

Differentiation of reproductive and competitive ability in the invaded range of *Senecio inaequidens*: the role of genetic Allee effects, adaptive and nonadaptive evolution

Susanne Lachmuth^{1,2}, Walter Durka³ and Frank M. Schurr^{1,4}

¹Plant Ecology and Nature Conservation, University of Potsdam, Maulbeerallee 2, 14469 Potsdam, Germany; ²Plant Ecology, Martin-Luther-University Halle-Wittenberg, Am Kirchtor 1, 06099 Halle, Germany; ³Helmholtz-Centre for Environmental Research – UFZ, Department Community Ecology (BZF), Theodor-Lieser-Str. 4, 06120 Halle, Germany; ⁴Institute for Physical Geography, Goethe-University Frankfurt am Main, Altenhoferallee 1, 60438 Frankfurt am Main, Germany

Summary

Author for correspondence:

Susanne Lachmuth

Fax: +49 345 5527228

Email: susanne.lachmuth@botanik.uni-halle.de

Received: 22 February 2011

Accepted: 24 May 2011

New Phytologist (2011) **192**: 529–541

doi: 10.1111/j.1469-8137.2011.03808.x

Key words: animal models, biological invasions, genetic Allee effects, interspecific competition, life history evolution, nonadaptive evolution, *r* and *K* selection, reproduction.

- Genetic differentiation in the competitive and reproductive ability of invading populations can result from genetic Allee effects or *r/K* selection at the local or range-wide scale. However, the neutral relatedness of populations may either mask or falsely suggest adaptation and genetic Allee effects.
- In a common-garden experiment, we investigated the competitive and reproductive ability of invasive *Senecio inaequidens* populations that vary in neutral genetic diversity, population age and field vegetation cover. To account for population relatedness, we analysed the experimental results with 'animal models' adopted from quantitative genetics.
- Consistent with adaptive *r/K* differentiation at local scales, we found that genotypes from low-competition environments invest more in reproduction and are more sensitive to competition. By contrast, apparent effects of large-scale *r/K* differentiation and apparent genetic Allee effects can largely be explained by neutral population relatedness.
- Invading populations should not be treated as homogeneous groups, as they may adapt quickly to small-scale environmental variation in the invaded range. Furthermore, neutral population differentiation may strongly influence invasion dynamics and should be accounted for in analyses of common-garden experiments.

Introduction

Evolutionary processes are increasingly being studied as factors determining the success of invasive plant species (Bossdorf *et al.*, 2005). Basically, these processes are assumed to be determined by the interplay of adaptive and nonadaptive evolution and effects of genetic diversity on plant fitness (Keller & Taylor, 2008; Gurevitch *et al.*, 2011). Empirical work on adaptive differentiation was, in particular, stimulated by theories on the evolution of increased competitive ability (Blossey & Nötzold, 1995). Much of this work compares genotypes from native and introduced populations of the same species (Moloney *et al.*,

2009). However, a major drawback of these native vs introduced comparisons is that genotypes from the native and the invaded range are regarded as two homogeneous groups. Population differentiation and adaptation to environmental conditions *within* invaded regions have so far mainly been investigated along latitudinal (Kollmann & Banuelos, 2004; Maron *et al.*, 2004; Dlugosch & Parker, 2008b; Montague *et al.*, 2008; Monty *et al.*, 2009) and altitudinal (Parker *et al.*, 2003; Monty & Mahy, 2009a; Poll *et al.*, 2009) gradients. Colautti *et al.* (2009) showed that latitudinal clines in phenotypic traits can obscure evolutionary inferences from common-garden comparisons of native and invasive populations. In addition to these large-scale

environmental gradients, selection pressures can be imposed by the local environmental conditions and the colonization process itself. This may lead to spatial variation within the invaded range, as well as among different invasion phases (Dietz & Edwards, 2006; Gurevitch *et al.*, 2011).

For instance, during the primary phase of an invasion, many invasive plant species preferably spread in low-competition habitats (Dietz & Edwards, 2006). This should lead to r selection for increased reproductive and colonization ability in recently colonized parts of the invaded range (MacArthur & Wilson, 1967; Travis & Dytham, 2002; Burton *et al.*, 2010; Phillips *et al.*, 2010). By contrast, in regions that have been invaded for a longer time, the species is likely to have entered the secondary phase of invasion which is characterized by increasing colonization of natural and semi-natural habitats and increasing population density. This should lead to K selection for increased competitive ability. Consequently, the strength of K vs r selection is likely to vary at the scale of the entire invaded range, depending on the age of the populations, that is the time span since the arrival of the species in a certain region (Phillips *et al.*, 2010). At the same time, selection at the local scale may cause K -selected genotypes to be more frequent in habitats with higher levels of interspecific competition. Nevertheless, to date, there has been a lack of work investigating how invasives evolve in response to interspecific competition. Yet, one may expect that variation in both intra- and interspecific competition will cause adaptive differentiation in the reproductive and competitive performance of invasive populations.

The reproductive and competitive performance of invasive populations is, however, also affected by nonequilibrium demographic situations arising during invasive spread (Keller & Taylor, 2008; Gurevitch *et al.*, 2011). A reduction in genetic diversity can occur during initial introduction or through subsequent bottlenecks caused by long-distance dispersal events (Austerlitz *et al.*, 2000). This may again lead to systematic variation within the invaded range, with genetic diversity decreasing towards the leading edge of the invasion (Austerlitz *et al.*, 2000; Saltonstall, 2003; Chun *et al.*, 2009; Excoffier *et al.*, 2009; Lachmuth *et al.*, 2010). Low genetic diversity may decrease the populations' adaptive potential and lead to inbreeding, resulting in reduced heterozygosity and inbreeding depression. Inbreeding has been shown to be important in wild populations (Keller & Waller, 2002) and may have negative effects on reproductive as well as competitive ability. Moreover, there is increasing evidence for positive effects of genetic diversity on fitness components through sampling effects, facilitation and niche partitioning (Hughes *et al.*, 2008), for example, on the colonization success of experimental populations (Crawford & Whitney, 2010). These various mechanisms causing positive effects of genetic diversity on fitness are subsumed as genetic Allee effects (Ellstrand &

Elam, 1993; Fischer *et al.*, 2000), in analogy with ecological Allee effects; that denote positive effects of population size on fitness (Allee, 1931).

Beyond affecting levels of genetic variation, the nonadaptive evolutionary processes of drift, mutation and recombination may cause neutral geno- and phenotypic divergence among invading populations (Keller & Taylor, 2008; Keller *et al.*, 2009). In particular, during range expansions, nonadaptive mechanisms may generate gradients in allele frequencies from the rear to the leading edge of the invasion (Edmonds *et al.*, 2004; Burton & Travis, 2008; Excoffier *et al.*, 2009). Statistical tests for adaptive evolution thus have to control for neutral differentiation arising from variation in the relatedness of populations. At the between-species level, this type of correction is well established in comparative mixed-effects analyses of macroevolutionary change (e.g. Housworth *et al.*, 2004). Moreover, at the between-individual level, mixed-effects models (so-called 'animal models') are widely used by quantitative geneticists, although the main intention of these analyses is to quantify additive genetic variance (the amount of phenotypic variation explained by relatedness) rather than to correct for relatedness (Lynch & Walsh, 1998). At the between-population level, however, analyses of phenotypic differentiation have mostly ignored relatedness.

A few studies have quantified neutral population differentiation and discussed its potential influence on evolutionary changes observed in invasive populations (Maron *et al.*, 2004; Dlugosch & Parker, 2007). Moreover, an increasing number of F_{ST} – Q_{ST} comparisons have aimed to disentangle adaptive from nonadaptive differentiation (Leinonen *et al.*, 2008). Meimberg *et al.* (2010) included a fixed effect of previously identified genetic lineages in the analysis of phenotypic traits. A further step was recently taken by Keller *et al.* (2009), who related phenotypic data to primary axes of genetic ordinations. However, none of the above-mentioned methods quantifies directly the phenotypic effects of relatedness. This can be achieved by including population relatedness directly in mixed model analyses of phenotypic data.

