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Genetic structure and diversity of the amphitropical disjunct grass *Leptochloa crinita* (formerly *Trichloris crinita*) and implications for restoration

R. Emiliano Quiroga[1,✉]; Paula Mathiasen[2]; M. Paula Quiroga[2]; Roberto J. Fernández[3]; Dayana G. Díaz[2] & Andrea C. Premoli[2]

[1] Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Catamarca. Catamarca, Argentina. [2] Instituto de Investigaciones en Biodiversidad y Medio Ambiente (INIBIOMA-CONICET), Universidad Nacional del Comahue. Bariloche, Río Negro, Argentina. [3] Facultad de Agronomía, Universidad de Buenos Aires e IFEVA-CONICET. Ciudad Autónoma de Buenos Aires, Argentina.

Abstract. Amphitropical species have disjunct distributions south and north of the equator. That is the case of *Leptochloa crinita*, a perennial grass found in dry and warm regions of South and North America, and recommended for rangeland restoration. We analyzed to what degree the distribution of the species in both subcontinents shaped its genetic differentiation and population variability. We collected seeds from 15 populations from South America and 7 from North America, and grew them in a common garden to evaluate adaptive variation. Three microsatellite markers and DNA sequences from one nuclear (ITS1-5.8S-ITS2) and one chloroplast region (rpl32-trnL) were used to analyze recent and historical gene flow, respectively. We used climatic niche models to infer past suitable habitats. We found significant genetic variability among populations within each subcontinent, but low genetic differences between populations from South vs. North America; these were detected only with microsatellites and not with DNA sequences. Results show that the species has diverged from a common gene pool in the recent past (~500-3300 generations ago [estimate: ~3000-20000 years]). Populations from South America had plants with more and taller inflorescences, more tillers and heavier seeds than the North American ones, which could represent adaptations to the less stressful environments that the species inhabits in South America. Climatic niche models indicate few potential habitats for the species in North America during the Last Glacial Maximum and Middle Holocene, in contrast to South America, where potential habitats seemed to be comparable or more extensive than at present. This and previous studies provide a view of the genetic resources of the species. Results suggest that —if necessary, and taking proper precautions— the admixture of *L. crinita* populations or the germplasm translocation between subcontinents are alternatives to consider in restoration practices.

[Keywords: biogeographic history, common garden, DNA sequences, microsatellites, North America, populations, South America]

Resumen. Estructura y diversidad genética en la gramínea disyunta anfitropical *Leptochloa crinita* (ex *Trichloris crinita*) e implicancias para la restauración. Las especies con distribución disyunta anfitropical se encuentran al sur y al norte del ecuador. Este es el caso de *Leptochloa crinita*, gramínea perenne que habita regiones secas y cálidas de Sudamérica y Norteamérica, que es recomendada para la restauración de pastizales. Se analizó en qué medida la diferenciación y la variabilidad genética de las poblaciones de *L. crinita* están influenciadas por su patrón de distribución en ambos subcontinentes. Se colectaron 15 poblaciones de Sudamérica y 7 de Norteamérica, cuyo potencial adaptativo fue evaluado en jardín común. Tres marcadores microsatélites y secuencias de ADN (ITS1-5.8S-ITS2, región nuclear; rpl32-trnL, región del cloroplasto) se utilizaron para analizar señales contemporáneas e históricas de flujo génico, respectivamente. Se usaron modelos de nicho climático para inferir la distribución de hábitats favorables en el pasado. Solo con microsatélites se encontró variabilidad genética significativa entre las poblaciones dentro de cada subcontinente, y escasa diferenciación entre poblaciones de Sudamérica y Norteamérica. La especie habría divergido en el pasado reciente a partir de un acervo genético común (hace ~500-3300 generaciones [estimación: ~3000-20000 años]). Las poblaciones sudamericanas presentaron plantas con inflorescencias más altas y numerosas, más macollos y semillas más pesadas que las norteamericanas; estas podrían representar adaptaciones a los ambientes menos estresantes que la especie habita en Sudamérica. Los modelos de nicho climático mostraron escasa disponibilidad de hábitats favorables para la especie en Norteamérica durante el Último Máximo Glacial y el Holoceno Medio. En cambio, en Sudamérica, la disponibilidad de hábitats parece haber sido similar o mayor a la de la actualidad. Estos conocimientos sobre *L. crinita* brindan una visión general de los recursos genéticos de la especie. Los resultados sugieren que, de ser necesario, y con las precauciones del caso, se podrían usan como prácticas de restauración la mezcla de poblaciones y la traslocación de germoplasma entre subcontinentes.

[Palabras clave: historia biogeográfica, jardín común, microsatélites, Norteamérica, poblaciones, Sudamérica]

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✉ quiroga.raul@inta.gob.ar
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Introduction

Rangelands cover more than a third of the continental areas, ranging from arid to sub-humid climates, and support half of the world's livestock and wildlife. A third of the human population lives in these environments; thus, their degradation represents a severe problem with global economic losses estimated at US$ 10 trillion per year (Sutton et al. 2016). In recent decades, efforts to reestablish vegetation cover, biodiversity and function of degraded rangelands have increased. The restoration of these environments implies taking into account the ecosystem services (e.g., forage for livestock, habitat for wildlife, erosion control, etc.) and the biology of the species to be reestablished. The latter should include intraspecific genetic structure and diversity in order to maximize evolutionary potential and minimize the risk of introducing inappropriate variants (Sgrò et al. 2011).

Widespread species may consist of populations (sensu Waples and Gaggiotti [2006]) (i.e., a group of individuals of the same species that co-occur in space and time and have an opportunity to interact with each other) with high phenotypic plasticity and/or with high genetic variability and differentially adapted genotypes, both characteristics that allow them to survive in different environments (Premoli and Mathiasen 2011; Kelly 2019). Wide-ranging species with geographical disjunction (sensu Thorne 1972) may present limitations to gene flow, which over time may increase the degree of divergence between them (Millar and Libby 1991). Geographic disjunctions may be the result of either long-distance dispersal or fragmentation of a previously continuous distribution by vicariance (Simpson et al. 2017). As in other parts of the world, a group of plant species in South and North America have amphitropical disjunct distribution (i.e., with populations inhabiting the two hemispheres but absent in the tropics) (Raven 1963; Simpson et al. 2017). Several authors pointed out the possibility that amphitropical distributions of genera and species in both American subcontinents were shaped by climatic oscillations at geological time scales (García et al. 1960; Raven 1963; Donoghue 2011; Villaverde et al. 2015). In addition, it has been found that most of the amphitropical disjunct species between South and North America show climatic niche shifts (Villaverde et al. 2017; Quiroga et al. 2021). This may be the result of natural selection acting differentially on the populations of these species and/or neutral forces such as genetic drift and isolation.

Here we focus on *Leptochloa crinita* (Lag.) P.M. Peterson and N.W. Snow [Poaceae; homotypic synonym: *Trichloris crinita* (Lag.) Parodi], one of the many species with natural disjunct distribution between hot arid and semi-arid environments of South and North America (Peterson et al. 2007). In central-northwestern Argentina, *L. crinita* is one of the main forage species of rangelands (Dalmasso 1994). Like other native grasses, it suffered the impact of overgrazing due to inadequate management of livestock herds (Anderson et al. 1980). Even though *L. crinita* is promoted for rangeland restoration (Quiroga et al. 2009; Kozub et al. 2017; USDA-NRCS 2020) and the existence of intraspecific variability has been acknowledged both within South America (Cavagnaro et al. 2006; Quiroga et al. 2010; Zabala et al. 2011) and within North America (Pezzani et al. 2006), the genetic structure and diversity between both subcontinents has not yet been explored. Advances in the knowledge of this ecologically important species could be relevant for other amphitropical disjunct species, considering that similar patterns of niche differentiation between desert species of South and North America were described recently: in North America, they tend to inhabit relatively drier and warmer environments than in South America (Quiroga et al. 2018, 2021).

Consideration of intraspecific genetic structure and diversity is important for species conservation and management, as is increasingly recognized in restoration ecology (Mijangos et al. 2015; Kozub et al. 2017). They are of particular interest for species under use in human activities —as *L. crinita* in ranching systems— and rangeland restoration (González and Látigo 1981; Passera et al. 1992; Quiroga et al. 2009; Pawelek et al. 2015). Previous studies explored the genetic variability in forage aptitude of Argentinean populations of the species using AFLP (amplified fragment length polymorphisms) (Cavagnaro et al. 2006). But genetic characteristics of populations of both subcontinents have not been compared so far. In addition, basic aspects of the species biogeographic history are not known, such as the degree of differentiation or the manner and time of divergence between South and North American populations.

In ecological restoration practice, it is widely discussed whether plant materials should be

from a diverse genetic pool or restricted to a local origin (e.g., McKay et al. 2005; Bucharova et al. 2017; Hancock et al. 2023). While the introduction of non-local germplasm may increase the genetic variability and evolutionary potential of recipient populations that have been 'genetically eroded' (Mijangos et al. 2015; Kettenring et al. al. 2014), in some cases, such genotypes can present poor adaptation to the local environment or cause outbreeding depression, resulting in lower ecological fitness (Johnson et al. 2010). Furthermore, climate change challenges the classic rule 'local is best' in seed sourcing for restoration, as local germplasm may be maladaptive under future climates (Jones 2013). For *L. crinita*, Kozub et al. (2017) suggested that although local adaptation is relevant, matching the local environmental factors could be of greater importance than using locally collected seed sources. They also pointed out that intraspecific variation could be exploited by using seeds from sites with similar environmental conditions as those in the restoration site. In any case, intraspecific variability in genetic and adaptive characteristics of target species is undeniably essential to defining seed-source populations and the restoration strategy.

