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The making of a rapid plant invader: genetic diversity and differentiation in the native and invaded range of *Senecio inaequidens*

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Abstract

To become invasive, exotic species have to succeed in the consecutive phases of introduction, naturalization, and invasion. Each of these phases leaves traces in genetic structure, which may affect the species' success in subsequent phases. We examined this interplay of genetic structure and invasion dynamics in the South African Ragwort (*Senecio inaequidens*), one of Europe's fastest plant invaders. We used AFLP and microsatellite markers to analyze 19 native African and 32 invasive European populations. In combination with historic data, we distinguished invasion routes and traced them back to the native source areas. This revealed that different introduction sites had markedly different success in the three invasion phases. Notably, an observed lag-phase in Northern Germany was evidently not terminated by factors increasing the invasiveness of the resident population but by invasive spread from another introduction centre. The lineage invading Central Europe was introduced to sites in which winters are more benign than in the native source region. Subsequently, this lineage spread into areas in which winter temperatures match the native climate more closely. Genetic diversity clearly increases with population age in Europe and less clearly decreases with spread rate up to population establishment. This indicates that gene flow along well-connected invasion routes counteracted losses of genetic diversity during rapid spread. In summary, this study suggests that multiple introductions, environmental preadaptation and high gene flow along invasion routes contributed to the success of this rapid invader. More generally, it demonstrates the benefit of combining genetic, historical, and climatic data for understanding biological invasions.

Keywords: biological invasions, climatic preadaptation, genetic diversity, lag phase, spread rate

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Introduction

Biological invasions are serious threats for global biodiversity (Sala *et al.* 2000; Millennium Ecosystem Assessment Board 2005). At the same time, they are a fascinating phenomenon and provide an opportunity to study how ecological and evolutionary processes are influenced by migration and sudden changes in environmental conditions (Sax *et al.* 2007). Richardson *et al.* (2000b) distinguished three major phases in the development of successful invasions: the species has to be dispersed to a new geographical region (introduction), form persistent populations in the novel environment (naturalization), and spread successfully (invasion). To pass each of these phases, the exotic species has to overcome barriers which – by influencing population genetic processes such as gene flow, genetic drift and selection – leave traces in genetic structure. Hence, patterns of neutral genetic diversity within and among invading populations provide valuable insights into introduction, naturalization, and invasion history (e.g. Prentis *et al.* 2009). On the other hand, the non-neutral genetic diversity of an exotic species can affect its

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demographic and evolutionary potential, and thereby its ability to overcome the next barrier in the invasion process.

In species that depend on sexual reproduction, genetic diversity may affect the demography of invading populations via genetic Allee effects (Allee 1931; Ellstrand & Elam 1993): low genetic diversity and heterozygosity can reduce the fitness of individuals thereby lowering population growth rates and increasing extinction probability (Courchamp *et al.* 1999). Furthermore, there is increasing evidence for positive ecological effects of genetic diversity through sampling effects as well as mechanisms such as facilitation (i.e. genotype interactions that benefit at least one of the participants and cause harm to neither) and niche-partitioning (Hughes *et al.* 2008). A recent study of Crawford & Whitney (2010) revealed that an augmentation of genetic diversity increases the colonization success of experimental populations. Additionally, genetic diversity is expected to increase the adaptive potential of introduced populations in the novel environment (Sakai *et al.* 2001). On the other hand, genetic drift and inbreeding in small founder populations can also cause phenotypic divergence between ranges and invasive populations (Barrett & Richardson 1986; Bossdorf *et al.* 2005).

The interplay of these multiple genetic and ecological factors and their relative importance for invasion success may vary between the three invasive phases. In the introduction phase, the amount of genetic diversity introduced strongly depends on the dispersal pathway (Wilson *et al.* 2009): Multiple introductions of large propagule numbers and sizes (*sensu* Lockwood *et al.* 2005) from different source populations (mass dispersal according to Wilson *et al.* 2009) are expected to increase the genetic diversity of introduced populations (e.g. Ross *et al.* 2008) and their probability to contain genotypes that are preadapted to the novel environment (Simberloff 2009; Wilson *et al.* 2009).

Environmental preadaptation (or niche matching) is often regarded as a prerequisite for successful naturalization and invasion (Pŷsek 1998; Richardson *et al.* 2000b). However, during the later phases of the invasion process exotic species may also shift for example their climatic niche (e.g. Broennimann *et al.* 2007). Often the transition from introduction via naturalization to invasion involves a considerable time lag. The processes causing these time lags may be adaptive, demographic (e.g. Allee effects and density-dependent dispersal), ecological (e.g. environmental variability and the delayed arrival of mutualists), or genetic (e.g. hybridization and inbreeding) (Richardson *et al.* 2000a; Sakai *et al.* 2001; Schurr *et al.* 2008).

Once an exotic species has entered the invasion phase, its mode of spread shapes the spatial genetic structure in the novel range. In particular, long-distance dispersal ahead of the continuous invasion front may form outlier populations with low genetic diversity (Austerlitz *et al.* 2000). Hence, the genetic diversity of a population should decrease with its spread rate (the velocity of spread from the introduction site up until population establishment). After population establishment, genetic diversity may then increase with population age due to gene flow from the core of the invasive range or due to admixture of invasion routes from different introduction sources. While these pre- and post-establishment processes have been studied with theoretical models (Austerlitz *et al.* 2000), we still have little empirical information about the relative importance of spread rate and population age for the genetic diversity of invasive populations. Yet, understanding the distribution of genetic diversity along invasion routes is important for understanding invasion dynamics. This is because genetically impoverished outlier populations might experience genetic Allee effects which can markedly slow down invasion rates (Lewis & Kareiva 1993; Taylor & Hastings 2005).

An indispensable prerequisite for the investigation of the processes outlined above is the reconstruction of spatio-temporal invasion dynamics. First of all, knowledge of the specific source areas of exotic species is needed to quantify the amount of genetic diversity introduced, to contrast environmental conditions in invasive and source areas, and to study genetic differentiation between them. Reconstructing the places of introduction, subsequent spread and admixture enables us to investigate genetic differentiation along invasion routes and the development of genetic diversity during the different invasion phases. Yet, despite the recent upsurge of interest in the population genetics and phylogeography of invasive species (e.g. Bossdorf *et al.* 2005; Dlugosch & Parker 2007; Chun *et al.* 2009), only very few studies have utilized historical information on introduction and spread (but see Neuffer & Hurka 1999; Genton *et al.* 2005).

Here, we study one of Europe's fastest plant invaders whose invasion history has been documented exceptionally well. The South African ragwort (*Senecio inaequidens* DC., Asteraceae) was introduced to several locations in Europe at the end of the 19th and beginning of the 20th century by means of wool transports. Starting from these introduction sites, several invasion routes have spread along traffic routes, and this spread has been described in detail (e.g. Böhmer 2001; Jeanmonod 2002).

By combining this knowledge about past invasion dynamics at the species level with analyses of current

genetic structure we aim to (i) identify the African source regions of the invading populations, and (ii) assign invasive populations to their introduction centers and thereby reconstruct the spatio-temporal invasion dynamics at the level of genetic clusters. We then use this reconstruction to (iii) compare climatic conditions between the native and invaded regions of the different clusters, (iv) quantify genetic differentiation at multiple spatial scales (between native and invaded range, between invasion routes, between populations of the same invasion route), and (v) quantify the effects of population age and spread rate on the genetic diversity of invading populations. While observational studies like ours cannot unequivocally identify causes of invasions, our analyses shed light on the genetic and ecological mechanisms that may have contributed to the successful introduction, naturalization, and invasion of *S. inaequidens* in Europe.

Materials and methods

Study species

Senecio inaequidens is a perennial ligneous herb native to the Drakensberg and Maloti Mountains in 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. Rare long-distance dispersal by extreme winds is thus expected to create fat-tailed distributions of seed dispersal distance in *S. inaequidens* (Nathan *et al.* 2008). In its native range the species occurs at elevations from sea level to 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 personal observation). *S. inaequidens* belongs to a species complex whose phylogeny and taxonomy is only partly resolved (Scott *et al.* 1998; Le Roux *et al.* 2006). Moreover, this *S. inaequidens/S. madagascariensis* complex comprises a tetraploid variety ($2n = 4\times = 40$) and two diploid ($2n = 2\times = 20$) varieties which occur largely allopatrically in Southern Africa (Lafuma *et al.* 2003). The geographical range of tetraploids lies in between the ranges of the two diploid varieties and mostly at higher elevations (Lafuma *et al.* 2003). Lafuma *et al.* (2003) suggested that tetraploid populations in the surroundings of Port Elizabeth derive from human-mediated colonization in the 20th century. Diploids (mostly referred to as *S. madagascariensis*) are expanding within Southern Africa (Werner *et al.* 1991) and are invasive in Australia (Scott *et al.* 1998; Radford *et al.* 2000), Hawaii (Le Roux *et al.* 2009) and South America (Lafuma *et al.* 2003). In Europe only tetraploid individuals have been found so far (Lafuma *et al.* 2003).

The wool, in which *S. inaequidens* seeds were introduced to Europe presumably originated from all over the Maloti and Drakensberg region, was gathered at several trading posts, and shipped to Europe from the harbors of Durban and Port Elizabeth (G. Tsekoa, wool trading post Molumong (Lesotho), Lafuma *et al.* 2003). In continental Europe, five primary introduction centers 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). In addition, we are aware of occurrences in Hanover (Germany, 1889), Mettmann (Germany, 1922), Leipzig (Germany, 1938), and Tilburg (Netherlands, 1939) that were presumably ephemeral (Kuhbier 1977; Ernst 1998). After introduction to Europe, *S. inaequidens* spread mainly along traffic routes such as roads and railways (see Data S1 for a summary of the invasion history). So far, it mostly invaded ruderal habitats, but it also increasingly colonizes grazed grasslands in Southern Europe (Garcia-Serrano *et al.* 2004). This development is particularly alarming due to alkaloid content poisonous for livestock (Dimande *et al.* 2007). *S. inaequidens* also occurs on fallow ground, in natural rocky habitats (Böhmer 2001), coastal dunes, and at open sites in pine forests (Werner *et al.* 1991; personal observation). Because of its ability to form dense stands and its exceptionally high invasion speed the species is considered as a potential threat to the native flora (Böhmer 2001).

