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ORIGINAL ARTICLE

Genetic differentiation of *Rosa rubiginosa* L. in two different Argentinean ecoregions

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Abstract We analyzed genetic differentiation of *Rosa rubiginosa* by RAPD from populations growing in two Argentinean ecoregions, Chaco Serrano and Patagonian Steppe. Leaf material was collected during the spring and summer of 2006. UPGMA dendrogram and PCoA clearly suggest a geographical differentiation of the provenances of *R. rubiginosa* populations. AMOVA analyses revealed high genetic variation within populations (71%) and low variation between populations (29%), in agreement with values estimated by the Shannon–Weaver index. Genetic differentiation between populations estimated by AMOVA was $\phi_{PT} = 0.29$ ($P < 0.001$). Nei's Gst (0.2205) was lower for interpopulation variation. The low interpopulation value obtained suggests genetic homogeneity between the populations. The presence of specific monomorphic bands accounts for the genetic differentiation between populations. The high percentage of within-population genetic diversity suggests the introduction of genetic variation into both ecoregions. From the present results we can conclude that we observed two independently established populations with high similarity between them and a strong intrapopulation differentiation. The empty niche hypothesis for explainging invasion success might explain the invasiveness of *R. rubiginosa* in Argentinean ecoregions.

Keywords Genetic diversity · RAPD · Exotic species · *Rosa rubiginosa* · Invasive species

Introduction

Rosa rubiginosa L. is a widespread shrub species, introduced to Argentina, and widely distributed in different environments and climatic conditions such as woodland and steppe. In the Patagonian region of Argentina (Bran et al. 2003, 2004), it has invaded protected areas from Neuquén to Chubut (National Park Nahuel Huapi) in woodlands of *Nothofagus* and *Austrocedrus*. Because of the economic impact it has produced, it is considered a weed.

Invasion of natural ecosystems by exotic plant species is recognized as a major threat to biodiversity (Vavra et al. 2007), and biological invasions may also occur at the gene level (Petit 2004). The most important aspect of an alien plant is how it responds to a new environment. The susceptibility of environments to invasion by species from other regions is "invasibility" (Davis et al. 2005). An invasive species is one that displays rapid growth and spread, allowing it to establish over large areas. The ability of an alien species to spread through a new environment is known as "invasiveness" (Hierro et al. 2005). Free from the vast and complex array of natural controls present in their native lands, exotic plants may experience rapid and unrestricted growth in a new environment. Some of these species become very abundant in their introduced range and cause serious environmental and economic problems. They can outcompete native species and change the

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structure and functioning of native communities and ecosystems (Bossdorf et al. 2005).

Evolution of invasive species can be rapid and therefore relevant to ecological studies. According to Bossdorf et al. (2005), there are several main reasons for this rapidity: first, evolution may occur by genetic drift and inbreeding in founder populations (Brown and Marshall 1981). Second, inter- or intraspecific hybridization in the introduced range may create novel genotypes (Ellstrand and Schierenbeck 2000). Third, invasions to novel environments often involve drastic changes in selection regimes that may cause adaptative behavior. Many of the species that become invasive do so after a lag time, perhaps after such evolutionary adjustments have taken place.

R. rubiginosa and *R. moschata* are considered nontimber forest products (NTFP) by the Food and Agriculture Organization (Chandrasekharan et al. 1996). Products from rose hips are relevant in rural economies (Tacón et al. 2006). In southern Argentina and Chile, *R. rubiginosa* is a representative food product with national and international trading tradition, consumed as a fruit ingredient in yoghurt, jam, and healthy beverages. A recent study by Damascos and Bran (2006) pointed out that in Argentina, *R. rubiginosa* has a wide distribution, while *R. moschata* is limited to Chile. The authors also clarified the nomenclatural problem, providing descriptive characteristics that allow identification of the species of the *Rosa* L. genus.

One hundred grams of fresh, edible rose hips contain approximately 500–2,200 mg of vitamin C. Vitamin C content varies according to collection site conditions, harvesting time, and drying process (Valladares et al. 1986). The seeds contain less than 10% w/w oil, of which 41 and 30% are linoleic acid and linolenic acid, respectively. Seeds have considerable value due to the presence of trans-retinoic acid, which has regenerative properties that are of interest in clinical and cosmetic applications (Moure et al. 2001).

In the context of plant invasions, molecular markers are important tools that provide information on both invasion pathways and the amount of genetic variation that has been introduced. According to Brown and Marshall (1981), the potential adaptative evolutionary change that may occur in an invasive species depends on the amount of genetic variation introduced.