Molecular markers can provide important information to estimate the genetic structure and diversity of a species difficult to obtain using other methods. For example, they can provide an assessment of the degree of relatedness between populations or past demographic events that modelled genetic patterns of isolation and divergence that exist today, which are relevant in restoration actions (Williams et al. 2014). In this work, we used two types of molecular markers: microsatellites and DNA sequences. Microsatellites are standard genetic markers for population genetic analysis. They consist of short sequences of nucleotides (typically 1-5 base pairs) that are repeated in tandem and are randomly or nearly randomly distributed in the genome (Li et al. 2002). They are usually considered as selectively neutral due to isolation and genetic drift, and because of their relatively high mutation rates can allow detecting genetic changes in relatively recent geological times. On the other hand, DNA sequences of the plastid and nuclear genomes have lower mutation rates and are more conserved than microsatellites. However, although DNA sequences are less variable than microsatellites, many studies have found intraspecific variability in the plastome (Harris and Ingram 1991), which have been widely used in phylogeographic analyses (Yang et al. 2021). Together, both types of molecular markers allow to analyze historical isolation of populations, which can be used to perform phylogenetic and biogeographic reconstructions (Avise 2004). In addition, the degree of divergence among populations estimated with neutral molecular markers can be compared to those estimated with quantitative traits that may have been subjected to natural selection, which measured in a controlled environment (i.e., common garden) allows the expression of the genetic background of populations and gives the possibility of exploring the influence of natural selection (Merilä and Crnokrak 2001; McKay and Latta 2002).

Our aim in this first exploratory genetic study including *L. crinita* populations from both South and North America was three-fold: a) to investigate the genetic differentiation and diversity at distinct hierarchical levels (i.e., populations and subcontinents); b) to estimate the influence of natural selection on populations of both subcontinents, and c) to infer the biogeographic history of the species. The hypothesis tested is that the amphitropical distribution of the species in both subcontinents shaped the genetic characteristics of *L. crinita* populations. Associated predictions were: a) we expect to find a marked genetic divergence on neutral markers between populations of both subcontinents due to current isolation and the influence of historical factors, and b) considering the differences found between the environments currently occupied by the species in South and North America (Quiroga et al. 2018), we also expect to find a differential effect of natural selection on the populations of each subcontinent.

Materials and Methods

Species

Leptochloa crinita is a perennial bunchgrass of summer growth with a C4 leaf anatomy (Nicora and Rúgolo de Agrasar 1987). Foliage reaches between 30 and 80 cm in height, with leaf blades of 15 to 25 cm long and 0.2 to 1.0 cm wide. Their dispersal unit (diaspora; for simplicity, hereafter: seed) is the fertile anthecium made up of the fruit (caryopse), the lemma and the palea. Seeds are arranged in inflorescences 5 to 18 cm long, with each

inflorescence located at the end of a 30-100 cm tall culm (Nicora and Rúgolo de Agrasar 1987). The species is strictly autogamous (Gutiérrez et al. 2016; Kozub et al. 2017) and tetraploid (2n=4x=40) (Kozub et al. 2019; Carloni et al. 2021).

Population collection

Populations of *L. crinita* were collected in northwestern Argentina (South America, 15 populations) and southwestern USA (North America, 7 populations) (Table 1; details of collection sites in Supplementary Material-Table S1). In both subcontinents, the minimum and maximum geographical distances between evaluated populations was ~5 km and ~1000 km, respectively. Sampling locations covered climatic differences between populations within each subcontinent. For each population, we collected seeds from ~20 mother plants, except for the Knox population that was too small, and thus seeds from only 5 plants were obtained. In each population, sampled plants were separated >5 m to avoid collect seeds from vegetative clones. Since our lower level of analysis was the population, seeds from each population were pooled. Chamical and Kinney populations are germplasm-registered at the Instituto Nacional de Tecnología Agropecuaria of Argentina (inta.gob.ar/variedades/chamical-inta) and the United States Department of Agriculture (USDA-NRCS 2020), respectively.

Common garden and sampling

As populations were collected in different years (2005, 2010, 2014), seeds that were collected first were germinated and planted

Table 1. Coordinates of the collection sites of South American (SA) and North American (NA) populations, and mean values of traits measured at the common garden. Statistical significance of populations comparison (n.s.: P>0.05; *P<0.05; **P<0.01; ***P<0.0001) and least significant difference (Fisher LSD) are shown below the list of populations. The last three rows show the mean values per subcontinent and statistical significance (P) of their comparison.

Tabla 1. Cordenadas de los sitios de colecta de las poblaciones sudamericanas (SA) y norteamericanas (NA), y valores medios de los caracteres evaluados en jardín común. Debajo de la lista de poblaciones se muestran los niveles de significancia estadística (n.s.: P>0.05; *P<0.05; **P<0.01; ***P<0.0001) y las diferencias mínimas significativas (LSD de Fisher). Las últimas tres filas muestran los valores medios por subcontinente y el nivel de significancia estadística (P) de su comparación.

Sub continent	Population	Latitude	Longitude	# Inflorescences	# Tillers	Infloresc. height (cm)	Canopy height (cm)	Weight of 1000 seeds (g)
NA	Bowie	32°17′ N	109°17′ W	10.8	32.4	81	37	0.17
NA	Kinney	29°19′ N	100°26′ W	12.6	39.0	82	47	0.19
NA	Knox	32°17′ N	106°46′ W	10.6	24.8	66	39	0.15
NA	Lucero	33°06′ N	106°32′ W	10.3	30.3	60	35	0.08
NA	San Simon	32°17′ N	109°16′ W	9.4	25.4	65	33	0.19
NA	Tornillo	31°24′ N	106°01′ W	9.2	29.0	63	37	0.15
NA	White Sands	32°28′ N	106°25′ W	10.0	26.4	77	41	0.13
SA	Amblayo	25°28′ S	65°50′ W	16.6	37.4	83	40	0.18
SA	Chamical	30°31′ S	66°08′ W	16.2	32.2	82	41	0.20
SA	Colpes	28°03′ S	66°13′ W	11.8	32.8	82	39	0.19
SA	HC	29°58′ S	63°29′ W	16.8	35.4	87	38	0.26
SA	HL	29°53′ S	64°28′ W	11.8	27.8	75	38	0.18
SA	RCH Ancha	34°16′ S	67°54′ W	13.0	35.6	84	40	0.25
SA	RCH Fina	34°16′ S	67°54′ W	12.8	29.8	82	39	0.20
SA	Recreo	29°19′ S	65°08′ W	27.5	49.3	89	45	0.20
SA	Recreo Salinas	29°31′ S	64°58′ W	21.8	45.8	89	43	0.25
SA	Salinas Grandes	30°36′ S	65°36′ W	13.2	30.8	74	39	0.21
SA	San Martín	29°13′ S	65°46′ W	27.2	37.6	87	41	0.18
SA	SC	31°24′ S	66°46′ W	17.4	35.0	80	38	0.15
SA	SL	31°31′ S	66°49′ W	8.0	26.5	82	35	0.22
SA	Tilimuqui	29°08′ S	67°26′ W	25.6	32.4	78	37	0.17
SA	El Tipán	28°59′ S	65°46′ W	19.0	38.8	89	46	0.19
LSD and P value between populations				7.5***	n.s.	14*	6***	0.06***
NA	(mean)			10.4	29.6	71	39	0.15
SA	(mean)			17.3	35.2	83	40	0.20
P value between subcontinents				**	*	***	n.s.	**

in 2006 in a first common garden located at INTA La Rioja (lat. 30°30'31'' S - long 66°07'12'' W; 409 m a. s. l.) arranged in 3 blocks, 8 plants per population per block, 0.5 m distance between plants. Then, seed samples from all the plants of each population were collected from this first trial, seeds were germinated and planted in 2011 in a second common garden at INTA Catamarca (lat. 28°28'27'' S - long. 65°43'54'' W; 513 m a. s. l.; same experimental design) into which the populations collected in 2010 were planted, and then in 2014 the last collected populations were added (same planting method). From this second common garden we collected leaf samples for molecular analyses (see below). It is important to note that the use of seeds harvested from a common garden is not a problem for this strictly autogamous species (Gutiérrez et al. 2016; Kozub et al. 2017; Marinoni et al. 2018). Finally, in order to measure size and productivity traits of plants of the same age, in 2018 a third common garden was established at INTA Catamarca (5 randomly distributed plants per population, 0.5 m distance between plants) using seeds harvested in the previous (second) common garden. At the end of the first growing season (one year old plants) we measured the following traits: number of inflorescences, number of tillers, inflorescence height, canopy height, and seed weight.

Molecular analyses

Leaf samples consisting of fresh tissue collected from plants of all provenances (from the second common garden, mentioned above) were subjected to a DNA extraction protocol adapted from Doyle (1990). Extracts were amplified by PCR for the analyses of DNA sequences and microsatellite (Supplementary Material-Appendix S1). The number of individuals that could be analyzed per population for each molecular marker is presented in Supplementary Material-Table S2 (one individual per population for DNA sequences, and 2 to 5 individuals per population for microsatellites). Nuclear and plastid DNA sequences were analyzed using universal primers of the regions ITS1-5.8S-ITS2 (internal transcribed spacer of the eukaryotic rRNA operon; primers ITS1 and ITS4; Wright et al. 2006) and rpl32-trnL (intergenic spacer between the rpl32 and trnl genes; primers trnL[UAG] and rpL32-F; Shaw et al. 2007), respectively. The amplified DNA fragments were sequenced directly. For microsatellites, a total of six microsatellites developed specifically for *L. crinita* by Kozub et al. (2018) were used but, only three of them amplified successfully; these were Mss8, Mss11 and Mss93 (Supplementary Material-Appendix S1). Amplified PCR products of DNA sequences and microsatellites were sent to Macrogen (Korea) for sequencing and genotyping, respectively. We aligned using MEGA11 software (Tamura et al. 2021) (alignments are available in Supplementary Material-Figure S1 and Supplementary Material-Figure S2). Thorough DNA sequence visual controls were performed to detect possible anomalies, as double picks, and corroboration of variable sites. Microsatellite genotypes were identified using the PEAK SCANNER v1.0 program (Applied Biosystems), assigning to each allele the size of the amplified fragment compared with a marker of standard molecular size (GS500 LIZ, Applied Biosystems). The patterns found reflected a diploid dose, and in no case more than two peaks were detected per microsatellite.