Sample collection

We sampled 51 tetraploid populations of *Senecio inaequidens*, 32 in Europe in 2006 and 19 Southern African populations in 2007 (Table 1). Since Lafuma *et al.* (2003) found that tetraploid African populations are the likely source of European invaders we concentrated sampling in Africa on the tetraploid range identified by their study. Additionally sampled diploid and mixed populations (Table 1) were identified and excluded prior to data analysis using flowcytometry and microsatellite analyses (results not shown). In Europe we sampled the four introduction sites of Bremen, Calais, Mazamet, and Verviers and presumably descendant populations across the current distribution in Austria, Belgium, France, Germany, the Netherlands, and Switzerland. We predominantly sampled European populations of known age, most of which were situated along traffic routes. The year of first occurrence within a 25 km radius was derived from the floristic literature and databases to calculate population age (Table 1). We

Table 1 Properties of sampled populations including sub-regions and invasion routes derived from STRUCTURE analyses: country codes A: Austria, B: Belgium, CH: Switzerland, D: Germany, F: France, L: Lesotho, NL: Netherlands and ZA: South Africa; Lat/Lon, geographical location; Alt, altitude; Temp, mean minimum winter temperature; Year 1st obs., year of first observation (in the invaded range); Dens, density of reproductive individuals per square meter; Ploidy, ploidy levels; N, sample size; *Hj*, gene diversity; *PLP* (5) and B_r (5); polymorphic marker proportion and band richness, respectively, with sample size rarefied to five individuals

Country	Location	Region	Route	Lat	Lon	Alt (m.a.s.l.)	Temp (°C)	Year 1st obs.	Dens	Ploidy	N	H_j	*PLP* (5)	*Br*(5)
ZA	Addo I	Africa-S	–	33.57S	25.69E	39	5.9	–	–	4	8	0.185	0.44	1.36
ZA	Addo II	Africa-S	–	33.44S	25.82E	177	5.6	–	–	4	5	0.217	0.41	1.406
ZA	Boskloof	Africa-N	–	28.50S	28.59E	1883	−2.7	–	1.5	4	11	0.172	0.50	1.346
ZA	Glen Reenen	Africa-N	–	28.50S	28.62E	1915	4.8	–	1.3	4	10	0.196	0.50	1.377
ZA	Golden Gate	Africa-N	–	28.51S	28.58E	1835	−2.7	–	–	4	6	0.192	0.38	1.347
ZA	Grahamstown I	Africa-S	–	33.32S	26.52E	626	5.1	–	–	4	8	0.212	0.52	1.411
ZA	Grahamstown II	Africa-S	–	33.29S	26.49E	650	−5.1	–	2.5	4	10	0.218	0.58	1.436
L	Ha Potiane	Africa-N	–	29.61S	27.76E	1780	−0.2	–	–	4	5	0.206	0.34	1.338
L	Letseng	Africa-N	–	28.98S	28.85E	3119	−2.7	–	–	4	5	0.196	0.35	1.352
L	Mafeteng	Africa-N	–	29.95S	27.29E	1641	−4.3	–	–	4	6	0.155	0.28	1.26
L	Makhomalong	Africa-N	–	29.28S	28.94E	2377	−3.2	–	–	4	5	0.196	0.35	1.352
L	Mothae	Africa-N	–	29.09S	28.85E	2897	−5.5	–	–	4	5	0.195	0.35	1.347
L	Oxbow	Africa-N	–	28.77S	28.64E	2490	−4.1	–	0.3	4	10	0.210	0.58	1.421
L	Rapo-Le-Boea	Africa-N	–	29.67S	27.92E	2379	−4.6	–	–	4	5	0.186	0.34	1.338
L	Sani Pass I	Africa-N	–	29.44S	29.14E	2456	−5.2	–	–	4	5	0.231	0.44	1.438
L	Sani Pass II	Africa-N	–	29.58S	29.28E	2875	−3.5	–	–	4	8	0.194	0.46	1.373
L	Sani Pass III	Africa-N	–	29.51S	29.18E	3042	−1.9	–	–	4	5	0.220	0.41	1.411
L	Semonkong	Africa-N	–	29.84S	28.04E	2190	−0.4	–	1.5	4	10	0.187	0.48	1.352
ZA	Zastron	Africa-N	–	30.27S	27.15E	1575	−0.8	–	0.8	4	8	0.172	0.37	1.295
ZA	Elliot	–	–	31.33S	27.86E	1461	0.5	–	3.7	2	9	NA	NA	NA
ZA	Lady Grey	–	–	30.80S	27.21E	1712	−2.5	–	–	2+4	6	NA	NA	NA
ZA	Langholm	–	–	33.43S	26.75E	295	8.0	–	–	2	8	NA	NA	NA
L	Malefiloune	–	–	28.76S	28.51E	1912	−1.4	–	–	2	5	NA	NA	NA
L	Masianokong	–	–	29.39S	27.55E	1536	−0.1	–	–	2	4	NA	NA	NA
L	Masuaneng	–	–	29.36S	29.07E	2205	−2.7	–	–	2	5	NA	NA	NA
L	Molumong	–	–	29.37S	29.00E	2740	−2.9	–	0.1	2	10	NA	NA	NA
D	Aachen	Europe-N	VER	50.77N	06.12E	184	−0.1	1972[1]	2.7	4	11	0.191	0.49	1.364
N	Amsterdam	Europe-N	VER	52.40N	04.81E	0	−1.4	1981[2]	3.5	4	7	0.189	0.37	1.318
CH	Basel	Europe-N	VER	47.57N	07.60E	255	0.0	1985[3]	1.0	4	10	0.167	0.42	1.304
D	Berlin	Europe-N	VER	52.46N	13.33E	45	−1.0	1993[4]	1.1	4	10	0.183	0.43	1.331
D	Brandenburg	Europe-N	VER	52.40N	12.57E	29	−2.6	1998[5]	5.9	4	11	0.174	0.43	1.317
D	Bremen	Europe-N	BRE	53.09N	08.78E	6	−1.8	1896[1]	11.4	4	11	0.185	0.46	1.351
F	Calais	Europe-N	CAL	50.97N	01.90E	5	1.9	1935[6]	14.1	4	10	0.169	0.38	1.28
D	Cologne	Europe-N	VER	50.94N	06.99E	43	−3.5	1978[7]	2.7	4	10	0.185	0.47	1.348
D	Cottbus	Europe-N	VER	51.75N	14.32E	72	−5.5	1996[5]	3.5	4	10	0.168	0.42	1.312
D	Deggendorf	Europe-N	VER	48.81N	12.97E	312	−0.9	1995[8]	4.4	4	10	0.131	0.28	1.203
D	Dortmund	Europe-N	VER	51.51N	07.43E	85	−3.3	1990[6]	2.6	4	11	0.183	0.46	1.338
CH	Genthod	Europe-N	MAZ	46.26N	6.16E	387	−1.9	2002[9]	–	4	5	0.154	0.23	1.233
F	Grenoble	Europe-N	MAZ	45.24N	05.66E	203	−2.8	NA	–	4	8	0.180	0.37	1.309
D	Gross Kreutz	Europe-N	VER	52.4 N	12.78E	33	−2.6	2001[10]	1.3	4	10	0.166	0.40	1.293
D	Hanover	Europe-N	BRE	52.37N	09.78E	54	−2.1	1976[1]	2.1	4	10	0.170	0.44	1.315
N	IJmuiden	Europe-N	VER	52.44N	04.56E	7	0.2	1992[11]	2.9	4	11	0.177	0.45	1.325
D	Karlsruhe	Europe-N	VER	49.00N	08.41E	114	−1.5	1988[12]	4.4	4	11	0.177	0.43	1.318
D	Kassel	Europe-N	VER	51.31N	09.52E	138	−2.3	1985[6]	–	4	8	0.175	0.38	1.308
D	Kiel	Europe-N	VER	54.31N	10.13E	4	−2.1	1991[13]	7.3	4	11	0.170	0.44	1.309
N	Kwade Hoek	Europe-N	VER	51.84N	04.00E	0	−0.8	1997[11]	I.I	4	10	0.150	0.31	1.237
CH	Lausanne	Europe-S	MAZ	46.53N	06.55E	403	0.7	1987[9]	0.8	4	10	0.124	0.28	1.204
F	Lunel	Europe-S	MAZ	43.68N	04.12E	9	−3.0	1986[14]	0.8	4	10	0.167	0.43	1.319
D	Mannheim	Europe-N	VER	49.45N	08.56E	106	1.5	1999[15]	0.1	4	9	0.166	0.39	1.297
F	Mazamet	Europe-S	MAZ	43.50N	02.37E	242	−1.6	1936[9]	2.5	4	12	0.172	0.49	1.336
D	Ohligse Heide	Europe-N	VER	51.16N	06.97E	72	1.2	NA	–	4	5	0.184	0.31	1.311
D	Passau	Europe-N	VER	48.57N	13.45E	302	−1.0	NA	–	4	5	0.186	0.31	1.311

Table 1 (Continued)

Country	Location	Region	Route	Lat	Lon	Alt (m.a.s.l.)	Temp (°C)	Year 1st obs.	Dens	Ploidy	N	H_j	*PLP* (5)	*Br*(5)
D	Regensburg	Europe-N	VER	49.01N	12.08E	340	−6.1	1989[16]	2.2	4	12	0.175	0.45	1.319
B	Verviers	Europe-N	VER	50.59N	05.85E	164	−4.6	1922[17]	2.8	4	10	0.200	0.48	1.378
A	Vienna	Europe-N	VER	48.22N	16.39E	161	−0.8	1991[18]	36.8	4	10	0.184	0.44	1.329
CH	Vilette	Europe-S	MAZ	46.50N	06.71E	392	−3.8	1996[9]	–	4	8	0.152	0.30	1.237
D	Weissenfels	Europe-N	VER	51.21N	11.97E	102	−2.5	1998[19]	8.3	4	11	0.160	0.39	1.284
D	Werl	Europe-N	VER	51.53N	07.86E	134	−3.6	NA	–	4	5	0.187	0.29	1.292

[1]Kuhbier 1977, [2]Ernst 1998, [3]Stöcklin *et al*. 2003, [4]König 1995, [5]Bornkamm & Prasse 1999, [6]Böhmer 2001, [7]Kehren 1995, [8]Düring 1997, [9]Jeanmonod 2002, [10]Bornkamm 2002, [11]FLORON database 2009, [12]Sebald *et al.* 1996, [13]Werner *et al*. 1991, [14]Guillerm *et al.* 1991, [15]SysTax database 2009, [16]Radkowitsch 1997, [17]Lafuma *et al.* 2003, [18]Melzer & Barta 1991, [19]Datenbank Farn- und Blütenpflanzen 2008, 2009.

estimated the density of reproductive individuals per square meter by averaging counts from 4 to 20 one square meter plots per population (Table 1). In each population, we collected seed and leaf material of 5–15 mother plants, resulting in a total of 438 sampled individuals. Leaves were dried and stored in silica gel.

