annuus* may vary either regionally or from year to year depending on weather conditions. Spatial and temporal variation in the strength and direction of selection on life-history traits was also observed in other annual species (Kalisz 1986, Kelly 1992). Due to contrasting selective pressures, the overall directional selection on phenology in *E. annuus* is probably only weak, which may then prevent the evolution of genotypes adapted to high altitudes. Evolution of locally adapted genotypes in *E. annuus* may be additionally hindered by presumably high extinction and colonization rates at high altitudes (Kawecki and Ebert 2004). Assuming that *E. annuus* plants are frequently killed by low winter temperatures, high-altitude sites are regularly re-colonized from lower altitudes, preventing any local adaptation. Similarly, such metapopulation processes may have limited local adaptation in another annual species *Chamaecrista fasciculata* (Galloway and Fenster 2000).

Little evidence for local adaptation to altitude was also found in the invasive species *Verbascum thapsus* (Parker et al. 2003) and *Pennisetum setaceum* (Williams et al. 1995) occurring across wide altitudinal gradients in their introduced ranges. However, both of these species displayed extensive phenotypic plasticity in their physiological and morphological characters. In *E. annuus*, the lack of genetic adaptation to altitude together with only moderate plasticity suggest that there is little potential for this species to spread beyond its current altitudinal limit in the Swiss Alps under unchanged climatic conditions. However, climate warming might promote the upward range expansion of *E. annuus* by reducing the risk of winter mortality and the risk of not being able to produce seeds before the end of the growing season.

ACKNOWLEDGMENTS

We thank Matthias Baltisberger and Jake Alexander for valuable comments on the manuscript; Hans Leuenberger for technical support at the ETH research stations in Chamau and Frübüel; Matthias Zeeman and Werner Eugster for providing the temperature data for the two stations; Antoine Guisan for allowing us to use his research plots at Dent de Morcles and Yann Hautier for maintenance of the plots.

Claudia Farrér, Karin Gafner, Aud Halbritter, Gabi Jakobs, Alexandra Käser, Deborah Scharfy and Pavel Trtik kindly helped with field and laboratory work. This project was financed by a grant from the ETH Research Fund.

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Chapter 2

Effects of competition and mowing on growth and reproduction of the invasive plant *Erigeron annuus*

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ABSTRACT

Disturbances that reduce competition or increase nutrient availability may facilitate the establishment of invasive plants. However, to persist in disturbed sites, the plants must be able to compensate for biomass loss caused by disturbances such as mowing. We studied how competition and mowing affect growth and reproduction of *Erigeron annuus*, a common invasive species in Switzerland. To investigate the influence of altitude upon these responses, the experiment was replicated in common gardens at altitudes of 400 m and 1000 m. Vegetative growth was negatively affected by competition at both sites, but especially at 1000 m. The number of capitula was not affected by competition at 400 m, but was reduced at 1000 m. Plants regrew vigorously after mowing in both gardens. The effects of mowing on reproductive output were positive at 400 m but negative at 1000 m. We conclude that while *E. annuus* can tolerate both competition and mowing at low altitudes, these factors hinder its spread at higher altitudes.

INTRODUCTION

Abundance of a plant species is determined by both its competitive ability relative to other plant species and its susceptibility to herbivores and pathogens (Crawley 1997). However, a species introduced into a new region may benefit because it escapes attack from specialised enemies. Under these conditions, selection might favour individuals that invest less in defence and more in growth leading to the evolution of increased competitive ability (EICA; Blossey and Notzold 1995). This hypothesis has been invoked to explain the successful spread of several invasive plants, such as *Lythrum salicaria* (Blossey and Notzold 1995) and *Solidago gigantea* (Jakobs et al. 2004). However, the EICA hypothesis does not appear to apply in the case of other successful invaders, including *Alliaria petiolata* (Bossdorf et al. 2004) and *Solidago canadensis* (van Kleunen and Schmid 2003), for which introduced populations were found to be less competitive than native ones.

Many species probably become successful invaders, not because they are more competitive than native species, but because they benefit more from nutrient-rich or disturbed conditions associated with human activities (Daehler 2003). Petryna et al. (2002) observed that establishment and persistence of alien plants in the mountain grasslands in central Argentina strongly depended on disturbances, such as aboveground biomass removal by cutting and burning, and soil disturbance. Establishment of *Centaurea diffusa*, one of the most problematic introduced plant species in western North America, was enhanced by high resource availability, but both the densities and size of *C. diffusa* only increased when competition was also reduced by partially removing resident grass vegetation (Seastedt and Suding 2007).

Some introduced plants might also compensate well for biomass loss due to mowing or grazing (Kimball and Schiffman 2003). For example, stem density and cover of the invasive plant *Lespedeza cuneata* were significantly increased by mowing (Brandon et al. 2004). However, the ability of a plant to compensate for biomass loss declines as competition increases or nutrient availability decreases; and it is also reduced if the loss occurs towards the end of the growing season, leaving insufficient time for the plant to recover (Maschinski and Whitham 1989). Therefore, we would not expect plants to respond positively to disturbances such as mowing at high altitudes, where growing season is short.

In this study we investigated how competition and mowing affect the growth and reproductive output of the winter annual *Erigeron annuus*. The species is predominantly

apomictic, producing large number of tiny, wind-dispersed seeds that are genetically identical to the mother plant. It is a native of North America, but was introduced and has become widespread in Europe. In Switzerland, it mostly occurs in the lowlands and is rarely found in the Alps above 1000 m (Becker et al. 2005). *E. annuus* is especially abundant in ruderal habitats, suggesting that frequent disturbances reducing competition from resident vegetation are necessary for its successful establishment; this is likely to be particularly important at higher altitudes (Petryna et al. 2002). On the other hand, ruderal sites occupied by *E. annuus* are often subjected to mowing (e.g. roadsides), and to persist in such environments plants must be able to compensate for any loss of biomass. Frey et al. (2003) reported that *E. annuus* regrows well after mowing. They did not study the effects on reproductive output, but observed strong plastic responses to the mowing treatment, particularly regarding leaf shape, to the extent that mown plants may have sometimes been misidentified as belonging to another taxon. However, plants may be less able to respond plastically under the poorer growth conditions at higher altitudes, making them less able to maintain their fitness.