Statistical analyses

Plant traits. Data of common-garden grown plants (number of inflorescences, number of tillers, inflorescence height, canopy height, and seed weight) were subjected to analysis of variance, considering both the subcontinent of origin and the populations nested within each subcontinent as fixed-effect factors (α=0.05; Fisher test for means comparison; INFOSTAT software) (Di Rienzo et al. 2020).

Population genetic diversity. Indicators of genetic diversity were obtained from 1) DNA sequences by calculating for each subcontinent the nucleotide diversity (polymorphism) (ARLEQUIN 3.5.2.2 software; Excoffier and Lischer 2010), and 2) microsatellite data by calculating for each population: number of different alleles (Na), number of effective alleles (Ne), Shannon index (I), number of unique or private alleles (Nap), expected heterozygosity (He) and observed heterozygosity (Ho) (GENALEX 6.503 software) (Peakall and Smouse 2012). Using the last two indices, the inbreeding coefficient was calculated for each population as:

$$Fis = (He - Ho) / He \qquad \text{Equation 1}$$

For microsatellite data, values of each indicator were compared between populations from South and North America using the non-parametric Kruskal Wallis test (α=0.05; INFOSTAT).

Population genetic structure. We used analysis of molecular variance (AMOVA) to evaluate genetic diversity at different hierarchical levels, i.e., within and among populations at each subcontinent, and between South and North America subcontinents. Data from DNA sequences and microsatellites were analyzed separately. For sequences, where one individual per population was analyzed, we assessed the variation between subcontinents (South America, North America) and within subcontinents (α=0.05; ARLEQUIN 3.5.2.2). For microsatellites, where 2 to 5 individuals per population were analyzed, we assessed the variation at each of the hierarchical levels explained above (α=0.05; GENALEX 6.503).

Heterogeneity in allele frequencies between groups for microsatellites was assessed with the Chi-square test (BIOSYS-1 software; Swofford and Selander 1981) under four different criteria: 1) the two subcontinents as populations (in a broad sense, South and North America); 2) all the populations of both subcontinents, pooled; 3) the South America populations, and 4) North America populations.

The isolation by distance model that correlates genetic and geographic distances was investigated with a Mantel test for populations within each subcontinent using microsatellite data (GENALEX 6.503). In addition, phylogenetic relationships among populations were estimated with the neighbour-joining method (NJ) for microsatellites (using the genetic chord distance) (Cavalli-Sforza and Edwards 1967) (POPULATIONS 1.2.32 software), and each of the two DNA sequences separately, ITS1-5.8S-ITS2 and rpl32-trnL (using Tamura 3-parameter genetic distance [Tamura 1992]; MEGA11 software).

Genetic groupings of individuals were obtained by the Bayesian algorithm model-based clustering method using microsatellite data with STRUCTURE 2.3.4 software (Pritchard et al. 2000; Hubisz et al. 2009). This software infers population structure using genotype data consisting of unlinked markers by identifying distinct genetic clusters, assigning individuals to such clusters, and identifying migrants and admixed individuals. For this analysis, we tested for the existence of several K groups (1 to 10). For each K, 10 runs of 300000 iterations were performed (burn-in length 50000; 'admixture model') (Pritchard et al. 2000; Hubisz et al. 2009). A summary graph of the 10 runs for each K value was made using CLUMPAK software (Kopelman et al. 2015). To select the most appropriate number of genetic groups, the method of Evanno et al. (2005) was implemented with STRUCTURE HARVESTER (Earl and VonHoldt 2012).

Molecular markers vs. quantitative traits divergence. Genetic differentiation in neutral markers using microsatellite data was calculated among the groups defined with the four criteria listed above by Fst and Rst statistics (Goodman 1997) using GENALEX 6.503. In turn, the Qst measure of genetic differentiation in quantitative traits (non-neutral, influenced by natural selection; Spitze 1993) was calculated among the same hierarchical groups using phenotypic data measured under common gardens (Pstat package in R) (Da Silva and Da Silva 2018). A comparison of Fst and Rst values with those of Qst was made considering the 95% confidence interval of the latter. When the measure of genetic differentiation in a neutral marker (Fst, Rst) is equal to the measure of genetic differentiation in a quantitative trait (Qst), there is no evidence that selection is acting on that trait. On the other hand, when Qst is greater than Fst or Rst, it means that selection favours the divergence of the traits between populations; finally, when the Fst or Rst are greater than Qst, it means that selection acts to stabilize the trait (Merilä and Crnokrak 2001; McKay and Latta 2002).

Population history. Using DNA sequence data, Tajima's D (Tajima 1989) was estimated by considering individuals from South and North America as two groups, in order to make inferences about their demography (ARLEQUIN 3.5.2.2). This parameter compares the total number of polymorphic sites in the sample and the proportion of nucleotidic differences between DNA sequences (Perfectti et al. 2009). Both values must be equal, resulting in a value close to zero, under a situation of neutral evolution (in the absence of selection, recombination, population subdivision, or change in population size). On the other hand, values significantly larger than zero indicate stabilizing selection and/or sudden demographic contraction (i.e., 'bottleneck'), while values significantly smaller than zero indicate selection in favour of new mutations and/or recent demographic expansion.

We used coalescence-based Bayesian analysis to reconstruct how and when the separation between *L. crinita* populations from South

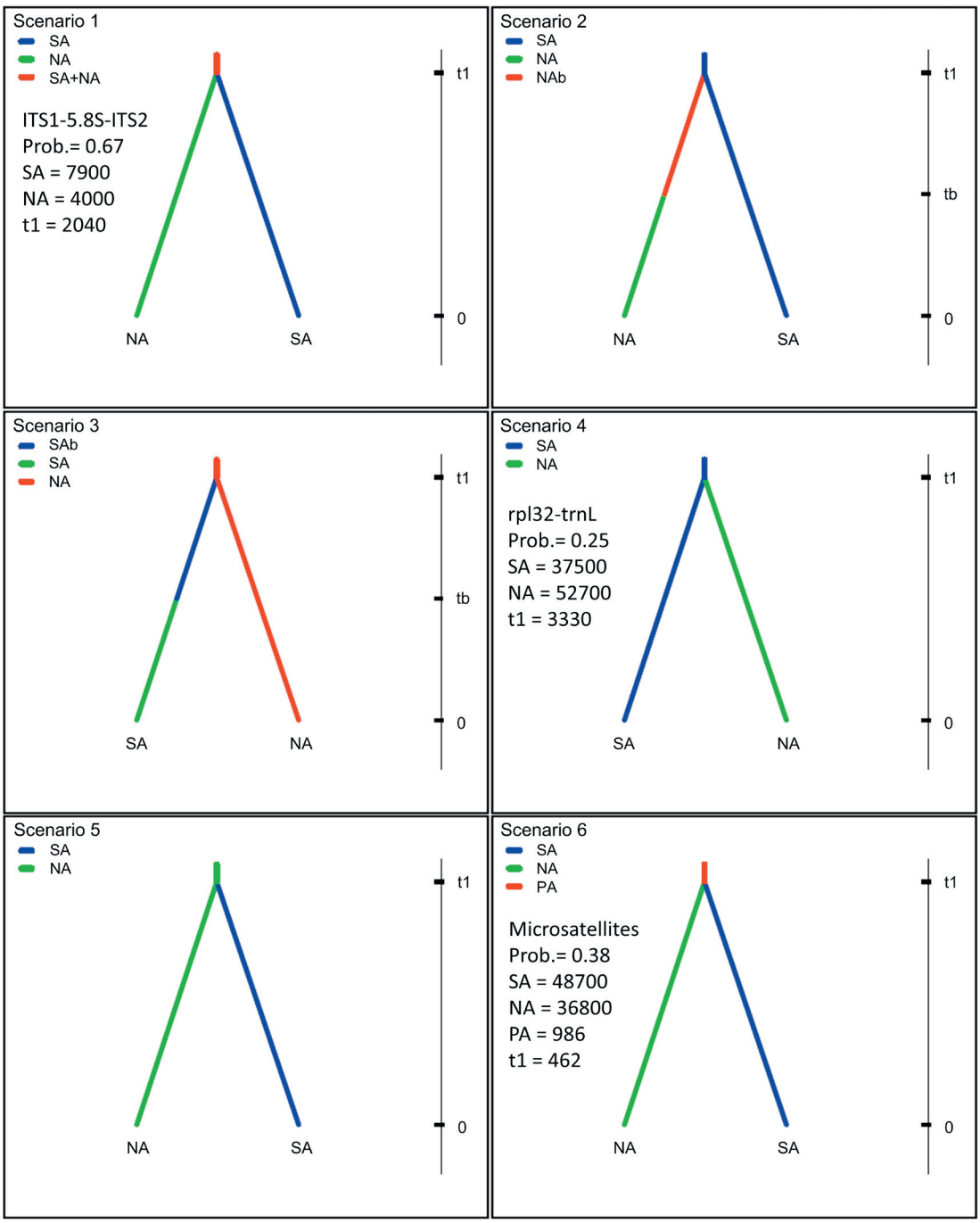

Figure 1. The six scenarios evaluated by Bayesian analysis. For selected scenarios (1 for ITS1-5.8S-ITS2, 4 for rpl32-trnL, 6 for microsatellites) we present probability values and medians of the parameters: current effective population size in South (SA) and North America (NA), effective population size of the ancestral population (PA), divergence time between the populations of South and North America, in number of generations (t1). The vertical axis represents the time in number of generations (0=time of populations sampling). Other parameters considered in non-selected scenarios were: effective population size of the South (SAb) and North American (NAb) population after a bottleneck, and time until which the bottleneck lasted, in number of generations (tb).