Molecular markers and genetic data analysis

We analyzed genetic variation of 438 samples for amplified fragment length polymorphisms (AFLP Vos *et al*. 1995) and one microsatellite locus (SE116, Le Roux & Wieczorek 2007). This resulted in presence-absence data for 187 AFLP loci and 31 microsatellite alleles.

Three microsatellite loci (SE 220, SE176, SE116) previously described from *S. madagascariensis* (Le Roux & Wieczorek 2007) were analyzed with all loci resulting in PCR products of the expected fragment length. However, polyploidy lead to complex banding patterns and only in SE116 alleles and stutter-bands could be distinguished unambiguously. Allele dosage could not be resolved, thus only the presence of alleles was recorded resulting in a presence-absence matrix of the 31 alleles. For the AFLP analyses, after screening 16 primer combinations, two primer combinations were selected: ACT (FAM)-CAG and ACA (VIC)-CAG. Fragment analysis of microsatellite and AFLP PCR products was performed simultaneously on an ABI 3130 genetic analyzer using GeneScan LIZ 500 as internal size standard. The detailed laboratory protocol is reported in the Data S1.

Genotyping of AFLP markers proceeded in several steps, similar to the procedure described in Whitlock *et al*. (2008). In a first step, a total of 298 loci were defined manually in the range of 50–500 bp in GeneMapper V. 3.7 (Applied Biosystems) and peak height data were exported. Second, reproducibility of AFLP fragments was checked on 118 samples which had twice been analyzed starting from the same DNA extraction. For each locus we manually adjusted an individual peak height threshold that minimized the error rate, with 50 relative fluorescence units as default value. We excluded all AFLP loci that were either monomorphic or had an individual error rate >10% of all the repeated samples or >50% of band frequency across the repeated samples, the latter condition being relevant only for rare fragments. Overall, 187 polymorphic AFLP loci were retained that had a mean phenotypic error rate per locus (Pompanon *et al*. 2005) of 2.9% across 22066 AFLP phenotypes (118 samples × 187 loci).

Genetic population structure was investigated by analyzing the AFLP data with a Bayesian cluster approach specifically adapted to dominant markers (STRUCTURE 2.2., Pritchard *et al*. 2000; Falush *et al*. 2007). With respect to our research objectives, we aimed to investigate genetic structure at different geographical scales conducting several analyses for nested subsets of data. To identify the African source regions of the European populations, analysis 1 contained all populations. Subsequently, we resolved the structure within continents by analyzing all African (analysis 2) and all European populations (analysis 3). Finally, single divergent populations in the European data set were removed (analysis 4) as recommended by Pritchard *et al*. 2007. From the results of analysis 1 we derived two sub-regions for the African (Africa-N and Africa-S) and European (Europe-N and Europe-S) sampling range respectively (Table 1) for use in further analyses. European populations were assigned to their respective invasion route (Bremen, BRE; Calais, CAL; Mazamet, MAZ; Verviers route, VER; Table 1) based on analyses 1 and 4.

In each of these four analyses we used STRUCTURE with cluster numbers (K) ranging from 1 to 15. For each value of K, we ran 20 replicate chains of 150 000 MCMC iterations and discarded the first 50 000 burn-in iterations. We used the recessive allele modus and applied the admixture model with correlated allele frequencies

without using a priori information on population origin. To determine the most likely number of clusters we followed the approach of Evanno *et al.* (2005) and identified the most likely K where ΔK (a measure for the second order rate of change in the likelihood of K) reached the maximum value (R-script 'structure-sum' by Ehrich (2006) adjusted to STRUCTURE 2.2). We used CLUMPP (Jakobsson & Rosenberg 2007) and *distruct* 1.1 (Rosenberg 2004) to produce bar plots of the individuals' assignment probabilities Q (Fig. 1). Geographical maps (Fig. 2) were produced by kriging [R packages 'fields' (Furrer *et al.* 2008) and 'maps' (Becker *et al.* 2008) (R Development Core Team 2008)].

Previous studies suggested that *S. inaequidens*' invasion of Central Europe was limited by winter frosts (Ernst 1998; Böhmer 2001). Hence, we used linear regression to test whether the assignment of native populations to the cluster invading Central Europe can be explained by minimum winter temperature (variable BioClim6 from http://www.worldclim.org (version 1.4), Hijmans *et al.* 2005). For this analysis, we arcsine-transformed the population assignment probability Q to this cluster averaged over all runs of analysis 1 for the most likely K. Moreover, we used linear models to compare minimum winter temperature between European populations colonized by the cluster and respective African source populations, and to relate the (log-transformed) age of these European populations to their minimum winter temperature.

Descriptive parameters of genetic variation within and among populations were obtained from the combined set of dominant AFLP and microsatellite data. Genetic variation at population level was calculated as gene diversity (H_j) based on a Bayesian estimation of allele frequencies (Zhivotovsky 1999) implemented in AFLPsurv (Vekemans *et al.* 2002) assuming Hardy-Weinberg equilibrium. Since samples sizes per population ranged from 5 to 12 (mean 8.6), we additionally used AFLPDiv (http://www.pierroton.inra.fr/genetics/labo/Software/Aflpdiv) to calculate band richness (B_r) and the proportion of polymorphic loci (*PLP*) (Coart *et al.* 2005). Both indices were corrected for varying sample size by rarefaction to the minimal sample size of five at the 5% level. We determined the number of private fragments in populations and regions. AFLPsurv was furthermore used to calculate pairwise F_{ST}

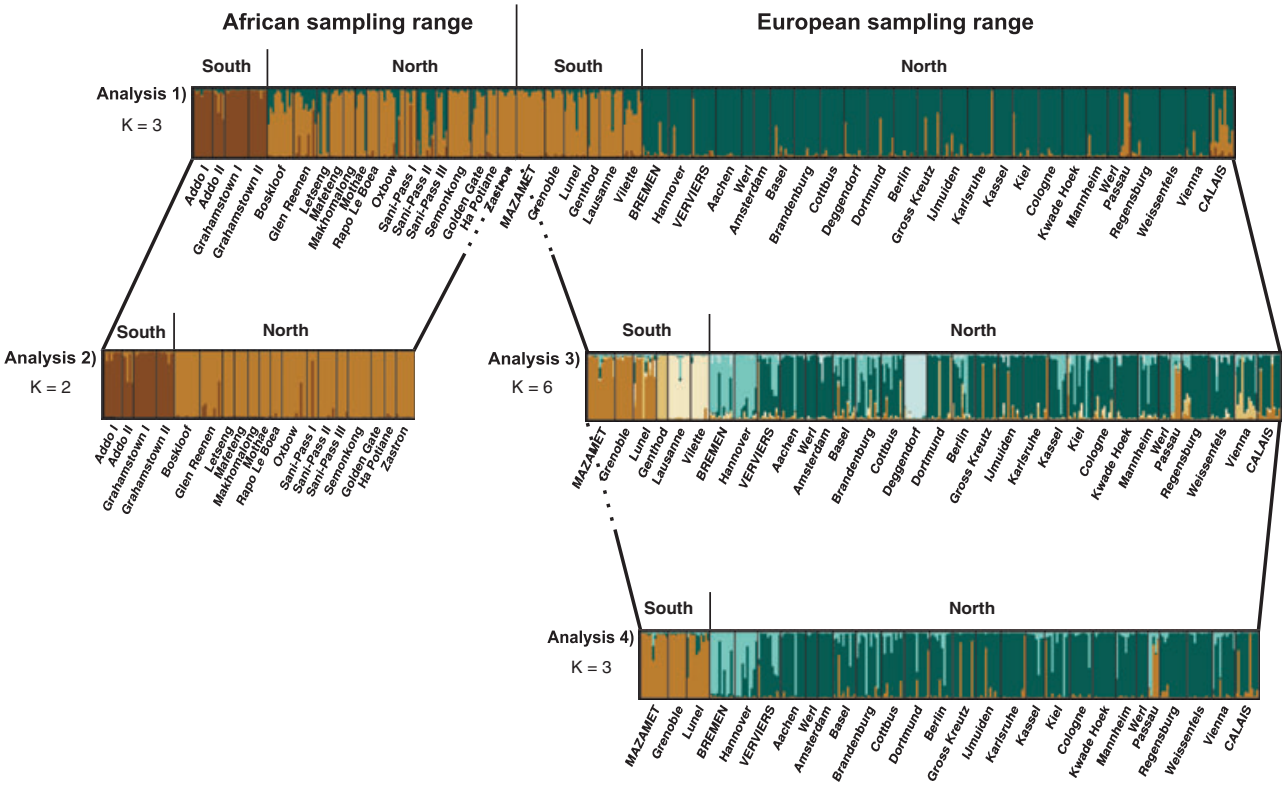

Fig. 1 Average cluster assignment probability Q in nested STRUCTURE analyses of AFLP data for individuals from native African and invasive European populations of *Senecio inaequidens*. Each bar represents one individual, and populations are separated by black lines. Analysis 1: including all African and European samples. Analysis 2: comprising African samples only. Analysis 3: including all European samples. Analysis 4: European samples without bottlenecked populations (Deggendorf, Genthod, Lausanne, Vilette). Populations are sorted alphabetically within clusters (as derived from analyses 1 and 4).