To investigate how *E. annuus* responds to competition and mowing, and whether these responses change with altitude, we conducted a common garden experiment at 400 m and at 1000 m. The lower altitude represented conditions in the main area of *E. annuus* distribution, whereas the higher altitude was close to the current altitudinal limit in Switzerland. We focused on the following questions: (i) How does *E. annuus* respond to competition and mowing? (ii) Is its fitness less affected at 400 m than at 1000 m? (iii) Does phenotypic plasticity in response to mowing differ between 400 m and 1000 m?

MATERIALS AND METHODS

Plant cultivation

Seeds were collected from 35 *E. annuus* plants growing along major roads in the Swiss Alps in August 2004. In the following year, they were sown in a common garden at 400 m in order to produce a new generation of seeds free of any maternal effects from their original sites; these were collected in August 2005. Although most populations of *E. annuus* exhibit a high degree of genetic variation, suggesting that sexual reproduction does occasionally occur (Edwards et al. 2006), the seeds from one mother plant are usually genetically identical; therefore, we refer to them as 'clones' hereafter.

In December 2005 seeds were germinated on wet filter paper in Petri dishes in a climate chamber (day/night temperatures of 25/16°C). After 10 days seedlings were transplanted into seedling trays filled with standard potting soil (Universalerde Capito, Landi Schweiz). To simulate winter conditions, seedling trays were kept for the next thirteen weeks in growth cabinets where day/night temperatures were progressively reduced from 20/12°C to 12/4°C. For the next five weeks the seedling trays were moved outdoors to the institute terrace, and they were transported to common gardens at 400 m on 3 May 2006 and at 1000 m on 5 May 2006.

Treatments

Common gardens were set up at the ETH research stations in Chamau (400 m) and Frübüel (1000 m) in canton Zug, Switzerland. At both sites a fenced area of about 65 m² was ploughed and divided into six plots (3.5 x 1.5 m), which were weeded regularly and treated with molluscicide pellets to protect against slugs. *E. annuus* seedlings were transplanted into two litre plastic pots filled with standard potting soil mixed with perlite (4:1) and slow release NPK fertilizer (Osmocote Exact Standard 8-9 months, Scotts; 0.5 kg per 100 l of soil and perlite mixture). The plants were watered regularly with tap water taken from the same source to ensure identical chemical composition. To reduce the risk of them drying out, the pots were set into the soil to 3/4 of their height. In each plot 35 pots (one per clone) were arranged randomly in four rows.

Three treatments (control, competition and mowing) were applied to two plots per garden. In the plots with the competition treatment, about 0.4 g of grass seeds (Schweizer Uni-Lawn, Eric Schweizer Samen) were sown into the pots at the time of seedling transplant (May 2006). The grass mixture contained 28% of *Lolium perenne*, 45% of *Festuca rubra*, and 27% of *Poa pratensis*. The density and species composition were typical for an ordinary garden lawn. The short stature of the grasses implies that their competitive effect on *E. annuus* was limited to below-ground resources. In the plots with mowing treatment, plants were clipped 5 cm above ground (about 90% of shoots removed), just as they were starting to flower, i.e. on 22-26 June at 400 m and on 6-12 July at 1000 m. This timing coincides with the main mowing season for extensively managed grasslands and roadsides in Switzerland.

Measurements

Plants were observed weekly to record the dates of important phenological changes

- bolting (stem height ≥ 3 cm), formation of flower buds, flowering, and seed set. Vegetative growth was assessed by measuring the number of stems and the number of leaves per stem when plants started to flower. Additionally, the final height of all the plants was measured after most of them had set seed. Reproductive output was assessed by collecting all inflorescences (stems with capitula without leaves). At each site the number of capitula was counted on 20 inflorescences in control and in mowing treatment. These were then dried at 70°C and weighed. Based on the linear relationships between inflorescence dry mass and the number of capitula, the total number of capitula was estimated for the rest of the plants. To assess plasticity in leaf shape, two leaves per clone were sampled from the upper part of the stems of mown plants in one plot per site, both before mowing and after plants had re-grown. Photographs of the leaves were used to calculate the ratio between length and width as shape parameter.

Data analysis

To analyse vegetative growth and reproductive output, we used analysis of variance. Treatment and growth site (i.e. common garden) were treated as fixed factors, whereas plot (nested within treatment and site) and clone were treated as random factors. To meet statistical requirements, the number of stems, the number of capitula per stem and the total number of capitula were square root transformed. The means of the treatments were compared with the control using Dunnett's method. Two-way analysis of variance was used to test the effects of mowing, growth site and their interaction on leaf morphology. All statistical analyses were performed in JMP 7.0.1 (SAS Institute Inc. 2007).

For the plant phenology data, we performed survival analyses. The numbers of days to bolting, formation of flower buds, flowering and seed set were analysed by fitting survival curves with a Weibull distribution separately for each phenological transition. If a phenological transition was not reached, the observation was censored. To test the fixed effects of treatment and growth site and the random effects of clones we fitted frailty models using the statistical package R 2.5.1 (The R Foundation for Statistical Computing 2007). Plots were not included because models with plots could not be fitted. The expected number of days needed for 50% of the plants to reach each phenological transition at either growth site was estimated separately for each treatment using survival models in JMP 7.0.1 (SAS Institute Inc. 2007).

Results

All plants survived and grew well, with performance being strongly affected by both the growth site and the experimental treatments (Table 1). At both sites, plants grown in competition with grasses had significantly fewer stems, but more capitula per stem than control plants (Table 2). Mown plants produced significantly more stems, but fewer leaves and capitula per stem than control plants (Table 2). In all treatments, the number of stems, the total number of leaves and the total number of capitula were lower at 1000 m than at 400 m (Table 2). Plants in control and competition tended to grow taller and have more leaves per stem at 1000 m than at 400 m, but this was not the case for the mown plants (i.e. significant treatment by site interactions in Table 1).

Leaf shape differed significantly between 400 m and 1000 m ($F=4.13$, $p=0.0440$). Before mowing leaves were narrower at 1000 m (length/width= 3.57 ± 0.08) than at 400 m (length/width= 3.09 ± 0.08). Mowing had a significant effect on *E. annuus* leaf shape ($F=21.09$, $p<0.0001$), but this effect differed between 400 m and 1000 m ($F=11.85$, $p=0.0008$). At 400 m the leaves produced after mowing (length/width= 3.79 ± 0.10) were narrower than before (Fig. 1), but this was not the case at 1000 m (length/width= 3.67 ± 0.09).