Figura 1. Seis escenarios evaluados mediante analisis bayesiano. Para los escenarios seleccionados (1 para ITS1-5.8S-ITS2, 4 para rpl32-trnL, 6 para microsatélites) se presenta los valores de probabilidad y las medianas de los parámetros: tamaño efectivo de la población en Sud (SA) y Norteamérica (NA) en la actualidad, y de la población ancestral (PA), tiempo de la divergencia entre las poblaciones de Sud y Norteamérica, en número de generaciones (t1). El eje vertical representa el tiempo en núumero de generaciones (0=momento de colecta de las poblaciones). Otros parámetros incluidos en escenarios no seleccionados fueron: tamaño efectivo poblacional luego de un cuello de botella genético en Sud (SAb) y Norteamérica (NAb), y tiempo de duración del cuello de botella, en número de generaciones (tb).

and North America occurred (DIYABC software; Cornuet et al. 2014). The analysis was carried out employing DNA sequences and microsatellite data separately. Given that the number of scenarios grows exponentially with the number of populations, two broad-sense populations were considered, separately grouping all the individuals from South America and all the individuals from North America. Six scenarios were compared (Figure 1), which evaluated different modes of separation between populations: vicariance from a common ancestral population (of a size similar to the sum of the populations of South and North America, Scenario 1; or from an ancestral population of unknown size, Scenario 6), dispersal from South to North America (with and without a bottleneck in the initiation of the population in North America, Scenarios 2 and 4, respectively), dispersal from North to South America (with and without a bottleneck in the initiation of the population in South America, Scenarios 3 and 5, respectively). The minimum value of the initial parameters in the different scenarios was set at 10; the maximum was set at 100000, except for the ITS1-5.8S-ITS2 marker for which it was set at 10000. Such large intervals for initial parameters were used due to the lack of ancestral information for the species (Bobo-Pinilla et al. 2016; López-González et al. 2021). For each scenario and parameter, we obtained the relative median of the absolute error (RMAE; values >0.3 indicate low precision of the estimate). The posterior probability of each scenario was calculated using a discriminant analysis with a logistic approach (Cornuet et al. 2014).

Past suitable habitats. We estimated the distribution of past suitable habitats for the species using MAXENT (Phillips 2005) and BIOCLIM (Booth 2018). In both cases, we first modelled the current potential distribution of the species for each subcontinent separately, using the same coordinates of species presence (177 for South America, 104 for North America) (gbif.org) and climatic data layers for the present (annual mean temperature, temperature annual range, mean temperature of the warmest quarter, annual precipitation and precipitation of the warmest quarter) as used in Quiroga et al. (2018; further details there). Then, we estimated suitable habitats for the species in the past by projecting the models calibrated for the present back to the climates of the Middle Holocene (~6000 years ago) and Last Glacial Maximum (~21000 years ago); all climate layers were obtained from the open access database Worldclim 1.4 (worldclim.org). We used the default settings of BIOCLIM (implemented in the DIVA-GIS software; Hijmans et al. 2001) and MAXENT, except that in MAXENT the fade-by-clamping option was used to limit extrapolation to areas with climates outside the range of those used in the calibration. Analyses were carried out for the American continent and nearby islands, including all South America and the portion of North America south of 49° N latitude (we excluded Alaska, Greenland and almost all of Canada, which have and had too cold climates for the species). The boundary between Colombia and Panama was considered the limit between subcontinents, and the Caribbean islands were considered part of North America (Quiroga et al. 2018).

Results

Plants from South American populations presented on average 66% more inflorescences, 19% more tillers, 17% taller inflorescences and 33% heavier seeds than the North American ones. Canopy height did not differ between plants of each subcontinent. Within each subcontinent, inflorescence and canopy height, as well as seed weight, differed significantly among populations. Also, inflorescence production varied among South American populations, but not among North American ones. The number of tillers per plant did not differ among populations of either subcontinent (Table 1).

Nucleotide diversity for DNA sequences was low in South (mean=0.00151 [standard deviation=0.00124] for ITS1-5.8S-ITS2; 0.00104 [0.00095] for rpl32-trnL) and North America (0.00000 [0.00000] for ITS1-5.8S-ITS2, 0.00089 [0.00093] for rpl32-trnL). South and North American populations showed similar levels of microsatellite genetic diversity (Na, Ne, I, Nap, He and Ho) (Table 2). In addition, no differences were found between subcontinents in the inbreeding coefficient (Fis), which showed negative values —indicating an excess of heterozygosity— for most of the populations of South (91%) and North America (86%) (Table 2).

The analysis of molecular variance did not show genetic structuring between subcontinents for the DNA sequences (P>0.05); most of the genetic variability in such sequences was within subcontinents

Table 2. Genetic diversity parameters estimated with microsatellite data for the populations of South America (SA) and North America (NA). Na: number of different alleles. Ne: number of effective alleles. I: Shannon index. Nap: number of private alleles; He: expected heterozygosity. Ho: observed heterozygosity. Fis: inbreeding coefficient. The last three rows show the mean values per subcontinent and statistical significance (P>0.05, n.s.) of their comparison.

Tabla 2. Índices de diversidad genética estimados con datos de microsatélites de las poblaciones sudamericanas (SA) y norteaméricanas (NA). Na: número de alelos diferentes. Ne: número efectivo de alelos. I: índice de Shannon. Nap: número de alelos privados. He: heterocigosis esperada. Ho: heterocigosis observada. Fis: coeficiente de endogamia. Las tres últimas filas muestran los valores medios por subcontinente y la significancia estadística de su comparación (P>0.05, n.s.).

Subcontinent	Population	Na	Ne	I	Nap	He	Ho	Fis
NA	Bowie	1.67	1.49	0.40	0.00	0.27	0.33	-0.22
NA	Kinney	1.33	1.22	0.35	0.67*	0.21	0.00	1.00
NA	Knox	1.00	0.76	0.13	0.00	0.07	0.08	-0.14
NA	Lucero	1.33	1.33	0.23	0.00	0.17	0.33	-1.00
NA	San Simon	1.33	1.33	0.23	0.00	0.17	0.33	-1.00
NA	Tornillo	1.33	1.33	0.23	0.00	0.17	0.33	-1.00
NA	White Sands	1.67	1.49	0.40	0.00	0.27	0.33	-0.22
SA	Amblayo	1.67	1.56	0.35	0.33*	0.21	0.33	-0.60
SA	Chamical	1.33	1.33	0.23	0.00	0.17	0.33	-1.00
SA	Colpes	1.33	1.31	0.22	0.00	0.16	0.27	-0.67
SA	El Tipán	1.67	1.36	0.35	0.00	0.23	0.13	0.42
SA	HC	1.00	1.00	0.00	0.00	0.00	0.00	—
SA	RCH Fina	1.33	1.33	0.23	0.33*	0.17	0.33	-1.00
SA	Recreo	1.00	0.76	0.13	0.00	0.07	0.08	-0.14
SA	Recreo Salinas	1.67	1.37	0.33	0.00	0.22	0.32	-0.46
SA	Salinas Grandes	1.33	1.33	0.23	0.00	0.17	0.33	-1.00
SA	San Martín	1.33	1.33	0.23	0.33*	0.17	0.33	-1.00
SA	SL	1.33	1.24	0.20	0.00	0.14	0.20	-0.43
SA	Tilimuqui	1.67	1.49	0.40	0.00	0.27	0.33	-0.22
NA	(mean)	1.38	1.28	0.28	0.095	0.19	0.25	-0.35
SA	(mean)	1.39	1.28	0.24	0.083	0.16	0.25	-0.57
P value between subcontinents		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*Private alleles were: 135, 153 y 149 in Mss11 for Amblayo, RCH Fina and San Martín, respectively in South America (SA); 170 and 176 in Mss93 for Kinney in North America (NA).

(94% and 100% for South and North America, respectively). For microsatellites, we found significant —but low— genetic variability between subcontinents (2%), and high variability among populations (55%) within each subcontinent (P<0.05).

We found relatively less differentiation of allele frequencies between subcontinents (only significant for Mss93, P<0.05) than among populations within both subcontinents (significant for Mss8 and Mss11 in South America, P<0.001; and for Mss93 and Mss11 in North America, P<0.01). Differentiation between populations was even more evident when all populations of both subcontinents were considered together (significant for all microsatellites, P<0.001).

Genetic distance between populations increased significantly with geographic distance in North America (R^2=0.22, P=0.01) but not in South America (R^2=0.004, P=0.16) (Supplementary Material-Figure S3). The phylogenetic trees obtained with microsatellite and sequence data did not show a separation of populations according to the subcontinent of origin (Supplementary Material-Figure S4). Similarly, the analyses of genetic affinity of individuals to distinct genetic clusters yielded a high degree of admixture (2 and 4 clusters selected; all clusters are shown in Supplementary Material-Figure S5). Yet, for microsatellite data, some populations formed defined clusters under both methods as Recreo-El Tipán-Kinney-Knox, Salinas Grandes-Tilimuqui and Bowie-White Sands (North American populations underlined here and in Supplementary Material-Figure S4 and Supplementary Material-Figure S5). In the same line, pairwise population Fst and Rst values did not show a distinctive pattern according to the subcontinent of origin (Supplementary Material-Table S3).

Among-population genetic divergence was high (Fst and Rst values between 0.49-0.60) regardless of the subcontinent of origin. However, genetic differentiation was low when comparing South America vs. North America as broad sense groups (Fst=0.02, Rst=0.07) (Table 3). Some Qst values obtained

Table 3. Fst and Rst values estimated from the microsatellite data and Qst values obtained for quantitative traits (with 95% confidence interval) measured in plants at the common garden. Qst values with asterisk (*) indicate significant differences with respect to the genetic divergence measured by microsatellites (Fst and Rst) and, therefore, show the effect of natural selection in the traits.

Tabla 3. Valores de Fst y Rst estimados de los datos de microsatélites y valores de Qst obtenidos de caracteres cuantitativos (intervalo de confianza del 95%) medidos en plantas del jardín común. Los valores de Qst con asterisco (*) señalan diferencias significativas respecto de la divergencia genética estimada mediante microsatélites (Fst y Rst) y, por lo tanto, el efecto de la selección natural.