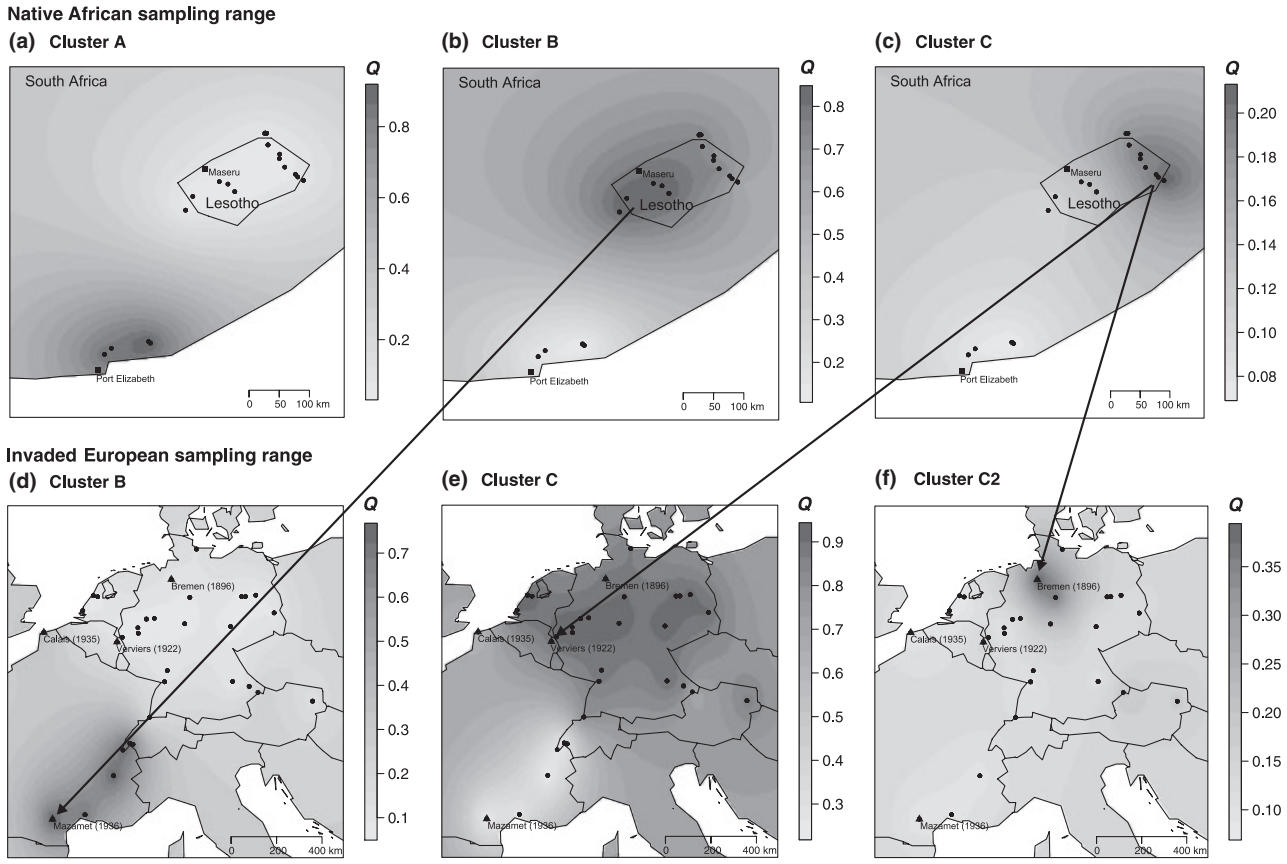

Fig. 2 Spatial interpolation (kriging) of assignment probabilities Q to single genetic clusters in the native African and invasive European range of *Senecio inaequidens*. Subplots (a–e) show results of STRUCTURE analyses 1 whereas (f) shows results of analysis 4 (see Fig. 1). Symbols indicate sampled populations (•) and sampled documented introduction sites (▲) as well as locations of bigger cities for orientation (■). Arrows connect these European sites to their most likely African origin.

values between populations following the approach of Lynch & Milligan (1994). To examine whether population structure followed a model of isolation by distance we regressed pairwise F_{ST} values against geographical distances, and used Mantel tests with 1000 permutations (R-package 'Ade4' (Dray & Dufour 2007)). These tests were performed separately for Africa, Europe, and for regions identified by the STRUCTURE analysis. The partitioning of genetic variation within and among continents and regions was estimated by analysis of molecular variance (AMOVA) (Excoffier *et al*. 1992) in Arlequin 3.11 (Excoffier *et al*. 2005).

To analyze differences in genetic diversity (H_j, B_r-1, and *PLP*, all arcsine-square-root-transformed) between populations from the native (Africa-N) and invaded range and between populations from different regions we used ANOVAS. For European populations of known age, we additionally calculated the spread rate up to population establishment as the geographical distance to the respective introduction site divided by the time lag between the first observation at introduction site and the first observation of the population. For these populations, we used linear-mixed effects models (lme, R-package 'nlme', Pinheiro *et al.* 2008) to test for effects of population age (log-transformed) and spread rate on genetic diversity with invasion route as a random effect. These maximal models were then simplified by stepwise-backward selection to obtain minimal adequate models (Crawley 2002).

Results

Source regions and invasion routes

The STRUCTURE analysis of the full data set (analysis 1) revealed three clusters (Fig. 1), of which cluster A encompasses the populations in the South African lowland (Africa-S, Fig. 2a). This analysis furthermore suggests that European *S. inaequidens* originate from two overlapping source regions (Figs 1 and 2): Cluster B is present in all African highland (Africa-N, Fig, 2b) populations and dominates the southern European pop-

ulations (Europe-S) that were colonized from Mazamet (Kuhbier 1977; Jeanmonod 2002), whereas cluster C comprises the Central European (Europe-N) populations and was also detected in the eastern African highland region. These eastern highland populations showed admixture of the clusters B and C (Fig. 2c) and are located at somewhat higher altitudes than populations from the western highlands ($F_{(1,13)} = 5$, $P < 0.05$). In the African range, the probability of population assignment (Q) to cluster C increases as minimum winter temperature decreases ($F_{(1,17)} = 14.1$, $P < 0.01$, Akaike's Information Criterion AIC: −10.8, Fig. 3). Minimum winter temperature is significantly higher in the Europe-N populations colonized by cluster C than in the eastern highlands source region ($F_{(1,36)} = 10.2$, $P < 0.01$, Fig. 3). Especially, all three European introduction sites of cluster C have moderate minimum winter temperatures compared to the native source region ($F_{(1,11)} = 15.3$, $P < 0.01$, Fig. 3) and the temperature is decreasing continuously with decreasing age (log) of populations subsequently colonized by this cluster ($F_{(1,22)} = 4.349$, $P < 0.05$). Moreover, none of the subsequently colonized populations falls clearly below the native temperature range (Fig. 3). Analysis 2 with African populations only identified two clusters and confirmed that the lowland populations (cluster A in analysis 1, Africa-S) are clearly distinct from the highland origin of European *S. inaequidens* (clusters B and C in analysis 1, Africa-N).

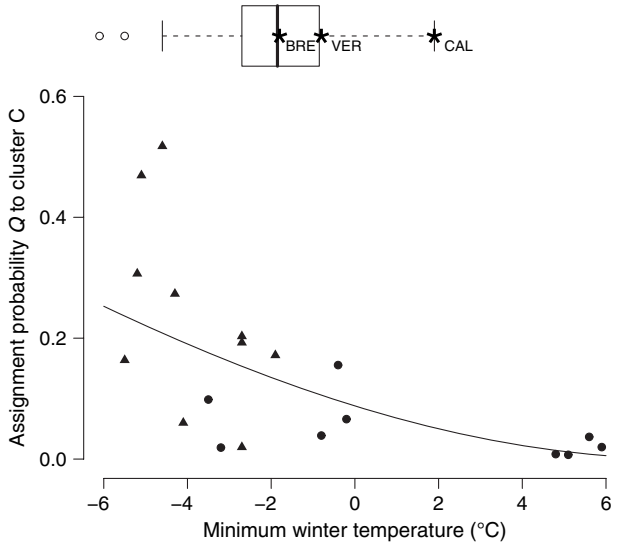

Fig. 3 Relationship between the mean minimum winter temperature of African populations and their mean assignment probability to cluster C invading Central Europe (Europe-N). Triangles (▲) mark the Eastern highlands within the Africa-N region which show the highest assignment probability to this cluster. The remaining African populations are indicated by points (•).Stars indicate the introduction sites in this cluster and the boxplot shows the temperature range of European populations assigned to this cluster (bold line: median, box: interquartile range, whiskers: typical range, points: outliers). Population assignment is based on STRUCTURE analysis 1.