All plants flowered and reproduced, except for two mown plants at 1000 m. Phenology was not influenced by competition (Fig. 2, Table 3), but seed set was postponed by mowing (Fig. 2). In all treatments, development was significantly slower at 1000 m than at 400 m (Table 3). Based on the estimated number of days for 50% of the plants to reach a phenological transition, the onset of seed production at 1000 m was delayed by 19, 22 and 50 days in control, competition and mowing treatment, respectively (Fig. 2).

Table 1. ANOVA results (F, p) for the fixed effects of treatment, growth site and their interaction on plant vegetative growth and reproductive output.

	Treatment (T)	Site (S)	T x S
df	2	1	2
nb stems	132.37 ***	51.46 ***	3.56
leaves per stem	586.82 ***	67.21 ***	31.50 **
total leaves	17.72 **	24.38 **	4.91 ^{0.051}
final height	27.79 ***	10.42 *	5.94 *
capitula per stem	16.79 **	2.99	0.52
total capitula	0.13	50.93 ***	3.17

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

Table 2. Means \pm SE of vegetative growth and reproductive output in different treatments at either growth site. Asterisks indicate comparisons with control significantly different at a level of 0.05.

	400 m			1000 m		
	control	competition	mowing	control	competition	mowing
nb stems	9.6 \pm 0.3	7.8 \pm 0.3*	21.8 \pm 0.7*	7.7 \pm 0.3	4.8 \pm 0.3*	14.3 \pm 0.5*
leaves per stem	25.2 \pm 0.3	26.1 \pm 0.4	15.2 \pm 0.2*	29.6 \pm 0.5	29.3 \pm 0.5	15.1 \pm 0.4*
total leaves	239.9 \pm 8.0	202.2 \pm 8.3*	327.9 \pm 9.8*	226.3 \pm 8.1	137.0 \pm 6.8*	210.7 \pm 7.9
final height (cm)	107.9 \pm 1.4	113.2 \pm 1.5*	104.5 \pm 1.5	117.8 \pm 1.3	118.5 \pm 1.6	102.6 \pm 1.1*
capitula per stem	350.8 \pm 24.6	464.5 \pm 34.1*	197.5 \pm 11.1*	266.8 \pm 13.8	447.7 \pm 42.4*	106.0 \pm 4.2*
total capitula	3188.6 \pm 166.6	3312.2 \pm 166.7	4087.6 \pm 195.6*	1946.9 \pm 83.1	1699.9 \pm 78.0*	1404.2 \pm 50.4*



Fig. 1. Leaves sampled from the mid stems of the same clones at 400 m (a) before and (b) after mowing, and at 1000 m (c) before and (d) after mowing.

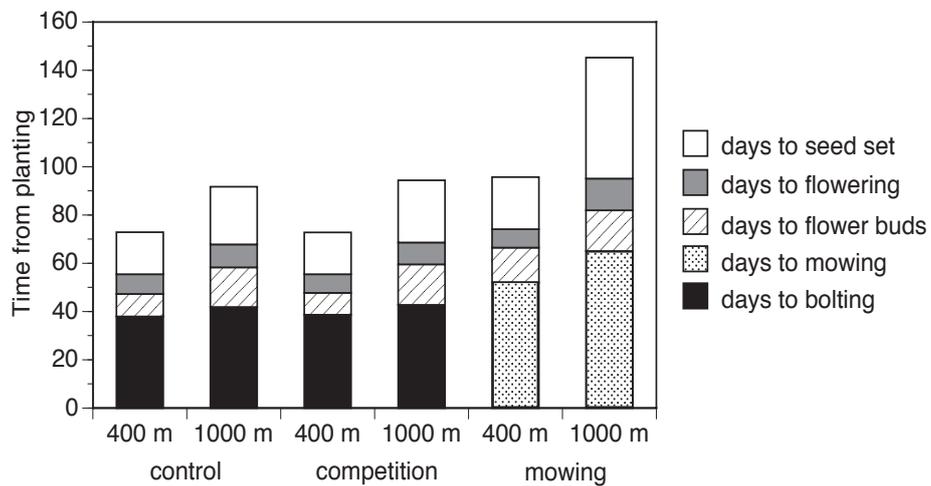


Fig. 2. Time estimates for 50% of the plants to reach different phenological transitions at 400 m and 1000 m, as derived from survival models. Plants were mown just as they were starting to flower.

Table 3. Results (χ^2 , p) derived from survival analyses for the fixed effects of treatment, growth site and their interaction on plant phenology.

	control + competition			mowing
	Treatment (T)	Site (S)	T x S	Site
df	1	1	1	1
days to bolting	4.20 *	75.13 ***	0.85	na
days to flower buds	3.44	865.07 ***	1.27	11.2 ***
days to flowering	2.71	867.57 ***	0.07	93.5 ***
days to seed set	0.82	1109.32 ***	1.36	4217.5 ***

*p<0.05, ***p<0.001, na – data not available

DISCUSSION

Response of *E. annuus* to competition

Vegetative growth was strongly reduced by competition, especially at 1000 m. Thus, compared to control plants, competition reduced the number of stems by 19% at 400 m, but by 38% at 1000 m. The fewer stems, however, did not lead to a lower reproductive output at 400 m, because more capitula were produced per stem. In contrast, at 1000 m competition reduced reproductive output by 13% compared to control plants. These results indicate that *E. annuus* can tolerate competition better at low altitudes than at higher altitudes. And competition could also reduce winter survival of *E. annuus*; in another experiment at 1000 m, we observed 81% winter mortality of *E. annuus* seedlings grown in competition with grasses compared to 27% for seedlings without competition (Chapter 1).

A limitation of our study is that competition took place in nutrient-rich conditions and was only imposed after the seedlings had established. Competitive interactions between plants might be influenced by the initial size of competing individuals, and the initially larger plants might be misjudged as competitively superior (Connolly et al. 2001, Gibson et al. 1999). The relative times at which plants establish may also play a role, as would be the case if the first plants to establish had a competitive advantage over those coming later (Ross and Harper 1972, White and Holt 2005). Our results do therefore not imply that *E. annuus* would be able to colonise and to persist in dense vegetation, as it might be outcompeted in the early life stages. However, in habitats with gaps available for its initial establishment it might compete well with its neighbours, especially at low altitudes.

Response of *E. annuus* to mowing

Mowing led to a vigorous regrowth of secondary stems in both common gardens. Mown plants produced 127% and 86% more stems than control plants at 400 m and at 1000 m, respectively. We could confirm the observation of Frey et al. (2003) that mown *E. annuus* plants produce narrower leaves, though this plastic response was not maintained under less favourable conditions at 1000 m. In addition, we found that leaf shape might also change as a response to altitude, as unmown plants had significantly narrower leaves at 1000 than at 400 m. Therefore, not only plants exposed to mowing, but also those originating from higher altitudes may have been mistakenly identified as a distinct taxon (i.e. *E. annuus* var. *septentrionalis*; Frey et al. 2003).