Groups	Fst	Rst	Qst (#inflorescences)	Qst (#tillers)	Qst (inflorescences heigth)	Qst (canopy height)
Two broad-sense populations (NA vs. SA)	0.02	0.07	0.92* (0.82-0.96)	0.72 (0.04-0.91)	0.93* (0.81-0.97)	0.45 (0.01-0.86)
All populations (NA and SA)	0.50	0.56	0.71* (0.68-0.85)	0.41 (0.40-0.72)	0.61* (0.58-0.81)	0.60* (0.57-0.81)
SA populations	0.51	0.54	0.71* (0.67-0.86)	0.47 (0.41-0.77)	0.37 (0.34-0.75)	0.55 (0.51-0.81)
NA populations	0.49	0.60	0.11 (0.08-0.67)	0.23 (0.18-0.71)	0.56 (0.42-0.83)	0.66 (0.55-0.86)

from the quantitative traits exceeded Fst and Rst values (outside the 95% confidence interval; 6 out of 16 comparisons) (Table 3), which suggests that the variation between populations in these traits was larger than that found in the neutral markers. On the other hand, for most of the comparisons (10 out of 16), the confidence intervals of Qst included the Fst and Rst values, suggesting absence of selection influence upon these traits (Table 3).

Tajima's D showed values that did not significantly differed from 0 (P>0.05) for the sequence rpl32-rtnL in both subcontinents and for ITS1-5-ITS2 in North America, indicating stability of population size in recent times. However, for ITS1-5.8S-ITS2 in South America a marginally significant negative value was obtained (D=-1.53, P=0.056), which would suggest some recent population expansion.

Of the six studied scenarios (vicariance —Scenarios 1 and 6— or dispersal with —Scenarios 2 and 3— and without a bottleneck on arrival to the other subcontinent —Scenarios 4 and 5—) (Figure 1), those more likely were Scenario 4 (for rpl32-trnL), Scenario 1 (ITS1-5.8S-ITS2) and Scenario 6 (microsatellites). For such alternative scenarios, estimated divergence times between South and North American populations were ca. 3300, 2000 and 500 generations, respectively (Figure 1; Supplementary Material-Table S4). Most of the estimated parameters (14 out of 16) for such scenarios presented acceptable error levels (RMAE<0.3) (Supplementary Material-Table S4).

Present distributions estimated for *L. crinita* with MAXENT and BIOCLIM showed extensive suitable areas in South and North America (Figure 2). In contrast, they led to modelled small (BIOCLIM) or null (MAXENT) suitable area for the species in North America during the Middle Holocene, and especially for the Last Glacial Maximum. This was not the case for South America, where they also modelled extensive suitable areas for such periods (Figure 2).

Discussion

Despite the extant geographical distance between the populations of South and North America, we found low genetic differentiation for our focus species between subcontinents, which were detected only with microsatellites (more recent signal), but not with DNA sequences (conserved, historical signal). This suggests that a common gene pool has probably diverged in the recent past. Although our study is not conclusive, since it was not possible to use more markers, we propose that it provides important insights into the genetic structure and diversity of *L. crinita* through its distribution range.

Genetic differentiation between subcontinents was lower than the differentiation of populations within each subcontinent. Higher genetic differentiation between populations was found as geographic distance increases in North America, but not in South America. The wide differentiation between populations within each subcontinent could have been favored for various factors which may include

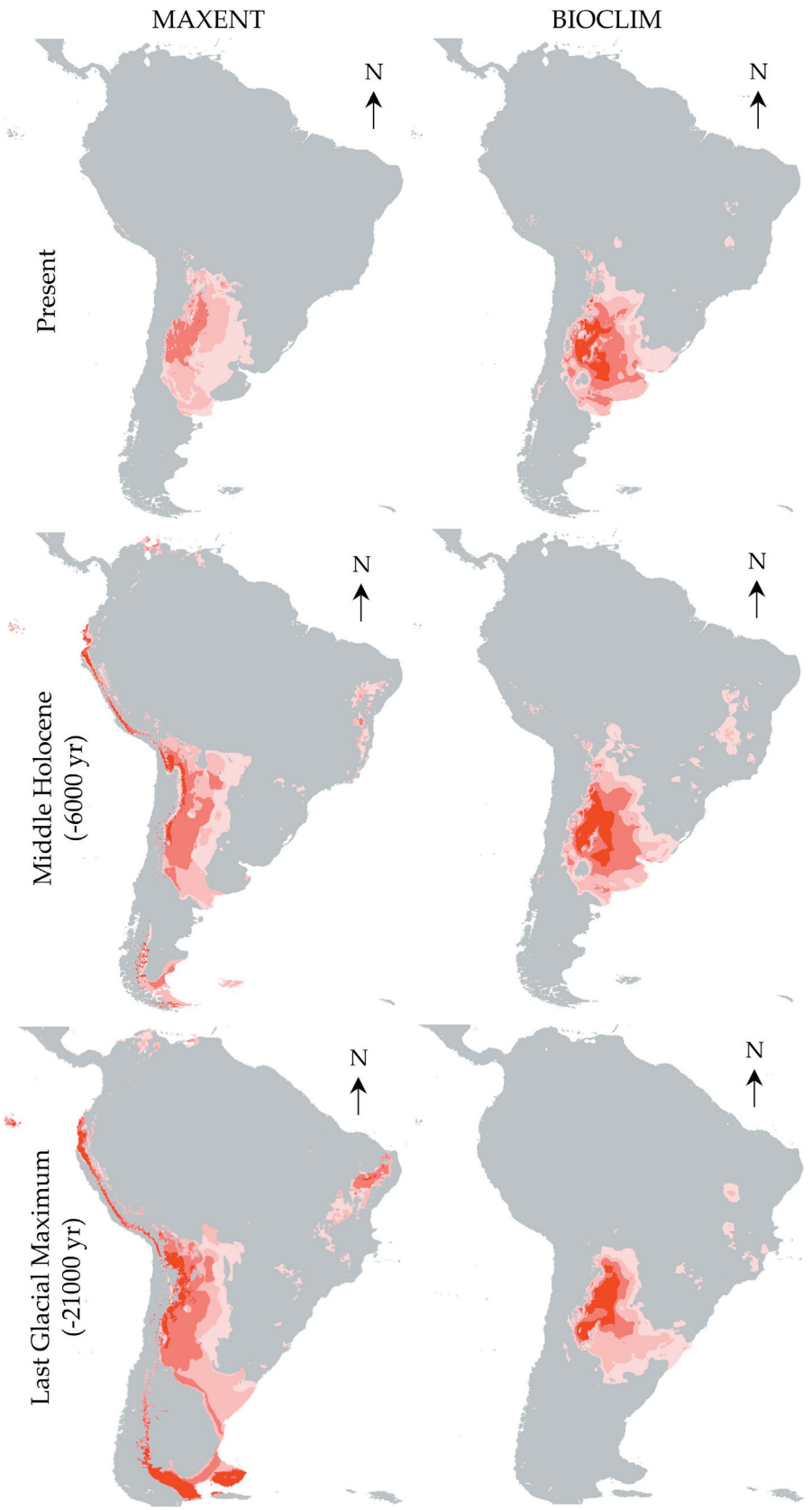
MAXENT
BIOCLIM
Present
Middle Holocene
(-6000 yr)
Last Glacial Maximum
(-21000 yr)
N

Figure 2. Potential habitats for *L. crinita* in the present and in the past (Middle Holocene —6000 years ago— and Last Glacial Maximum —21000 years ago—) modeled separately in South and North America, using MAXENT and BIOCLIM. Red: high suitability. Pink: medium suitability. Light pink: low suitability. Gray: unsuitable.

Figura 2. Hábitats potenciales para *L. crinita* en el presente y en el pasado (Holoceno Medio —hace 6000 años— y Último Máximo Glacial —hace 21000 años—) modelados de forma separada en Sudamérica y en Norteamérica, utilizando MAXENT y BIOCLIM. Rojo=altamente adecuado. Rosa: moderadamente adecuado. Rosa claro: poco adecuado. Gris: no adecuado.

their reproduction mode (autogamy), broad distribution (Loveless and Hamrick 1984), herbaceous life form and climatic variability (Gamba and Muchhala 2020). Such genetic differentiation can explain, at least partially, the broad distribution of the species in each subcontinent; another explanation could be the phenotypic plasticity (Premoli and Mathiasen 2011; Kelly 2019) that was reported in previous studies for the species (Greco and Cavagnaro 2003; Quiroga et al. 2013; Marinoni et al. 2022), although this was not investigated in the present work. Regarding the low genetic differentiation between subcontinents, Loveless and Hamrick (1984) suggested that the geographic range of a species is generally a poor predictor of its genetic structure, and that other historical or ecological factors —such as those already mentioned in this paragraph— can interact in complex ways shaping genetic patterns. In this sense, our finding of a possible recent divergence between *L. crinita* populations from South and North America could be one of the main causes of the shallow genetic structure between subcontinents.

We found that natural selection differentially influenced populations in the two subcontinents. South American populations manifested evidence of natural selection on inflorescence number, while for North American populations, no such evidence was detected on any of the studied traits (Table 3). These results suggest that traits of

L. crinita have been differentially shaped in each subcontinent, which is likely a result of adaptive and genetically based responses to different environmental conditions and biogeographical histories. Populations from South America had plants with more and taller inflorescences, more tillers, and heavier seeds than North American ones (Table 1). These characteristics could represent adaptations to the less stressful environments on which the species thrives in South America (Quiroga et al. 2018). In addition, another difference between subcontinents observed in situ by the first author during field collections revealed that populations in North America were smaller and more isolated than those in South America. Such characteristics of the North American populations are consistent with the observed pattern of genetic isolation-by-distance, which indicates lower gene flow as populations are more distant. Also, while neutrality tests reflected a certain stability of population sizes in recent geological times, some degree of expansion was measured for South American populations.

Longer and relatively more stable biogeographic history for *L. crinita* in South America (Figure 2) (i.e., a more continuous local presence) could have possibly caused a significant adaptive among-population divergence in such continent than in North America. Whereas many plant taxa exhibit amphitropical disjunct distributions between South and North America (Raven 1963; Wen and Ickert-Bond 2009), the biogeographic history of these species do not fit a single pattern of origin. South-to-north dispersal patterns predominate for amphitropical disjunct species of desert areas (Frost et al. 2017), contrary to north-to-south ones for disjuncts from temperate or polar regions (Raven 1963; Simpson et al. 2017).