For the European populations (analysis 3) the ΔK-analysis identified six clusters, of which two comprise a single population (Deggendorf, Genthod) and one comprises two populations (Lausanne and Vilette) (Fig. 1). These populations had the lowest values of genetic diversity observed (Table 1) and were spatially isolated in their respective regions which taken together suggests that they were strongly genetically bottlenecked. After removal of these presumably bottlenecked populations, analysis 4 identified three clusters which contain the introduction sites Mazamet (a subset of cluster B in analysis 1, Europe-S), Verviers (cluster C1, a subset of cluster C in analysis 1, Europe-N), and Bremen (cluster C2, a subset of cluster C in analysis 1, Europe-N) and their presumable descendent populations, respectively (Figs 1 and 2d–f). The introduction site Calais was assigned to the Verviers cluster, but formed a distinct cluster at $K = 5$. This is consistent with Calais containing separately introduced genotypes that subsequently admixed *in situ* with the Verviers invasion route. The Verviers route (cluster C1) also comprises all remaining Central European populations with the exception of Hanover (which has high assignment probability to cluster C2 (Bremen), Figs 1 and 2e, f). Some individuals in the C1 populations have low but non-zero assignment probabilities to the Bremen and Mazamet clusters, respectively (see Fig. 1, analysis 4). Thus, when analyses 1 and 4 are combined, three clusters can be distinguished in Europe, which correspond largely to the reported invasion routes from Mazamet, Verviers and Bremen (Fig. 2). Since Calais is a confirmed additional introduction centre (Jeanmonod 2002) and distinct from the Verviers cluster (see above) four invasion routes were distinguished in further analyses.

Population structure

The hierarchical AMOVA comprising all samples (Table 2) showed little differentiation between continents (4.2%), but considerable differentiation among populations (14.8%), whereas the majority of genetic variation resided within populations (81.1%). Separate analyses including either of the two European regions and the potential African source region (Africa-N) suggest that the Europe-S region is somewhat less differentiated from the African source region (2.4%) than the Europe-N region (4.6%). In contrast, the Africa-S populations are more strongly differentiated from the Africa-N region (12.9%). Genetic population structure was mainly based on band frequency differences as indi-

Table 2 Hierarchical analyses of molecular variance among and within the native and invaded regions of *S. inaequidens* (AMOVA; Excoffier *et al.* 1992)

	Over all F_{ST}	Percentage of variation		
Source of variation			Among populations	Within populations
Both continents pooled	0.17		17.01	82.99
Africa	0.14		13.54	86.46
Europe	0.16		16.23	83.77
		Among continents	Among populations within continents	Within populations
Africa + Europe	0.19	4.15	14.8	81.05
Africa – North + Europe – North	0.16	4.6	11.09	84.31
Africa – North + Europe – South	0.17	2.43	14.39	83.19
		Among regions	Among populations within regions	Within populations
Africa: North + South	0.20	12.88	7.62	79.5
Africa: North	0.10		9.61	90.39
Africa: South	0.06		6.01	93.99
Europe: North + South	0.20	6.25	13.65	80.1
Europe: North	0.12		12.39	87.61
Europe: South	0.26		25.65	74.35

Significance was tested on 1000 permutations. All differences are significant at $P < 0.001$.

cated by a low number of private fragments (0, 4, 1, and 1 polymorphic bands were only found in Africa-N, Africa-S, Europe-N, and Europe-S, respectively).

Genetic distances were significantly correlated with geographic distances in the entire African sample ($r = 0.6$, Mantel-$P < 0.01$, slope of regression of F_{ST} values against geographical distances = 0.0001) and the Africa-N region ($r = 0.4$, Mantel-$P < 0.01$, slope = 0.0002). A weaker but still significant correlation was found for the entire European sample ($r = 0.3$, Mantel-$P < 0.05$, slope = 0.00006) and the Europe-N region ($r = 0.3$, Mantel-$P < 0.05$, slope = 0.00005).

Genetic diversity within populations

Genetic diversity of populations (*Hj*, *PLP* and B_r) varied between 0.12 and 0.23 (mean 0.18), 0.23–0.58 (mean 0.41) and 1.20–1.44 (mean 1.33) per population, respectively (Table 1). European populations showed a decrease in *Hj* and B_r compared to the Africa-N source populations (*Hj*: $F_{(1,45)} = 16.3$, $P < 0.001$, B_r: $F_{(1,45)} = 14.6$, $P < 0.001$, see also Fig. 4a). This also held after omitting the four strongly bottlenecked European populations (Deggendorf, Genthod, Lausanne, Vilette, Table 1). In contrast, *PLP* did not differ significantly between native and invasive populations ($F_{(1,45)} = 0.4$, $P > 0.05$, see also Fig. 4b). Within Africa, the lowland populations (Africa-S) were slightly more diverse than the highland populations (Africa-N), but the difference was not significant. In Europe, southern populations (Europe-S) were significantly less diverse than the Central European (Europe-N) ones except for *PLP* (*Hj*: $F_{(1,30)} = 6.2$, $P < 0.05$, B_r: $F_{(1,30)} = 4.7$, $P < 0.05$, *PLP*: $F_{(1,30)} = 3.2$, $P > 0.05$; Fig. 4). The different diversity measures showed the same temporal development along the four European invasion routes. All of them increased with population age (*Hj*: $\chi^2_{(1)} = 9.4$, $P < 0.01$, B_r: $\chi^2_{(1)} = 11.7$, $P < 0.001$, *PLP*: $\chi^2_{(1)} = 11.5$, $P < 0.001$, Fig. 5a, b), whereas spread rate was dropped from the minimum adequate model. A comparison of models containing either log-transformed population age or spread rate as explanatory variable further established the higher explanatory power of population age for all three measures [AIC differences: *Hj*: 2.4, B_r: 4.1, *PLP*:5.6, note that population age and spread rate were correlated (Spearman's $\rho = -0.63$, $P > 0.001$)]. Still, spread rate as a single explanatory variable did have a significant negative effect on genetic diversity (*Hj*: $\chi^2_{(1)} = 7.1$, $P < 0.01$, B_r: $\chi^2_{(1)} = 7.6$, $P < 0.01$, *PLP*: $\chi^2_{(1)} = 5.86$, $P < 0.05$, Fig. 5c, d).

Discussion

Introduction, naturalization, and invasion history

Our results go beyond the previous identification of the Maloti and Drakensberg mountains as the source area of European *S. inaequidens* (Lafuma *et al.* 2003) by more finely resolving the ancestry of different European

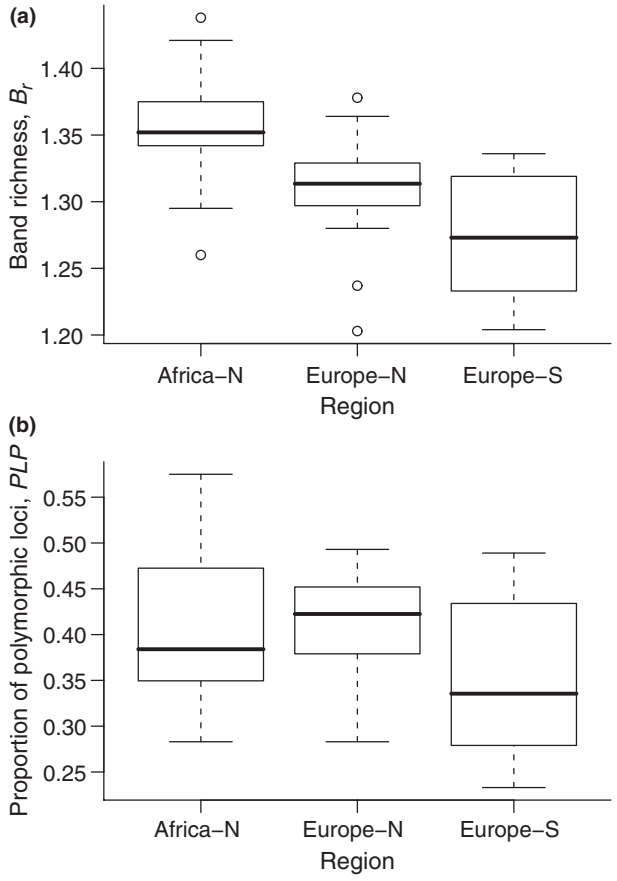

Fig. 4 Band richness B_r (5) (a) and proportion of polymorphic loci *PLP* (5) (b) of populations in the Africa-N source region and European sub-regions. The definition of regions was derived from the results of STRUCTURE analysis 1. Both measures were rarefied to the minimum sample size of five individuals. See Fig. 3 for an explanation of the statistics indicated by the boxplots.

provenances. While Southern European (Europe-S) populations may originate from all over the African highlands (Africa-N), the ancestors of Central European (Europe-N) populations are likely to stem from the Eastern part of these highlands (Figs 1 and 2). This might be explained by Central European introduction sites receiving seed-carrying wool only from the Eastern highlands. However, this explanation seems unlikely because wool from all over the Africa-N region was mixed at various stages of transport (G. Tsekoa, wool trading post Molumong, Lesotho) and because all sampled highland populations had high assignment probabilities to cluster B that invaded Southern Europe (Europe-S; Figs 1 and 2).

An alternative explanation for the differentiation within Europe is that preadapted genotypes from the Eastern highlands were selected after introduction to Central Europe. In the native range, assignment probability to cluster C that invaded the Europe-N region increases with the severity of winter frosts (Fig. 3). If African *S. inaequidens* show local adaptation to climatic conditions, cluster C should be adapted to cold winters (whereas climatic requirements of the widespread cluster B would be less well defined). Although climatic local adaptation (i.e. non-neutral genetic differentiation) of the different clusters needs to be tested experimentally, our finding provides evidence against the hypothesis that cluster C shifted or extended its temperature niche towards lower minimum winter temperatures. For *S. inaequidens*, it was previously stated that introduced plants had to face winter temperatures that were lower than those in their native range (Heger & Böhmer 2005) and that this initially restricted the species' spread in Central and Northern Europe (Ernst 1998; Böhmer 2001). However, our results show that all three introduction sites in the Europe-N region exhibit rather moderate winter temperatures compared to the source region of the invading cluster C (Eastern highlands within Africa-N, Fig. 3). From these introduction sites, the cluster then spread into areas with continuously decreasing temperatures which match the native climate better (Fig. 3). Indeed, warm winter temperatures (or correlated environmental variables such as low summer precipitation) may preclude cluster C from expanding into Southern Europe. Clearly, our simple and correlative bioclimatic model (Fig. 3) cannot identify a specific climatic determinant of genetic differentiation in *S. inaequidens*. Yet, our analysis constitutes a step towards understanding the distribution and the consequences of climatic differentiation within species ranges, which is increasingly claimed (Davis & Shaw 2001). Thus, our analysis suggests climatic niche matching as a specific mechanism of preadaptation, which has been claimed to be important for *S. inaequidens*' invasion of Central Europe (Bossdorf *et al.* 2008).