The random amplification of polymorphic DNA (RAPD) technique (Williams et al. 1990) is a common, well proven tool used in genetic studies and is a suitable method for detecting total variation and genetic differentiation between and within populations (Jürgens et al. 2007). Estimates derived by RAPD are very similar to those from other methods (AFLP, ISSR) and may be directly comparable (Nybom 2004). The RAPD technique has been broadly applied for the following: to characterize and evaluate phylogenetic relationships in *Rosa* (Millan et al. 1996); to demonstrate apomixis in *Rosa* (Werlemark 2000); to determine relationships among Nordic dogroses (*Rosa* L. sect. *Caninae*, Rosaceae) (Olsson et al. 2000); to study the effects of meiosis on the transmittal of different characters in *Rosa* sect. *Caninae* (Werlemark and Nybom 2001); to evaluate genetic resources in minor fruits (Nybom et al. 2003); and to assess genetic diversity (Nybom 2006). Using RAPD markers, Werlemark et al. (1999) analyzed the skewed distribution in a pair of reciprocal crosses between species of *Rosa* sect. *Caninae*.

Previous studies on *R. rubiginosa* in Argentina evaluated fruit availability (Bran et al. 2003; Bran et al. 2004) related to the economic use of rose hips in suburban areas in Bariloche (Ladio and Rapoport 2002). The ethnobotanical use of rose hips has been described in aboriginal communities from Northwest Patagonia (Ladio and Lozada 2001; Ladio 2005; Ladio et al. 2007) as has its medicinal use as a nontimber forest product in San Luis (Aguirre et al. 2007). The relationship between *R. rubiginosa* and environmental or anthropic variables was studied by Damascos and Gallopin (1992). The degree of disturbance, the openness of the shrub stratum, and the average annual rainfall were the main factors influencing the invasion by *R. rubiginosa.*

We investigated two populations of the alien and invasive *Rosa rubiginosa*, growing in two different Argentinean ecoregions—Chaco Serrano and Patagonian Steppe—by RAPD analysis. We aim to better understand the genetic differentiation of this widespread exotic shrub species.

Materials and methods

Studied species

Description *Rosa rubiginosa* (*Rosa eglanteria* L.) is an introduced species to Argentina and grows as an erect or scrambling shrub. The crushed foliage has a sweet apple-like fragrance, a distinctive character for *R. rubiginosa* (Damascos and Bran 2006). The branches present prickles usually mixed with glandular hairs. It is a deciduous shrub of variable height (up to 2.5 m tall). The stems have numerous curved thorns and bristles. The leaves are bright green with 5–7 leaflets ovate or obovate to ±circular, 10–25 mm long, 10–15 mm wide, and with toothed margins. The upper surface is glabrous or hairy and the lower surface usually presents at least a few simple hairs mixed with glandular hairs. Small clusters of pink flowers (20–40 mm) with glandular-hairy peduncles are followed by conspicuous hips. The fruits or hips are orange-red, ovoid to globose, 15–20 mm long and with prickles.

Rosa rubiginosa plants are reproduced mainly from seeds. The fruits are dispersed mainly by birds, but also by horses and cattle when used as a forage plant.

Species of the genus *Rosa* exhibit a polyploid series with the basic chromosome number of 7. Section *Caninae* contains only polyploid species with $2n = 28$, 35, or 42 and with pentaploidy as the most common level. *R. rubiginosa* exhibits $2n = 35$ (Werlemark and Nybom 2001). At the peculiar meiosis only seven bivalents are formed while the rest of the chromosome occurs as univalent. The egg cells obtain seven chromosomes from the bivalents plus the univalent, whereas the viable pollen cells only obtain seven chromosomes from the bivalent formation (Werlemark and Nybom 2001).

Habitat The habitat preferences of *R. rubiginosa* have been described by Bran et al. (2003) and Damascos and Gallopin (1992). It prefers loam soils and a humid to subhumid rainfall regime and is associated mainly with disturbed areas, with higher abundance in the vicinity of populated areas or transit corridors.

Study area and sampling

R. rubiginosa was sampled from two different ecoregions of Argentina. Experimental site 1 included samples from Potrero de los Funes-El Volcán (PV) in Dry Chaco (in Spanish, *Chaco Seco*, subregion Chaco Serrano, San Luis province). Experimental site 2 included samples from Andacollo-La Primavera (ALP) in Patagonian Steppe (in Spanish, *Estepa Patagónica*; Neuquén province). Table 1 shows the characteristics of the two sites studied. At both sites, *R. rubiginosa* samples were harvested along a line transect of 6 km. In this study, the term "population" refers to individuals of one species growing in one of the sampling sites.

Plant material Leaf material—young meristematic tissue—was collected from 40 plants of *R. rubiginosa* during the spring and summer of 2006 and stored at −80°C for 1 or 2 days before DNA extraction. Approximately 20 individual plants per population were collected along a transect of 6 km, with a minimum distance of 100 m between plants to minimize the chance of sampling closely related or genetically identical individuals.

DNA isolation and RAPD methods

According to Khanuja et al. (1999), the presence of certain metabolites can hamper DNA isolation procedures and reactions such as DNA restriction, amplification, and cloning. We used young meristematic tissue because it is characterized by a high content of genomic material and low levels of polyphenols and polysaccharides that interfere in the amplification reaction by polymerase chain reaction (PCR).

