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HIGHLY REDUCED GENETIC DIVERSITY OF *ROSA RUBIGINOSA* L. POPULATIONS IN THE INVASIVE RANGE

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Rosa rubiginosa (Rosaceae) populations introduced to Argentina successfully invade various habitats, forming extensive impenetrable thickets. To investigate the consequences of founder events and to track the native origin of Argentinean populations, the genetic diversity of invasive *R. rubiginosa* populations was compared with that of native populations in Europe, and genetic similarity was assessed between groups. We sampled 13 Argentinean populations and 20 native populations in Germany and Spain, and we applied two molecular marker techniques (simple sequence repeats and random amplification of polymorphic DNA [RAPD]). Genetic diversity within the invasive range was clearly lower than it was in the native range. Principle coordinate analysis and between-class analysis did not reveal the exact European origin of the invasive populations, but our data suggest that at least one Argentinean population originated in Germany. Overall, the strong similarity of RAPD and allelic phenotypes throughout Argentina suggests a limited number of introduction events, that the species spread through human transport, and that the few genetic phenotypes present in the species were conserved largely unaltered as a result of mainly asexual reproduction.

Keywords: Rosaceae, genetic diversity, invasive shrub, native source population, South America.

Online enhancement: appendix.

Introduction

A single seed or ramet might be sufficient to establish a reproducing colony (Baker 1955). This fact is specifically exemplified by invasive populations that are usually founded by a small number of individuals carrying only a fraction of the original genetic variation (Barrett et al. 2008). Among colonizing plants, selfing species are known to be highly common (Price and Jain 1981). Populations of selfing plants have the advantage of growing fast after establishment, yet further spread should be facilitated by recombination and microevolution (Eckert 2002) since invasive species have to adapt to changing local environmental conditions during range expansion (Barrett et al. 2008). Studies on the genetic diversity of invasive populations in comparison with populations in their native range can provide valuable insights into genetic bottleneck processes (Barrett and Kohn 1991; Carter and Sytsma 2001; Bossdorf et al. 2008), yet comparative studies on alien plants are still rare (Lambrinos 2001; Novak and Mack 2005). Moreover, the circumstances of the immigration history of an invasive species must be investigated, since multiple introductions into the new environment may reduce founder effects (Pappert et al. 2000; Bartlett et al. 2002; Novak and Mack 2005). Examples are provided by the rangeland weed *Centaurea diffusa* Lam., for which multiple introductions have facilitated its invasion throughout North America (Marrs et al. 2008), and by *Cortaderia selloana* Asch and Graebn., the extensive use of which in landscaping has triggered an expansion of its invasive range (Okada et al. 2007).

Rosa rubiginosa L. (Rosaceae) represents a suitable species for the study of founder event effects, as it is native to Eurasia and has been introduced to several countries in the southern hemisphere, including Argentina (Weber 2003). *Rosa rubiginosa* (sweet briar) can be expected to be a good colonizer since it is a self-fertile, apomictic, clonal species; then again, it is also able to reproduce sexually, therefore enabling recombination and microevolution. In this study, native and invasive *R. rubiginosa* populations throughout an extreme geographic and climatic range were investigated. This shrub invades disturbed and seminatural communities in Argentina (Bran et al. 2004)\, building monodominant stands, some of which cover hundreds of square meters. Overall, among-population genetic diversity is expected to be higher than within-population genetic diversity in selfing species; however, without knowing the number of introduction events, genetic diversity cannot be foreseen solely on the mode of reproduction (Novak and Mack 2005). The immigration history of this invasive plant in

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Argentina is still unresolved, and the four following possible scenarios underpin the ongoing debate on the subject: Spanish (scenario 1) or German (scenario 2) colonizers may have introduced *R. rubiginosa* directly to Argentina, or either of these colonizers (scenarios 3 and 4, respectively) introduced *R. rubiginosa* to Chile, and from there it was brought to Argentina (Damascos 1992; Joublan et al. 1996). The introduction of the plant to Chile probably occurred before its introduction to Argentina, since the first record of *R. rubiginosa* in Chile dates back to 1875 (Matthei 1995) while the first introduction to Patagonia occurred around 1910 (Damascos 1992).

We used two different molecular marker techniques to study genetic diversity as a measure for founder events in *R. rubiginosa*, namely, random amplification of polymorphic DNA (RAPD) and microsatellites. Simple sequence repeats (SSR) or microsatellites are codominantly inherited, making them valuable tools for the analysis of migration patterns and founder events (Besnard et al. 2007; Okada et al. 2007). The main advantage of SSR is the associated identification of individual alleles, which indicates the level of heterozygosity. However, as *R. rubiginosa* is pentaploid, thereby having usually two identical copies of at least one allele from each locus (Nybom et al. 2004, 2006), the status of heterozygosity cannot be determined. Therefore, we also performed RAPD analysis, which is an established standard dominant marker system and which might be better suited than microsatellites to the study of polyploid genotypes (Budak et al. 2005).

The aim of our study was to address the following questions: (1) Do invasive *R. rubiginosa* populations have lower levels of genetic diversity than native populations? (2) Can we derive clues on the native origin of these invasive populations by analyzing the most likely source populations with the help of molecular markers? (3) Do results differ for SSR and RAPD markers?

Material and Methods

Study Species and Study Region

The shrub *Rosa rubiginosa*, in the family Rosaceae, belongs to the section *Caninae* (DC.) Ser., which is characterized by its unique heterogamous meiotic system (Wissemann 1999). *Rosa rubiginosa* is pentaploid, with 35 chromosomes; a fifth of these chromosomes are transmitted through pollen (Täckholm 1920, 1922; Blackburn and Harrison 1921). During *Canina* meiosis, two pairing genomes form bivalents and an additional three nonpairing genomes form univalents. The subsequent migration of the chromosomes in female and male meiosis differ insofar as the functional egg cell is tetraploid, with one bivalent genome and three univalent genomes (28 chromosomes), whereas the pollen grain is haploid, with one bivalent genome (seven chromosomes). Therefore, sexual reproduction is secured in spite of uneven chromosome numbers. In addition, *R. rubiginosa* is able to produce apomictic seeds, and it also spreads vegetatively by root suckers (Werlemark 2000).