Invasive spread in Europe

In the STRUCTURE analysis of invasive populations, four small populations (Deggendorf, Genthod, Lausanne, Vilette) formed distinct clusters. We suspect that these distinct clusters are due to founder effects during invasive spread rather than representing additional introduction events. This is because all of these populations were situated at the leading edges of the invasion, were isolated from other populations (personal observation S Lachmuth), and showed very low genetic diversity (Table 1).

The vast majority of invasive populations was clearly assigned to genetic clusters that coincided with reported introduction centers. Two of the four invasion routes spread extensively with Mazamet dominating

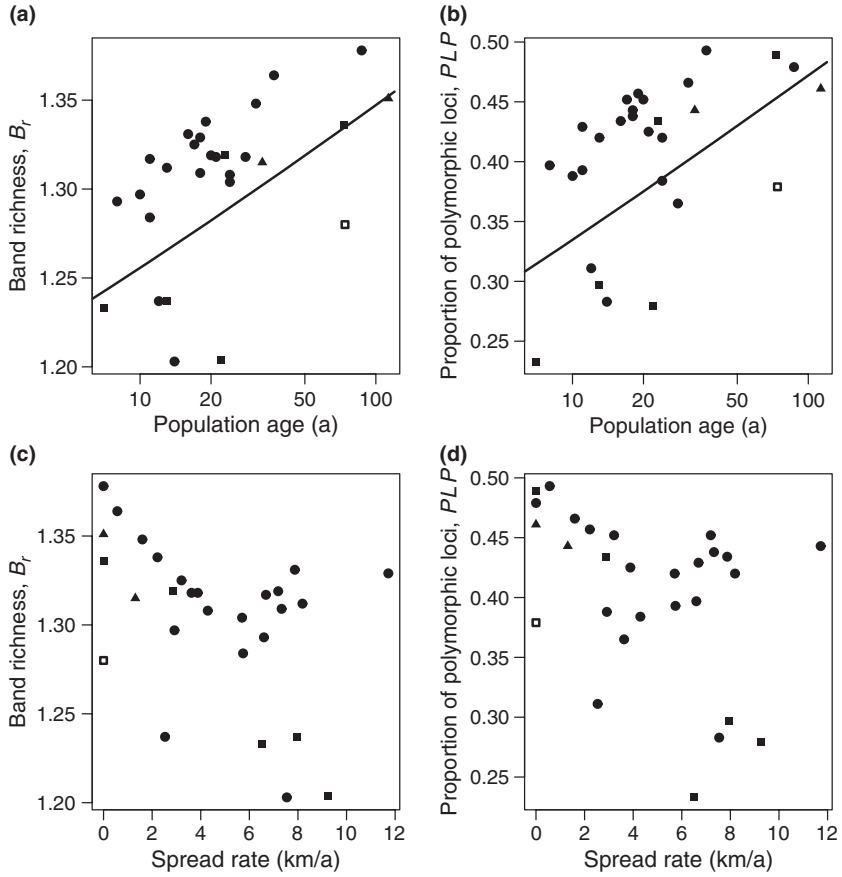

Fig. 5 Influence of population age (a and b) and spread rate (c and d) on band richness B_r (5) (a and c) and proportion of polymorphic loci *PLP* (5) (b and d) in the European range of *S. inaequidens*. Symbols represent invasion routes (▲: Bremen, □: Calais, ■: Mazamet, •: Verviers), and the line indicates the prediction of the corresponding linear mixed-effects models for the significant effect of population age. Note that the *x*-axis of (a and c) is plotted on a log scale. Both measures of genetic diversity were rarefied to the minimum sample size of five individuals and show a complex relationship to spread rate with initial steep decline and greater scatter at higher spread rates.

the southern (Europe-S) and Verviers dominating the Central European (Europe-N) region. The population at Calais could be identified as an introduction site, but apparently expanded neither into our sampling area nor into Central France (INRA 2007). Moreover, it seems to be highly admixed with the Verviers cluster. Bremen, as the oldest introduction site, seems to have colonized a single sampled population (Hanover, Figs 1 and 2) whereas all other sampled populations in its surroundings are dominated by the Verviers cluster (Fig. 2).

The abovementioned findings paint a differentiated picture of *S. inaequidens*' spread across Europe: even though the populations at Verviers and Mazamet established 25 and 40 years after the Bremen population (Table 1), their descendants colonized a much greater part of Europe (Fig. 2). These differences in the spread of individual invasion routes require a reinterpretation of the invasion dynamics of *S. inaequidens*: in contrary to what has been suggested previously (Ernst 1998; Böhmer 2001), the observed 80 years lag phase between introduction to Bremen and spread in surrounding areas was not terminated by demographic, evolutionary or climatic changes that increased the invasiveness of the Bremen population. Instead, the observed lag only ended when the rapidly spreading Verviers route 'overran' the slowly expanding Bremen route. This illustrates how the combination of historical and molecular data can point to the mechanisms generating lag phases in biological invasions.

The history of *S. inaequidens* strikingly exemplifies that the invasion of one and the same species may fail or succeed at different stages of the invasion process (Richardson *et al*. 2000b): some introductions failed (e.g. to the cities of Hanover, Mettmann, Leipzig and Tilburg; Ernst 1998), some resulted in naturalized but stable or slowly spreading populations (Calais and Bremen, Fig. 2), and some developed into rapid invasions

(Verviers and Mazamet, Fig. 2). The success of the Verviers route might be related to the fact that the Verviers population has the highest genetic diversity (Table 1), which may reflect a previously high propagule pressure (*sensu* Lockwood *et al.* 2005). On the other hand, the successful introduction site Mazamet exhibits comparably low genetic diversity. Alternative explanations for the differential success of introductions may be differences in reproductive and dispersal ability of introduced genotypes or differences in the availability, connectivity, and stability of habitat. These questions certainly require empirical investigation for which this now highly resolved system provides a promising basis. Irrespective of the underlying causes, the detected differences among introduction sites emphasize that invasion is not simply a species property: without the introductions to Verviers and Mazamet, *S. inaequidens* would probably not be a rapid invader in Europe. This shows how introductions to multiple sites can increase the probability that some introduced populations pass all barriers from introduction to invasion and thereby enhance species-level invasion success (Lockwood *et al.* 2005; Simberloff 2009).

Our reconstruction of *S. inaequidens'* invasion history suggests a re-examination and potential re-interpretation of previous studies that investigated phenotypic differentiation in this species (Scherber *et al.* 2003; Prati & Bossdorf 2004; Lafuma & Maurice 2007; Bossdorf *et al.* 2008; Caño *et al.* 2009; Garcia-Serrano *et al.* 2009; Monty & Mahy 2009). Studies that included diploid African populations or tetraploid populations that are only distantly related to invasive genotypes may lead to erroneous postulation of adaptive evolution during invasion. In general, the invasive populations should not be treated as one homogeneous group, but their ancestry from the different introduction sites has to be considered. Hence, this study can serve as a guideline for the design of future sampling schemes (Keller & Taylor 2008) and management programs.

Dynamics of genetic diversity during a rapid invasion

We found rather high genetic diversity in most introduction sites (Table 1) and low genetic differentiation between continents (Table 2). Interestingly, only gene diversity H_j and band richness B_r are significantly lower in introduced populations, while proportion of polymorphic loci *PLP* is unaltered. Dlugosch & Parker (2008) found that introduction events affect different measures of genetic diversity in different ways: heterozygosity (representing unevenness of allele frequencies) does not vary between species with single and multiple introductions, whereas losses in allelic richness (representing loss of alleles) can be counteracted by multiple introductions and subsequent admixture. We found no substantial admixture among invasion routes of *S. inaequidens* in Europe but the wool containing *S. inaequidens* seeds has presumably been imported to the different introduction centers in high quantities over many decades. This probably ensured mass-dispersal (Wilson *et al.* 2009) and counteracted a loss of alleles and consequently a reduction of *PLP*.

Once a species has started to spread, genetic diversity is generally expected to decrease towards the invasion front, due to founder events and genetic drift in small founding populations (Austerlitz *et al.* 2000). This is particularly expected in plant species spreading by rare long-distance dispersal events (Austerlitz *et al.* 2000). A decrease of genetic diversity with distance from presumed introduction sites was found in North-American populations of *Phragmites australis* (Saltonstall 2003) and *Lythrum salicaria* (Chun *et al.* 2009) as well as in French populations of *Ambrosia artemisiifolia* (Genton *et al.* 2005). In contrast, high admixture of genetic material from multiple introductions prevented a decrease of diversity even in peripheral invasive populations of *Phalaris arundinacea* in North America and it was suggested that this fostered the species' continued range expansion (Lavergne & Molofsky 2007). To our knowledge, however, no previous study investigated how genetic diversity varies with population age and the spread rate up to population establishment. When analyzing these relationships, we found that genetic diversity significantly increases with population age (Fig. 5a, b), and decreases with spread rate (Fig. 5c, d). Still, population age is a better predictor than spread rate, which is only significant when tested as single explanatory variable. These findings are consistent with spread by stratified or fat-tailed dispersal (Shigesada *et al.* 1995; Clark *et al.* 2001) where long-distance dispersal events form outlying foci that are initially isolated from a continuous front proceeding by diffusive spread. This mode of spread was also suggested for the invasion of *S. madagascariensis* in Hawaii (Le Roux *et al.* 2009). High spread rates mainly result from long-distance jumps which very likely involve founder effects and genetic drift (Austerlitz *et al.* 2000). Our finding of a decrease in genetic diversity with increasing spread rate indicates these reductions of genetic diversity during initial rapid spread. The significant positive effect of population age on genetic diversity suggests that population growth and gene flow along invasion routes efficiently counteract these losses over time. Further support for substantial gene flow comes from the low population differentiation in the invaded range. Gene flow is certainly promoted by the fact that *S. inaequidens* invaded along railways and roads which form a highly connected habitat.