Although different protocols were assayed, DNA was isolated using slight modifications of the methods of Holm (1995) and Doyle and Doyle (1987). Initial steps followed Holm's protocol, which uses two extraction buffers and multiple chloroform-isoamilic washes. The precipitation step and final procedures were according to Doyle and Doyle's protocol. The quality of the obtained DNA was verified by spectrophotometric quantification and analysis on agarose gels (1%), and only good DNA was used.

RAPD reactions were performed in 25 µl reaction mixture containing 20 ng of plant genomic DNA, PCR buffer containing 2 mM $MgCl_2$, and 13 selected primers from kit A (Operon Technologies) and 1.6 U of *Taq* polymerase (Manzur et al. 2006). Alternatively, 1 U of commercial *Taq* polymerase was used (GoTaq DNA Polymerase, Promega). Amplifications were carried out in a Perkin–Elmer Gene Amp 2400 PCR System thermocycler under the following conditions: an initial cycle at 94°C for 5 min; 40 cycles of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C; and a final cycle of 7 min at 72°C. Amplification products were run in a 3% agarose gel, TAE buffer, at 60 V for 50–60 min, and stained with ethidium bromide solution. Fragment size was determined by comparison with molecular weight standards (100 bp DNA Ladder, Fermentas). Duplicate amplifications were conducted to ensure reproducible results and minimize errors. Gels were photographed under UV light with a digital camera.

Table 1 Characteristic of the sites studied

	Potrero de los Funes, El Volcán (PV)	Andacollo, La Primavera (ALP)
Coordinates	S 33°13′59.3″, W 66°13′31.0″; S 33°14′24.4″, W 66°10′49.5″	S 37°15′32.24″, W 70°36′57.4″; S 37°12′51.71″, W 70°37′18.4″
Precipitation (mm)	>700	>700
Soil	Sandy loam with poor organic matter content	Loam
Altitude (m)	990	1,350

Statistical analysis

RAPD analysis was carried out by identifying the homologous bands present. The presence or absence of each RAPD fragment was treated as a binary character (coded as 1 or 0) and used to construct the original data matrix. Polymorphic markers were used for the entire data set.

Genetic variation parameters were estimated using the POPGENE version 1.32 (32-bit) software package (Yeh et al. 1997), and the following indices were estimated: mean observed number of alleles per locus, effective number of alleles per locus, percentage of polymorphic loci, and Nei's (1973) gene diversity (h).

Nei's original measures of genetic identity and genetic distance (Nei 1972) were estimated by both POPGENE version 1.32 (32-bit) and by GenAlEx 6 (Peakall and Smouse 2006) in order to compare results.

Phenetic similarity was analyzed by classification techniques (clustering analysis) that were applied to a similarity matrix constructed by using the Jaccard's coefficient (Jaccard 1908) from the NTSYSpc program, version 2.1e (Rohlf 1997). The unweighted pair-group method of arithmetic averages (UPGMA) was used to construct the dendrogram by NTSYSpc 2.1e.

In addition, principal coordinate analysis (PCoA) was extracted from Euclidean distance matrix to analyze the data set by GenAlEx 6.

Genetic diversity of the RAPD locus was estimated by the Shannon–Weaver information index (Shannon and Weaver 1949) as follows:

$$H = -\sum_{i=1}^{k} Pi \ln Pi$$

where Pi is the frequency of the ith band in a given population, and k is the number of RAPD bands. H is the population's RAPD diversity for each primer. These data were averaged to obtain estimates of within-population RAPD diversity (H_o). The average diversity for all populations (H_{pop}) was calculated at two levels: for each primer as the average of H and over all primers as the average of H_o. RAPD diversity for the species (H_{sp}) was calculated using pooled band frequencies of all individuals. Consequently, it was possible to estimate the proportion of diversity within populations (H_{pop}/H_{sp}) and between populations [$(H_{sp} - H_{pop})/H_{sp}$] (Gauer and Cavalli-Molina 1999). Total diversity and partition of the variation were also estimated through Nei's diversity statistics (Nei 1973). An analysis of molecular variance (AMOVA) was conducted to estimate variance components at several hierarchical levels, partitioning the variation between geographical regions, between populations, and within populations using GenAlEx 6. AMOVA analysis was computed from a matrix of pairwise distances between individuals using Euclidean distance. Since RAPD markers are generally dominant, calculations of pairwise genetic distance for binary data follow the method of Huff et al. (1993). In order to estimate population genetic structures, we have calculated ϕ_{PT} via AMOVA, a measure of population genetic differentiation for binary data that is analogous to F_{ST}. Variance components were tested statistically by nonparametric randomization test using 999 permutations.

Results

Study area

Several studies conducted in the Argentinean Patagonia pointed out habitat preferences for *R. rubiginosa* (Damascos and Gallopin 1992; Bran et al. 2003). However, no studies are available at present about the habitat preferences in San Luis province. Potrero de los Funes (site 1, PV), situated in a hilly area (990 m above sea level), was selected because this location might be the site of introduction of *R. rubiginosa* in San Luis province. Bran et al. (2003) assessed the distribution and abundance of *R. rubiginosa* from Neuquén province and reported a high frequency in La Primavera, included in the present study (site 2).