Here, we use this approach to account for population relatedness in analyses of a common-garden experiment that tested for adaptive and nonadaptive differentiation in competitive and reproductive ability within the invaded European range of the South African ragwort (*Senecio inaequidens*). In this experiment, genotypes from 22 invasive populations were exposed to different levels of interspecific competition by the native grass *Festuca brevipila*. We used this experiment to test the following (not mutually exclusive) hypotheses: (1) small-scale r/K selection causes genotypes from low-competition environments to reproduce more in the absence of competition and to be more sensitive to competition; (2) large-scale r/K selection causes similar effects in young populations at the

invasion front (Phillips *et al.*, 2010); (3) genetic Allee effects cause competitive and reproductive ability to increase with the genetic diversity of populations; (4) and non-adaptive evolution causes competitive and reproductive differentiation that can be attributed to population relatedness. These analyses help to understand how adaptive evolution at different spatial scales, effects of genetic diversity and neutral genetic processes interact to affect the invasion dynamics of one of Europe's fastest plant invaders.

Materials and Methods

Study species

Senecio inaequidens DC. is a perennial ligneous herb native to Lesotho and South Africa. The species is self-incompatible (López-García & Maillet, 2005; Lafuma & Maurice, 2007), insect pollinated and produces large amounts of seeds that are well dispersed by wind. In the native region, the *S. inaequidens/madagascariensis* species complex comprises tetraploid and at least two different diploid cytotypes (Lafuma *et al.*, 2003). The invasive European range, however, seems to comprise only tetraploids, which originate from two overlapping source regions in the Drakensberg and Maloti Mountains (Lafuma *et al.*, 2003; Lachmuth *et al.*, 2010). In the native range, *S. inaequidens* occurs at altitudes between sea level and 2850 m in a wide range of naturally or anthropogenically disturbed habitats, such as river banks, rocky slopes, heavily grazed or recently burned grasslands, and road verges (Hilliard, 1977; S. Lachmuth, pers. obs.).

Seeds of *S. inaequidens* were accidentally introduced in wool transports to several locations in Europe in the late 19th and early 20th centuries. In continental Europe, five primary expansion centres have been reported and dated: Bremen (Germany, 1896), Verviers (Belgium, 1922), Calais (France, 1935), Mazamet (France, 1936) and Verona (Italy, 1947) (see Kuhbier, 1977; Ernst, 1998; Jeanmonod, 2002; and citations therein). The spatiotemporal development of the invasions from these introduction sites (except Verona) has been reconstructed with a combination of molecular analyses and historical records by Lachmuth *et al.* (2010). These molecular analyses and flow cytometry analyses confirming tetraploidy comprised all populations studied here.

Senecio inaequidens originally invaded ruderal habitats along traffic routes, but recently it has started to colonize heavily grazed grasslands in southern Europe (Scherber *et al.*, 2003; Garcia-Serrano *et al.*, 2004). This development is particularly alarming as the species contains large amounts of alkaloids that are poisonous to livestock (Dimande *et al.*, 2007). *Senecio inaequidens* is also increasingly colonizing fallow ground, rocky habitats (Böhmer, 2001), coastal dunes and pine forest clearings (Werner

et al., 1991; S. Lachmuth, pers. obs.). Its ability to form dense stands and its exceptionally high invasion speed make the species a potential threat to the native European flora (Böhmer, 2001).

Source populations

We investigated 22 European populations of *S. inaequidens* that belong to four different invasion routes expanding from Bremen (BRE), Calais (CAL), Mazamet (MAZ) and Verviers (VER) (Fig. 1a). The key characteristics of these populations are listed in Supporting Information Table S1 (for further details, see Lachmuth *et al.*, 2010). The investigated populations mainly occupy ruderal sites, but differ in vegetation cover, which we use as a proxy for competition intensity. For each population, we estimated the vegetation cover in 12–20 plots of 1 m², and found that the mean vegetation cover per population ranged from 47% to 100%. The age of the populations was calculated in relation to the year of first occurrence within a 25-km radius, as derived from floristic literature, and ranged from 6 to 111 yr at the time of sampling (Lachmuth *et al.*, 2010). Genetic diversity was calculated as the band richness B_r of each population, which varied between 1.20 and 1.38 [B_r was calculated from amplified fragment length polymorphism (AFLP) and microsatellite data, correcting for varying sample size by rarefaction to the minimum sample size of five at the 5% level using AFLPDiv, <http://www.pierroton.inra.fr/genetics/labo/Software/Aflpdiv>; see Lachmuth *et al.*, 2010]. When interpreting the results presented below, it must be taken into account that all investigated population characteristics show some degree of correlation (population age and genetic diversity, Spearman's $\rho = 0.4$; population age and field vegetation cover, $\rho = 0.18$; genetic diversity and field vegetation cover, $\rho = -0.32$).

Population relatedness

Pairwise population relatedness was derived from AFLP and microsatellite data obtained from samples of the field populations (Lachmuth *et al.*, 2010). As these anonymous markers are not necessarily neutral, we identified markers potentially under diversifying selection by running BayesScan (Foll & Gaggiotti, 2008) with default settings on 191 markers polymorphic for the investigated populations. Three strongly bottlenecked populations (DEG, LAU and KWH) were excluded from this analysis to avoid false positives (Foll & Gaggiotti, 2008). Setting the threshold of the Bayes factor to a very conservative value of 100 ($\log_{10}BF = 2.0$) excluded six potentially non-neutral markers. The remaining markers were used to estimate the pairwise relatedness of individuals following the approach of Ritland (1996) in Mark 3.1 (Ritland, 1996, available at <http://genetics.forestry.ubc.ca/ritland/programs.html>). Pairwise

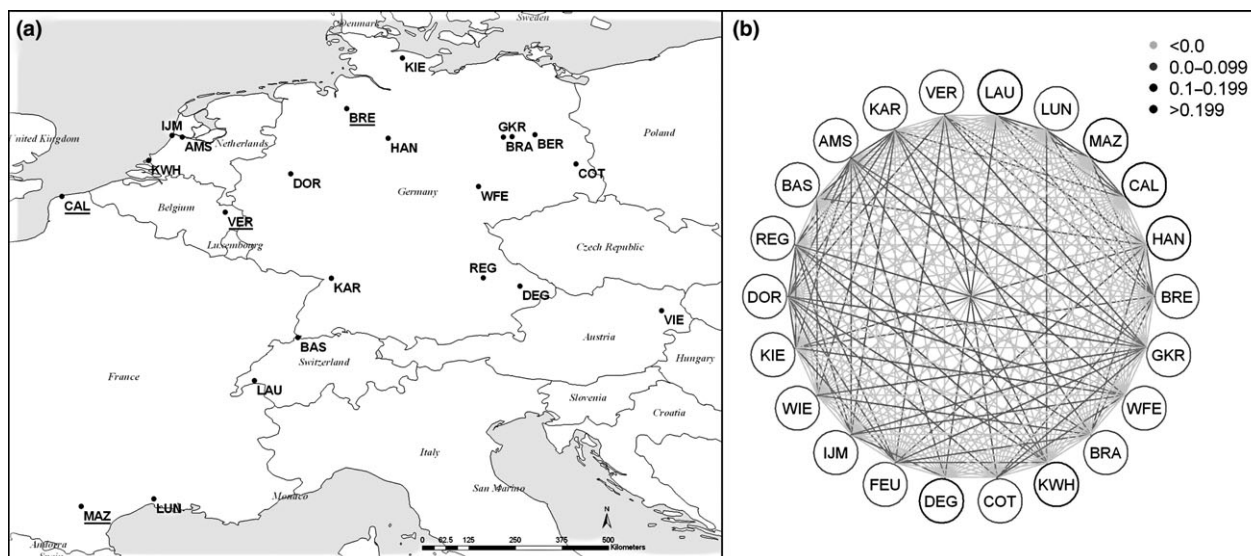


Fig. 1 (a) Geographical distribution of the sampled *Senecio inaequidens* populations including four documented centres of introduction (underlined). (b) Network diagram of population relatedness with grey scale of the lines representing mean relatedness coefficients between all individuals of a population and all individuals of the respective other population.

population relatedness was then calculated as the mean relatedness coefficients of all individuals of a population to all individuals of the respective other population (Fig. 1b). As relatedness coefficients obtained from dominant markers are less reliable for polyploids than for diploids, we applied pairwise Mantel tests to test the consistency of our matrix with matrices obtained from three other approaches: kinship coefficients for dominant markers (Hardy, 2003) in *SPAGeDi* 1.3 (Hardy & Vekemans, 2002; $r = 0.93$, $P < 0.001$); relatedness coefficients for dominant markers (Hardy, 2003) in *SPAGeDi* 1.3 ($r = 0.93$, $P < 0.001$); and relatedness coefficients obtained by the method of Lynch & Milligan (1994) in *AFLPsurv* (Vekemans *et al.*, 2002; $r = 0.33$, $P < 0.001$).