Conclusions

By reconstructing the invasion history of *S. inaequidens* in Europe we showed that different introduction centers had very different invasion success. Our results suggest that multiple introductions, environmental preadaptation, and the maintenance of high genetic diversity throughout the invasion process contributed to the eventual success of this rapid invader. More generally, this study demonstrates the benefit of combining genetic, historical, and climatic data for understanding the introduction, naturalization, and invasion of exotic species.

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References

Allee WC (1931) *Animal Aggregations, A Study in General Sociology*. University of Chicago Press, Chicago.

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.

Barrett SCH, Richardson BJ (1986) Genetic attributes of invading species. In: *Ecology of Biological Invasions: An Australian Perspective* (eds Groves RH, Burdon JJ), pp. 21–33, Australian Academy of Science, Canberra.

Becker RA, Wilks AR (Original S Code) *et al.* (2008) *maps: Draw Geographical Maps*. R package version 2.0-40.

Böhmer HJ (2001) Das Schmalblättrige Greiskraut (*Senecio inaequidens* DC. 1837) in Deutschland – eine aktuelle Bestandsaufnahme. *Floristische Rundbriefe*, **35**, 47–54.

Bornkamm R (2002) Die weitere Ausbreitung von *Senecio inaequidens* DC. in Berlin und dem südwestlich angrenzenden Brandenburg in den Jahren 1998-2001. *Verhandlungen des Botanischen Vereins Berlin-Brandenburg*, **135**, 25–40.

Bornkamm R, Prasse R (1999) Die ersten Jahre der Einwanderung von *Senecio inaequidens* DC. in Berlin und dem südwestlich angrenzenden Brandenburg. *Verhandlungen des Botanischen Vereins Berlin-Brandenburg*, **132**, 131–139.

Bossdorf O, Auge H, Lafuma L *et al.* (2005) Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, **144**, 1–11.

Bossdorf O, Lipowsky A, Prati D (2008) Selection of preadapted populations allowed *Senecio inaequidens* to invade Central Europe. *Diversity and Distributions*, **14**, 676–685.

Broennimann O, Treier UA, Müller-Schärer H *et al.* (2007) Evidence of climatic niche shift during biological invasion. *Ecology Letters*, **10**, 701–709.

Caño L, Escarré J, Vrieling K, Sans FX (2009) Palatability to a generalist herbivore, defence and growth of invasive and native *Senecio* species: testing the evolution of increased competitive ability hypothesis. *Oecologia*, **159**, 95–106.

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.

Clark JS, Lewis M, Horvath L (2001) Invasion by extremes: population spread with variation in dispersal and reproduction. *The American Naturalist*, **157**, 537–554.

Coart E, Van Glabeke S, Petit RJ, Van Bockstaele E, Roldan-Ruiz I (2005) Range wide versus local patterns of genetic diversity in hornbeam (*Carpinus betulus* L.). *Conservation Genetics*, **6**, 259–273.

Courchamp F, Clutton-Brock T, Grenfell B (1999) Inverse density dependence and the Allee effect. *Trends in Ecology and Evolution*, **14**, 405–410.

Crawford KM, Whitney KD (2010) Population genetic diversity influences colonization success. *Molecular Ecology*, **19**, 1253–1263.

Crawley MJ (2002) *Statistical Computing. An Introduction to Data Analysis Using S-Plus*, John Wiley, New York, NY.

Datenbank Farn- und Blütenpflanzen (2008), Environmental protection office Saxony-Anhalt, Germany.

Davis MB, Shaw RG (2001) Range shifts and adaptive responses to Quaternary climate change. *Science*, **292**, 673–679.

Dimande AFP, Botha CJ, Prozesky L *et al.* (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 (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431–449.

Dray S, Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software*, **22**, 1–20.

Düring C (1997) *Senecio inaequidens* DC. auch in Nordostbayern in Ausbreitung. *Hoppea*, **58**, 385–388.

Ehrich D (2006) AFLPDAT: a collection of R functions for convenient handling of AFLP data. *Molecular Ecology Notes*, **6**, 603–604.

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.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes – application to human mitochondrial-DNA restriction data. *Genetics*, **131**, 479–491.

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics*, **1**, 47–50.

Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, **7**, 574–578.

FLORON database (2009), FLORON foundation, Leiden, The Netherlands. From: http://www.natuurloket.nl.

Furrer R, Nychka D, Sain S (2008) *fields: tools for spatial data*. R package version 5.01. From: http://www.image.ucar.edu/Software/Fields.

Garcia-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 malacitanus* in Mediterranean climate. *Canadian Journal of Botany-Revue Canadienne de Botanique*, **82**, 1346–1355.

Garcia-Serrano H, Caño L, Escarré J, Fleck I, Sans FX (2009) Physiological comparison of alien *Senecio inaequidens* and *S. pterophorus* and native *S. malacitanus*: implications for invasion. *Flora*, **204**, 445–455.

Genton BJ, Shykoff JA, Giraud T (2005) High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple sources of introduction. *Molecular Ecology*, **14**, 4275–4285.

Guillerm JL, Le Floc'h E, Maillet J, Boulet C (1991) The invading weeds within the Western Mediterranean Basin. In: *Biological Invasions in Europe and the Mediterranean Basin* (eds di Castri F, Hansen AJ, Debussche M), pp. 61–84, Kluwer, Dordrecht.

Heger T, Böhmer HJ (2005) The invasion of Central Europe by *Senecio inaequidens* DC– A complex biogeographical problem. *Erdkunde*, **59**, 34–49.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hilliard OM (1977) *Compositae in Natal*. University of Natal Press, Pietermaritzburg.

Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M (2008) Ecological consequences of genetic diversity. *Ecology Letters*, **11**, 609–623.

INRA (2007) Hypermedia for Plant Protection- Weeds. From: http://www.dijon.inra.fr/bga/hyppa/hyppa-a/seniq_ah.htm.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801–1806.

Jeanmonod D (2002) Nouvelles stations pour le *Senecio inaequidens* DC. ou la conquête irrésistible du séneçon du Cap. *Saussurea*, **32**, 43–50.

Kehren W (1995) Ausbreitungstendenzen von *Senecio inaequidens* DC. im Stadtbereich von Köln-Deutz (MTB 5007 Köln). *Floristische Rundbriefe*, **29**, 177–180.

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.

König P (1995) *Senecio inaequidens* – nun auch in Berlin. *Verhandlungen des Botanischen Vereins Berlin-Brandenburg*, **128**, 159–163.

Kuhbier H (1977) *Senecio inaequidens* DC.- ein Neubürger der nordwestdeutschen Flora. *Abhandlungen des Naturwissenschaftlichen Vereins Bremen*, **38**, 383–396.

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.

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.

Lavergne S, Molofsky J (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 3883–3888.

Le Roux JJ, Wieczorek AM (2007) Isolation and characterization of polymorphic microsatellite markers from fireweed, *Senecio madagascariensis* Poir (Asteraceae). *Molecular Ecology Notes*, **7**, 327–329.

Le Roux JJ, Wieczorek AM, Ramadan MM, Tran CT (2006) Resolving the native provenance of invasive fireweed (*Senecio madagascariensis* Poir.) in the Hawaiian Islands as inferred from phylogenetic analysis. *Diversity and Distributions*, **12**, 694–702.

Le Roux JJ, Wieczorek AM, Tran CT, Vorsino AE (2009) Disentangling the dynamics of invasive fireweed (Senecio madagascariensis Poir. species complex) in the Hawaiin Islands. *Biological Invasions*, DOI 10.1007/s10530-009-9635-9.

Lewis MA, Kareiva P (1993) Allee dynamics and the spread of invading organisms. *Theoretical Population Biology*, **43**, 141–158.

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.

Melzer H, Barta T (1991) Neues zur Flora des Burgenlandes, von Niederösterreich und Wien. *Linzer biologische Beiträge*, **23**, 575–592.

Millennium Ecosystem Assessment Board (2005) *Ecosystems and Human Well-Being: General Synthesis*. Island Press, Washington DC.

Monty A, Mahy G (2009) Clinal differentiation during invasion: *Senecio inaequidens* (Asteraceae) along altitudinal gradients in Europe. *Oecologia*, **159**, 305–315.

Nathan R, Schurr FM, Spiegel O, Steinitz O, Trakthenbrot A, Tsoar A (2008) Mechanisms of long-distance seed dispersal. *Trends in Ecology and Evolution*, **23**, 638–647.

Neuffer B, Hurka H (1999) Colonization history and introduction dynamics of *Capsella bursa-pastoris* (Brassicaceae) in North America: isozymes and quantitative traits. *Molecular Ecology*, **8**, 1667–1681.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core team (2008) *nlme: Linear and Nonlinear Mixed Effect Models*. R package version 3.1-89.

Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: causes consequences, and solutions. *Nature Reviews Genetics*, **6**, 847–859.

Prati D, Bossdorf O (2004) A comparison of native and introduced populations of the South African Ragwort *Senecio inaequidens* DC. in the field. In: *Results of Worldwide Ecological Studies* (eds Breckle SW, Schweizer B, Fangmeier A), pp. 353–359, Verlag Günther Heimbach, Stuttgart.

Prentis PJ, Sigg DP, Raghu S *et al.* (2009) Understanding invasion history: genetic structure and diversity of two globally invasive plants and implications for their management. *Diversity and Distributions*, **15**, 822–830.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Pritchard JK, Xiaoquan W, Falush D (2007) Documentation for structure software: Version 2.2. From: http://pritch.bsd.uchicago.edu/software/structure22/readme.pdf.

Pŷsek P (1998) Is there a taxonomic pattern to plant invasions? *Oikos*, **82**, 282–294.

R Development Core Team (2008) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Form: http://www.R-project.org.

Radford IJ, Muller P, Fiffer S, Michael PW (2000) Genetic relationships between Australian fireweed and South African and Madagascan populations of *Senecio madagascariensis* Poir. and closely related *Senecio* species. *Australian Systematic Botany*, **13**, 409–423.

