

The role of reproductive modes in shaping genetic diversity in polyploids: a comparative study of selfing, outcrossing, and apomictic *Paspalum* species

A. Verena Reutemann^{1, †}; Mara Schedler^{2, †}; Diego H. Hojsgaard³; Elsa A. Brugnoli¹; Alex L. Zilli¹; Carlos A. Acuña¹, Ana I. Honfi⁴ and Eric J. Martínez^{1*}.

¹ Grupo de Genética y Mejoramiento de Especies Forrajeras, Instituto de Botánica del Nordeste (CONICET-UNNE), Corrientes, Argentina. verena.reutemann@agr.unne.edu.ar (A.V.R.); abrugnoli@agr.unne.edu.ar (E.A.B.); azilli@agr.unne.edu.ar (A.L.Z.); cacuna@agr.unne.edu.ar (C.A.A.); eric@agr.unne.edu.ar (E.J.M.)

² Estación Experimental Agropecuaria Montecarlo, Instituto Nacional de Tecnología Agropecuaria (INTA), Misiones, Argentina. schedlermara@gmail.com

³ Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. hojsgaard@ipk-gatersleben.de

⁴ Programa de Estudios Florísticos y Genética Vegetal, Instituto de Biología Subtropical (CONICET-UNaM), Misiones, Argentina. Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones (FCEQyN-UNaM), Misiones, Argentina. ahonfi@fceqyn.unam.edu.ar

* Correspondence: eric@agr.unne.edu.ar

[†] These authors contributed equally to this work.

Abstract: Exploring the genetic diversity and reproductive strategies of *Paspalum* species is essential for advancing forage grass improvement. We compared morpho-phenological, molecular, and genotypic variation in five tetraploid *Paspalum* species with contrasting mating systems and reproductive modes. Contrary to previous findings, selfing (*Paspalum regnellii* and *P. urvillei*) and outcrossing (*P. durifolium* and *P. ionanthum*) species exhibited similar phenotypic diversity patterns, with low intrapopulation variability and no morphological differentiation among populations. The apomictic species (*P. intermedium*) exhibited low intrapopulation phenotypic variation but high population differentiation, indicative of genetic drift and local adaptation. Outcrossing species showed greater intrapopulation genotypic variation than selfing species, which displayed a high population structure due to restricted pollen migration. The apomictic species exhibited the lowest intrapopulation molecular diversity, forming uniclinal populations with high interpopulation differentiation, highlighting the fixation of distinct gene pools via apomixis. This is the first report about genetic diversity in populations of sexual allopolyploid species of *Paspalum*. Population structure in these allotetraploid *Paspalum* species is primarily shaped by how reproductive modes, mating systems, and geographic distribution influence gene flow via pollen and seeds. Our findings contribute significantly to the conservation and genetic improvement of forage grasses, particularly for developing cultivars with enhanced adaptability and productivity.

Keywords: apomixis, mating systems, outcrossing, *Paspalum*, population structure, selfing

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Last-name

Received: date

Revised: date

Accepted: date

Published: date



Copyright: © 2024 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Exploring the population genetic diversity of *Paspalum* L. species is essential for conceiving strategies for germplasm collection, conservation, and advancing genetic improvement and cultivar development in this genus [1–2]. Understanding key traits like ploidy levels and reproductive modes, and their role in the population patterns of genetic diversity can assist the development of such strategies [1].

Most angiosperms reproduce through two main modes: sexual and asexual, through either vegetative reproduction or apomixis [3–4]. Sexual reproduction leads to genetically heterogeneous populations as new genotypes emerge through meiotic recombination and random syngamy [5]. In addition, sexual reproduction avoids mutation accumulation by natural selection of deleterious combinations [6]. Additionally, sexually reproducing plants experience regular genetic exchange between individuals and populations via pollen and seed dispersal, reducing interpopulation differentiation and the potential impact of genetic drift [5].

On the contrary, apomictic plants show reduced genetic variation due to the absence of meiotic segregation and recombination, as demonstrated in numerous studies [7–8]. Thus, apomictic plants produce offspring genetically identical to the mother plant through seeds [7]. There are two types of apomixis: sporophytic and gametophytic, which differ in how the clonal embryos are formed [3, 7]. In sporophytic apomixis, also called polyembryony, an embryo originates from the nucellus or the integuments independently of the gametophytic development [3]. In gametophytic apomixis, an unreduced female gametophyte is formed in which the egg cell develops into an embryo without fertilization (parthenogenetically), and the seeds are developed either autonomously (without pollination) or requiring fertilization of the central cell (pseudogamy) for successful endosperm development [7]. Therefore, another expected consequence of apomixis is the formation of genetically uniform (uniclonal) populations that become differentiated through stochastic and/or adaptive events [9–12]. This usually results in multiple origins and founding events, leading to high population differentiation through the fixation of divergent genotypes [13–16]. Apomixis has long been seen as an unparalleled natural tool to exploit plant breeding, with potential influence on global farming systems [1,17].