Experimental design

Senecio inaequidens seeds were collected in the field from July to November 2007, dried at room temperature for 6 wk and then stored at 4°C. For the experiment, we used seeds from five randomly chosen maternal seed families per population. We conducted the experiment in a common-garden of Potsdam University (Germany, 52.41°N, 13.02°E, 41 m asl). As a competitor, we chose *Festuca brevipila*, which is a typical dominant grass in dry grasslands of Central Europe. Hence, this species is an important competitor for *S. inaequidens* colonizing this endangered natural habitat type. *F. brevipila* tussocks were collected at two field sites in Potsdam (Maulbeerallee, 52.404°N, 13.024°E; Neues Palais, 52.403°N, 13.017°E). Linear mixed-effects models for all response variables with sampling site as single explanatory variable did not show

significant effects of grass provenance. Between 8 and 11 April 2008, we reduced the tussocks to a size of 2–3 cm in diameter and planted them in pots of 16 × 16 × 16 cm³ size containing a mixture of 75% sand and 25% compost soil. The grass was then allowed to establish for 2 months before the start of the experiment. We applied three levels of interspecific competition by *F. brevipila*: bare ground (no grass), scarce grass cover (two tussocks per pot) and dense grass cover (four tussocks per pot). For statistical analyses, we used the competitor biomass per pot at the end of the experiment (see the section entitled 'Statistical analyses'), as this continuous variable describes competition intensity more precisely than the coarse treatment factor. *S. inaequidens* seedlings were germinated and raised in a shaded glasshouse 2 wk before the start of the experiment. For germination, 30–45 seeds per seed family were distributed on randomly placed pots with sterilized substrate and a constant water supply.

From 12 to 16 June, these seedlings were transplanted into the prepared pots. Each pot received five seedlings of the same seed family. Both seedlings and grass tussock were located at defined regularly spaced positions. Each competition treatment was applied once per seed family (i.e. five times per population). Overall, we planted 1725 seedlings in 345 randomly distributed pots. During the first week of the experiment, dead seedlings were replaced as their death was most probably caused by transplanting.

Throughout the experiment, the plants were watered daily, except for rainy days. From 29 June to 2 July, we measured the initial size of the seedlings from the ground to the tip of the longest leaf. This measurement of initial size was then included in all statistical analyses to control

for potential maternal effects. The reproductive status (flowering/nonflowering) of all plants was recorded every second day to detect the time to flowering. A plant was considered to be flowering as soon as at least one disc floret of one flowerhead had opened. As a measure of reproductive output, we counted all flowering and withered flowerheads at the day of harvest. From 30 September to 23 October, the above-ground biomass of surviving *S. inaequidens* plants was harvested, dried at 105°C for 8 h and stored at room temperature. The above-ground grass biomass was harvested and dried from 24 to 31 October. Before weighing, both *S. inaequidens* and grass biomass were dried again for 3 h at 105°C and cooled to room temperature in the closed ovens.

Statistical analyses

To statistically account for the relatedness of our study populations, we used statistical models derived from so-called 'animal-models' developed in quantitative genetics (Lynch & Walsh, 1998; Bates & Vazquez, 2009). Animal models are mixed-effects models in which the correlation structure of individual-level random effects is determined by kinship coefficients derived from multigenerational pedigrees. For plant populations, complex pedigree information is usually not available. Instead of incorporating relatedness at the individual level, we used the pairwise relatedness of populations to weight random effects at the population level. We fitted these animal models in a generalized linear mixed modelling framework using the R-package '*pedigreemm*' (Bates & Vazquez, 2009) in R 2.12.2 (R Development Core Team, 2011). However, we adapted the *pedigreemm* code so that it could directly use a relatedness matrix instead of a pedigree (see Methods S1). To assess the effect of accounting for population relatedness, we repeated all *pedigreemm* analyses with mixed models (*mm*) that do not account for relatedness. For normally distributed response variables, we used linear mixed-effects models (R-package nlme; Pinheiro *et al.*, 2011) and, for binomially distributed responses, we used generalized linear mixed models (R-package lme4; Bates *et al.*, 2011). In the special case of zero between-population and constant within-population relatedness, *pedigreemm* models are identical to the respective *mm* models. Any differences between *pedigreemm* and *mm* analyses can therefore be attributed to the inclusion of population relatedness.

We used these statistical models to analyse the variation in survival of 1600 plants (after removing individuals with missing values from the dataset). For the 1494 surviving plants (93.4%), we analysed the reproductive state (flowering/nonflowering) and, for 1489 plants, the log_e-transformed above-ground biomass; 620 flowering plants (41.4% of the surviving plants) were included in the analyses of the log_e-transformed number of flowerheads at the time of harvest, and a subset of 599 flowering plants was used to

analyse log_e-transformed time to flowering (the time span between measurement of initial size and start of flowering, in days). Although the reproductive state and survival were analysed with a binomial error distribution, the remaining response variables had normal errors following the respective transformations. The maximal models for survival, above-ground biomass, reproductive status and time to flowering comprised the continuous fixed effects of above-ground competitor biomass in interaction with the population characteristics population age, band richness and field vegetation cover, respectively. To correct for large-scale climatic variation, differences in dates of sowing and measurements, and maternal effects, we incorporated latitude, duration of experiment (time span between measurements of initial size and measurement of the response variable) and initial seedling size as covariates. The maximal model for the number of flowerheads additionally included *S. inaequidens* biomass and its interactions with the population characteristics (age, genetic diversity and field vegetation cover) to account for a potential trade-off between growth and reproduction. For all analyses, *S. inaequidens* biomass, competitor biomass, population age, genetic diversity ($B_r - 1$), initial seedling size and duration of experiment were log_e transformed. Moreover, these models comprised the nested random effects of pot within seed family within population. *pedigreemm* analyses estimated the random effect of population based on the population relatedness matrix (see the section entitled 'Population relatedness'). To obtain minimal adequate models for each response variable, we simplified the maximal model by removing all nonsignificant terms ($P > 0.05$) in a stepwise-backward procedure based on likelihood ratio tests. Main effects included in significant interactions, as well as the two potentially confounding variables of duration of experiment and initial size, were retained in the minimal adequate models. In a first step of model simplification, we used the chi-squared approximation to the likelihood ratio test statistic. However, this approximation may falsely retain insignificant variables (Pinheiro & Bates, 2000). Subsequently, we thus calculated unbiased P values for all remaining model terms. These unbiased P values were obtained by comparing likelihood ratio statistics with a distribution of likelihood ratios obtained for replicate simulations of new response data, with the simpler of the two models compared in the test (see Pinheiro & Bates, 2000). For model simplification, we fitted all models by maximum likelihood (ML), but, to reliably quantify random effects, we additionally fitted the minimal adequate models with restricted maximum likelihood (REML; Bolker *et al.*, 2009).

As the initial size of the seedling might vary systematically between populations, we analysed its response to the fixed effects of population age, genetic diversity, field vegetation cover and latitude, with seed family within population being included as nested random terms.