In Germany and Spain, *R. rubiginosa* grows up to 3 m in height, mostly forming sparse populations of less than 20 individuals (H. Zimmermann, unpublished observation). It occurs on dry grasslands and scrub communities in lowlands as well as in montane regions over 1000 m a.s.l. In the south of Argentina (Patagonia), *R. rubiginosa* individuals outgrow their ancestors in Europe in both number and size, and they outcompete native species (Damascos 1992). This species, with its squarrose branches, may climb trees up to heights of 10 m. Populations here consist of thousands of shrubs forming dense thickets in a broad variety of disturbed habitats along roads, on fallows, in *Austrocedrus chilensis* Florin and Boutelje forests of the lower montane zone, and in *Nothofagus* Blume forest clearings. In central Argentina, *R. rubiginosa* is invasive in the sense that it replaces natural plant communities (Richardson et al. 2000), but it is not as widespread there as it is in Patagonia. It can be found in various disturbed habitats, such as lower-montane *Fagara coco* Engl. forests, which experience recurring fire events, or *Pinus* L. plantations, as well as in higher-montane grazed scrub-grassland communities (up to 2000 m a.s.l.). The earliest herbarium record of *R. rubiginosa* in the province of Córdoba, in central Argentina, dates from 1957 (Museo Botanico Córdoba, 20433). Therefore, the limited distribution of the species here in comparison to that in Patagonia is probably due to its comparatively later introduction rather than to less favorable abiotic or biotic conditions.

Sampling

For this study, 14 invasive *R. rubiginosa* populations in central Argentina (Córdoba; six populations), Patagonia (Río Negro and Neuquén; seven populations), and Chile (Concepción, one population) were sampled in 2006 and 2007, as were 20 native populations in Germany (13 populations) and Spain (seven populations; fig. 1; table 1). Populations were located using local floras (Castroviejo 1998; Fukarek and Henker 2005) and expert knowledge (see "Acknowledgments"). A population was defined as a group of plants separated from their closest conspecific by more than 4 km. Population sizes were examined by estimating the area covered by *R. rubiginosa*, which usually varied between 0.03 km^2 and >3 km^2 in South America, whereas in Europe it was between 0.009 km^2 and 0.09 km^2. For South America, in most cases, 10 to 16 individuals were randomly sampled, while in Europe, sample size per population was often less than 10 individuals, mostly due to the small population sizes (table 1). In order to achieve comparable results, distances between sampled individuals were at least 3 m. In South America, random coordinates were located with a GPS in areas that contained *R. rubiginosa* populations. At the given coordinates, study plots of 50×50 m were established and subdivided into 25 100-m^2 grid cells. In the center of each cell, we sampled the leaves of one *R. rubiginosa* shrub. Therefore, in South America, samples were collected from areas no larger than 0.0025 km^2, whereas in Europe, because of the sparse population density, sampling was always conducted over the maximum population extension (up to 0.09 km^2). Leaves were immediately stored in silica gel.

DNA Extraction and Final Sample Sizes

Total DNA was extracted from 25 mg of dried leaf material using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions (QIAGEN 2006). DNA concentrations were standardized to 10 ng μL^{-1}. The final RAPD analysis covered a total of 86 individuals from the German populations, 32 individuals

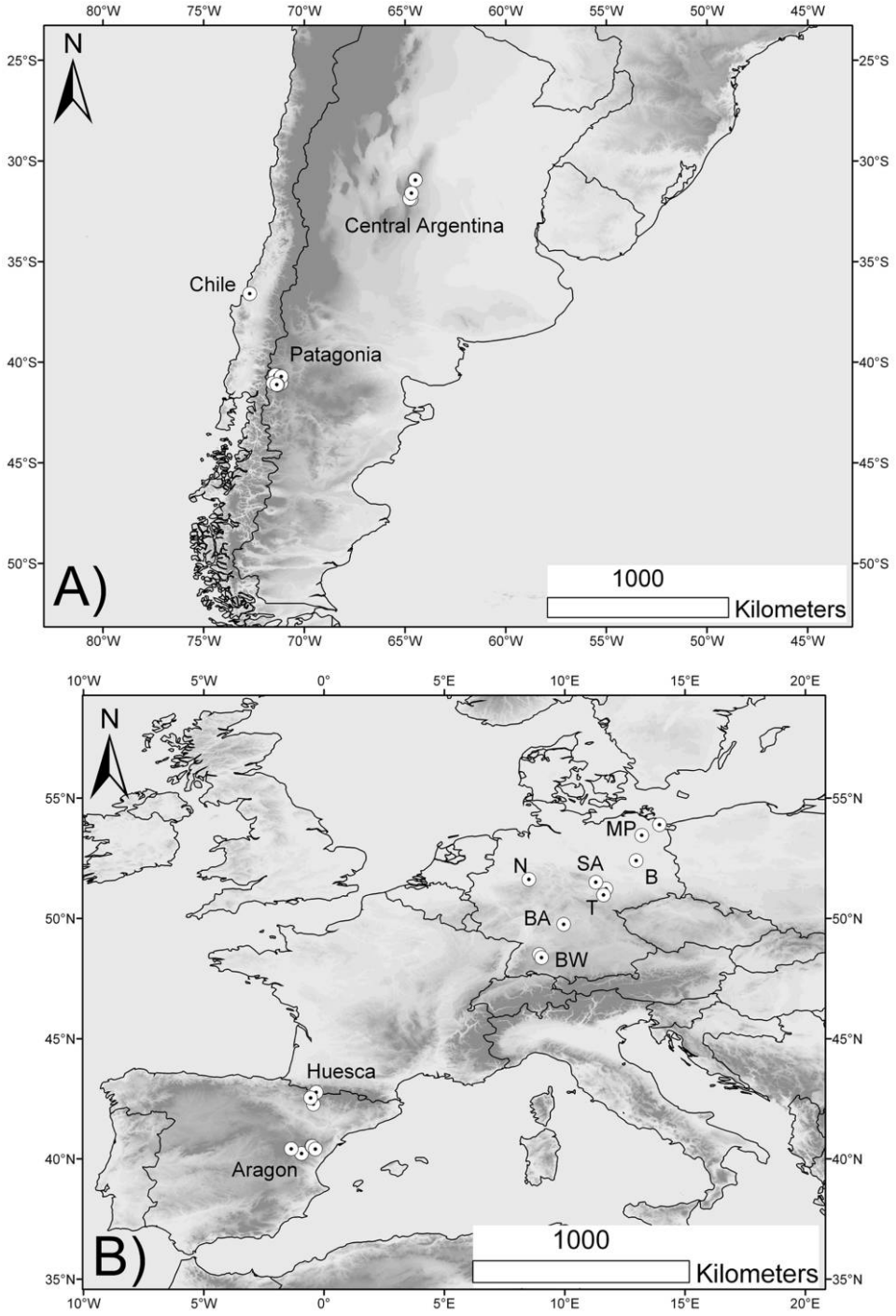

Fig. 1 Sampled populations of *Rosa rubiginosa* (circles) in (*A*) Argentina and Chile and (*B*) Germany and Spain. For Germany, the provinces are labeled as follows: *BW* = Baden Württemberg, *BA* = Bayern, *N* = Niedersachsen, *T* = Thüringen, *SA* = Sachsen-Anhalt, *B* = Brandenburg, and *MP* = Mecklenburg-Vorpommern.

from the Spanish populations, and 141 individuals from Argentina and Chile (table 1). However, as RAPD analyses revealed that the South American populations were highly homogenous, the number of individuals in the South American population was reduced to five for the subsequent microsatellite analysis (see "Statistical Analysis" and table 1).