Table 1 shows the soil characteristics and rainfall regime for the two studied areas. Site 2, Andacollo-La Primavera (ALP), exhibits similar characteristics (Bran et al. 2003) to the ones present in site 1. Both studied areas are disturbed areas, with a comparable rainfall regime and similar soil characteristics, and sampling was performed along a low-transit road.

Specific bands for R. rubiginosa

In order to compare the molecular characteristics of *R. rubiginosa* with those reported from plants of European origin, we used primer OPB-7, a reported primer that generates specific markers for *R. rubiginosa* (Werlemark et al. 1999). Figure 1 shows the pattern of amplified fragments obtained for samples from both experimental sites using primer OPB-7. The pattern obtained was present in most of the samples from both sites. Two bands, reported as typical for *R. rubiginosa* (OPB-7, 450 and 900 bp) were observed in our samples.

RAPD analysis

Genomic DNA from 32 *R. rubiginosa* samples was amplified using 13 primers for RAPD analysis, and 89 fragments were scored. The size of the amplified fragments

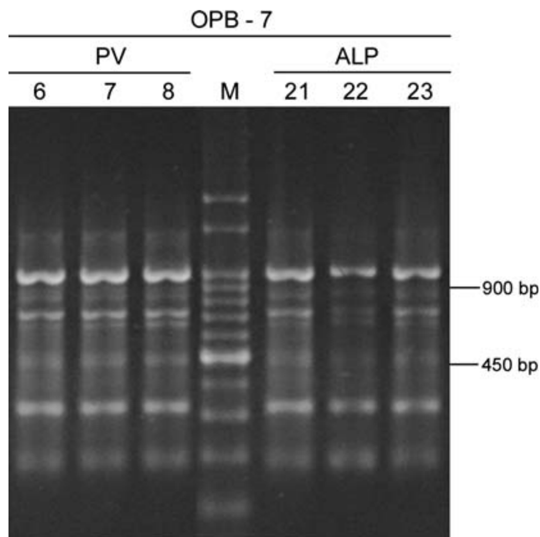

Fig. 1 Pattern of amplified fragments obtained using primer OPB-7. Representative samples were included from site PV: PV6, PV7, and PV8 and site ALP: ALP21, ALP22, and ALP23

Table 2 Number of monomorphic and polymorphic RAPD bands obtained; percentage of polymorphic bands (PPB) using OPA primers for *R. rubiginosa*. *PV* Population from Potrero de los Funes, El Volcán; *ALP* population from Andacollo, La Primavera

Primers	Number of amplified bands	Number of polymorphic bands (PPB)		Total number of polymorphic bands
		PV	ALP	
OPA-3	7	4 (57%)	3 (42%)	7
OPA-5	6	6 (100%)	1 (16%)	6
OPA-6	5	3 (6%)	3 (6%)	5
OPA-7	10	8 (8%)	7 (7%)	10
OPA-8	2	2 (100%)	1 (5%)	2
OPA-9	9	4 (44%)	7 (77%)	9
OPA-10	5	3 (6%)	5 (1%)	5
OPA-13	19	13 (68%)	10 (52%)	19
OPA-14	5	1 (2%)	4 (8%)	5
OPA-15	7	4 (57%)	5 (71%)	7
OPA-17	5	2 (4%)	3 (6%)	5
OPA-18	6	2 (33%)	5 (83%)	6
OPA-19	4	3 (75%)	0 (0%)	3
Average	6.92	4.23 (61%)	4.15 (59%)	6.84 (98%)

ranged from 100 to 2,500 base pairs (bp). The number of obtained amplified fragments ranged from 1 to 19, with an average per primer of 6.92 fragments. Table 2 summarizes these data and Table 3 shows similar results obtained with POPGENE 1.32. The percentage of polymorphic bands (PPB) for each primer ranged from 0 to 100%. The average yield of polymorphic markers across primers was 61%, ranging from 100% (e.g., OPA-5 and OPA-8) to 20% (OPA-14) for *R. rubiginosa* genotypes from the PV site. The samples from ALP were amplified with an average yield of 59%, ranging from 100% (e.g., OPA-10) to 0% (OPA-19). Similar results for mean values of allelic variation and Nei's genetic diversity were scored for the two populations analyzed (Table 3).

Table 3 Genetic variation parameters and Nei's genetic diversity in the analyzed population

Population	na	ne	*h*	*P* (%)
PV	1.6292	1.4522	0.2456	62.92
ALP	1.5955	1.3055	0.1831	59.55
PV-ALP	1.9888	1.4637	0.2767	100.00

Comparisons of mean values of allelic variation and genetic diversity, grouped according to experimental sites and total samples

na Observed number of alleles, *ne* effective number of alleles, *h* Nei's gene diversity, *P* percentage of polymorphic loci

Large variations were observed between the two studied groups. For instance, primer OPA-5 exhibits maximum value in the PV group and a low yield (16%) in the ALP site. The largest difference was observed for primer OPA-19, with 75% of PPB in PV and none in ALP populations.