The effects of mowing on reproductive output were positive at 400 m, but negative at 1000 m due to stronger decrease in the number of capitula per stem. Mown plants produced 28% more capitula than control plants at 400 m, but 28% fewer capitula at 1000 m. Thus, at 400 m mown *E. annuus* plants overcompensated both in terms of vegetative growth and reproductive output. Similar responses have been observed in other monocarpic plants, but only when they were grown without competition in high nutrient environments (Huhta et al. 2000, Maschinski and Whitham 1989). However, ruderal sites frequently occupied by *E. annuus* are often characterised by high nutrient availability and reduced competition, and therefore, spread of *E. annuus* would probably not be prevented by mowing in the lowlands.

In contrast, mowing might hinder *E. annuus* establishment at higher altitudes. Furthermore, mowing also strongly delayed plant phenology. Seed set in mown plants was postponed to the end of September, while control plants reproduced at the beginning of August. Hence, there might not be enough time for seed germination and rosette formation before the onset of winter, which might strongly reduce the number of newly established plants. However, in another experiment we observed that the seed germination might be postponed to the following spring and that the annual life cycle was changed to biennial (Chapter 1). Thus, in disturbed sites at higher altitudes mowing might delay but not prevent the spread of *E. annuus*.

ACKNOWLEDGMENTS

We thank Hans Leuenberger for technical support at the ETH research stations in Chamau and Frübüel. Britta Jahn, Albert Kölbener, Miluse Kousalova, Daniel

Schläpfer and Pavel Trtik kindly helped with fieldwork. This project was financed by a grant from the ETH Research Fund.

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Chapter 3

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

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ABSTRACT

We investigated whether local adaptation has been important in enabling the invasive apomictic species *E. 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 *E. annuus* distribution and growth performance along the altitudinal gradient. We then used amplified fragment length polymorphism (AFLP) to assess genetic variation within and among *E. annuus* populations originating from different altitudes. We also set up two common gardens at contrasting altitudes to compare performance of differently distributed genotypes. Populations of *E. annuus* were seldom found above 1000 m, but plant growth performance did not decrease with increasing altitude. However, there was a strong decline with altitude in the genotypic diversity within populations. In addition, highland (711-1100 m) populations were more differentiated ($G_{st}=0.55$) than lowland (200-530 m) populations ($G_{st}=0.33$). In the common gardens locally restricted genotypes performed less well than more widespread ones, and also were less likely to reproduce. We found no evidence for on-going adaptive changes and we suggest that there is no very strong selection acting on particular genotypes. This leads us to assume that the patterns in the distribution of genotypic diversity in *E. annuus* are governed by processes of genotype formation, dispersal and extinction to a large extent neutrally.

INTRODUCTION

Many ecological factors influence whether or not an introduced species becomes invasive. Apart from the traits of an introduced species, the susceptibility of the habitat to invasion (i.e. invasibility) and propagule pressure are also important in determining its spread (Colautti et al. 2006, Dietz and Steinlein 2004). In addition, there is now increasing evidence that adaptive evolution plays an important role in enabling some plant species to become successful invaders (Lambrinos 2004, Lee 2002, Sakai et al. 2001). For example, rapid adaptive changes after introduction were observed in aggressive invaders, such as *Solidago gigantea* in Europe (Weber and Schmid 1998), or *Tamarix ramosissima* in North America (Sexton et al. 2002).

Recently, it has been emphasised that the importance of various mechanisms promoting the spread of an introduced species may change during the course of an invasion (Dietz and Edwards 2006, Theoharides and Dukes 2007). Broad environmental tolerance due to high phenotypic plasticity is likely to be particularly important during the early stages of invasion, as it may increase the range of habitats into which an introduced species can become naturalized (Sexton et al. 2002). However, for further range expansion into habitats with more limiting conditions, such as higher altitudes in mountainous regions, local adaptation may be necessary (Dietz and Edwards 2006).

Many introduced species initially suffer from reduced genetic variation due to founder effects and genetic bottlenecks, but this disadvantage seems to be overcome over longer timescales (Dlugosch and Parker 2008), and so locally adapted genotypes may evolve. On the other hand, genetic variation may be lost as species expand their range from the lowlands to higher altitudes (Ohsawa and Ide 2008), and thus their potential to adapt to adverse environmental conditions might be limited (Hoffmann and Blows 1994). In addition, a swamping effect of gene flow from lowland populations could cause further maladaptation in populations at higher altitudes (Kirkpatrick and Barton 1997).

In this study we investigate whether adaptive evolution has been important in permitting the spread of *Erigeron annuus* in the Swiss Alps. *E. annuus* is a winter annual that is native to North America. Nowadays, however, it is abundant in many parts of Europe including Switzerland, where it occurs mainly in the lowlands but has been reported up to an altitude of 1790 m (Becker et al. 2005). Although *E. annuus* reproduces predominantly via seeds that are genetically identical to the mother plant

(apomixis), most populations contain several genotypes and it is thought that sexual reproduction occurs occasionally (Edwards et al. 2006).

In a previous study of genetic variation in *E. annuus* in its native and introduced range, only a few genotypes (strictly, RAPD phenotypes) were recorded very frequently, and these tended to be more common in the introduced range in Europe (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 is probably advantageous during the early stages of invasion of *E. annuus* in Europe. However, in the longer term, genotypes capable of occasional sexual reproduction may be favoured because these would permit closer adaptation to local conditions (Edwards et al. 2006).

To test these ideas, 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 differently distributed genotypes in two common gardens, representing the altitude at which the species is most abundant (400 m) and the altitudinal limit (1000 m). With this approach we were able to address the following questions: (i) Does the growth performance of *E. annuus* plants change along the altitudinal gradient? (ii) Does genetic variation within and among *E. annuus* populations change along the altitudinal gradient? (iii) Are there any trends in the altitudinal distribution of genotypes? and (iv) Are there differences in performance of genotypes collected from different altitudes?

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 of altitude ranging from 1008 m to 2478 m. The sites surveyed ranged in altitude from 243 m to 2065 m, and included car parks, roadsides, railway stations, industrial areas and dumpsites. At sites where *E. annuus* was present, plant density was estimated.