Modelling potential habitats for the species in North America during the Last Glacial Maximum suggested the absence (MAXENT) or a lower availability (BIOCLIM) of favorable sites with respect to those in South America, which, in turn, were more extensive than present ones (Figure 2). Various authors showed that during the Last Glacial Maximum temperatures decreased more in North America than in South America (Otto-Bliesner et al. 2006; Schneider von Deimling et al. 2006; Ehlers and Gibbard 2007). This could help explain a recent disjunction (i.e., after the Last Glacial Maximum, as suggested by our Bayesian analyses [Figure 1] and the low divergence yielded by molecular markers). In this sense, climatic data used in the modelling showed that sites with current presence of *L. crinita* in both subcontinents differed in their past mean annual temperatures. In North America, they were, on average, 6 °C and 1 °C colder during the Last Glacial Maximum and Middle Holocene than at present, whereas in South America they were only 3 °C and 0.5 °C colder than today, respectively. On the other hand, precipitation in each subcontinent did not show a marked variation during these periods, even though rainfall was more restrictive at all times in North America than in South America (Supplementary Material-Table S5). It is known that some species adapted to hot arid and semi-arid environments in North America migrated towards the south during such cold periods, while others favored by the orography of the region were able to persist in refuges with relatively favorable climates (Brown and Makings 2014; Sánchez-del Pino et al. 2020). This is consistent with the modelled low availability of favorable habitats for *L. crinita* in North America in the past (Figure 2).

Among the scenarios selected by Bayesian analysis, Scenario 4 (for rpl32-trnL) suggests that North American populations arrived by dispersal from South America ca. 3300 generations ago, whilst Scenarios 1 (ITS1-5.8S-ITS2) and 6 (microsatellites) suggest that current disjunct populations diverged by vicariance from an ancestral population between ca. 2000 and ca. 500 generations ago, respectively (Figure 1). Previous work on other genera and species of American amphitropical disjunct plants indicates that most of these disjunctions would have occurred by long-distance dispersal during the last 8 million years (since the Late Miocene, and more frequently in the Quaternary) (Raven 1963; Peterson and Columbus 1997; Peterson and Morrone 1997; Wen and Ickert-Bond 2009; Amarilla et al. 2015). The main dispersal mode suggested for such disjunctions is the attachment of diaspores to migrant birds (Raven 1963; Schenk and Saunders 2017). In this sense, although we did not find studies that have evaluated the dispersal mode of *L. crinita*, the characteristics of its diaspores suggest the action of wind (anemochory) and attachment to animals (e.g., birds, mammals and epizoochory, sensu Sádlo et al. [2018]) as the main dispersal modes. We hereby present partial support for the dispersal from

South to North America (Scenario 4) and also for a process of vicariance (Scenarios 1 and 6); therefore, our results based on a limited number of genetic markers are not conclusive regarding the mode and origin of the divergence of *L. crinita* populations. However, we did find some consistency in the timing of divergence between South and North American populations, which would have occurred between ca. 3000 and ca. 20000 years ago -after the Last Glacial Maximum using a mean generation time of 6 years (Supplementary Material-Appendix S2). A recent divergence, in geological-evolutionary times, is suggested by the relatively low differentiation at molecular markers between South and North America, as well as the modelling of the past suitable habitats (Figure 2). These results are consistent with the recent disjunction (<1-2 million years) estimated for most of the American amphitropical species (Wen and Ickert-Bond 2009; Simpson et al. 2017).

Climatic oscillations since the Pliocene (last ~5 million years) in North and South America could have forced distributional changes of genera, species and populations. In fact, Amarilla et al. (2015) demonstrated that the currently disjunct genus *Munroa* (Poaceae) with distinct species in arid and semi-arid regions of both subcontinents, underwent distributional and evolutionary changes (speciation) through that period. Furthermore, Peterson et al. (2012, 2022) pointed out that *L. crinita* is related to other grasses of the genera *Leptochloa* and *Afrothichloris*, both originated mostly in the Southern Hemisphere. In this sense, our results are not conclusive, although they are more suggestive for the origin of *L. crinita* in South America than in North America. Adding a layer of complexity, it is not possible to discard that other events of disjunction and conjunction may have occurred during previous climatic cycles (i.e., glacial and interglacial); for example, by long-distance dispersal or latitudinal and altitudinal population movements in both subcontinents (Jackson and Overpeck 2000; Hewitt 2011). The genetic clusters we obtained are consistent with this idea, since they presented a considerable degree of admixture (i.e., each cluster has representatives of both subcontinents) (Supplementary Material-Figure S5). Our work suggests that *L. crinita* consists of a common gene pool with low divergence between South and North America, which since the time of disjunction only allowed the accumulation of a reduced number of mutations and private alleles within each subcontinent.

Unlike what was found by Kozub et al. (2017) and what could be expected from an autogamous species, we found heterozygosity excess in almost all the studied populations (10 out of 11 from South America, 6 out of 7 from North America; Table 2). A possible explanation for our result could be the incidence of evolutionary bottlenecks, which occur mainly because random genetic drift that eliminates many low-frequency alleles and reduce the mean number of alleles per locus, causing allelic diversity to decrease faster than observed heterozygosity. However, our results are not consistent with this explanation since we did not detect sudden demographic contractions in the past (see Results, Population history). On the other hand, the high values of observed heterozygosity could be due to the effects of natural selection. Although microsatellites are neutral markers, the action of selection on nearby loci on chromosomes could result in a selective sweep, increasing the frequency of certain genotypes (Nielsen et al. 2006). Another explanation could be based on what was described by Loveless and Hamrick (1984), by pointing out that autogamous species (such as *L. crinita*) generally present reduced observed heterozygosity and low intra-familial diversity, but a high diversity between families within populations. Therefore, it is possible that excess heterozygosity in our study may reflect the sampling scheme at the time of seed collection from populations, leading us to study plants corresponding to genetically distinct families.

The low genetic differentiation, short estimated divergence time and the slight to moderate traits differences between *L. crinita* populations from South and North America suggest that germplasm translocation between subcontinents could be an alternative to consider in restoration practices if necessary (taking proper precautions). Following the scheme of Weeks et al. (2011) for decision making, according to the objective and species to be used in restoration, the admixture of *L. crinita* populations could be a viable option for seed sourcing, as well as the translocation of genotypes/populations (Bucharova et al. 2019; Höfner et al. 2021; Quiroga 2022).

References

Amarilla, L. D., J. O. Chiapella, V. Sosa, N. C. Moreno, and A. M. Anton. 2015. A tale of North and South America: Time and mode of dispersal of the amphitropical genus *Munroa* (Poaceae, Chloridoideae). Botanical Journal of the Linnean Society 179:110-125. https://doi.org/10.1111/boj.12304.

Anderson, D. L., J. A. Del Aguila, A. Marchi, J. C. Vera, and E. L. Oriente. 1980. Manejo del pastizal natural y producción ganadera. Parte 1. Páginas 1-61. Editorial INTA. Buenos Aires, Argentina.

Avise, J. C. 2004. Molecular markers, natural history and evolution 2nd ed. Sunderland: Sinauer.

Bobo-Pinilla, J., S. B. B de León, J. S. Colomar, G. Fenu, G. Bacchetta, J. P. de Giles, and M. M. Martínez-Ortega. 2016. Phylogeography of Arenaria balearica L.(Caryophyllaceae): evolutionary history of a disjunct endemic from the Western Mediterranean continental islands. PeerJ 4:e2618. https://doi.org/10.7717/peerj.2618.

Booth, T. H. 2018. Why understanding the pioneering and continuing contributions of BIOCLIM to species distribution modelling is important. Austral Ecology 43:852-860. https://doi.org/10.1111/aec.12628.

Brown, D. E., and E. Makings. 2014. A guide to North American grasslands. Desert Plants 29:1399-160.

Bucharova, A., W. Durka, N. Hölzel, J. Kollmann, S. Michalski, and O. Bossdorf. 2017. Are local plants the best for ecosystem restoration? It depends on how you analyze the data. Ecology and Evolution 7:10683-10689. https://doi.org/10.1002/ece3.3585.

Bucharova, A., O. Bossdorf, N. Hölzel, J. Kollmann, R. Prasse, and W. Durka. 2019. Mix and match: regional admixture provenancing strikes a balance among different seed-sourcing strategies for ecological restoration. Conservation Genetics 20:7-17. https://doi.org/10.1007/s10592-018-1067-6.

Carloni, E. J., R. E. Quiroga, K. Grunberg, and A. C. Premoli. 2021. Nivel de ADN-Ploidía en poblaciones sudamericanas y norteamericanas de la gramínea nativa disyunta *Trichloris crinita* (Chloridoideae, Poaceae). Revista FAVE Ciencias Agrarias 20:220-227. https://doi.org/10.14409/fa.v20i1.10260.

Cavagnaro, P. F., J. B. Cavagnaro, J. L. Lemes, R. W. Masuelli, and C. B. Passera. 2006. Genetic diversity among varieties of the native forage grass *Trichloris crinita* based on AFLP markers, morphological characters, and quantitative agronomic traits. Genome 49:906-918. https://doi.org/10.1139/g06-060.

Cavalli-Sforza, L. L., and A. W. Edwards. 1967. Phylogenetic analysis. Models and estimation procedures. American Journal of Human Genetics 19:233-257.

Cornuet, J. M., P. Pudlo, J. Veyssier, A. Dehne-García, M. Gautier, et al. 2014. DIYABC v2. 0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. Bioinformatics 30:1187-1189. https://doi.org/10.1093/bioinformatics/btt763.

Da Silva, S. B., and A. Da Silva. 2018. Pstat: An R package to assess population differentiation in phenotypic traits. The R Jorunal 1:447-454. https://doi.org/10.32614/RJ-2018-010.