Figure 2 shows the pattern of amplified fragments obtained for samples from both experimental sites using primers OPA-7 (Fig. 2a) and OPA-13 (Fig. 2b). An homogeneous pattern of amplified bands was observed for each site with either primer, with exceptions (e.g., PV9). Samples from the ALP site show, in general, a lower number of bands. The polymorphic bands of 1,250 and 700 bp obtained with OPA-7 were present in most of the samples from site PV, but absent in samples PV9, PV16, PV17, PV19, and PV20. These markers were observed in site ALP, but absent in samples ALP21, ALP28, ALP 29, and ALP 30. Sample PV1 exhibited a different pattern.

Primer OPA-13 rendered a higher number of bands in both groups (Fig. 2b). However, samples from site 1, PV (upper panel), exhibited markers of higher molecular weight, which were absent in samples from the ALP site (lower panel).

Cluster analysis

To estimate the level of polymorphism in each population and the similarity among *R. rubiginosa* individuals, a molecular dendrogram was constructed. The molecular dendrogram denoted two subclusters into which samples from each population (PV or ALP) were clearly grouped (Fig. 3). This observation correlates with the geographical provenances of the evaluated populations. Group I (GP I) associated the individuals sampled from experimental site 1 (PV), whereas individuals sampled from experimental

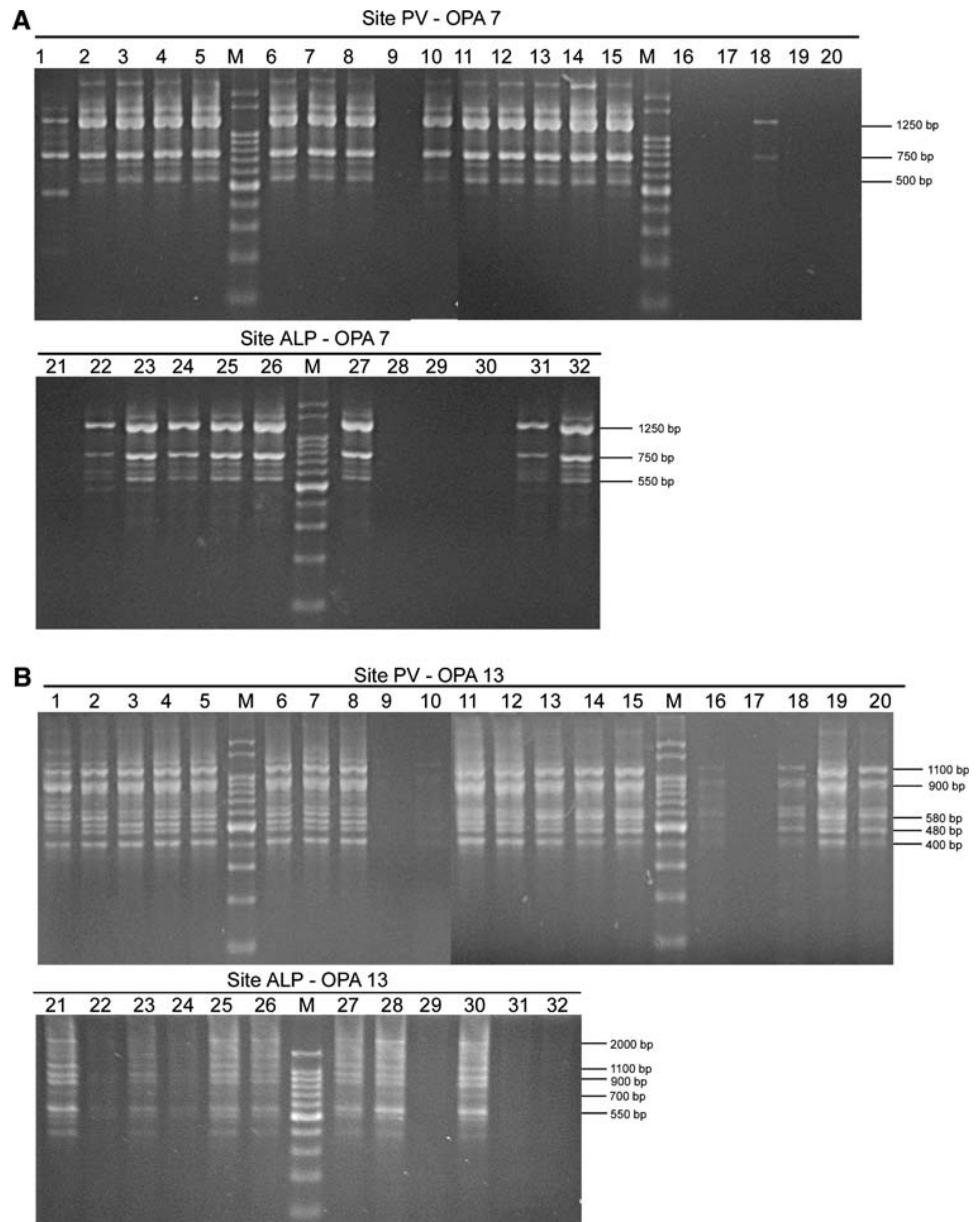

Fig. 2 RAPD bands produced by primers OPA-7 (**a**) and OPA-13 (**b**) for samples from the PV (*upper panels*) and ALP sites (*lower panels*). *M* Molecular weight marker

site 2 (ALP) were associated in group II (GP II). The sample PV9 was outside of the two population clusters.