In addition to reproductive modes, mating systems also play a pivotal role in shaping the genetic structure of plant populations [18]. Selfing tends to evolve when the advantages of transmission and reproductive assurance outweigh the disadvantages associated with inbreeding depression and reduced fitness caused by the increased homozygosity of deleterious alleles [19]. However, selfing is expected to evolve at the cost of reduced genetic variation [18, 20–21]. Several studies have shown that selfing species exhibit lower intrapopulation variation but increased interpopulation differentiation than outcrossers due to genetic drift and limited gene flow [21–28]. More recently, comparative population genetic studies of related plant species have confirmed this pattern [29–30].

Beyond reproduction modes and mating systems, environmental and ecological factors also influence the spatial genetic structure of plant species [31–33]. Genetic diversity results from a complex interplay of intra- and interpopulation dynamics, as well as climatic, historical, and geological factors [5, 34]. Understanding these dynamics is crucial for developing local conservation strategies [35] and promoting sustainable agroecosystems [36].

Paspalum is one of the few genera in which sexual and asexual reproductive modes have been characterized side by side for more than 70 years [2]. Sexuality in *Paspalum* species is frequent and is characterized by the double fertilization of a reduced embryo sac of the *Polygonum* type [37]. Over 330 species have been described for this genus [38], however only a few of them have been studied at a population level. Cytoembryological and cytological analyses of 72 *Paspalum* species showed that 68% of them had some potential for apomixis, 73.6% showed self-fertility, 43.1% were self-sterile, and 75% were polyploids [39]. Nevertheless, these species only represent 21.8% of the total number of species in the genus, and most studies involve singular plants rather than populations. The study of reproduction modes and mating systems in *Paspalum* species offers valuable insights into the genetic mechanisms underlying population genetic structure, as observed in previous works on this genus [16, 30, 40–43]. This knowledge is essential for the efficient use of these tropical grasses in breeding programs and conservation plans [1]. Therefore, we selected five *Paspalum* species with contrasting reproductive modes and mating systems, commonly found in natural grasslands of northeastern Argentina.

Paspalum durifolium Mez and *P. ionanthum* Chase are allotetraploids ($2n = 4x = 40$) that form complexes with higher ploidy numbers [44–47]. These tetraploids are sexual and predominantly outcrossers due to a gametophytic self-incompatibility system (GSI) [48]. Although some tetraploids showed apomictic embryo sacs, no apomictic seeds have been reported [48]. *Paspalum regnellii* Mez and *P. urvillei* Steud. are allotetraploid species ($2n = 4x = 40$) [49–51]. Both species behave mainly as selfers, but varying degrees of cross-pollination have been reported [48]. Finally, *P. intermedium* Munro ex Morong is an agamic complex with self-sterile sexual diploids ($2n = 2x = 20$) and self-fertile facultative-apomictic autotetraploids ($2n = 4x = 40$) [44, 52]. Species like *P. ionanthum*, *P. regnellii*, and *P. urvillei* hold promise for domestication or genetic improvement as forage species due to their desirable agronomic traits. In contrast, *P. durifolium* and *P. intermedium* typically form large tussocks, which are less suitable for grazing and have lower forage value but have potential as ornamental crops.

In this study, we addressed the following questions: (1) Do populations of self-fertile *Paspalum regnellii* and *P. urvillei* exhibit lower genetic diversity than self-sterile populations *P. durifolium* and *P. ionanthum*? (2) Is genetic diversity lower in apomictic *P. intermedium* compared to self-fertile and self-sterile *Paspalum* species? (3) Are the differences in the genetic structure among *Paspalum* populations related to their mode of reproduction or mating system?

2. Results

2.1. Phenotypic variation within populations

We described the variation within each population using the mean and standard deviation (Table 1). The coefficient of variation for morphological traits indicated low variation within populations for most traits. Exceptions included internode length (NL) in the PIN7 population of *P. intermedium*, as well as the PD1 and PD4 populations of *P. durifolium*, and the vegetative period (VP) in PU2, PU3, PU4, and PU5 populations of *P. urvillei* (Supplementary Data, Table S1).