RAPD Amplification

An initial screening of 60 RAPD primers resulted in the selection of six of them (Roth, Karlsruhe, Germany; table 2). Amplification of double-stranded DNA was performed in 10 μL containing 0.8 μL DNA, 0.6 μL primer (10 pmol μL^{-1}), 1 μL 2 mM deoxyribonucleotide triphosphates (dNTPs; QBio-

Table 1

Origin and Number of *Rosa rubiginosa* Samples Analyzed Using Random Amplification of Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR) Markers

Country, region, province, identification	Latitude	Longitude	No. samples	H_p		J	
				RAPD	SSR	RAPD	SSR
Argentina:							
Central Argentina:							
Córdoba:							
i	−31.60	−64.67	5 (10)	.01 (.02)	.00	.08	.00
a	−31.63	−64.68	5 (7)	.00 (.01)	.00	.00	.00
b	−31.88	−64.76	5 (10)	.00 (.01)	.00	.05	.00
p	−31.91	−64.68	5 (10)	.03 (.04)	.00	.19	.02
o	−30.91	−64.49	4 (10)	.01 (.02)	.00	.07	.02
c	−30.95	−64.47	5 (10)	.01 (.01)	.00	.05	.00
Patagonia:							
Río Negro:							
l	−41.05	−71.54	5 (8)	.01 (.01)	.00	.05	.00
t	−41.12	−71.36	5 (10)	.00 (.01)	.00	.02	.00
A	−41.13	−71.32	5 (10)	.00 (.01)	.00	.03	.00
Neuquén:							
s	−41.04	−71.15	5 (10)	.00 (.003)	.00	.00	.00
f	−40.72	−71.14	5 (10)	.00 (.01)	.00	.03	.00
w	−40.67	−71.31	5 (10)	.01 (.01)	.00	.08	.00
n	−40.65	−71.43	5 (10)	.01 (.01)	.00	.06	.00
Chile:							
Chile:							
Concepción:							
*	−36.60	−72.71	5 (16)	.00 (.02)	.00	.03	.00
Germany:							
Germany:							
Brandenburg:							
T	52.39	12.97	6	.06	.02	.36	.12
Sachsen-Anhalt:							
N	51.22	11.72	10	.06	.01	.30	.05
Thüringen:							
H	51.49	11.29	4	.01	.00	.08	.00
K	51.42	10.99	3	.06	.005	.20	.05
J	50.96	11.61	10	.05	.02	.22	.09
Niedersachsen:							
G	51.61	8.51	5	.02	.02	.15	.12
Bayern:							
W	49.74	9.96	7	.05	.02	.28	.13
Baden-Württemberg:							
R	48.49	8.94	2	.02	.02	.29	.33
B	48.36	9.04	3	.06	.03	.51	.26
Mecklenburg-Vorpommern:							
I	53.44	13.20	6	.02	.00	.11	.00
U	53.88	13.93	10	.06	.02	.27	.08
M	54.00	13.85	6	.02	.00	.14	.00
D	54.46	12.51	14	.002	.003	.01	.02
Spain:							
Spain:							
Aragon:							
P	40.21	−.92	6	.08	.05	.54	.33
F	40.53	−.48	4	.06	.05	.41	.36
Q	40.40	−.35	4	.03	.05	.26	.34
E	40.43	−1.39	5	.07	.07	.43	.42
Huesca:							
O	42.53	−.54	5	.12	.06	.49	.34
V	42.61	−.48	5	.10	.06	.56	.35
Z	42.76	−.32	3	.07	.05	.58	.39
Total			187 (259)				

Note. The sample identification refers to the principal coordinate analysis plot (fig. 3). Latitude and longitude are given in decimal degrees. Numbers in parentheses for the South American data give the total number of samples for South America (SSR analyses were conducted with the reduced numbers of samples only; see "Material and Methods" for explanation). H_p is the genetic diversity within populations (see also fig. 2), and J is the mean Jaccard dissimilarity of each population for the RAPD and the SSR data sets.

Table 2

Sequences of Random Amplification of Polymorphic DNA Primers and Numbers of Polymorphic Bands Amplified

Primer identification	Sequence	No. polymorphic bands
Roth A-04	5′-AATCGGGCTG-3′	11
Roth A-08	5′-GTGACGTAGG-3′	6
Roth A-09	5′-TCGGCGATAG-3′	6
Roth A-12	5′-TCGGCGATAG-3′	5
Roth A-18	5′-AGGTGACCGT-3′	2
Roth A-20	5′-GTTGCGATCC-3′	3
Total		33

gene, Heidelberg, Germany), 1 μL polymerase buffer with 1.5 mM $MgCl_2$ (Qbiogene), 0.1 μL *Taq* polymerase (5 U μL^{-1}; QBiogene), and 6.5 μL double-distilled H_2O. The reaction mixture was initially denatured at 94°C for 2 min, which was followed by 36 cycles of 12 s of denaturation at 94°C, 45 s of annealing at 36°C, 2 min of elongation at 72°C, and a final cooling step at 4°C in a Mastercycler (Eppendorf, Hamburg, Germany).

Polymerase chain reaction (PCR) products were separated on a 2% agarose gel with a TAE (tris-acetate-EDTA) buffer system at 150 V for 150 min with a 100-bp DNA ladder Plus (Gene Ruler, Fermentas, St. Leon-Rot, Germany) as a size standard. The agarose gel was stained with ethidium bromide and photographed under ultraviolet light. Each individual was scored for the presence (1) or absence (0) of bands. In order to avoid genotyping errors, each sample was run in at least two independent RAPD-PCR reactions. We checked for contaminations with a negative control, and previous amplified samples served as references (Bonin et al. 2004).