Principal coordinate analysis (PCoA)

Calculated pairwise Euclidean distances were used as input for PCoA. The first three principal coordinate axes accounted for 45.12, 17.93, and 12.30% of total variation (cumulative value 75.35%). Considering the three principal coordinates, populations were plotted as a broad scatter and clustered independently one from each other. UPGMA clustering results were confirmed by the pattern of PCoA plotting (Fig. 4). The PCoA illustrates a separation between the evaluated populations. However, population PV exhibited a higher association among individuals than population ALP.

Genetic variation within populations

Within-population variation of *R. rubiginosa* was estimated using the Shannon–Weaver index for the 13 studied primers. The estimated values of genetic diversity within populations (H_o) for each primer and the mean values for all primers are shown in Table 4. The higher genetic diversity was obtained with primer OPA-13 for both populations (PV and ALP) and reached a maximum value for

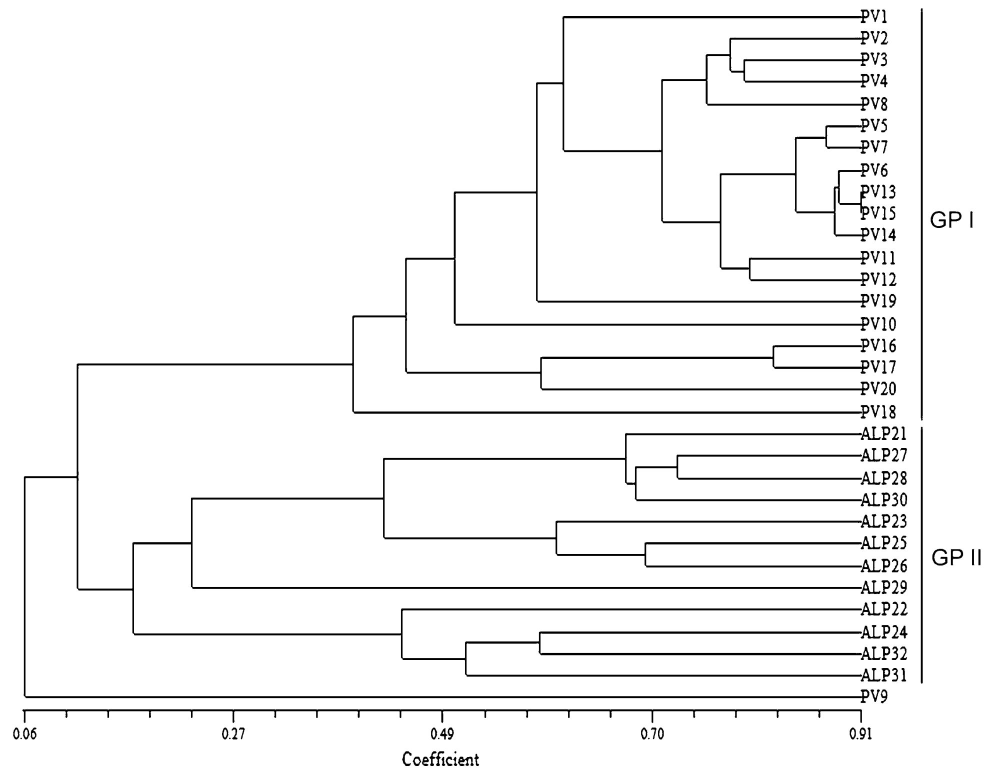

Fig. 3 UPGMA dendrogram visualizing the genetic relationship among 32 samples of *R. rubiginosa*, based on the Jaccard's similarity coefficient

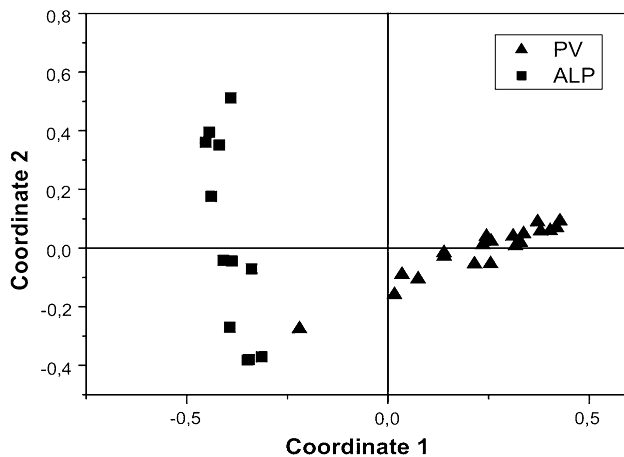

Fig. 4 Principal coordinate analysis (PCoA) of *R. rubiginosa* provenances from the PV and ALP sites

the PV population (2.03). The lowest genetic diversity was obtained with primers OPA-17 and OPA-18 for the PV site and primers OPA-17 for the ALP site. The estimated value of genetic diversity was similar for both populations ($H_o = 1.17$ and 1.10 for PV and ALP, respectively) and the average RAPD marker diversity for *R. rubiginosa* was $H_{sp} = 1.50$.