Results

Effects of population characteristics and experimental competition corrected for population relatedness

The effects of population characteristics and experimental competition on key fitness measures in the common-garden are summarized in Table 1. According to the minimal adequate *pedigreemm* models, survival and *S. inaequidens* biomass were not affected by any of the population characteristics (age, genetic diversity or field cover), but decreased with competitor biomass (survival, $\chi^2_{(1)} = 4.1$, $P < 0.05$; biomass, $\chi^2_{(1)} = 144.3$, $P < 0.001$); 41.7% of the surviving plants started to flower during the experiment, and the percentage of flowering plants increased with genetic diversity ($\chi^2_{(1)} = 8.2$, $P < 0.01$; Fig. 2) and decreased with competitor biomass ($\chi^2_{(1)} = 111.8$, $P < 0.001$). The time to flowering was influenced by the interaction of competitor biomass and field vegetation cover ($\chi^2_{(1)} = 11.9$, $P < 0.001$; Fig. 3a,b), whereas time to flowering generally increased with competitor biomass; this increase was less pronounced and, in some cases, turned into a decrease for populations with high field vegetation cover (Fig. 3a). Hence, populations that had been exposed to more intense competition in the field were less sensitive to experimental competition. At the same time, populations experiencing intense competition in the field showed lower reproduction in the first year, as we found a negative effect of field vegetation cover on the number of flowerheads ($\chi^2_{(1)} = 16.7$, $P < 0.001$; Fig. 4). This finding is unlikely to result from a negative maternal effect of field cover, as we controlled for the initial size of the seedling which, itself, was independent of vegetation cover and other population characteristics (Table 1). This result thus supports the idea of selection against investment in reproduction in populations with a highly competitive environment. The number of flowerheads furthermore increased with latitude ($\chi^2_{(1)} = 5.9$, $P < 0.05$) as well as with *S. inaequidens* biomass ($\chi^2_{(1)} = 841.0$, $P < 0.001$), and was negatively affected by competitor biomass ($\chi^2_{(1)} = 11.0$, $P < 0.001$).

Influence of population relatedness

Accounting for population relatedness clearly increased the percentage of variance explained by the random effect of population: this percentage ranged from 1.4% to 2.2% in classical *lme* and from 23.0% to 56.2% in *pedigreemm* analyses (Fig. 5). For all response variables, the inclusion of relatedness predominantly decreased the variance attributed to pots and residuals (Fig. 5), changed parameter estimates and reduced the significance of fixed effects (Table S2). For instance, *mm* analysis suggests a significant positive effect of genetic diversity on *S. inaequidens* biomass ($\chi^2_{(1)} = 8.1$, $P < 0.01$), which is no longer significant ($\chi^2_{(1)} = 5.4$,

$P = 0.052$) when accounting for population relatedness. Furthermore, the *mm* analysis for the number of flowerheads yielded a significant negative effect of population age ($\chi^2_{(1)} = 6.2$, $P < 0.05$) and a significant interaction between *S. inaequidens* biomass and genetic diversity ($\chi^2_{(1)} = 6.1$, $P < 0.05$). This interaction seems to indicate that genetically diverse populations have a higher allometric exponent for flowerhead number (Table S2, Fig. 6a), but lower reproduction of small plants (Fig. 6b). Yet, the fact that these effects were not significant in the corresponding *pedigreemm* analysis indicates that they are not independent of population relatedness.

Discussion

In the common-garden experiment, *S. inaequidens* populations from sites with different vegetation density showed reproductive differentiation that was consistent with adaptation to local levels of interspecific competition. By contrast, apparent large-scale *r/K* differentiation between populations of different age, and positive effects of genetic diversity on competitive and reproductive ability, lost statistical support when correcting for population relatedness and hence invasion history. In the following, we discuss how our findings on differentiation with respect to population age, vegetation cover, genetic diversity and population relatedness advance current knowledge. Subsequently, we highlight potential consequences of these findings for the invasion dynamics of *S. inaequidens* and for the study of evolution during invasions in general.

Local vs large-scale adaptation to competitive regimes

In the last decade, an increasing number of theoretical and conceptual studies have addressed the spatiotemporal variation in *r* vs *K* selection during biological invasions (Travis & Dytham, 2002; Dietz & Edwards, 2006; Phillips *et al.*, 2008, 2010; Phillips, 2009). Empirical tests of these hypotheses are still scarce and evidence for increased *r* selection towards the leading edge mainly comes from the cane toad *Bufo marinus* in Australia (Phillips *et al.*, 2008; Alford *et al.*, 2009). For *S. inaequidens*, Monty & Mahy (2009b) identified the evolution of a larger pappus and resulting lower plume load, which may lead to increased dispersal ability, towards the leading edge of the invasion route expanding from Mazamet towards the high Pyrenees. This result strengthens the assumption that *r* selection is acting on young populations. Nevertheless, the study was restricted to only one invasion route and did not correct for population relatedness.

In this study, we tested whether *S. inaequidens* shows small- and large-scale variation in reproductive and competitive ability that can be related to vegetation cover and population age, respectively. Our finding that vegetation

Table 1 Analyses testing for differentiation in key fitness proxies of *Senecio inaequidens*

Fixed effects	log _e initial seedling size (cm)	Survival	log _e <i>S. inaequidens</i> biomass (g)
Intercept	1.094	3.446	-11.849
log _e competitor biomass (g)	nt	-0.114*	-0.276***
Latitude °N	ns	ns	ns
log _e population age (a)	ns	ns	ns
log _e genetic diversity	ns	ns	ns
Field vegetation cover (%)	ns	ns	ns
log _e competitor biomass : log _e population age	nt	ns	ns
log _e competitor biomass (g) : log _e genetic diversity	nt	ns	ns
log _e competitor biomass (g) : field vegetation cover (%)	nt	ns	ns
Potentially confounding variables			
log _e initial seedling size (cm)	nt	1.590***	2.437***
log _e duration of experiment (d)	nt	-0.238	1.822
Random effect variances			
Population	0.059 (22)	2.320 (22)	2.284 (22)
Population/family	0.021 (108)	0.000 (108)	0.000 (108)
Population/family/pot	nt	3.476 (320)	0.454 (320)
Residuals	0.094 (1600)	ne (1600)	5.099 (1489)
Fixed effects	Proportion flowering plants	log _e time to flowering (d)	log _e no. of flowerheads
Intercept	23.505	4.407	-31.559
log _e competitor biomass (g)	-0.223***	0.056 ^a	-0.039***
Latitude °N	ns	ns	0.059*
log _e population age (a)	ns	ns	ns
log _e genetic diversity	1.713**	ns	ns
Field vegetation cover (%)	ns	0.001 ^a	-0.02***
log _e competitor biomass : log _e population age	ns	ns	ns
log _e competitor biomass (g) : log _e genetic diversity	ns	ns	ns
log _e competitor biomass (g) : field vegetation cover (%)	ns	-0.0005***	ns
log _e <i>S. inaequidens</i> biomass (g)	nt	nt	1.002***
log _e <i>S. inaequidens</i> biomass (g) : log _e population age (a)	nt	nt	ns
log _e <i>S. inaequidens</i> biomass (g) : log _e genetic diversity	nt	nt	ns
log _e <i>S. inaequidens</i> biomass (g) : field vegetation cover (%)	nt	nt	ns
Potentially confounding variables			
log _e initial size of seedling (cm)	2.175***	-0.137***	0.298
log _e duration of experiment (d)	4.798***	nt	6.282***
Random effect variances			
Population	0.000 (22)	0.012 (22)	1.011 (22)
Population/family	0.000 (108)	0.000 (108)	0.000 (108)
Population/family/pot	0.33 (319)	0.002 (240)	0.091 (244)
Residuals	ne (1494)	0.032 (599)	0.764 (620)

^aTerms in significant interaction: ne, not estimated (results directly from linear predictor); ns, not significant; nt, not tested; levels of significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

The table gives parameter estimates for significant fixed effect terms (bold) and variance estimates for random effects (with the number of groups at each random effect level in parentheses). The results shown are based on the minimal adequate models obtained from *pedigreemm* analyses with the random effect of population weighted for population relatedness. Unbiased P values were obtained by comparing likelihood ratio statistics with a distribution of likelihood ratios obtained for replicate simulations of new response data with the simpler of the two models compared in the test (see Pinheiro & Bates, 2000). All likelihood ratio tests for fixed effects had one degree of freedom. Dropping the random effect of family in cases in which it was estimated to be very small (cf. Bolker *et al.*, 2009) did not change the given parameter estimates or significance values.

cover explains competitive and reproductive differentiation independent of relatedness suggests repeated local-scale adaptation to competitive regimes (rather than the secondary spread of a wave of K -selected genotypes). On the contrary,

large-scale differentiation with respect to population age does not have sufficient statistical support. Younger populations of *S. inaequidens* seem to invest more in flowerhead production in the first year (Table S2), as expected under r