Dalmasso, A. D. 1994. Fenología de cinco gramíneas de interés forrajero: *Pappophorum caespitosum*, *Trichloris crinita*, *Setaria leucopila*, *Digitaria californica*, *Diplachne dubia*. Multequina 3:9-34.

Di Rienzo, J. A., F. Casanoves, M. G. Balzarini, L. González, M. Tablada, and C. W. Robledo. 2020. InfoStat versión 2018. Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL: infostat.com.ar.

Donoghue, M. J. 2011. Bipolar biogeography. Proceedings of the National Academy of Sciences 108:6341-6342. https://doi.org/10.1073/pnas.1103801108.

Doyle, J. J. 1990. Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Earl, D. A., and B. M. VonHoldt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359-361. https://doi.org/10.1007/s12686-011-9548-7.

Ehlers, J., and P. L. Gibbard. 2007. The extent and chronology of Cenozoic global glaciation. Quaternary International 164:6-20. https://doi.org/10.1016/j.quaint.2006.10.008.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564-567. https://doi.org/10.1111/j.1755-0998.2010.02847.x.

Frost, L. A., S. M. Tyson, P. Lu-Irving, N. O'Leary, and R. G. Olmstead. 2017. Origins of North American arid-land Verbenaceae: More than one way to skin a cat. American Journal of Botany 104:1708-1716. https://doi.org/10.3732/ajb.1700292.

Gamba, D., and N. Muchhala. 2020. Global patterns of population genetic differentiation in seed plants. Molecular Ecology 29:3413-3428. https://doi.org/10.1111/mec.15575.

García, E., C. Soto, and F. Miranda. 1960. Larrea y clima. Anales del Instituto de Biología de la Universidad Nacional Autónoma de México 31:133-171.

Greco, S. A., and J. B. Cavagnaro. 2003. Effects of drought in biomass production and allocation in three varieties of *Trichloris crinita* P. (Poaceae) a forage grass from the arid Monte region of Argentina. Plant Ecology 164:125-135. https://doi.org/10.1023/A:1021217614767.

González, C. L., and G. V. Látigo. 1981. Rootplowing, front-end stacking, and seeding effects on herbaceous plant species composition. Journal of Range Management 34:460-465. https://doi.org/10.2307/3898098.

Goodman, S. J. 1997. RST Calc: a collection of computer programs for calculating estimates of genetic differentiation

from microsatellite data and determining their significance. Molecular Ecology 6:881-885. https://doi.org/10.1046/j.1365-294X.1997.00260.x.

Gutiérrez, H. F., G. A. Richard, M C. Cerino, and J. F. Pensiero. 2016. Sistema reproductIvo de *Trichloris* (poaceae, chloridoideae, chlorideae). Boletín de la Sociedad Argentina de Botánica 51:111-122. https://doi.org/10.31055/1851.2372.v51.n1.14421.

Hancock, N. M., F. Encinas-Viso, and L. M. Broadhurst. 2023. A documented paradigm shift in seed sourcing: attitudinal changes to using local native seed for ecological restoration. Restoration Ecology 31:e13845. https://doi.org/10.1111/rec.13845.

Harris, S. A., and R. Ingram. 1991. Chloroplast DNA and biosystematics: the effects of intraspecific diversity and plastid transmission. Taxon 40:393-412. https://doi.org/10.2307/1223218.

Hewitt, G. M. 2011. Quaternary phylogeography: the roots of hybrid zones. Genetica 139:617-638. https://doi.org/10.1007/s10709-011-9547-3.

Hijmans, R. J., L. Guarino, M. Cruz, and E. Rojas. 2001. Computer tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. Plant Genetic Resources Newsletter 127:15-19.

Höfner, J., T. Klein-Raufhake, C. Lampei, O. Mudrak, A. Bucharova, and W. Durka. 2021. Populations restored using regional seed are genetically diverse and similar to natural populations in the region. Journal of Applied Ecology 59: 2234-2244. https://doi.org/10.1111/1365-2664.14067.

Hubisz, M. J., D. Falush, M. Stephens, and J. K. Pritchard. 2009. Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources 9:1322-1332. https://doi.org/10.1111/j.1755-0998.2009.02591.x.

Jackson, S. T., and J. T. Overpeck. 2000. Responses of plant populations and communities to environmental changes of the late Quaternary. Paleobiology 26:194-220. https://doi.org/10.1017/S0094837300026932.

Johnson, R., L. Stritch, P. Olwell, S. Lambert, M. E. Horning, and R. Cronn. 2010. What are the best seed sources for ecosystem restoration on BLM and USFS lands? Native Plants Journal 11:117-131. https://doi.org/10.2979/NPJ.2010.11.2.117.

Jones, T. A. 2013. When local isn't best. Evolutionary Applications 6:1109-1118. https://doi.org/10.1111/eva.12090.

Kelly, M. 2019. Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. Philosophical Transactions of the Royal Society B 374:20180176. https://doi.org/10.1098/rstb.2018.0176.

Kettenring, K. M., K. L. Mercer, C. Reinhardt Adams, and J. Hines. 2014. Application of genetic diversity–ecosystem function research to ecological restoration. Journal of Applied Ecology 51:339-348. https://doi.org/10.1111/1365-2664.12202.

Kopelman, N. M., J. Mayzel, M. Jakobsson, N. A. Rosenberg, and I. Mayrose. 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Molecular Ecology Resources 15:1179-1191. https://doi.org/10.1111/1755-0998.12387.

Kozub, P. C., K. Barboza, F. Galdeano, C. L. Quarin, J. B. Cavagnaro, and P. F. Cavagnaro. 2017. Reproductive biology of the native forage grass *Trichloris crinita* (Poaceae, Chloridoideae). Plant Biology 19:444-453. https://doi.org/10.1111/plb.12549.

Kozub, P. C., K. Barboza, J. B. Cavagnaro, and P. F. Cavagnaro. 2018. Development and characterization of SSR markers for *Trichloris crinita* using sequence data from related grass species. Revista de la Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo 50:1-16.

Kozub, P. C., M. L. Peñas, P. E. Novo, and P. F. Cavagnaro. 2019. Molecular cytogenetic characterization of the native forage grass *Trichloris crinita*. Crop Science 59:1-13. https://doi.org/10.2135/cropsci2018.12.0731.

Li, Y. C., A. B. Korol, T. Fahima, A. Beiles, and E. Nevo. 2002. Microsatellites: genomic distribution, putative functions, and mutational mechanisms: a review. Molecular Ecology 11:2453-2465. https://doi.org/10.1046/j.1365-294X.2002.01643.x.

López-González, N., J. Bobo-Pinilla, N. Padilla-García, J. Loureiro, S. Castro, B. M. Rojas-Andrés, and M. M. Martínez-Ortega. 2021. Genetic similarities versus morphological resemblance: unraveling a polyploid complex in a Mediterranean biodiversity hotspot. Molecular Phylogenetics and Evolution 155:107006. https://doi.org/10.1016/j.ympev.2020.107006.

Loveless, M. D., and J. L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics 15:65-95. https://doi.org/10.1146/annurev.es.15.110184.000433.

Marinoni, L., J. M. Zabala, M. Parra-Quijano, R. J. Fernández, and J. F. Pensiero. 2018. Genetic and environmental variation of seed weight in *Trichloris* species (Chloridoideae, Poaceae) and its association with seedling stress tolerance. Plant Ecology and Diversity 11:173-184. https://doi.org/10.1080/17550874.2018.1449262.

Marinoni, L., J. M. Zabala, R. E. Quiroga, G. A. Richard, and J. F. Pensiero. 2022. Seed weight and trade-offs: an experiment in false rhodes grasses under different aridity conditions. Plants 11:2887. https://doi.org/10.3390/plants11212887.

McKay, J. K., and R. G. Latta. 2002. Adaptive population divergence: markers, QTL and traits. Trends in Ecology and Evolution 17:285-291. https://doi.org/10.1016/S0169-5347(02)02478-3.

McKay, J. K., C. E. Christian, S. Harrison, and K. J. Rice. 2005. How local is local? — A review of practical and conceptual issues in the genetics of restoration. Restoration Ecology 13:432-440. https://doi.org/10.1111/j.1526-100X.2005.00058.x.

Merilä, J., and P. Crnokrak. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. Journal of Evolutionary Biology 14:892-903. https://doi.org/10.1046/j.1420-9101.2001.00348.x.

Mijangos, J. L., C. Pacioni, P. B. Spencer, and M. D. Craig. 2015. Contribution of genetics to ecological restoration. Molecular Ecology 24:22-37. https://doi.org/10.1111/mec.12995.

Millar, C. I., and W. J. Libby. 1991. Strategies for conserving clinal, ecotypic, and disjunct population diversity in widespread species. Pp. 149-170 *in* D. A. Falk and K. E. Holsinger (eds.). Genetics and Conservation of Rare Plants, Oxford University Press, New York, USA. https://doi.org/10.1093/oso/9780195064292.003.0010.

Nicora, E. G., and Z. E. Rúgolo de Agrasar. 1987. Los géneros de gramíneas de América austral. 611 páginas. Editorial Hemisferio Sur, Buenos Aires, Argentina.

Nielsen, E. E., M. M. Hansen, and D. Meldrup. 2006. Evidence of microsatellite hitch-hiking selection in Atlantic cod (*Gadus morhua* L.): implications for inferring population structure in nonmodel organisms. Molecular Ecology 15: 3219-3229. https://doi.org/10.1111/j.1365-294X.2006.03025.x.

Otto-Bliesner, B. L., E. C. Brady, G. Clauzet, R. Tomas, S. Levis, and Z. Kothavala. 2006. Last glacial maximum and Holocene climate in CCSM3. Journal of Climate 19:2526-2544. https://doi.org/10.1175/JCLI3748.1.

Passera, C. B., O. Borsetto, R. J. Candia, and C. R. Stasi. 1992. Shrub control and seeding influences on grazing capacity in Argentina. Journal of Range Management 45:480-482. https://doi.org/10.2307/4002906.