The Shannon–Weaver index, calculated from the relation between H_{sp} (1.50) and H_{pop} (1.13), allocated 74% of total variation within populations.

Table 4 Genetic diversity in *R. rubiginosa* populations and partitioning of genetic diversity within and between populations (Shannon-Weaver index) using 13 primers

Primers	H		H_{pop}	H_{sp}	H_{pop}/H_{sp}	$(H_{sp} - H_{pop})/H_{sp}$
	PV	ALP				
OPA-3	1.36	1.10	1.23	1.55	0.79	0.21
OPA-5	1.72	0.00	0.86	1.73	0.50	0.50
OPA-6	1.10	1.10	1.10	1.50	0.73	0.27
OPA-7	1.88	1.91	1.89	2.07	0.91	0.09
OPA-8	0.69	0.00	0.35	0.68	0.51	0.49
OPA-9	1.37	1.79	1.58	1.94	0.82	0.18
OPA-10	1.10	1.23	1.16	1.23	0.95	0.05
OPA-13	2.03	1.94	1.99	2.30	0.86	0.14
OPA-14	0.00	1.33	0.67	1.02	0.66	0.34
OPA-15	1.37	1.56	1.47	1.81	0.81	0.19
OPA-17	0.69	0.86	0.78	1.37	0.57	0.43
OPA-18	0.69	1.50	1.10	1.13	0.97	0.03
OPA-19	1.18	0.00	0.59	1.18	0.50	0.50
	H_o					
Average	1.17	1.10	1.13	1.50	0.74	0.26

Partitioning of variation and genetic divergence between populations

The Shannon–Weaver index, calculated from the relation between H_{sp} (1.50) and H_{pop} (1.13), allocated 26% of total

Table 5 AMOVA analysis carried out on 89 RAPD loci in 32 *R. rubiginosa* samples from PV and ALP. Degrees of freedom (*df*), sum of squares (SS), variance components, fraction of total variation, associated significance level (P, $n = 999$ permutations) and ϕ-statistics are shown

Source of variation	*df*	SS	Variance	Total (%)	ϕ-statistics	*P* value
Between populations	1	16.818	0.966	29		
Within populations	30	69.62	2.32	71	0.294	0.001

variation between populations rather than within them. Nei's Gst (0.2205), estimated by POPGENE 1.32 (32-bit), was in accordance with the results from the Shannon–Weaver index, attributing 22% of variation between populations.

Pairwise comparisons between populations conducted by AMOVA at two levels revealed a highly significant ($P < 0.001$) differentiation of genetic variability occurring between populations from these provenances (Table 5). Thus, 71% of genetic variability was distributed within populations and 29% between the populations (Table 5). These results are in accordance with those estimated from the Shannon–Weaver's index. Furthermore, the recorded population genetic differentiation ϕ-statistic (ϕ_{PT}) and the associated significance level from AMOVA (Table 5) can be used to determine genetic distance between populations (Huff et al. 1993). In the present study, ϕ_{PT} showed low interpopulation differentiation for *R. rubiginosa,* with $\phi_{PT} = 0.29$. All estimates of genetic differentiation provided similar values.

Measures of genetic identity and genetic distance (Nei 1972) were estimated by using POPGENE 1.32 v (32-bit) and GenAlEx 6 for comparison. Both programs provided equivalent results, thus suggesting genetic proximity between both populations in study. The obtained values were $I_{NEI} = 0.85$ and $D_{NEI} = 0.157$.

Discussion

R. rubiginosa is an introduced exotic species in Argentina that combines the negative effect of a weed with a potential economic value. The aim of the present study was to use RAPD to evaluate genetic diversity and differentiation in populations of *Rosa rubiginosa* from two different Argentinean ecoregions. We selected for this study two populations; site 1 was selected because it was the place of introduction of *R. rubiginosa* in San Luis and site 2 was previously evaluated by other authors (Bran et al. 2003, 2004). Both studied areas are disturbed areas, with a comparable rainfall regime and similar soil characteristics and sampling was performed along a low-transit road.

RAPD markers were applied to characterize genetic variation in *Rosa rubiginosa* by using 13 primers. The RAPD markers generated in the two studied populations provided 61% of polymorphic markers for plants growing from Dry Chaco and 59% for plants growing from Patagonian Steppe. A similar rate (52.1%) was achieved for *Prunus mahaleb* (Rosaceae) (Jordano and Godoy 2000), while Jürgens et al. (2007) found a high rate (81.7–83.2%) in *Rosa canina*. A monomorphic band (350 bp) was consistently observed in the PV group when using primer OPA-19. A number of differential bands were present in only one of the two populations. These markers indicated genetic differentiation between populations.