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. In 2004 these plants were also tagged with the coloured wire. A total of 46 sites ranging from 243 m to 1762 m were sampled. These sites, which are referred to here as populations were at least 2 km apart and could be separated into four distinct regions: (i) the northern region near the city of Zurich, (ii) the western region near the city of Bern, (iii) the southern region near the city of Bellinzona, and (iv) the eastern region near the city of Chur. The sampled leaves were placed in the field in paper bags and preserved in silica gel; in the laboratory, they were lyophilised and stored at -80°C until used for DNA extraction.

Morphological measurements and seed germination test

In August 2004 twenty-six sites (325-965 m) were revisited to measure the plants that were tagged during the leaf sampling. The following morphological traits were measured for 3-10 plants at each site: height, number of stems, number of branches per stem, and stem diameters. All of the plants measured eventually produced seeds, though, at the time of seed collection, seeds were only available for 213 plants.

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

Common garden experiment

Seedlings from 197 mother plants were grown in seedling trays for one month and then transplanted into 1-liter plastic pots filled with standard potting soil (Universallerde Capito, Landi Schweiz) mixed with perlite (4:1). Three seedlings were planted per pot with two pots per mother plant. After hardening one pot per mother plant was placed into common gardens at 400 m (10 June 2005) and at 1000 m (16 June 2005). The common gardens were set up at the ETH research stations in Chamau (400 m) and Frübüel (1000 m) in canton Zug. Pots were arranged randomly into six plots (3.5 x 1.5 m). The plants were watered regularly using tap water from the same source and protected from slugs using molluscicide.

To assess plant growth, number of stems, rosette diameter and plant height were measured when plants were harvested (29 September - 5 October 2005). The phenological stage of each plant was assessed as (i) bolting, (ii) forming flower buds, (iii) flowering, and (iv) 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. Total of 171 plants could be assigned to 43 different genotypes using amplified fragment length polymorphisms (AFLPs).

DNA extraction

Total genomic DNA was extracted from 16–20 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 an 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 was quantified for a subset of samples with a Mini-Fluorometer TBS-380 (Turner Biosystems).

AFLP analyses

Amplified fragment length polymorphisms (AFLPs) were generated using a protocol of Vos et al. (1995) with the following minor modifications. Total genomic DNA (150–300 ng) was digested with *EcoRI* and *MseI* restriction enzymes (New England Biolabs - NEB). The consequent ligation of *EcoRI* and *MseI* 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: *EcoRI*+ACC/*MseI*+CCC, *EcoRI*+ACC/*MseI*+CCA, *EcoRI*+ACC/*MseI*+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 - *EcoRI*+*ACC/MseI*+*CCC* and *EcoRI*+*ACC/MseI*+*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 (*EcoRI*+*ACC/MseI*+*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.

To reduce genotyping and scoring errors, several precautions were taken, as suggested by Bonin et al. (2004), Pompanon et al. (2005), and Bonin et al. (2007). 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

To assign individuals to the different genotypes, we used the software GenoType (Meirmans and Van Tienderen 2004). For this purpose, 57 polymorphic markers were used. Because a small proportion of peaks could not be clearly scored as present or absent, some data were 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 with increasing number of the differences accepted between two individuals to identify them as belonging to the same clone. Thus, in order to eliminate the scoring errors, we may have slightly underestimated the observed genotypic diversity. Nevertheless, the main findings were consistent independent of the number of difference accepted to identify two individuals as clonemates.

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 (H_t) represents the sum of the genotypic diversity within (H_s) and among populations (D_{st}). Genotypic diversity within populations was corrected for the sample size as follows: $H_s = n / (n-1) (1 - \sum p^2)$, where n is the sample size and p is the genotype frequency. The coefficient of gene differentiation (G_{st}) was calculated as follows: $G_{st} = (H_t - H_s) / H_t$.

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.

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.

Statistical analyses

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. 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 m and 1000 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. All the statistical analyses were performed in 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 124 sites surveyed (243-2065 m). The species was mainly confined to the lowlands, and was found at only five sites above 1000 m, the highest being at 1762 m (Fig. 1). The average plant density of *E. annuus* stands did not decline with increasing altitude.

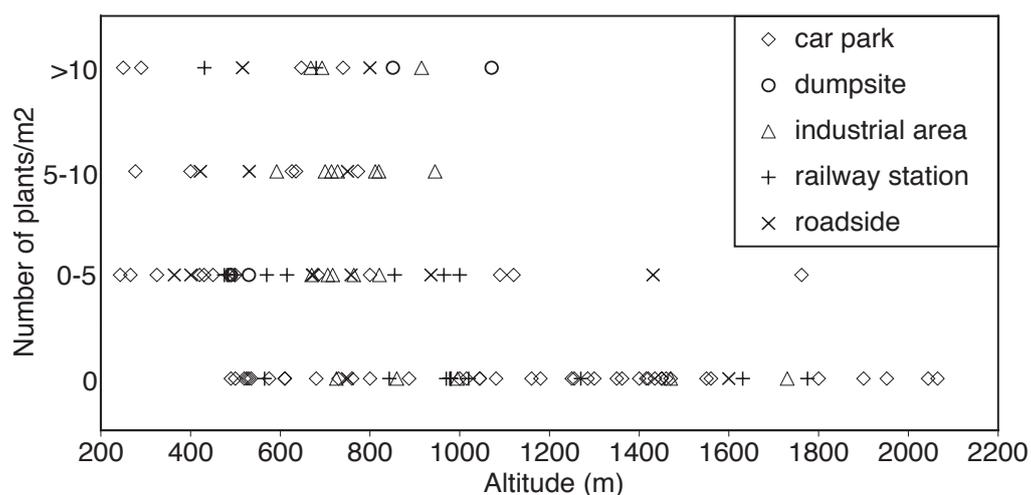


Fig. 1. Presence of *E. annuus* along several major roads crossing the Swiss Alps. Each point represents one site.