SSR Amplification

Sequence-tagged microsatellite sites were obtained from Plant Research International, Wageningen, the Netherlands (Esselink et al. 2003). We chose eight primer pairs for our analysis, which resulted in a total of 69 alleles (table 3). Amplification of doubled-stranded DNA was performed in 25 μL of reaction medium containing 1 μL DNA, 5 pmol fluorescence-labeled forward primer and 5 pmol reverse primer (metabion international, Martinsried, Germany), 2.5 μL 2 mM dNTPs (QBiogene), 2.5 μL polymerase buffer with 1.5 mM $MgCl_2$ (Qbiogene), 1 μL *Taq* polymerase (Fermentas), and 16.8 μL double-distilled H_2O. The reaction mixture was initially denatured at 94°C for 3 min followed by 28 cycles (for primers RhD201 and RhE2b, 35 cycles) with 30 s of denaturation at 94°C, 30 s of annealing at 50°C, a 60-s elongation step at 72°C, and a final elongation at the same temperature for 3 min in a Mastercycler (Eppendorf). PCR products were diluted 1 : 5 with double-distilled H_2O in order to minimize the interference of salts.

PCR products were separated using capillary electrophoresis (MegaBace 1000, Amersham Bioscience, Uppsala, Sweden), using MegaBACE-ET ROX 400 (Amersham Bioscience) as a size standard and were detected via fluorescence emission. We used the MegaBace Fragment Profiler Software 1.2 (Amersham Bioscience) to translate the fluorescence signals into peaks and to assign peak location to an allele. Because the maximum number of peaks at one locus was four, it was not possible to detect the exact genotype. Thus, all analyzes were performed with the allelic phenotype of *R. rubiginosa*.

Statistical Analysis

Genetic diversity. The six RAPD primers (table 2) produced two to 11 informative bands, each resulting in a total of 41 scorable bands of lengths between 390 and 1400 bp. Among them, 33 bands were polymorphic and were included in the analysis (table 2). Because *R. rubiginosa* populations cannot be assumed to be in Hardy-Weinberg equilibrium due to the species' mixed mating type and to the unique meiosis described above, RAPD and SSR data were analyzed on the basis of band and allelic phenotypes, respectively, using the program RAPDIV (Whitkus et al. 1998; De Cock et al. 2008), which does not assume Hardy-Weinberg equilibrium. To our knowledge, there is no genetic software that analyzes genotypes of pentaploid organisms. Hence, genetic diversity was calculated with Shannon-Weaver information statistics using the Brillouin formula in order to eliminate any bias inferred by the finite sample size. Band diversity (H) and allelic diversity were calculated with the total number of individuals scored for the band or allele (N) and the number of individuals in the alternative categories (n_i): $H = (1/N) * (\log N! - \Sigma \log n_i!)$. Genetic diversity within a group was defined as the average diversity over all RAPD bands or alleles, and it was calculated with the program RAPDDIV at the following three hierarchical levels: population ($\overline{H}_p$), region ($\overline{H}_r$), and continent ($\overline{H}_c$). In this way, our case study contributes to the knowledge on the genetic situation of invasive plant populations at multiple scales, as was requested by Ward (2006). Diversity between continents was then calculated as the difference between total diversity and the average within-continent diversity, divided by the total diversity ($G_c = (H_{tot} - \overline{H}_c)/H_{tot}$). Diversity between regions is represented by the average within-continent diversity minus the average regional diversity, divided by the total diversity ($G_r = (\overline{H}_c - \overline{H}_r)/H_{tot}$). Finally, diversity between populations is represented as a ratio between average regional diversity and total diversity ($G_p = \overline{H}_r/H_{tot}$).

Table 3

Number of Alleles Amplified with Microsatellite Primers (Esselink et al. 2003)

Primer identification	Repeat sequence	Fragment size (bp)	No. alleles
RhD201	$(TCT)_{33}$	201–236	13
RhD206	$(TCT)_{14}$	113–139	14
RhD221	$(TCT)_{21-1}$	163–233	7
RhB303	$(GA)_{11}$	150–178	4
RhEO506	$(CAG)_6(CCA)_{18-7}$ $(CAG)_6$	180–242	10
RhP519	$(TGA)_{11-1}$	198–232	3
RhE2b	$(TGT)_{20-6}$	151–195	8
RhAB26	$(GT)_{18-2}(GA)_{17}$	150–178	10
Total			69

South American samples appeared to have highly homogenous RAPD band patterns. Accordingly, estimates of RAPD band diversity for the South American populations did not differ significantly when the total number of samples or the randomly reduced numbers of five samples per population were analyzed (ANOVA, $P = 0.3$; table 4), confirming that RAPD-based assessments are relatively insensitive to sampling intensity (Nybom and Bartish 2000). As an additional measure of within-population diversity, we calculated the mean Jaccard dissimilarities for each population with 999 permutations (Oksanen et al. 2008; R Development Core Team 2008). For the RAPD data set sampling, numbers were bootstrapped in order to account for the different sampling numbers per population; thus, mean Jaccard dissimilarities were calculated for different population sizes (see fig. A1 in the online edition of the *International Journal of Plant Sciences*). Hence, for comparability of the two molecular marker techniques, only reduced sample numbers were used for further analyses in both cases (i.e., principal coordinate analysis and between-class analysis; see below).

Principal coordinate analysis and between-class analysis. A principal coordinate analysis (PCoA) was performed on square-root-transformed Jaccard dissimilarities in order to detect similarities between individuals and populations (Oksanen et al. 2008; R Development Core Team 2008). This ordination technique was performed with the RAPD and the SSR data sets separately. In order to measure the degree of concordance between the PCoA results of the RAPD and the SSR genetic data sets, a Procrustes analysis was performed (Oksanen et al. 2008). To this end, the first three axes of the ordination were scaled and rotated for maximum similarity for each sample, and the distance to each original sample score was calculated. The statistical significance of the Procrustean fit was assessed with a permutation test, which calculates the correlation (r) of the data sets (Peres-Neto and Jackson 2001).

As an alternative approach, genetic relationships between countries were further visualized with a between-class analysis (Dray and Dufour 2007; R Development Core Team 2008). A PCoA was performed using the same distance matrix as in the individual samples described above (square-root-transformed Jaccard dissimilarities). PCoA scores were then integrated into a between-class analysis, which groups individuals into countries/regions by maximizing the between-group variance. Groups are represented by stars, where the center of the star is the weighted mean of each group. An ellipse was drawn around each center, representing 1.5 times the dispersion from the center. The statistical significance of the between-class analysis was assessed with a permutation test (Monte-Carlo, 999 permutations, $P < 0.05$).