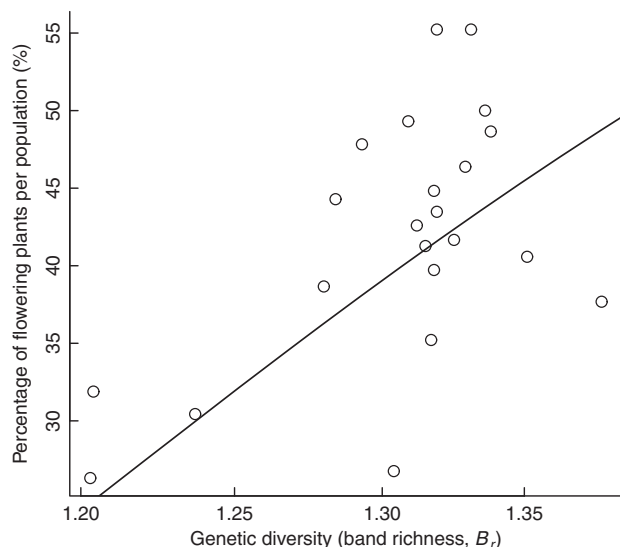


Fig. 2 Relationship between the percentage of *Senecio inaequidens* individuals that flowered in the common-garden experiment and the genetic diversity (band richness) of the respective source population. The result was obtained from a *pedigreemm* analysis with the random effect of population weighted for population relatedness.

selection at the invasion front (Phillips *et al.*, 2010). However, the inclusion of population relatedness in the more conservative *pedigreemm* analysis revealed that this apparent age effect might simply arise from neutral differentiation between populations. It is further possible that we failed to detect stronger effects of population age because we did not sample right at the invasion front (the youngest investigated population was 6 yr old at the time of sampling). After the invasion front has passed a certain region, adaptation to local environmental conditions is assumed to become increasingly

important compared with selection imposed by the colonization process itself (Phillips *et al.*, 2010).

At the local scale, we found that genotypes from highly competitive environments delay their time to flowering less in response to experimental competition, and that they produce less flowerheads under all levels of competition (Table 1, Figs 3, 4). These findings are in line with the expectation that intense competition should lead to *K* selection against high reproductive investment in early life stages (MacArthur & Wilson, 1967), and that plasticity of reproductive traits is disadvantageous in highly competitive environments (Van Kleunen & Fischer, 2005). The adaptive advantage of reduced reproduction in high-competition environments remains to be clarified, as we did not find an increase in above-ground biomass or survival. It is, however, possible that resources are instead allocated to below-ground biomass or long-term survival.

The fact that the effect of vegetation cover persists when controlling for population relatedness suggests repeated independent adaptation to local competitive regimes. Under an alternative scenario of a second wave of advance (proceeding more slowly) of more competitive but less reproductive (and eventually dispersive) forms, populations with adaptations to more competitive environments should be closely related and vegetation cover should drop out of the *pedigreemm* analyses.

Future empirical studies of adaptive differentiation could complement our analysis by quantifying traits determining long-distance seed dispersal (Nathan *et al.*, 2008) as a further life history component that can evolve during invasions (Travis & Dytham, 2002; Travis *et al.*, 2010). This would further allow empirical investigation of the recent theoretical prediction that life history evolution during range

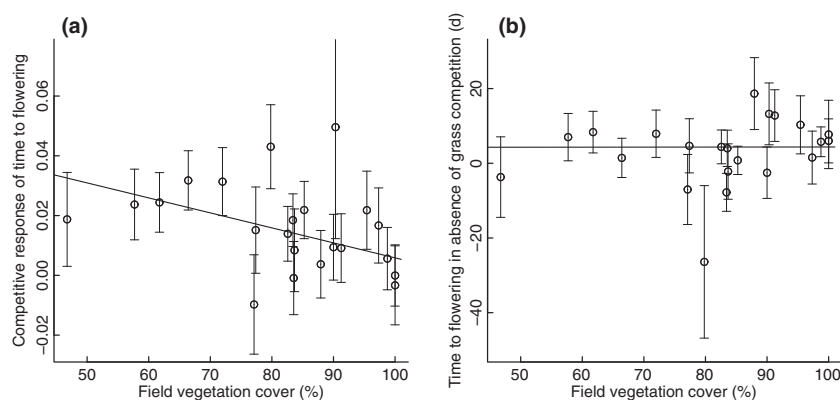


Fig. 3 Relationship between vegetation cover in *Senecio inaequidens* populations and (a) the response of time to flowering to experimental competition by *Festuca brevipila*, as well as (b) the time to flowering in the absence of competition by *F. brevipila*. For each population, a linear mixed-effect regression of time to flowering against competitor biomass was used to quantify response to competition (as the regression slope) and time to flowering in the absence of competition (as the regression intercept). The points show these slopes and intercepts, respectively, and whiskers show the corresponding standard errors. Lines represent predictions of the minimal adequate *pedigreemm* model estimated for all populations (note that *pedigreemm* and linear mixed-effects models additionally corrected for initial seedling size, which, for plotting, was set to its overall median).

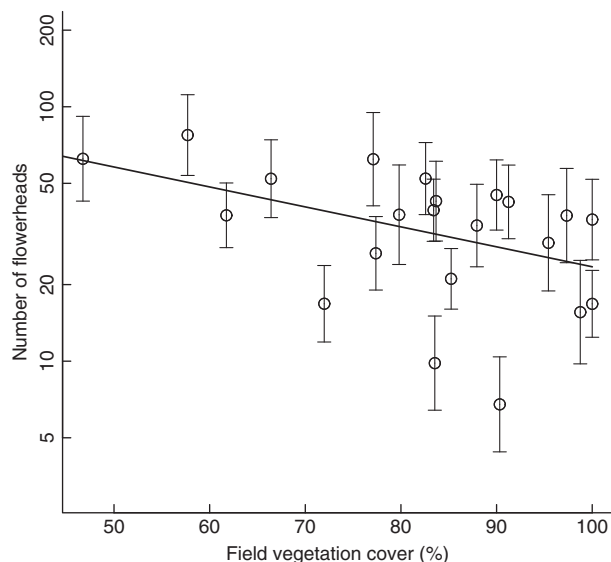


Fig. 4 Relationship between number of flowerheads of *Senecio inaequidens* individuals in the common-garden experiment and the field vegetation cover of the source populations. Points represent population means and bars the associated standard errors. The line shows the prediction of the minimal adequate *pedigreemm* model with the random effect of population weighted for population relatedness. All explanatory variables, except field vegetation cover, were set to their overall mean.

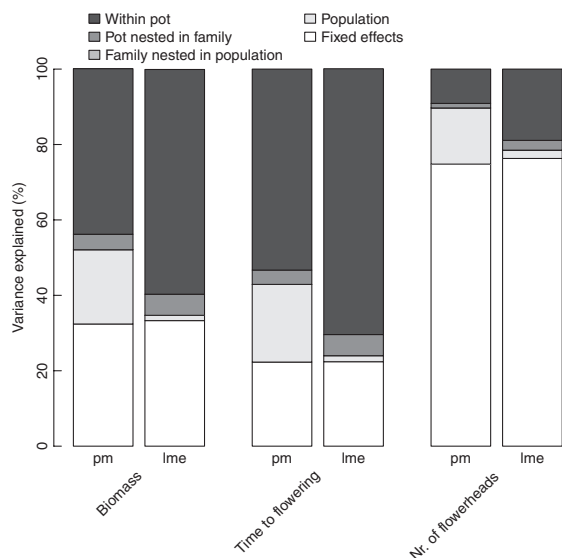


Fig. 5 Variance attributed to nested random effects of *Senecio inaequidens* population, seed family and pot, and to residuals in the respective minimal adequate models resulting from *pedigreemm* (pm) and *lme* (lme) analyses of above-ground biomass, time to flowering and number of flowerheads. *pedigreemm* analyses described the random effect of population based on a matrix of pairwise population relatedness.

expansions may be constrained by competitive interactions with other species (Burton *et al.*, 2010). In particular, acceleration in the rate of spread through the evolution of

increased dispersal ability at the leading edge may be reduced in the presence of interspecific competitors. Moreover, reciprocal transplant experiments would provide more direct tests for the adaptive value of the r - K differentiation reported here (Moloney *et al.*, 2009). In addition, transplant experiments could be used to test for adaptive differentiation with respect to other environmental conditions, such as soil conditions or climate.