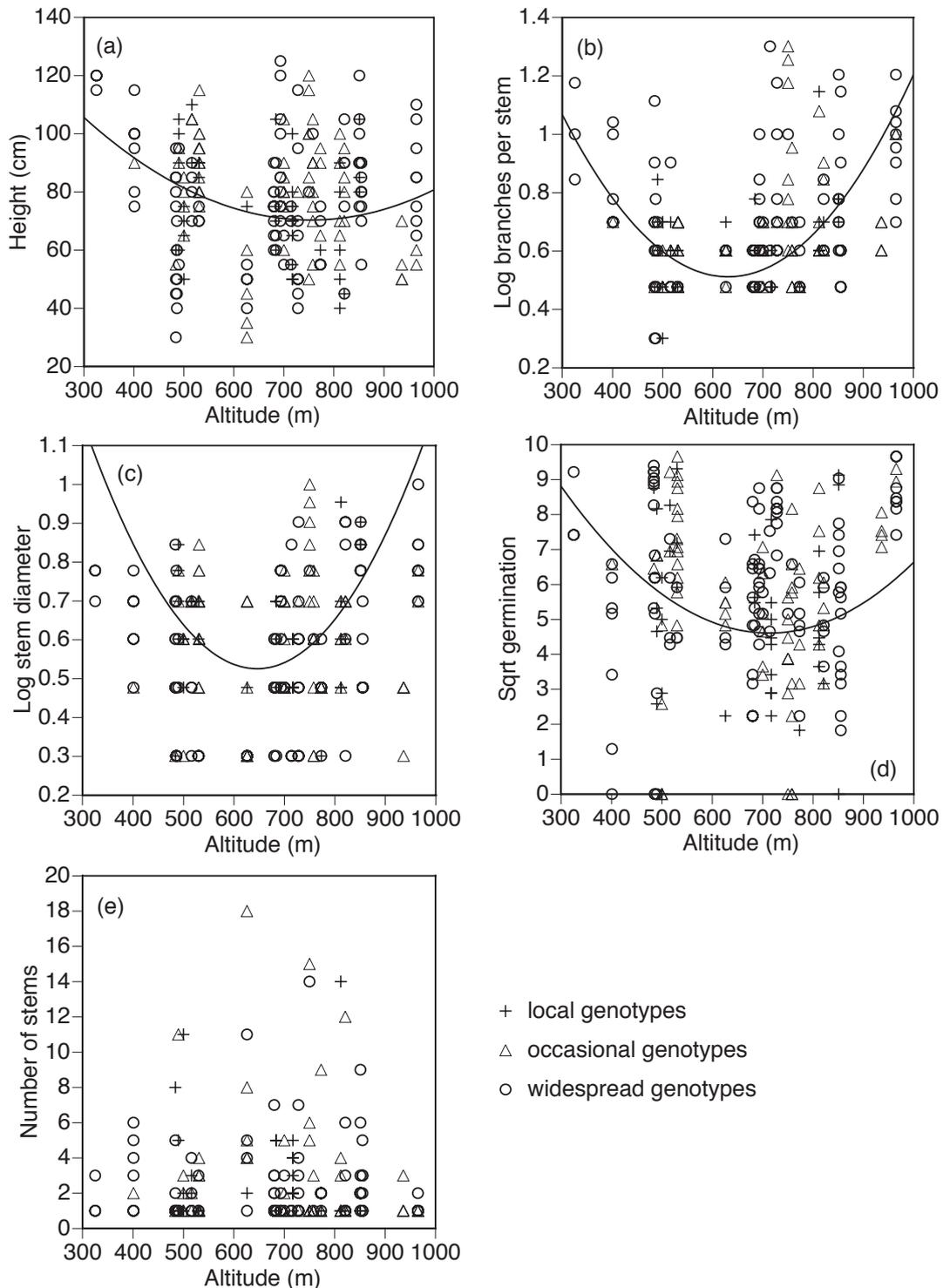


Fig. 2. Quadratic relationships between altitude and (a) plant height ($R^2=0.04$, $p=0.0072$), (b) number of branches per stem (log transformed, $R^2=0.19$, $p<0.0001$), (c) stem diameter (mm) (log transformed, $R^2=0.08$, $p<0.0001$), (d) germination rates of the seeds collected in the field and germinated in the greenhouse (square root transformed, $R^2=0.05$, $p=0.0027$), and (e) number of stems (not significant). The symbols represent the different genotypes.

The growth performance was investigated for a total of 218 plants from 26 natural populations (325-965 m). All 218 plants flowered and produced seeds. The germination test for 213 plants showed that 95% of the plants produced viable seeds. All morphological traits and seed germination rates varied widely within populations. The number of stems was unrelated to altitude (Fig. 2e) but other morphological traits and seed germination rates showed weak but significant quadratic relationships with altitude (Fig. 2a-d). For these, the minimum of all the fitted curves tended to be at mid-altitudes.

Genetic variation along the altitudinal gradient

Fifty-seven (61%) of the 94 selected markers 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 populations were identified to be 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. 3a). However, the dissimilarity among clones within a population was not related to altitude (Fig. 3b).

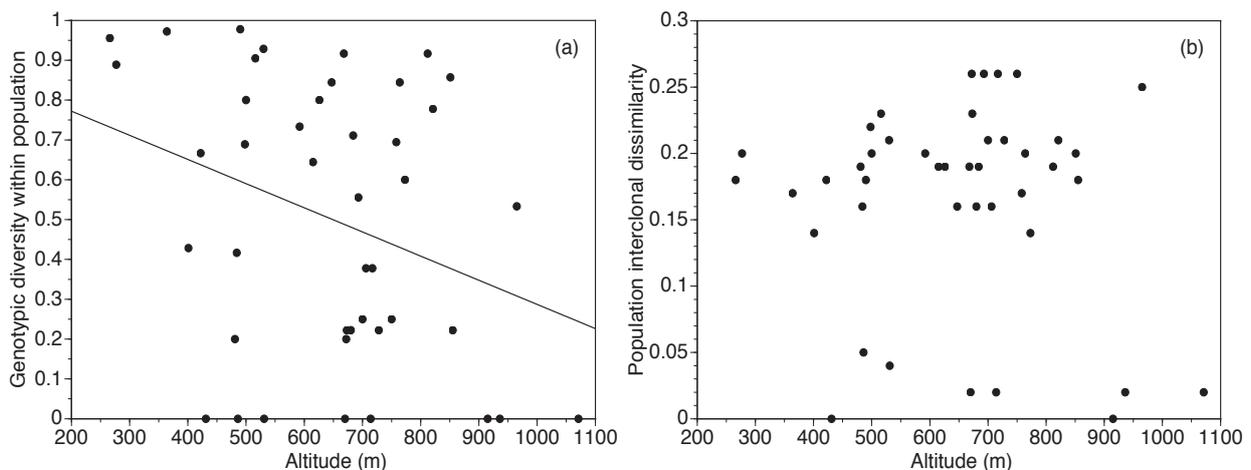


Fig. 3. Genetic variation of 43 populations along the altitudinal gradient: (a) genotypic diversity within each population ($R^2=0.10$, $p=0.0378$), (b) population interclonal dissimilarity represented by simple mismatch coefficient.