Radkowitsch A (1997) *Senecio inaequidens* DC- ein Beitrag zur Verbreitung in Deutschland unter besonderer Berücksichtigung von Bayern. *Hoppea*, **58**, 389–404.

Richardson DM, Allsopp N, D'Antonio CM, Milton SJ, Rejmánek M (2000a) Plant invasions – the role of mutualisms. *Biological Reviews*, **75**, 65–93.

Richardson DM, Pŷsek P, Rejmánek M *et al.* (2000b) Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions*, **6**, 93–107.

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4**, 137–138.

Ross C, Auge H, Durka W (2008) Genetic relationships among three native North-American *Mahonia* species, invasive *Mahonia* populations from Europe, and commercial cultivars. *Plant Systematics and Evolution*, **275**, 219–229.

Sakai AK, Allendorf FW, Holt JS *et al.* (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305–332.

Sala OE, Chapin FS, Armesto JJ *et al.* (2000) Biodiversity – Global biodiversity scenarios for the year 2100. *Science*, **287**, 1770–1774.

Saltonstall K (2003) Microsatellite variation within and among North American lineages of *Phragmites australis*. *Molecular Ecology*, **12**, 1689–1702.

Sax DF, Stachowicz JJ, Brown JH *et al.* (2007) Ecological and evolutionary insights from species invasions. *Trends in Ecology & Evolution*, **22**, 465–471.

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.

Schurr FM, Steinitz O, Nathan R (2008) Plant fecundity and seed dispersal in spatially heterogeneous environments: models, mechanisms and estimation. *Journal of Ecology*, **96**, 628–641.

Scott LJ, Congdon BC, Playford J (1998) Molecular evidence that fireweed (*Senecio madagascariensis,* Asteraceae) is of South African origin. *Plant Systematics and Evolution*, **213**, 251–257.

Sebald O, Seybold S, Philippi G, Wörz A (1996) *Die Farn- und Blütenpflanzen Baden-Württembergs*. Ulmer, Stuttgart.

Shigesada N, Kawasaki K, Takeda Y (1995) Modeling Stratified Diffusion in Biological Invasions. *American Naturalist*, **146**, 229–251.

Simberloff D (2009) The role of propagule pressure in biological invasions. *Annual Review of Ecology Evolution and Systematics*, **40**, 81–102.

Stöcklin J, Schaub P, Ojala O (2003) Häufigkeit und Ausbreitungsdynamik von Neophyten in der Region Basel: anlass zur Besorgnis oder Bereicherung? *Bauhinia*, **17**, 11–23.

SysTax database (2009), University of Ulm, Germany, From: http://www.biologie.uni-ulm.de/cgi-bin/query_all/details.pl?id=31439&stufe=A&typ=PFL.

Taylor CM, Hastings A (2005) Allee effects in biological invasions. *Ecology Letters*, **8**, 895–908.

Vekemans X, Beauwens T, Lemaire M, Roldan-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.

Vos P, Hogers R, Bleeker M *et al.* (1995) Aflp – A New Technique for Dna-Fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.

Werner DJ, Rockenbach T, Hölscher ML (1991) Herkunft, Ausbreitung, Vergesellschaftung und Ökologie von *Senecio inaequidens* DC. unter besonderer Berücksichtigung des Köln-Aachener Raumes. *Tuexenia*, **11**, 73–107.

Whitlock R, Hipperson H, Mannarelli M, Butlin RK, Burke T (2008) An objective, rapid and reproducible method for scoring AFLP peak-height data that minimizes genotyping error. *Molecular Ecology Resources*, **8**, 725–735.

Wilson JRU, Dormontt EE, Prentis PJ, Lowe AJ, Richardson DM (2009) Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology & Evolution*, **24**, 136–144.

Zhivotovsky LA (1999) Estimating population structure in diploids with multilocus dominant DNA markers. *Molecular Ecology*, **8**, 907–913.

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Supporting information

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

Data S1 Supporting information including a description of the invasion hisotry of *Senecio inaequidens* in Europe and the detailed laboratory protocol of the AFLP and microsatellite analyses.

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Supporting information

Invasion history of S. inaequidens in Europe

In the following, we briefly summarize the spread of *S. inaequidens* in the European sampling range with special emphasis on records in the vicinity of the four documented introduction sites (Bremen, Verviers, Calais, and Mazamet). In the surroundings of Bremen and in Hanover, the species was recorded in the 1960s and 1970s (Kuhbier 1977). In 1985 the species was reported from Kassel (Böhmer 2001). In the vicinity of the Verviers introduction site, *S. inaequidens* was regarded as naturalised in the Vesdre Valley in 1955 and reached Germany's Western border in the 1970s (Werner *et al.* 1991). Subsequently, spread was documented into Northern Switzerland (Jeanmonod 2002), South-Eastern Germany (Radkowitsch 1997), and North Western Germany. In the latter area, admixture with the Bremen provenance around 1990 and subsequent rapid spread towards Eastern Germany has been proposed (Werner *et al.* 1991, Böhmer 2001). *S. inaequidens* rapidly colonised the Netherlands in the 1960s (see Kuhbier 1977 for detailed description) and connected to the Western German range in Osnabrück (W.H.O. Ernst, VU University Amsterdam). We are not aware of eastward spread from Calais into the study area, but *S. inaequidens* was reported to colonise the surrounding dune areas (Kuhbier 1977). In the vicinity of Mazamet the species spread towards the Mediterranean coast reaching the region of Montpellier around 1975 (Kuhbier 1977). Subsequently, it was observed to proceed northwards along the Rhone valley and further eastwards reaching Lake Genève in Switzerland around 1995 (Jeanmonod 2002). Nowadays the species is distributed all over Europe from Spain to the British Isles in the West to Norway, Belarus and Hungary in the East (Heger & Böhmer 2006, W.H.O. Ernst, VU University Amsterdam).

AFLP and microsatellite analysis

DNA was extracted from 5 mg dry leaf tissue with the DNeasy 96 Plant Kit (QIAGEN). Three microsatellite loci (SE 220, SE176, SE116) previously described from *S. madagascariensis* (Le Roux & Wieczorek 2007) were analyzed in one multiplex PCR reaction using the multiplex PCR kit (QIAGEN) following manufacturers prescriptions in a total volume of 10 µl. Forward primers where labelled with PET fluorescent dye.

We analyzed amplified fragment length polymorphisms (AFLP, Vos *et al*. 1995) using, in short, the following protocol. For restriction and ligation 6 µl of template DNA (appr. 100 ng DNA) were combined with 5 µl of restriction-ligation (RL) reaction mix containing 0.05 µl H_2O (HPLC-grade, Merck), 0.55 µg BSA (New England Biolabs, NEB), 0.11 M NaCl, 5 u *Eco*RI (NEB), 1 u *Mse*I (NEB), 1.1 µl T4 DNA ligase buffer (NEB), 67 u T4 DNA ligase (NEB), 1 µl *Mse*I adapter (50 mM) and 1 µl *Eco*RI adapter (5 mM). The reaction was incubated 2 h at 37 °C and diluted 1:5. For preselective amplification 4 µl of RL template were combined with 16 µl of preselective PCR reaction mix containing 1.5 ng/µl of *Mse*I- and *Eco*RI preselective primers each, 200 µM dNTPs (Roth), 2 µl PCR buffer + $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.8 u Taq polymerase (Fermentas) and 9.64 µl H_2O. The thermocycler protocol was 72.0°C (2 min) followed by 20 cycles of 94.0°C (20sec), 56.0°C (30sec) and 72.0°C (2 min) and a final step at 60.0°C (30 min). For selective amplification, 1 µl of the preselective PCR products (diluted 1:10) was added to 2.2 µl of Multiplex PCR kit (QIAGEN) and 0.6 µl of *Mse*I (5 mM) and 0.6 µl *Eco*RI (1 mM) selective primers. The thermocycler protocol was 94.0°C (2 min) followed by 10 cycles of 94.0°C (20 sec), 66.0°C (30 sec, decreasing 1°C per cycle) and 72.0°C (2 min) and 20 cycles of 94.0°C (20 sec), 56.0°C (30 sec) and 72.0°C (2 min), and a final step at 60.0°C (30 min).

References

Böhmer HJ (2001) Das Schmalblättrige Greiskraut (*Senecio inaequidens* DC. 1837) in Deutschland - eine aktuelle Bestandsaufnahme. *Floristische Rundbriefe*, **35**, 47-54.

Heger T, and Böhmer HJ (2006) NOBANIS – Invasive Alien Species Fact Sheet – *Senecio inaequidens.* – From: Online Database of the North European and Baltic Network on Invasive Alien Species – NOBANIS www.nobanis.org.

Jeanmonod D (2002) Nouvelles stations pour le *Senecio inaequidens* DC. ou la conquête irrésistible du séneçon du Cap. *Saussurea*, **32**, 43-50.

Le Roux JJ, Wieczorek AM (2007) Isolation and characterization of polymorphic microsatellite markers from fireweed, *Senecio madagascariensis* Poir. (Asteraceae). *Molecular Ecology Notes*, **7**, 327-329.

Kuhbier H (1977) *Senecio inaequidens* DC.- ein Neubürger der nordwestdeutschen Flora. *Abhandlungen des Naturwissenschaftlichen Vereins Bremen*, **38**, 383-396.

Radkowitsch A (1997) Senecio inaequidens DC. - ein Beitrag zur Verbreitung in Deutschland unter besonderer Berücksichtigung von Bayern. *Hoppea*, **58**, 389-404.

Vos P, Hogers R, Bleeker M *et al.* (1995) AFLP - a new technique for DNA-fingerprinting. *Nucleic Acids Research*, **23**, 4407-4414.

Werner DJ, Rockenbach T, Hölscher ML (1991) Herkunft, Ausbreitung, Vergesellschaftung und Ökologie von *Senecio inaequidens* DC. unter besonderer Berücksichtigung des Köln-Aachener Raumes. *Tuexenia*, **11**, 73-107.