Pawelek, K. A., F. S. Smith, A. D. Falk, M. K. Clayton, K. W. Haby, and D. W. Rankin. 2015. Comparing three common seeding techniques for pipeline vegetation restoration: a case study in South Texas. Rangelands 37:99-105. https: //doi.org/10.1016/j.rala.2015.03.007.

Peakall, R., and P. E. Smouse. 2012. GENALEX 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537-2539. https://doi.org/10.1111/j.1471-8286.2005.01155.x.

Perfectti, F., F. X. Picó, and J. M. Gómez. 2009. La huella genética de la selección natural. Revista Ecosistemas 18:10-16.

Peterson, P. M., and J. T. Columbus. 1997. Allelic variation in the amphitropical disjunct *Scleropogon brevifolius* (Poaceae: Eragrostideae). Biollania 6:473-490.

Peterson, P. M., and O. Morrone. 1997. Allelic variation in the amphitropical disjunct *Lycurus setosus* (Poaceae: Muhlenbergiinae). Madroño 44:334-346.

Peterson, P. M., J. T. Columbus, and S. J. Pennington. 2007. Classification and biogeography of New World grasses: Chloridoideae. Aliso 23:580-594. https://doi.org/10.5642/aliso.20072301.43.

Peterson, P. M., K. Romaschenko, N. Snow, and G. Johnson. 2012. A molecular phylogeny and classification of *Leptochloa* (Poaceae: Chloridoideae: Chlorideae) sensu lato and related genera. Annals of Botany 109:1317-1329. https://doi.org/10.1093/aob/mcs077.

Peterson, P. M., K. Romaschenko, Y. Herrera Arrieta, and M. S. Vorontsova. 2022. Phylogeny, classification and biogeography of *Afrothrichloris*, *Apochiton*, *Coelachyrum*, *Dinebra*, *Eleusine*, *Leptochloa*, *Schoenefeldia* and a new genus, *Schoenefeldiella* (Poaceae: Chloridoideae: Cynodonteae: Eleusininae). Journal of Systematics and Evolution 60:630-639. https://doi.org/10.1111/jse.12803.

Pezzani, F., C. Montaña, and R. Guevara. 2006. Associations between arbuscular mycorrhizal fungi and grasses in the successional context of a two-phase mosaic in the Chihuahuan Desert. Mycorrhiza 16:285-295. https://doi.org/10.1007/s00572-006-0044-y.

Phillips, S. J. 2005. A brief tutorial on Maxent. ATT Research 190:231-259.

Premoli, A. C., and P. Mathiasen. 2011. Respuestas ecofisiológicas adaptativas y plásticas en ambientes secos de montaña: *Nothofagus pumilio*, el árbol que acaparó los Andes australes. Ecología Austral 21:251-269.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959. https://doi.org/10.1093/genetics/155.2.945.

Quiroga, E., L. Blanco, and E. Orionte. 2009. Evaluación de estrategias de rehabilitación de pastizales áridos. Ecología Austral 19:107-117.

Quiroga, R. E., R. A. Golluscio, L. J. Blanco, and R. J. Fernández. 2010. Aridity and grazing as convergent selective forces: an experiment with an Arid Chaco bunchgrass. Ecological Applications 20:1876-1889. https://doi.org/10.2111/08-044.1.

Quiroga, R. E., R. J. Fernández, R. A. Golluscio, and L. J. Blanco. 2013. Differential water-use strategies and drought resistance in *Trichloris crinita* plants from contrasting aridity origins. Plant Ecology 214:1027-1035. https://doi.org/10.1007/s11258-013-0228-4.

Quiroga, R. E., A. C. Premoli, and R. J. Fernández. 2018. Climatic niche shift in the amphitropical disjunct grass Trichloris crinita. PLoS One 13:e0199811. https://doi.org/10.1371/journal.pone.0199811.

Quiroga, R. E., A. C. Premoli, and R. J. Fernández. 2021. Niche dynamics in amphitropical desert disjunct plants: Seeking for ecological and species-specific influences. Global Ecology and Biogeography 30:370-383. https://doi.org/10.1111/geb.13215.

Quiroga, R. E. 2022. Variabilidad intraespecífica regional y continental en *Trichloris crinita* (Poaceae): aspectos de su nicho ecológico y diferenciación genética relevantes para la restauración de pastizales. Tesis doctoral. Escuela para Graduados, Facultad de Agronomía, Universidad de Buenos Aires, Argentina. Pp. 189.

Raven, P. H. 1963. Amphitropical relationships in the floras of North and South America. The Quarterly Review of Biology 38:151-177. https://doi.org/10.1086/403797.

Sádlo, J., M. Chytrý, J. Pergl, and P. Pyšek. 2018. Plant dispersal strategies: a new classification based on the multiple dispersal modes of individual species. Preslia 90:1-22. https://doi.org/10.23855/preslia.2018.001.

Sánchez-del Pino, I., A. Alfaro, R. H. Andueza-Noh, A. Mora-Olivo, M. Chávez-Pesqueira, et al. 2020. High

phylogeographic and genetic diversity of Tidestromia lanuginosa supports full-glacial refugia for arid-adapted plants in southern and central Coahuila, Mexico. American Journal of Botany 107:1296-1308. https://doi.org/10.1002/ajb2.1536.

Schneider von Deimling, T., A. Ganopolski, H. Held, and S. Rahmstorf. 2006. How cold was the last glacial maximum? Geophysical Research Letters 33:1-5. https://doi.org/10.1029/2006GL026484.

Sgrò, C. M., A. J. Lowe, and A. A. Hoffmann. 2011. Building evolutionary resilience for conserving biodiversity under climate change. Evolutionary Applications 4:326-337. https://doi.org/10.1111/j.1752-4571.2010.00157.x.

Shaw, J., E. B. Lickey, E. E. Schilling, and R. L. Small. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. American Journal of Botany 94:275-288. https://doi.org/10.3732/ajb.94.3.275.

Schenk, J. J., and K. Saunders. 2017. Inferring long-distance dispersal modes in American amphitropically disjunct species through adaptive dispersal structures. American Journal of Botany 104:1756-1764. https://doi.org/10.3732/ajb.1700178.

Simpson, M. G., L. A. Johnson, T. Villaverde, and C. M. Guilliams. 2017. American amphitropical disjuncts: Perspectives from vascular plant analyses and prospects for future research. American Journal of Botany 104:1600-1650. https://doi.org/10.3732/ajb.1700308.

Spitze, K. 1993. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. Genetics 135: 367-374. https://doi.org/10.1093/genetics/135.2.367.

Sutton, P. C., S. J. Anderson, R. Costanza, and I. Kubiszewski. 2016. The ecological economics of land degradation: Impacts on ecosystem service values. Ecological Economics 129:182-192. https://doi.org/10.1016/j.ecolecon.2016.06.016.

Swofford, D. L., and R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72:281-283. https://doi.org/10.1093/oxfordjournals.jhered.a109497.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123: 585-95. https://doi.org/10.1093/genetics/123.3.585.

Tamura, K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. Molecular Biology and Evolution 9:678-687. https://doi.org/10.1093/oxfordjournals.molbev.a040752.

Tamura, K., G. Stecher, and S. Kumar. 2021. MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38:3022-3027. https://doi.org/10.1093/molbev/msab120.

Thorne, R. F. 1972. Major disjunctions in the geographic ranges of seed plants. The Quarterly Review of Biology 47: 365-411. https://doi.org/10.1086/407399.

USDA-NRCS. 2020. Release Brochure for Kinney Germplasm false Rhodes grass [*Trichloris crinita* (Lag.) Parodi]. USDA-Natural Resources Conservation Service, E. 'Kika' de la Garza Plant Materials Center, Kingsville, Texas 78363. URL: tinyurl.com/4usvpam8.

Villaverde, T., M. Escudero, M. Luceño, and S. Martín-Bravo. 2015. Long-distance dispersal during the middle–late Pleistocene explains the bipolar disjunction of *Carex maritima* (Cyperaceae). Journal of Biogeography 42:1820-1831. https://doi.org/10.1111/jbi.12559.

Villaverde, T., P. González-Moreno, F. Rodríguez-Sánchez, and M. Escudero. 2017. Niche shifts after long-distance dispersal events in bipolar sedges (*Carex*, Cyperaceae). American Journal of Botany 104:1765-1774. https://doi.org/10.3732/ajb.1700171.

Weeks, A. R., C. M. Sgrò, A. G. Young, R. Frankham, N. J. Mitchell, et al. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evolutionary Applications 4:709-725. https://doi.org/10.1111/j.1752-4571.2011.00192.x.

Waples, R. S., and O. Gaggiotti. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Molecular Ecology 15:1419-1439. https://doi.org/10.1111/j.1365-294X.2006.02890.x.

Wen, J., and S. M. Ickert-Bond. 2009. Evolution of the Madrean-Tethyan disjunctions and the North and South American amphitropical disjunctions in plants. Journal of Systematics and Evolution 47:331-348. https://doi.org/10.1111/j.1759-6831.2009.00054.x.

Williams, A. V., G. Nevill, and S. L. Krauss. 2014. Next generation restoration genetics: applications and opportunities. Trends in Plant Science 19:529-537. https://doi.org/10.1016/j.tplants.2014.03.011.

Wright, S., J. Keeling, and L. Gillman. 2006. The road from Santa Rosalia: a faster tempo of evolution in tropical climates. Proceedings of the National Academy of Sciences 103:7718-7722. https://doi.org/10.1073/pnas.0510383103.

Yang, Y. Y., X. J. Qu, R. Zhang, G. W. Stull, and T. S. Yi. 2021. Plastid phylogenomic analyses of Fagales reveal signatures of conflict and ancient chloroplast capture. Molecular Phylogenetics and Evolution 163:107232. https://doi.org/10.1016/j.ympev.2021.107232.

Zabala, J. M., P. Widenhorn, and J. F. Pensiero. 2011. Germination patterns of species of the genus *Trichloris* in arid and semiarid environments. Seed Science and Technology 39:338-353. https://doi.org/10.15258/sst.2011.39.2.07.