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	13	120	24	0.93	0.40	0.57
Bern	7	60	18	0.91	0.57	0.38
Bellinzona	15	126	31	0.93	0.70	0.24
Chur	11	98	26	0.92	0.44	0.52

Nearly half of the total genotypic diversity occurred among populations ($G_{st}=0.46$). However, lowland populations (200-530 m) were significantly less differentiated ($G_{st}=0.33$) than those at higher altitudes (711-1100 m; $G_{st}=0.55$). The G_{st} values were also different for the four regions (Table 1), though only 3% of the total genotypic diversity was found among the regions ($G_{st}=0.03$).

Genotype distribution and growth performance along the altitudinal gradient

Based on the number of the populations in which the genotypes occurred, the following three groups of genotypes were distinguished: (i) local genotypes found in only one population, (ii) occasional genotypes found in two to four populations, and (iii) widespread genotypes found in five or more populations. There were 36 local genotypes, 17 occasional genotypes, found 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 ($F=6.86$, $p=0.0015$). However, the decline in numbers of genotypes with altitude ($F=9.16$, $p=0.003$) 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. genotype by altitude interaction not significant).

There was also significant variation among the genotype groups in the number of individuals per genotype in a population ($F=4.55$, $p=0.0138$). Closer analysis showed that this was because local genotypes tended to be represented by fewer individuals per population (mean 1.6) than occasional (mean 3.4) and widespread (mean 3.8)

genotypes. However, the number of individuals per genotype was unrelated to altitude, both across all the 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).

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 m and 1000 m), but generally grew larger and produced more aboveground biomass at 400 m than at 1000 m (Table 2, Fig. 4). 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. 4). 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. 5). 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).

Table 2. F ratios and significances of ANOVA – effects of genotype group with three levels (local, occasional, widespread genotypes), growth site with two levels (400 m and 1000 m) and their interaction on genotype performance in 2005.

	Site (S)	Genotype group (GG)	S x GG
rosette diameter	40.26 ***	2.34	1.57
nb stems	51.37 ***	4.23 *	0.30
final height	296.29 ***	1.93	2.59
dry biomass	163.40 ***	5.55 **	0.97

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

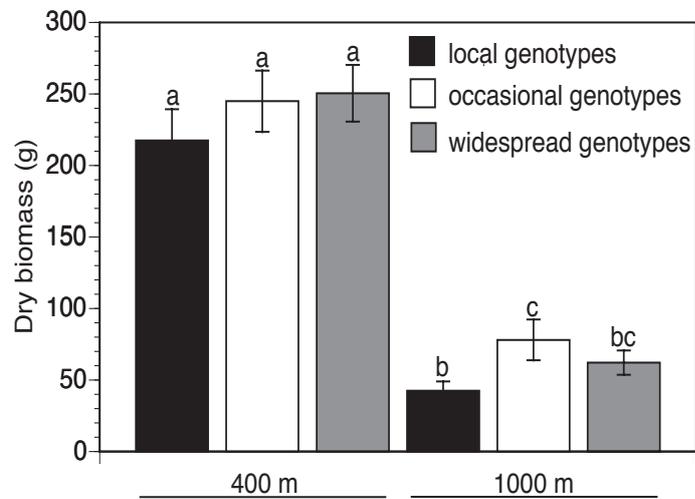


Fig. 4. Mean aboveground dry biomass produced by local, occasional and widespread genotypes in the common gardens at 400 m and 1000 m in 2005. Means (\pm SE) not connected by the same letter are significantly different.

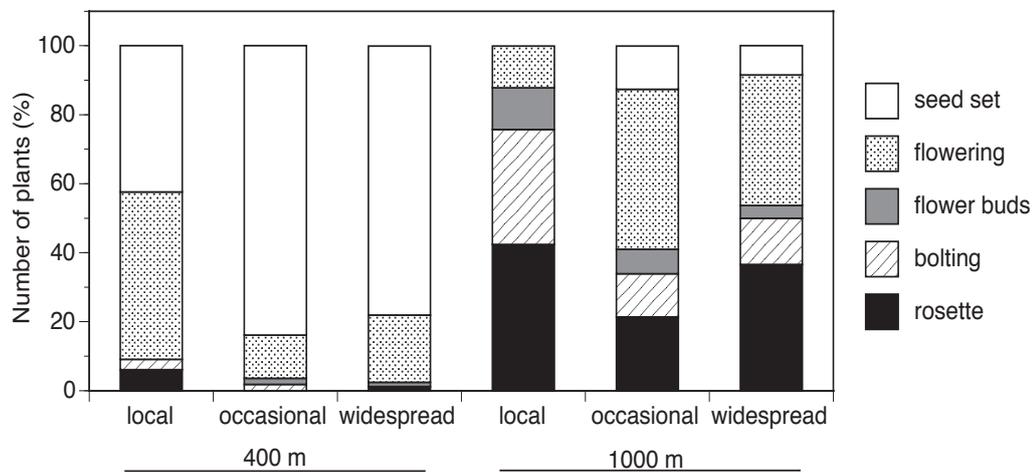


Fig. 5. The final phenological stage (rosette, bolting, flower buds, flowering, seed set) that the plants belonging to local, occasional and widespread genotypes reached at the end of the common garden experiments at 400 m and 1000 m in September/October 2005.

DISCUSSION

***E. annuus* abundance in the Swiss Alps**

In the field survey *E. annuus* declined rapidly in abundance above 1000 m, although it was found at sites as high as 1762 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, the performance of *E. annuus* in the field did not decrease with altitude, and even plants growing at the altitudinal limit produced abundant viable seed. 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 1200 m on the island of Réunion (Baret et al. 2004). However, in that case the authors observed a switch 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 trunk roads that presented no obstacle to dispersal of tiny, wind-blown seeds. It seems more likely that seedlings have a reduced chance of establishing at higher altitudes due to greater winter mortality (Chapter 1). As a result high altitude populations are less persistent and the proportion of occupied sites declines with altitude.

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

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

Although somatic mutations are an important source of genetic variation in apomictic species (Ellstrand and Roose 1987), 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 ($G_{st}=0.46$) 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 can form effective 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 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.