Genetic Allee effects

Based on classical mixed models (*mm*) analyses alone, one would have diagnosed *S. inaequidens* with clear genetic Allee effects, indicated by positive effects of genetic diversity on biomass, proportion of flowering plants and the allometric exponent of flowerhead production (Table S2, Fig. 6). However, in the more conservative *pedigreemm* analyses, correcting for neutral population differentiation, most of these apparent Allee effects become insignificant and only the positive effect on the proportion of flowering plants remains (Fig. 2). Nevertheless, the fact that the bottlenecked populations DEG and KWH produced the smallest amounts of biomass and the smallest number of flowerheads per biomass (data not shown) encourages direct testing for genetic Allee effects by experimental crosses (Bailey & McCauley, 2006) in a larger number of unrelated bottlenecked populations. In fact, inbreeding depression may be promoted by *S. inaequidens*, being a predominantly outcrossing species with a history of high genetic variation within native populations (Lachmuth *et al.*, 2010). Deleterious alleles are therefore likely to have accumulated. Tetraploidy, however, reduces the probability that recessive deleterious alleles become homozygous. Moreover, bottlenecks experienced during invasion can help to purge deleterious alleles (Facon *et al.*, 2011).

So far, only few empirical studies have addressed the effects of genetic diversity or inbreeding on fitness in invading populations (but see O'Neil, 1994; Daehler, 1999; Van Kleunen & Johnson, 2005; Bailey & McCauley, 2006; Facon *et al.*, 2011). This is surprising, as decreased fitness is well documented from genetically impoverished wild populations of rare and declining species (Lande, 1988; Ellstrand & Elam, 1993). Moreover, for many invasive species, a loss of genetic diversity in invading compared with native populations has been reported (Dlugosch & Parker, 2008a; Lachmuth *et al.*, 2010). Finally, many conceptual and theoretical articles have emphasized the important role played by Allee effects in slowing down invasions (Sakai *et al.*, 2001; Liebhold & Bascompte, 2003; Lockwood *et al.*, 2005; Taylor & Hastings, 2005; Tobin *et al.*, 2007; Kanarek & Webb, 2010). Our results underline that urgently needed empirical tests for genetic Allee effects in invasive species should, however, correct for neutral population differentiation.

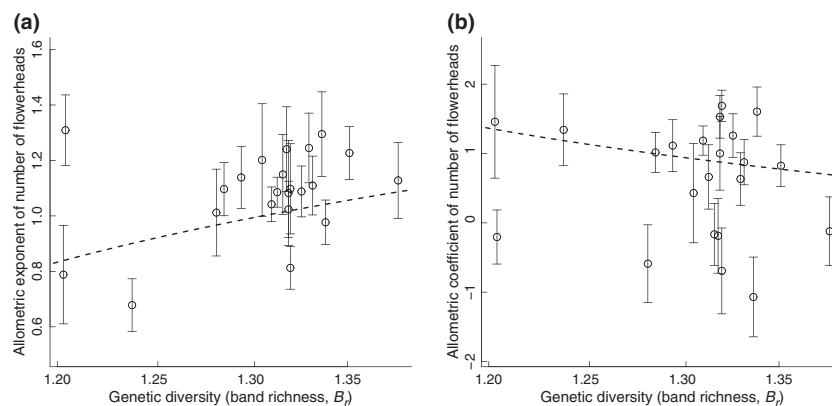


Fig. 6 Relationship between genetic diversity (band richness) of *Senecio inaequidens* populations and (a) the allometric exponent of the number of flowerheads, as well as (b) the allometric coefficient of the number of flowerheads. For each population, a linear mixed-effect regression of the number of flowerheads against *Senecio inaequidens* biomass was used to quantify the allometric exponent (as the regression slope) and the allometric coefficient (as the regression intercept). The points show these slopes and intercepts, respectively, and whiskers show the corresponding standard errors. Dashed lines represent predictions of the minimal adequate *lme* model estimated for all populations (note that the linear mixed-effects models additionally corrected for competitor biomass, initial seedling size and duration of the experiment, which, for plotting, were set to their overall medians). This interaction of *S. inaequidens* biomass and genetic diversity was not significant in *pedigree* analyses with the random effect of population weighted for population relatedness.

Effects of population relatedness

We found that population relatedness explains a large amount of variance in important fitness proxies of *S. inaequidens* and that a conservative approach considering neutral differentiation may attenuate the postulation of adaptive evolution or genetic Allee effects. These results are congruent with the findings of Keller *et al.* (2009), who found strong correlations between neutral genetic variance represented by the first two axes of genetic ordinations and phenotypic clines with gradients in geography and climate in the native and invaded ranges of two *Silene* species. The two statistical approaches are complementary as they capture different aspects of the genetic data. Comparing the results of both approaches may help to understand the influence of demographic history on the genetic and phenotypic differentiation of invading populations.

Recently, there has been increasing awareness of the importance of nonadaptive evolution during invasions (Parker *et al.*, 2003; Keller & Taylor, 2008; Excoffier *et al.*, 2009; Phillips *et al.*, 2010). Nevertheless, we are not aware of any previous analyses of phenotypic population differentiation that have statistically accounted for gradual variation in population relatedness. Our results emphasize the importance of such correction and suggest that extensions of the animal model are a useful tool for this purpose. One must be aware that our animal model analyses assume that neutral differentiation is genetically additive and described by the estimated relatedness matrix; violation of this assumption might bias hypothesis tests (see Whitlock, 2008 for a related discussion in the context of Q_{ST} – F_{ST} comparisons). Nevertheless, this assumption seems to be substantially

more realistic than the assumption of classical common-garden analyses that all populations are completely unrelated. Finally, we point out that the between-population variance estimated by our analyses has biological meaning in itself (even though this was not the focus of this paper). Notably, our finding that between-population genetic variance explains a large proportion of life history variation (Fig. 5) suggests that future admixtures between unrelated populations can substantially increase the adaptive potential of the descendent populations.

Consequences for invasion dynamics

The fact that population relatedness explains large amounts of variance in important fitness proxies, and consequently population growth rates, has important implications for invasion dynamics (Gurevitch *et al.*, 2011). As stochastic processes generally play an important role during colonization processes (Keller & Taylor, 2008; Excoffier *et al.*, 2009), the population dynamics are likely to fluctuate in response to bottlenecks, genetic drift and the admixture of previously isolated genetic material. As long as local adaptation in the invaded range is still low, invading populations are assumed to benefit from admixture, leading to increased genetic variation, the creation of novel genotypes and the masking of deleterious mutations (Verhoeven *et al.*, 2010). However, the latter seems to be less important for *S. inaequidens*, as genetic Allee effects do not seem to play an important role, except maybe for extremely bottlenecked populations. In the long term, as the species becomes closer to demographic equilibrium in the invaded range, we expect the influence of adaptive evolution to increase. However,

even in the current stage of the invasion, we already find signs of adaptation to local competitive regimes. Assuming a fitness advantage of the less plastic *K* strategists selected in competitive environments, this adaptive response may not only increase the number of habitats invaded, but also have negative impacts of *S. inaequidens* on the local community. The result emphasizes that adaptive evolution can occur in relatively short time spans and at small spatial scales.

To evaluate the relative importance of different adaptive as well as stochastic processes, common-garden experiments should be combined with demographic field studies to quantify the effects of these processes under natural environmental variation and with modelling approaches to evaluate their effects on the spatiotemporal invasion dynamics (e.g. Kuparinen & Schurr, 2007; Kuparinen *et al.*, 2010).

Conclusions

This study suggests that small-scale adaptation to competitive regimes and large-scale variation in population relatedness have played an important role for the course and success of the invasion of *S. inaequidens* in Europe. The ability to adapt to local competitive regimes is likely to foster the spread of *S. inaequidens* into more natural habitats. In general, our finding of small-scale competitive differentiation underlines that adaptive evolution can act rapidly and on small spatial scales. Simple comparisons of genotypes from native vs introduced ranges of invasive species are unlikely to capture such small-scale and short-term differentiation. More emphasis should thus be placed on rapid local adaptation within invaded ranges. Our results furthermore demonstrate that failure to account for variation in population relatedness can lead to erroneous conclusions about adaptive and nonadaptive causes of phenotypic differentiation. Neutral population differentiation is not just a nuisance in tests for adaptive evolution, but may itself play an important role in shaping variation in fitness and population dynamics during invasions.