Two different analyses—the UPGMA dendrogram and the PCoA—clearly suggest a geographical differentiation of the provenances of *R. rubiginosa* populations at a regional scale. The obtained polymorphic molecular markers (89 bands scored), evaluated by cluster analysis, allow the identification of two different groups.

AMOVA analyses of *R. rubiginosa* from two areas of study revealed high genetic variation within populations (71%) and low variation between populations (29%). These results are in agreement with the values estimated from the Shannon–Weaver index (Table 4). In general, the AMOVA statistic confirmed the PCoA and cluster analyses projections. Similar values with RAPD markers were obtained with the Shannon–Weaver index for *Rosmarinus tomentosus* (66 and 34%, Martín and Hernandez Bermejo 2000), *Ilex paraguariensis* (85 and 15%, Gauer and Cavalli-Molina 1999). As well, similar values with RAPD markers were obtained with AMOVA for *Rosa canina* (76–87 and 24–13%, Jürgens et al. 2007), *Pilgerodendron uviferum* (81.45 and 18.55%, Allnutt et al. 2003), and *Pinus mugo* (83.39 and 15.36%; Monteleone et al. 2006). Values correspond to intra- and interpopulation genetic diversities, respectively.

Our results suggest a higher relevance of the outcrossing rate and an efficient seed dispersal system for intrapopulation genetic variation in *R. rubiginosa.*

Genetic differentiation between *R. rubiginosa* populations estimated by AMOVA was $\phi_{PT} = 0.29$ ($P < 0.001$). Nei's Gst for RAPD-based studies (0.2205) was lower for interpopulation variation. Nybom (2004) reported that the value for AMOVA-derived genetic differentiation among populations (ϕ_{PT}) is almost identical for different dominant marker-based methods (RAPD, AFLP, STMS), but they found a large discrepancy between ϕ_{PT} and Gst values. Thus, comparison of genetic differentiation values among populations from different studies must be treated carefully. The low interpopulation value that was obtained suggests considerable genetic homogeneity between the

two system populations that were evaluated. However, these populations exhibited specific monomorphic bands that account for genetic differentiation between both populations.

Nybom (2004) reported a relatively high value of genetic differentiation among populations in the case of annuals ($\phi_{PT} = 0.62$), a moderate value for short-lived perennials ($\phi_{PT} = 0.41$), and lowest values for long-lived perennials ($\phi_{PT} = 0.25$). For *R. rubiginosa*—a shrub species—the ϕ_{PT} value obtained in our study (0.29) fell within the last group. Similar values were reported for *R. canina* (range 0.103–0.35) (Jürgens et al. 2007).

Breeding system, seed dispersal, life form, and geographical distribution are decisive factors when determining levels of genetic variation and its partitioning among and within populations (Jürgens et al. 2007). RAPD analyses (Allnutt et al. 2003; Jordano and Godoy 2000; Jürgens et al. 2007; Nybom 2004) have shown that outcrossing species are generally characterized by a high level of intrapopulation diversity and low differentiation among populations. Our present data are in agreement with these observations. Efficient seed dispersal for *R. rubiginosa* is provided by an assortment of frugivorous birds and mammals that usually remove and disperse a high proportion of the fruit every year.

The hypotheses to explain exotic plant invasion success, including the lack of natural enemies, evolution of invasiveness, empty niche and novel weapons, have been revised by Hierro et al. (2005). Several mechanisms have been proposed to explain the variation in community susceptibility to invasions by studying exotics in their introduced range; hypotheses address plant invasion biology, disturbance, species richness, and propagule pressure (Hierro et al. 2005). The hypothesis that species-rich systems are less susceptible to invasion (Gilpin 1994; Morton et al. 1996; Moore et al. 2001) is based on the idea that species richness correlates with community saturation so that establishment by a new species is more difficult in saturated communities. Damascos and Gallopin (1992), in a study on *Rosa rubiginosa,* evaluated the risk of invasion for vegetal communities in the Andino-Patagonic region of Argentina and consider that the number of exotic species present in assemblages is often higher in species-rich systems. *R. rubiginosa* is an r strategist species that occupies environments with an altered vegetation structure (Damascos and Gallopin 1992). Moore et al. (2001) and Damascos and Gallopin (1992) assumed that the number of exotic species is correlated with the probability of successful invasion.

The current widespread distribution of *R. rubiginosa* in Argentina is not only due to its adaptative potential but also to several accompanying factors, including (1) natural or anthropic degradation of natural woodland, (2) the presence of ungulate herbivores and their effects in facilitating exotic plant invasions, (3) the palatability of the fruit of *R. rubiginosa* foraged by native species and domestic ungulates, which in turn transport it to different sites favoring seed dispersion. The observed high percentage of within-population genetic diversity suggests that an important amount of genetic variation has been introduced into both ecoregions. At the same time, the intrapopulation genetic diversity allows a higher adaptation capability in the introduced species. From the present results we can conclude that we observed two independently established populations with high similarity between them and a strong intrapopulation differentiation. The empty niche hypothesis for invasion success might explain the invasibility of *R. rubiginosa* in Argentinean ecoregions.

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