Table 4

Analysis of Genetic Diversity of *Rosa rubiginosa* for Both the Random Amplification of Polymorphic DNA (RAPD) Data Set and the Simple Sequence Repeats (SSR) Data Set

	RAPD *N*		
Level, genetic diversity	Total	Reduced	Reduced SSR *N*
Population:			
$\overline{H}_p$	.03	.03	.01
G_p	.59	.62	.64
Region:			
H_r:			
Chile	.02	.00	.00
Patagonia	.03	.03	.00
Central Argentina	.07	.07	.02
Germany	.11	.11	.06
Spain	.17	.17	.11
$\overline{H}_r$	.08	.09	.05
G_r	.13	.14	.18
Continent:			
H_c:			
South America	.06	.05	.01
Europe	.15	.15	.09
$\overline{H}_c$	.10	.11	.06
G_c	.27	.23	.18

Note. Total diversity (H_{tot}) is 0.14 (for all data) and 0.15 (for the reduced data set) for the RAPD data set and 0.08 for the SSR data set. Genetic diversity is partitioned as follows: within regions (H_r) and within continents (H_c); average genetic diversity within populations ($\overline{H}_p$), within regions ($\overline{H}_r$), and within continents ($\overline{H}_c$); and average genetic diversity between populations ($G_p = \overline{H}_r/H_{tot}$), between regions ($G_r = \overline{H}_c - \overline{H}_r/H_{tot}$), and between continents ($G_c = H_{tot} - \overline{H}_c/H_{tot}$). Within-population diversity (H_p) is shown in table 1 and fig. 2.

Results

Genetic Diversity

Average genetic diversity between populations (G_p; table 4) was high for both marker types, ranging between 0.59 and 0.64. The average genetic diversity distributed within *Rosa rubiginosa* populations ($\overline{H}_p$) was 0.03 with the RAPD marker and 0.01 with the SSR marker (table 4). *Rosa rubiginosa* populations in Spain showed the highest within-population diversity ($H_p = 0.12$), followed by those in Germany and in Argentina (0.06 and 0.04, respectively; table 1; fig. 2). Consequently, at the higher hierarchical levels "region" and "continent," genetic diversity in Chile, Patagonia, and central Argentina was lower than it was in Germany or Spain (H_r). With both marker types, genetic diversity in South America was lower than it was in Europe (H_c; table 4).

Genetic diversity between continents (G_c) proved to be nearly twice as high as that between regions (G_r) for the RAPD data set, whereas the two values for the SSR data set were almost equal (table 4).

Mean Jaccard dissimilarity coefficients largely corresponded to the within-population diversity values calculated above, with populations in South America reaching maximum values of only 0.08 whereas European populations reached values of up to 0.58 (table 1). Bootstrapping proved that estimates of population diversity were invariant to differences in sample size (fig. A1).

Similarity between Individuals and Populations

Most South American stands of *R. rubiginosa* were composed of genetically identical samples. In figure 3, only three out of a total of 69 possible data points (4%; indicated by triangles and asterisks) represent unique RAPD phenotypes. The other data points represent between two and 20 samples.

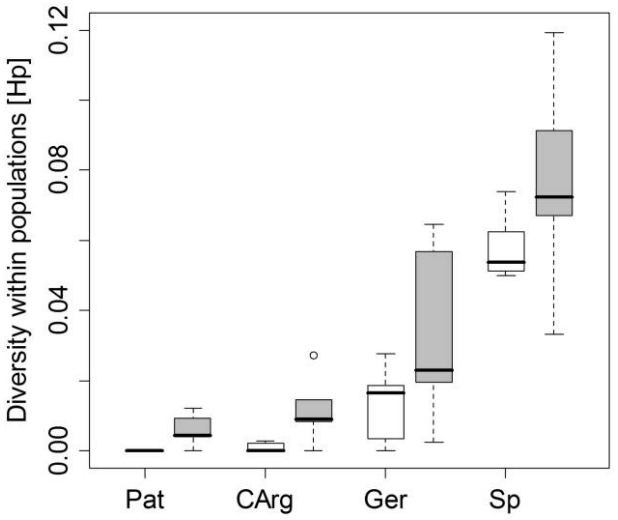

Fig. 2 Box plot of genetic diversity within *Rosa rubiginosa* populations (H_p) of each region (*Pat* = Patagonia, *CArg* = central Argentina, *Ger* = Germany, *Sp* = Spain); white boxes represent the simple sequence repeats data set, and gray boxes represent the random amplification of polymorphic DNA data set.

Not only did individuals belonging to the same population share the same phenotype, but individuals growing 1000 km apart did as well. In Europe, with the exception of two individuals from distant locations (northern and southern Germany), identical phenotypes were usually restricted to a given population or geographical region, and 65% of the samples possessed unique RAPD phenotypes (fig. 3).

The first axis of the PCoA (fig. 3; 25% of total variance) separated the South American samples from the European samples. However, one population from central Argentina (*p*) showed RAPD phenotypes that were similar to the European samples, and one individual was even identical to an individual from Saxony-Anhalt. Samples from Germany and Spain were intermingled and could not be separated by either the second axis (fig. 3*A*; 11% of total variance) or the third axis (fig. 3*B*; 9% of total variance). The Chilean population grouped with the Argentinean cluster (triangles on the right-hand side of fig. 3) without having any apparent preference to either central Argentina or Patagonia. Overall, the PCoA did not reflect the underlying geographical pattern.

The SSR data set was far more homogenous than the RAPD data set (fig. 4). Only one individual from South America possessed an unique phenotype. The other data points represented between three and 63 samples. This contrasts with the European samples, in which 35% of the phenotypes were unique; however, unlike the results of the RAPD data set, individuals that were rather geographically distant (e.g., individuals from Spain and from Germany) had identical allelic phenotypes. The central Argentinean individual, which had the same RAPD phenotype in the RAPD data set as the German samples from Saxony-Anhalt, again grouped with German populations and was identical to individuals from Lower Saxony (*G*) and Brandenburg (*T*). Two other individuals from the same central Argentinean population (*p*) had the same SSR phenotype as an individual from Mecklenburg–Western Pomerania (*U*). All samples from Chile had the same SSR phenotype and were identical to central Argentinean and Patagonian individuals.