Acknowledgements

The study was funded by the German Environmental Foundation (DBU), the Hans-Sauer-Foundation, the German Academic Exchange Service (DAAD), the graduate programme of the Federal State of Brandenburg and the European Union through Marie Curie Transfer of Knowledge Project FEMMES (MTKDCT-2006-042261). We thank S. R. Keller and two anonymous referees for very constructive comments on the manuscript, and G. Seidler for producing the map. M. Burkart, K. Kläring, R. Meiling and workers from Potsdam Botanical Garden supported us in organizational and technical matters. A. Eulenburg, I. Hoppe, M. Huth and A. Rubo assisted with experimental work.

References

- Alford RA, Brown GP, Schwarzkopf L, Phillips BL, Shine R. 2009. Comparisons through time and space suggest rapid evolution of dispersal behaviour in an invasive species. *Wildlife Research* 36: 23–28.
- Allee WC. 1931. *Animal aggregations, a study in general sociology*. Chicago, IL, USA: University of Chicago Press.
- Austerlitz F, Mariette S, Machon N, Gouyon PH, Godelle B. 2000. Effects of colonization processes on genetic diversity: differences between annual plants and tree species. *Genetics* 154: 1309–1321.
- Bailey MF, McCauley DE. 2006. The effects of inbreeding, outbreeding and long-distance gene flow on survivorship in North American populations of *Silene vulgaris*. *Journal of Ecology* 94: 98–109.
- Bates D, Maechler M, Bolker BM. 2011. *lme4: linear mixed-effects models using S4 classes*. R package version 0.999375-39. [WWW document]. URL <http://cran.r-project.org/web/packages/lme4/index.html> [accessed 31 May 2011].
- Bates D, Vazquez AI. 2009. *pedigreemm: pedigree-based mixed-effects models*. R package version 0.2-4. [WWW document]. URL <http://cran.r-project.org/web/packages/pedigreemm/index.html> [accessed 31 May 2011].
- Blossey B, Nötzold R. 1995. Evolution of increased competitive ability in invasive nonindigenous plants – a hypothesis. *Journal of Ecology* 83: 887–889.
- Böhmer HJ. 2001. Das Schmalblättrige Greiskraut (*Senecio inaequidens* DC. 1837) in Deutschland – eine aktuelle Bestandsaufnahme. *Floristische Rundbriefe* 35: 47–54.
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHM, White JS. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution* 24: 127–135.
- Bossdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D. 2005. Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* 144: 1–11.
- Burton OJ, Phillips BL, Travis MJM. 2010. Trade-offs and the evolution of life-histories during range expansion. *Ecology Letters* 13: 1210–1220.
- Burton OJ, Travis MJM. 2008. Landscape structure and boundary effects determine the fate of mutations occurring during range expansions. *Heredity* 101: 329–340.
- Chun YJ, Nason JD, Moloney KA. 2009. Comparison of quantitative and molecular genetic variation of native vs. invasive populations of purple loosestrife (*Lythrum salicaria* L., Lythraceae). *Molecular Ecology* 18: 3020–3035.
- Colautti RI, Maron JL, Barrett SCH. 2009. Common garden comparisons of native and introduced plant populations: latitudinal clines can obscure evolutionary inferences. *Evolutionary Applications* 2: 187–199.
- Crawford KM, Whitney KD. 2010. Population genetic diversity influences colonization success. *Molecular Ecology* 19: 1253–1263.
- Daehler CC. 1999. Inbreeding depression in smooth cordgrass (*Spartina alterniflora*, Poaceae) invading San Francisco Bay. *American Journal of Botany* 86: 131–139.
- Dietz H, Edwards PJ. 2006. Recognition that causal processes change during plant invasion helps explain conflicts in evidence. *Ecology* 87: 1359–1367.
- Dimande AFP, Botha CJ, Prozesky L, Bekker L, Rösemann GM, Labuschagne L, Retief E. 2007. The toxicity of *Senecio inaequidens* DC. *Journal of the South African Veterinary Association-Tydskrif Van Die Suid-Afrikaanse Veterinere Vereniging* 78: 121–129.
- Dlugosch KM, Parker IM. 2007. Molecular and quantitative trait variation across the native range of the invasive species *Hypericum canariense*: evidence for ancient patterns of colonization via pre-adaptation? *Molecular Ecology* 16: 4269–4283.