We constructed the neighbour joining tree for all 64 genotypes, similar to that by Edwards et al. (2006) based on RAPD data. The grouping of the genotypes did not correspond to genotype distribution along the altitudinal gradient. Unlike Edwards et al. (2006), we found no tendency for the widespread genotypes to be closely related to each other. However, this result should be taken with caution as the topology of the neighbour joining tree, constructed with dominant markers, is affected by both the population genetic structure and the number of the markers employed (Hollingsworth and Ennos 2004). Thus, the unresolved star-shaped topology of the neighbour joining tree that we obtained may have been due to relatively low number of AFLP markers we used.

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

Hancock and Wilson (1976) hypothesised that the high genotypic diversity in *E. annuus* is maintained by environmental heterogeneity. They argued that different *E. annuus* genotypes may grow better in different microsites and that the relative frequency of genotypes therefore reflects the frequency of these microsites. In an experiment designed to investigate this hypothesis, Stratton (1994) showed that the pattern of variation in relative fitness of *E. annuus* genotypes was inconsistent with the pattern of variation observed in the environment, i.e. 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. Based on these results, Stratton and Bennington (1998) concluded that such a fine grained spatial variation together with temporal variation in selection could not maintain the observed genetic variation in *E. annuus*. Furthermore, there was little evidence that density-dependent selection occurs in *E. annuus* and no evidence for frequency-dependent selection (Bennington and Stratton 1998).

Our study was conducted at a much larger spatial scale than those of Stratton (1994) and Stratton and Bennington (1998), but we also 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. On the other hand, in our study we did not investigate *E. annuus* in the early stages of the life cycle. Indeed, in another experiment winter survivorship was the most important episode of selection in *E. annuus* (Stratton 1992). Therefore, we can not exclude the possibility that selection on seedlings affects the observed patterns in the distribution and abundance of genotypes in *E. annuus*.

If the adaptation of genotypes to local conditions were important, then we might expect that genotypes with a restricted distribution perform better under local conditions than more widespread genotypes. 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 less well than widespread genotypes, producing fewer stems and being less likely to produce viable seed. 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 produces many poorly adapted individuals.

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 was described as a 'general purpose' genotype (Baker 1965). For example, the invasive grass *Pennisetum setaceum* has greater altitudinal distribution than any other grass on the island of Hawaii (Williams et al. 1995). However, little genetic variation was found in *P. setaceum*, and thus its invasive success is apparently due to high phenotypic plasticity (Poulin et al. 2007, Poulin et al. 2005, Williams et al. 1995). A general purpose genotype rather than local adaptation also appears to have allowed *Verbascum thapsus* to spread to higher elevation in the Sierra Nevada (Parker et al. 2003). In the case of *E. annuus*, Stratton and Bennington (1998) suggested that the unpredictable temporal variation and the fine-grained spatial variation in selection would favour the evolution of generalist genotypes.

CONCLUSIONS

Our study showed that neither plant density nor growth performance of *E. annuus* decreased with increasing altitude in the Swiss Alps. We found no evidence for on-going adaptive changes; however, *E. annuus* plants maintained relatively high fitness up to the current altitudinal limit of the species distribution, probably due to phenotypic plasticity. These findings lead us to suggest that there is no very strong selection acting on particular genotypes, and that the patterns in the distribution of genotypic diversity in *E. annuus* are governed by processes of genotype formation, dispersal and extinction to a large extent neutrally.

ACKNOWLEDGEMENTS

We thank Aria Minder, Alex Widmer and Sophie Karrenberg for advice on AFLP analyses, Claudia Michel for assistance in the laboratory, Daniel Schläpfer for help in constructing the phylogenetic tree, and Pavel Trtik for help in the field. This project was financed by the Stiftung Rübél and by a grant from the ETH Research Fund.

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Acknowledgments

I am very grateful to Peter Edwards, Sabine Güsewell and Matthias Baltisberger for supervising my thesis, for their insightful comments and discussions, and to Jürg Stöcklin for being the co-examiner.

I am very thankful to Peter for his continuous enthusiasm and inspiration, and for offering me the opportunity to do this PhD and so to gain invaluable life experience, from which I learnt enormously. I would especially like to thank Sabine who supported me and encouraged me endlessly throughout my PhD. She was always there to talk and to give priceless advice, and she made my PhD life easier many times.

I am grateful to Claudia Michel for showing me all the miracles one can do with the leaves in the molecular lab, to Alex Widmer for introducing me to ecological genetics, and to Aria Minder for our many discussions (not only) about AFLP.

I thank Hans Leuenberger and the staff at the ETH research stations in Chamau and Frübüel for technical support and for helping me to set up the common gardens, Antoine Guisan for allowing me to use his research plots at Dent de Morcles and Yann Hautier for maintenance of the plots.

Many thanks to Alexandra Käser, Aud Halbritter, Claudia Farrér and Marcel Reinhard who participated in the project during their semester works, and to all the other people who helped me with field and laboratory work.

This project was supported financially by the Stiftung Rübel and the ETH Research Fund.

I would also like to thank all members of the Plant Ecology and the Plant Ecological Genetics groups for making the former Geobotanical Institute a friendly workplace. I am grateful to René Graf for his warm welcome at Zürichbergstrasse 38 and for fixing all my technical queries, to Hans-Heini Vogel and Karsten Rohweder for solving all my IT problems, to Erika Aeschbach for helping me with administrative issues, and to Martin Fotsch for providing me with gardening material.

I am thankful to all my colleagues with whom I could spend refreshing and funny lunch breaks, and who offered me help and kept me in a good spirit during my PhD, in particular to Albert Kölbener, Carmen Rothenbühler, Dieter Ramseier,

Evelyn Underwood, Georg von Arx, Hans Göransson, Harry Olde Venterink, Jake Alexander, Luciola Lannes, Myriam Poll, Steffi Lorenz, Stephanie Halsdorf and Tim Seipel. Special thanks to Debbie Scharfy for her friendship and for all our tea-times, and to Daniel Schläpfer for introducing me not only to PAUP, InDesign etc. but also to rowing, Silvesterlauf and Rumkugel.

I am very grateful to all my friends and my family for their entertainment and great support, and for not asking me too often how many pages of my thesis I had already written. A special thank to my mother Miluse Kousalova who spent most of her holiday time helping me in the field.

Finally, I wish to express my deepest gratitude to my husband Pavel Trtik with whom I could share all joys and sorrows, who always found a way to make me laugh even in the most difficult times, and without whom I would have never found the courage to knock on the door of the Geobotanical Institute.

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