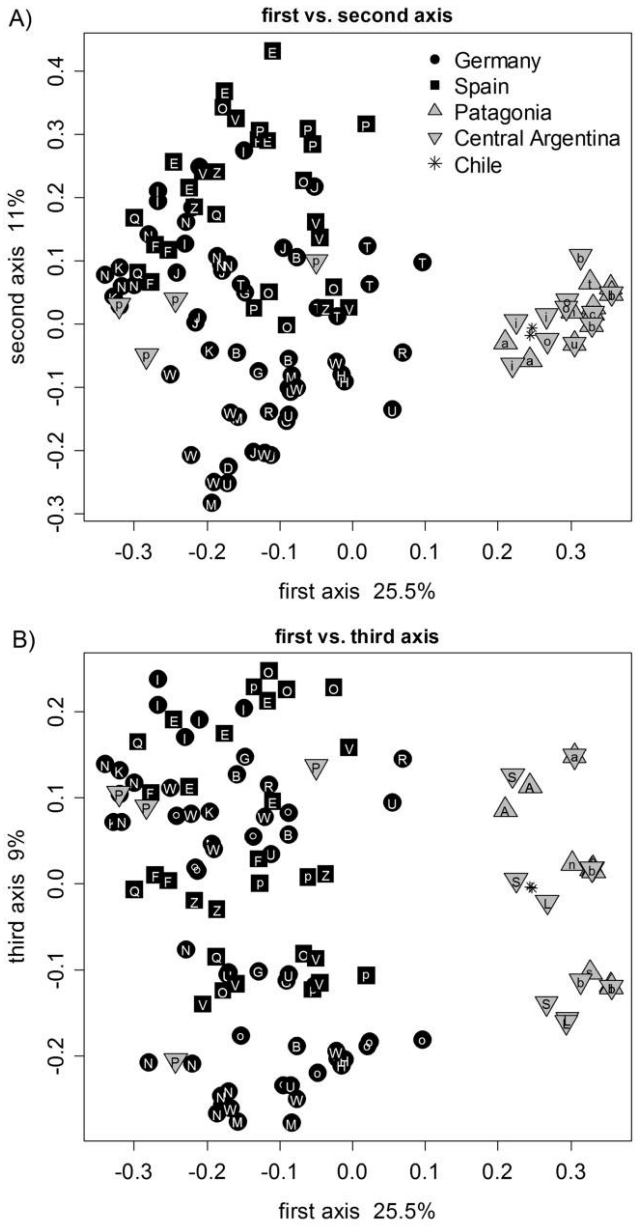

Fig. 3 Principal coordinate analysis of the random amplification of polymorphic DNA (RAPD) data set of *Rosa rubiginosa* consisting of 33 polymorphic bands amplified using six RAPD primers. In *A* and *B*, the first axis explains 25.5% of the total variance; in *A*, the second axis explains 11%; and in *B*, the third axis explains 9%. Regions are represented by symbols, and letters correspond to the population identifications in table 1. Because of the strong homogeneity of the South American samples, only three out of 69 samples have individual RAPD phenotypes; the rest could not be separated by the principal coordinate analysis. Population *p* from central Argentina groups with the European samples, and one individual from this population shares the same RAPD phenotype as population *N* from Germany.

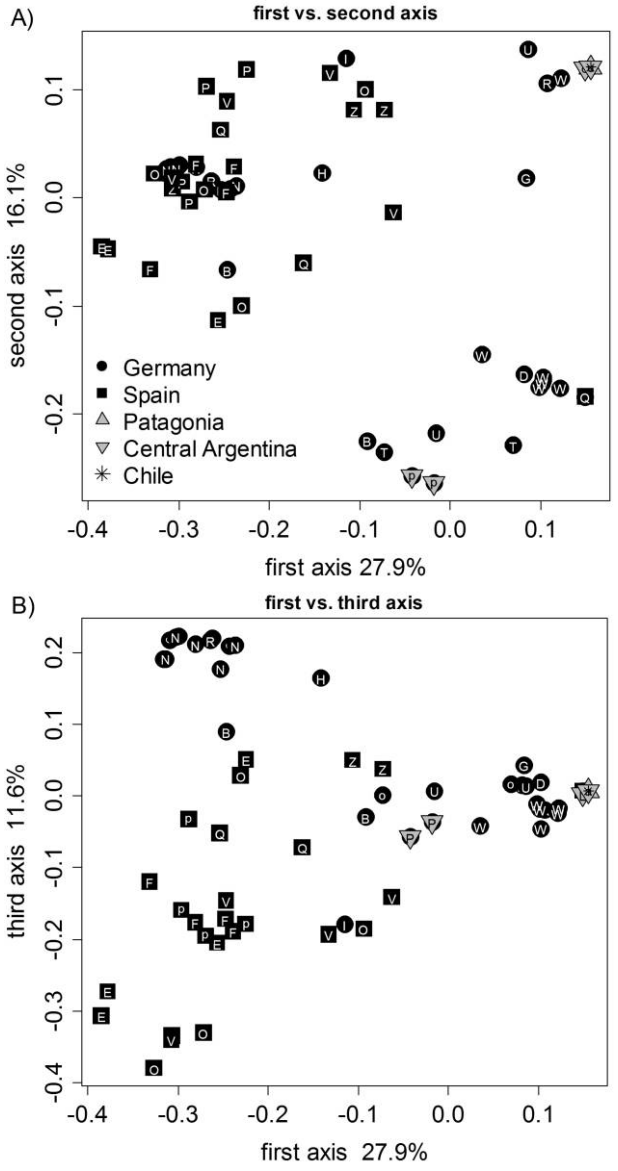

Fig. 4 Principal coordinate analysis of the simple sequence repeats (SSR) data set of *Rosa rubiginosa* consisting of 69 alleles from eight microsatellite loci. In *A* and *B*, the first axis explains 27.9% of the total variance; in *A*, the second axis explains 16.1%; and in *B*, the third axis explains 11.6%. Regions are represented by symbols, and letters correspond to the population identifications in table 1; however, the identification of the South American samples cannot properly be recognized, since 91% of the South American samples share the same SSR phenotype and could not be separated by the principal coordinate analysis. The South American group on the right-hand side (in both *A* and *B*) includes the Chilean samples. The only South American samples that are separated from this group are those in population *p* from central Argentina, which shares the same SSR phenotype with populations *T*, *G*, and *U* from Germany.

The first three axes of the PCoA of the SSR data set explained 56% of the total variance (fig. 4). The first axis grouped the Spanish samples together with a group of mostly central German samples apart from the rest of the German and South American samples (fig. 4; 28% of total variance). The Argentinean population *p* that clustered together with the German samples in the PCoA of the RAPD data set was equally as separated from the remaining South American populations on the second axis (fig. 4*A*; 16% of the total variance). In this analysis, Spanish and German samples were not intermingled, although they had been on the third axis of the RAPD data set (fig. 4*B*; 12% of total variance). These samples fell into a cloud of Spanish samples that was widely spread in the ordination space, as well as into two more concentrated German fractions. The German fraction in the left-hand corner consists mostly of central German populations, but two individuals from southern Germany were also placed there. The German fraction in the lower right-hand corner consists of populations from all over Germany; hence, similar to the RAPD data, the SSR data failed to capture any geographic pattern. PCoA results of the RAPD and the SSR genetic data sets were significantly similar ($P < 0.001$), with a Procrustes correlation of 0.66.