- Dlugosch KM, Parker IM. 2008a. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* 17: 431–449.
- Dlugosch KM, Parker IM. 2008b. Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecology Letters* 11: 701–709.
- Edmonds CA, Lillie AS, Cavalli-Sforza LL. 2004. Mutations arising in the wave front of an expanding population. *Proceedings of the National Academy of Sciences, USA* 101: 975–979.
- Ellstrand NC, Elam DR. 1993. Population genetic consequences of small population-size – implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217–242.
- Ernst WHO. 1998. Invasion, dispersal and ecology of the South African neophyte *Senecio inaequidens* in The Netherlands: from wool alien to railway and road alien. *Acta Botanica Neerlandica* 47: 131–151.
- Excoffier L, Foll M, Petit RJ. 2009. Genetic consequences of range expansions. *Annual Review of Ecology and Evolutionary Systematics* 40: 481–501.
- Facon B, Hufbauer RA, Tayeh A, Loiseau A, Lombaert E, Vitalis R, Guillemaud T, Lundgren JG, Estoup A. 2011. Inbreeding depression is purged in the invasive insect *Harmonia axyridis*. *Current Biology* 21: 424–427.
- Fischer M, van Kleunen M, Schmid B. 2000. Genetic Allee effects on performance, plasticity and developmental stability in a clonal plant. *Ecology Letters* 3: 530–539.
- Foll M, Gaggiotti O. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics* 180: 977–993.
- García-Serrano H, Escarré J, Sans FX. 2004. Factors that limit the emergence and establishment of the related aliens *Senecio inaequidens* and *Senecio pterophorus* and the native *Senecio malacianus* in Mediterranean climate. *Canadian Journal of Botany-Revue Canadienne de Botanique* 82: 1346–1355.
- Gurevitch J, Fox GA, Wardle GM, Inderjit, Taub D. 2011. Emergent insights from the synthesis of conceptual frameworks for biological invasions. *Ecology Letters* 14: 407–418.
- Hardy OJ. 2003. Estimation of pairwise relatedness between individuals and characterization of isolation-by-distance processes using dominant genetic markers. *Molecular Ecology* 12: 1577–1588.
- Hardy OJ, Vekemans X. 2002. SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2: 618–620.
- Hilliard OM. 1977. *Compositae in Natal*. Pietermaritzburg, South Africa: University of Natal Press.
- Housworth EA, Martins EP, Lynch M. 2004. The phylogenetic mixed model. *American Naturalist* 163: 84–96.
- Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M. 2008. Ecological consequences of genetic diversity. *Ecology Letters* 11: 609–623.
- Jeanmonod D. 2002. Nouvelles stations pour le *Senecio inaequidens* DC. ou la conquête irrésistible du séneçon du Cap. *Saussurea* 32: 43–50.
- Kanarek AR, Webb CT. 2010. Allee effects, adaptive evolution, and invasion success. *Evolutionary Applications* 3: 122–135.
- Keller SR, Sowell DR, Neiman M, Wolfe LM, Taylor DR. 2009. Adaptation and colonization history affect the evolution of clines in two introduced species. *New Phytologist* 183: 678–690.
- Keller SR, Taylor DR. 2008. History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. *Ecology Letters* 11: 852–866.
- Keller LF, Waller DM. 2002. Inbreeding effects in wild populations. *Trends in Ecology & Evolution* 17: 230–241.
- Kollmann J, Banuelos MJ. 2004. Latitudinal trends in growth and phenology of the invasive alien plant *Impatiens glandulifera* (Balsaminaceae). *Diversity and Distributions* 10: 377–385.
- Kuhbier H. 1977. *Senecio inaequidens* DC. – ein Neubürger der nordwestdeutschen Flora. *Abhandlungen des Naturwissenschaftlichen Vereins Bremen* 38: 383–396.
- Kuparinen A, Savolainen O, Schurr FM. 2010. Increased mortality can promote evolutionary adaptation of forest trees to climate change. *Forest Ecology and Management* 259: 1003–1008.
- Kuparinen A, Schurr FM. 2007. A flexible modelling framework linking the spatio-temporal dynamics of plant genotypes and populations: application to gene flow from transgenic forests. *Ecological Modelling* 202: 476–486.
- Lachmuth S, Durka W, Schurr FM. 2010. The making of a rapid plant invader: genetic diversity and differentiation in the native and invaded range of *Senecio inaequidens*. *Molecular Ecology* 19: 3952–3967.
- Lafuma L, Balkwill K, Imbert E, Verlaque R, Maurice S. 2003. Ploidy level and origin of the European invasive weed *Senecio inaequidens* (Asteraceae). *Plant Systematics and Evolution* 243: 59–72.
- Lafuma L, Maurice S. 2007. Increase in mate availability without loss of self-incompatibility in the invasive species *Senecio inaequidens* (Asteraceae). *Oikos* 116: 201–208.
- Lande R. 1988. Genetics and demography in biological conservation. *Science* 241: 1455–1460.
- Leinonen T, O'Hara RB, Cano JM, Merliä J. 2008. Comparative studies of quantitative trait and neutral marker divergence: a meta-analysis. *Journal of Evolutionary Biology* 21: 1–17.
- Liebholt A, Bascompte J. 2003. The Allee effect, stochastic dynamics and the eradication of alien species. *Ecology Letters* 6: 133–140.
- Lockwood JL, Cassey P, Blackburn T. 2005. The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution* 20: 223–228.
- López-García MC, Maillet J. 2005. Biological characteristics of an invasive South African species. *Biological Invasions* 7: 181–194.
- Lynch M, Milligan BG. 1994. Analysis of population genetic-structure with RAPD markers. *Molecular Ecology* 3: 91–99.
- Lynch M, Walsh B. 1998. *Genetics and the analysis of quantitative traits*. Sunderland, MA, USA: Sinauer.
- MacArthur RH, Wilson EO. 1967. *The theory of island biogeography*. Princeton, NJ, USA: Princeton University Press.
- Maron JL, Vila M, Bommarco R, Elmendorf S, Beardsley P. 2004. Rapid evolution of an invasive plant. *Ecological Monographs* 74: 261–280.
- Meimberg H, Milan NF, Karatassiou M, Espeland EK, McKay JK, Rice KJ. 2010. Patterns of introduction and adaptation during the invasion of *Aegilops triuncialis* (Poaceae) into Californian serpentine soils. *Molecular Ecology* 19: 5308–5319.
- Moloney KA, Holzapfel C, Tielbörger K, Jeltsch F, Schurr FM. 2009. Rethinking the common garden in invasion research. *Perspectives in Plant Ecology, Evolution and Systematics* 11: 311–320.
- Montague JL, Barrett SCH, Eckert CG. 2008. Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (*Lythrum salicaria*, Lythraceae). *Journal of Evolutionary Biology* 21: 234–245.
- Monty A, Lebeau J, Meerts P, Mahy G. 2009. An explicit test for the contribution of environmental maternal effects to rapid clinal differentiation in an invasive plant. *Journal of Evolutionary Biology* 22: 917–926.
- Monty A, Mahy G. 2009a. Clinal differentiation during invasion: *Senecio inaequidens* (Asteraceae) along altitudinal gradients in Europe. *Oecologia* 159: 305–315.
- Monty A, Mahy G. 2009b. Evolution of dispersal traits along an invasion route in the wind-dispersed *Senecio inaequidens* (Asteraceae). *Oikos* 119: 1563–1570.
- Nathan R, Schurr FM, Spiegel O, Steinitz O, Trakhtenbrot A, Tsoar A. 2008. Mechanisms of long-distance seed dispersal. *Trends in Ecology & Evolution*, 23: 638–647.

- O'Neil P. 1994. Genetic incompatibility and offspring quality in the tristylous plant *Lythrum salicaria* (Lythraceae). *American Journal of Botany* 81: 76–84.
- Parker IM, Rodriguez J, Loik ME. 2003. An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbasicum thapsus*. *Conservation Biology* 17: 59–72.
- Phillips BL. 2009. The evolution of growth rates on an expanding range edge. *Biology Letters* 5: 802–804.
- Phillips BL, Brown GP, Shine R. 2010. Life-history evolution in range-shifting populations. *Ecology* 91: 1617–1627.
- Phillips BL, Brown GP, Travis MJM, Shine R. 2008. Reid's paradox revisited: the evolution of dispersal kernels during range expansion. *American Naturalist* 172: S34–S48.
- Pinheiro J, Bates D. 2000. *Mixed-effects models in S and S-PLUS. Statistics and computing series*. New York, NY, USA: Springer-Verlag.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team. 2011. *nlme: linear and nonlinear mixed effects models*. R package version 3.1-98. [WWW document]. URL <http://cran.r-project.org/web/packages/nlme/index.html> [accessed 31 May 2011].
- Poll M, Naylor BJ, Alexander JM, Edwards PJ, Dietz H. 2009. Seedling establishment of Asteraceae forbs along altitudinal gradients: a comparison of transplant experiments in the native and introduced ranges. *Diversity and Distributions* 15: 254–265.
- R Development Core Team. 2011. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing [WWW document]. URL <http://www.R-project.org> [accessed on 31 May 2011].
- Ritland K. 1996. Estimators for pairwise relatedness and individual inbreeding coefficients. *Genetical Research* 67: 175–185.
- Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC *et al.* 2001. The population biology of invasive species. *Annual Review of Ecology and Systematics* 32: 305–332.
- Saltonstall K. 2003. Genetic variation among North American populations of *Phragmites australis*: implications for management. *Estuaries* 26: 444–451.
- Scherber C, Crawley MJ, Porembski S. 2003. The effects of herbivory and competition on the invasive alien plant *Senecio inaequidens* (Asteraceae). *Diversity and Distributions* 9: 415–426.
- Taylor CM, Hastings A. 2005. Allee effects in biological invasions. *Ecology Letters* 8: 895–908.
- Tobin PC, Whitmore SL, Johnson DM, Bjornstad ON, Liebhold AM. 2007. Invasion speed is affected by geographical variation in the strength of Allee effects. *Ecology Letters* 10: 36–43.
- Travis MJM, Dytham C. 2002. Dispersal evolution during invasions. *Evolutionary Ecology Research* 4: 1119–1129.
- Travis MJM, Smith HS, Ranwala SMW. 2010. Towards a mechanistic understanding of dispersal evolution in plants: conservation implications. *Diversity and Distributions* 16: 690–702.
- Van Kleunen M, Fischer M. 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist* 166: 49–60.
- Van Kleunen M, Johnson SD. 2005. Testing for ecological and genetic Allee effects in the invasive shrub *Senna didymobotrya* (Fabaceae). *American Journal of Botany* 92: 1124–1130.
- Vekemans X, Beauwens T, Lemaire M, Roldán-Ruiz I. 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology* 11: 139–151.
- Verhoeven KJF, Macel M, Wolfe LM, Biere A. 2010. Population admixture, biological invasions and the balance between local adaptation and inbreeding depression. *Proceedings of the Royal Society B* 278: 2–8.
- Werner DJ, Rockenbach T, Hoelscher ML. 1991. Herkunft, Ausbreitung, Vergesellschaftung und Ökologie von *Senecio inaequidens* DC unter besonderer Berücksichtigung des Köln-Aachener Raumes. *Tuexenia* 11: 73–107.
- Whitlock MC. 2008. Evolutionary inference from Q_{ST} . *Molecular Ecology* 17: 1885–1896.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Characteristics of the investigated *Senecio inaequidens* populations

Table S2 Parameter estimates for explanatory terms included in the minimal adequate mixed-effects models (*lme/lmer*) and corresponding *pedigreemm* models

Methods S1 R function for fitting linear mixed-effects models or generalized linear mixed models that account for genetic relatedness among grouping factors such as populations.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.