The general pattern of the between-class analysis is comparable to that of the PCoA (fig. 5). The German and Spanish ellipses overlap, and a few South American samples reach into the German group. The groups explained the same fraction of the total variance for the RAPD (fig. 5*A*) and SSR (fig. 5*B*) data sets (26%). Both between-class analyses were equally significant ($P = 0.001$). In both graphs, the Germany and Spain ellipses overlapped slightly. The Argentinean ellipse did not overlap with the European one; however, some of the samples from Argentina did reach into the German ellipse.

Discussion

Comparison of Genetic Diversity between the Native and the Invasive Ranges

Invasive *Rosa rubiginosa* populations in Argentina and Chile proved to have lower levels of genetic diversity than did European native populations. As is expected for a selfing species, we found higher levels of genetic diversity among populations than within populations. Our data are not in line with those of Aguirre et al. (2009), who found 79% of the genetic diversity of *R. rubiginosa* within populations; however, this discrepancy might be the effect of a low number of studied populations and a completely different sampling design. A study on invasive *Erigeron annuus* L. populations (Edwards et al. 2006) found nearly half of the mean number of RAPD phenotypes in its invasive in relation to its native range, whereas we found only 15% RAPD phenotypes and 3% SSR phenotypes in invasive *R. rubiginosa* populations. Within South America, we also failed to find any clear differentiation between invasive populations of Patagonia, central Argentina, and Chile. Weak or no genetic differentiation accompanied by a lack of geographical structuring might be the result of introductions at multiple locations from a single source population (Okada et al. 2007). Since *R. rubiginosa* has low levels of recombination (Nybom et al. 2006), and because it may produce apomictic seeds (Werlemark 2000) as well as root suckers, it is possible that, within a given population, one genotype could be conserved over large periods of time. However, we assume that apomixis plays a minor role in seed

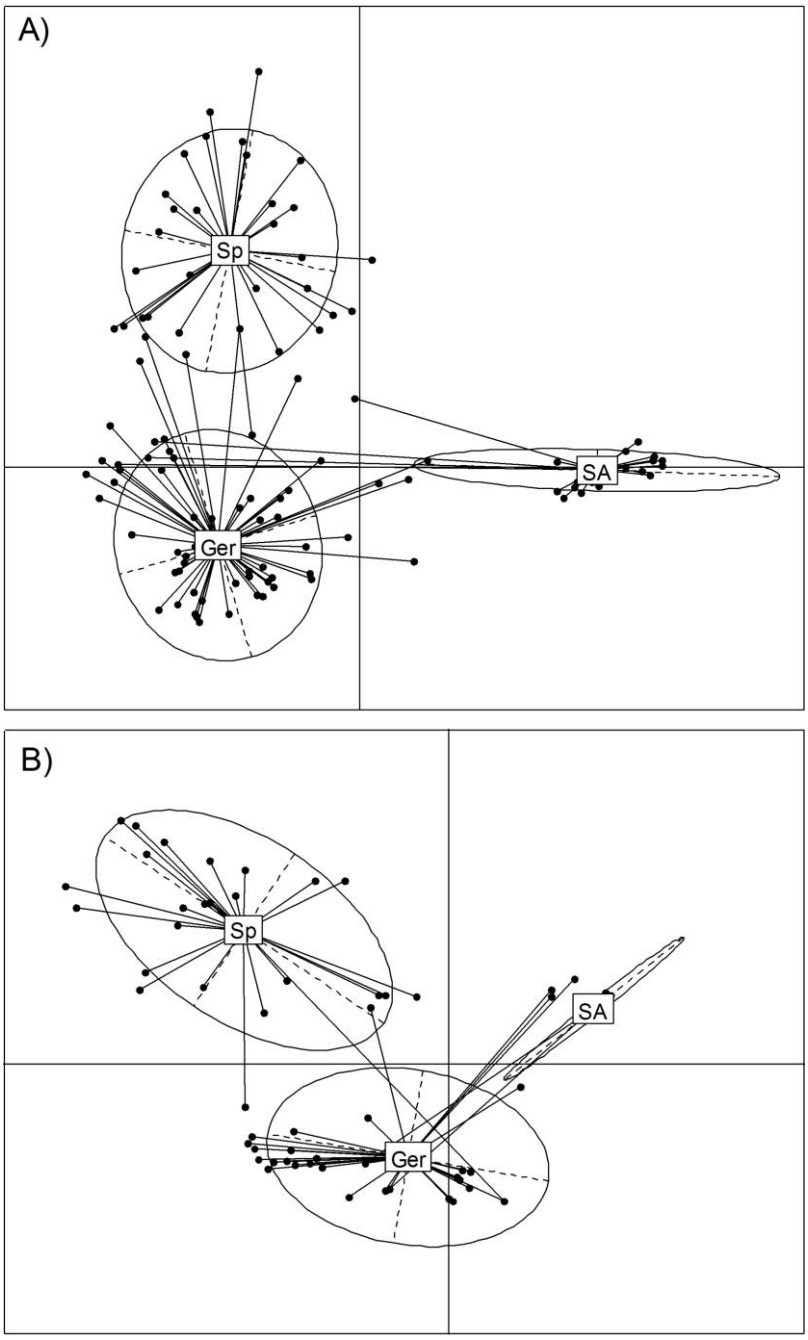

Fig. 5 Between-class analysis of (*A*) the random amplification of polymorphic DNA data set and (*B*) the simple sequence repeats data set. Groups are represented by stars, wherein the center represents the weighted mean of each group and the surrounding ellipse represents 1.5 times the dispersion from the center. Both graphs show no overlap between *Rosa rubiginosa* populations from South America and Spain (*Sp* = Spain, *Ger* = Germany, *SA* = South America). The variance explained by the grouping is 26%. The permutation test (999 permutations) revealed a significant grouping ($P = 0.001$) for both analyses.

production, since the number of viable seeds produced by apomixis is clearly lower than that produced by xenogamy (only 5% are produced by apomixis; Wissemann and Hellwig 1997). Instead, we found similar or even higher numbers of seeds per rosehip in the invasive populations (mean number of seeds: Argentina, 22; Europe, 17). Since we observed connected ramets over several meters in the field, as well as impenetrable thickets, vegetative growth may play a dominant role in the reproductive success of *R. rubiginosa*. In fact, Amsellem et al. (2001) found an increase in asexual reproduction in invasive populations of *Rubus alceifolius* Poir. in comparison to its native range.

General theory suggests that outcrossing species should be capable of adapting to a greater range of environmental con-

ditions as a result of an increased level of ecotypic differentiation (Lambrinos 2001). However, the genetically poorly differentiated *R. rubiginosa* is vigorously expanding in a wide variety of habitats and climates throughout Argentina and is a clear exception to the general theory. In contrast to the findings of Tranel and Wassom (2001) for the weed *Xanthium strumarium* L., we could not find any indications of selection leading to local adaptation. However, this is in line with Besnard et al. (2007), who also found no difference in invasion success for two subspecies of *Olea europaea* L. in spite of their differing genetic diversity: one taxon with significantly low genetic diversity was as successful an invader as the other more genetically diverse olive tree subspecies. For invasive *Butomus umbellatus* L. populations, sexual reproduction and genetic variation do not seem to provide colonization advantage either (Kliber and Eckert 2005).

One potential explanation for the successful invasion of *R. rubiginosa* in South America, in spite of its low genetic diversity, is the introduction of a unique, a highly adapted, or an aggressive genotype (Saltonstall 2002) to Argentina/Chile. Unfortunately, it is unclear from where such an outstanding genotype may have originated (see below). Another explanation is that deleterious alleles were purged in the process of genetic drift and subsequent selection (Parisod et al. 2005), or that single genotypes may also possess high phenotypic plasticity (Sultan 2000; Richardson and Pyšek 2006). Flexible biomass allocation, for instance, enables invasive clonal plant species to successfully invade different habitat types because of phenotypic plasticity (Barney et al. 2005; Geng et al. 2007). Loomis and Fishman (2009) also concluded that phenotypic plasticity may provide the flexibility necessary for the establishment of *Hieracium aurantiacum* L., since they found virtually no genetic variability in invasive populations throughout North America (0.035 clonal diversity). In both its native and its invasive ranges, *R. rubiginosa* shows a highly variable morphology in response to the given environmental conditions, for example, radiation and wind (H. Zimmermann, personal observation; Weber 1995). As such, we believe that *R. rubiginosa* benefits from the apparent phenotypic plasticity observed in the field.

Origin of the Invasive Populations

Low levels of genetic diversity in South American *R. rubiginosa* populations do support the idea of few introductions into Argentina, and we failed to identify the exact origin of the Argentinean populations. In order to narrow down their origin, additional genetic data from the entire native range are needed, although the original populations may already be extinct. However, one population was closely related to German populations but not to Spanish ones. Perhaps this central Argentinean population was introduced more recently, directly from Germany by German immigrants. Aguirre et al. (2009) provide further evidence for more than one introduction of *R. rubiginosa* populations to Argentina, as they discovered two independently established populations, in Patagonia and in central Argentina.

Because the Chilean and Argentinean populations contain similar or even identical RAPD bands or microsatellite alleles, an introduction via Chile is possible. However, more Chilean populations are needed to prove this scenario of introduction. Remarkably, populations from central Argentina and from Patagonia were very similar. These two regions are 1000 km apart and, during our fieldwork, we did not find any roses in between these two ranges, nor are there any records of such in the available literature. This suggests that seeds or root suckers of *R. rubiginosa* were deliberately transported from one region to the other, presumably from Patagonia to central Argentina. In Patagonia, where *R. rubiginosa* is much more widespread, it is already accepted in the local culture, its image can be found on postcards, and its fruits are commercially exploited, it is likely that plants were transported to other areas of Argentina as a Patagonian souvenir.

Organelle markers, rather than nuclear markers, may be better suited to detecting migration histories (Budak et al. 2005); unfortunately, however, genetic variation of chloroplast markers within dog roses is very low (Wissemann and Ritz 2005; Bruneau et al. 2007). Because of the pronounced maternal overload caused by *Canina* meiosis, egg cells inherit four-fifths of total genetic material from the mother. Thus, nuclear markers may, in this case, behave similarly to the strictly uniparentally inherited organelle DNA (Olsson et al. 2000; Nybom et al. 2001). Other nuclear markers, such as the highly polymorphic amplified fragment length polymorphism (AFLP) technique, have proved useful in the reconstruction of relationships within different dog rose species (De Cock et al. 2008; Koopman et al. 2008). Given that we have already used two different nuclear marker systems, we doubt that more markers would reveal any new insights in this case.

Comparison of RAPD and SSR Markers

Results of the RAPD and the SSR analyses were largely comparable. This is in line with Rao et al. (2008), who found RAPD and SSR markers to be equally successful in identifying progenies of *Citrus* L. hybrids. RAPD markers do not evolve in the same way as SSR markers (Ghislain et al. 2006); nevertheless, only minor differences in the magnitude of differentiation were detected, and the overall pattern was congruent. While SSR markers are supposed to be highly polymorphic (Parker et al. 1998), RAPD markers had even higher levels of polymorphism in this case study and, unlike SSR markers, they were suited to distinguishing different phenotypes among the distant European samples. A previous RAPD study of seven rose taxa reported by Olsson et al. (2000) also failed to reveal a considerably higher number of polymorphic primers (they found 11) after an initial screening of 120 primers, implying that the genus *Rosa* is rather monomorphic.

In both the RAPD and the SSR data sets, according to the PCoA analyses, one Argentinean population matched some of the German populations. Unfortunately, the RAPD and SSR analyses revealed a match with German populations that were located in different provinces in Germany. This could be due to the fact that more loci and alleles are needed or that the interpretations of both genetic markers are based on phenotypes and not genotypes. We found a maximum of four different peaks in our analyis of the pentaploid *R. rubiginosa*, and thus, we could not detect the exact genotypes with SSR. Apparently, at least one allele of each locus has

two identical copies, as was also shown by Nybom et al. (2004, 2006). This implies that in the case of SSR analysis, two phenotypically identical individuals had the same set of alleles but not necessarily the same combination of alleles. In this case, the less cost-intensive RAPD analysis did not significantly deviate from the SSR analysis.

Conclusions

Despite lower levels of genetic diversity, *Rosa rubiginosa* populations in Argentina do not seem to suffer founder effects, as ongoing range expansion is still threatening biodiversity and reducing possibilities of agricultural use. Human transport is probably the main migration driver for this species within South America, and it must be prevented by raising awareness of the ecological damage of *R. rubiginosa*, which is already classified as a noxious weed in South Africa, New Zealand, and Australia (Parsons and Cuthbertson 2001; Bellingham et al. 2004; Nel et al. 2004). The exact European origins of the invasive populations have not been detected, but this could be achieved with an enlarged sampling strategy in the native range. Knowledge of the origins of the populations and, consequently, these populations' respective environmental backgrounds would help to determine this species' invasion dynamics (Milne and Abbott 2000, 2004; Novak and Mack 2001). On the basis of our results, a Spanish origin seems unlikely; as such, future research should focus on central Europe.

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