Table 1. Mean and standard deviation for each phenotypic trait in five *Paspalum* species.

Species	Pop	n	H	Hw	LL	LW	ShL	NL	IL
<i>P. intermedium</i>	PIN2	14	187.9±17.2	100.7±9.2	34.2±4.2	1.2±0.1	26.4±3.0	26.3±3.2	25± 1.5
	PIN4	18	228.1±17.2	131.1±9.6	45.8±5.5	1.9±0.1	40.2±5.6	36.6±3.7	37.3±2.7
	PIN7	18	110.3±21.1	71.4±14.1	23.6±2.9	1.2±0.1	29.4±2.4	11.0±4.1	22.4±2.4
	PIN8	18	173.8±31.3	85.6±9.9	35.8±7.5	1.4±0.3	29.4±5.5	23.9±3.6	26.9±3.5
	PIN9	18	225.7±25.6	106.8±14.7	38.6±10.2	1.4±0.3	34.1±3.8	28.3±5.2	35.4±4.8
<i>P. durifolium</i>	PD3	16	118.1±17.4	57.9±6.9	24.4±6.4	0.9±0.1	11.7±2.9	23.2±5.3	19.7±2.4
	PD4	16	111.3±21.3	53.6±5.7	20.1±3.0	0.8±0.1	10.5±2.3	18.9±6.1	18.7±2.3
	PD5	19	114±16.8	58.3±6.3	25.6±5.2	0.8±0.1	10.7±2.4	19.5±4.6	18.7±2.3
	PD1	19	107.5±19.6	58.7±9.7	25.3±4.8	0.8±0.1	11.6±2.9	16.5±5.7	18.7±2.0
	PD2	16	118.7±14.2	60.2±12.0	25.7±4.9	0.8±0.1	9.8±1.4	21.8±4.4	18.9±2.4
<i>P. ionanthum</i>	PI3	19	109.7±21.2	67.7±7.4	29.3±5.0	0.7±0.1	12.1±2.0	25.8±5.3	10.4±1.7
	PI4	20	121.0±18.8	69.1±7.1	31.4±5.2	0.8±0.1	8.4±1.5	25.8±4.7	11.1±1.3
	PI5	19	126.3±21.0	66.3±9.7	31.0±3.6	0.8±0.1	9.0±2.1	28.5±5.3	11.8±1.6
	PI1	19	96.5±6.7	67.8±7.7	26.1±3.0	0.8±0.1	13.9±2.4	23.0±2.3	10.7±1.3
	PI2	19	102.1±20.8	72.3±7.6	32.3±5.7	0.8±0.1	15.3±3.1	22.0±6.4	11.9±1.8
<i>P. regnellii</i>	PR2	18	183.3±12.8	92.8±7.6	34.5±5.3	2.3±0.2	14.9±3.2	18.8±2.6	12.4±1.8
	PR1	20	185.6±11.7	95.5±2.8	33.8±3.5	2.3±0.2	18.0±2.6	17.9±2.00	14.1±2.2
	PR3	20	185±10.9	99.0±9.8	34.7±5.4	2.3±0.3	15.8±1.9	19.2±2.0	13.8±2.3

	PR4	20	184.5±12.2	101.2±7.2	35±5.3	2.4±0.3	14.3±2.2	19.0±3.0	14.4±2.3
	PR5	19	197.9±8.5	112.1±24.2	37.9±5.5	2.2±0.1	16.0±1.7	20.3±2.4	15.5±2.4
<i>P. urvillei</i>	PU1	16	199.1±8.4	135.3±10.4	43.3±5.1	1.6±0.1	20.8±2.1	20.4±2.1	28.4±4.9
	PU3	18	216.4±11.2	118.1±9.6	36±2.7	1.2±0.1	20.2±2.4	19.4±3.0	29.4±3.3
	PU4	17	213.1±15.6	117±9.6	34.4±5.4	1.2±0.1	22.7±2.5	20.7±2.1	31.4±3.2
	PU5	17	206.7±12.2	124.9±11.4	39.7±5.6	1.4±0.2	20.4±2.4	19.5±2.8	29.5±4.0
	PU2	20	196±13.2	119±12.5	35.8±4.4	1.4±0.2	19.6±2.0	18.3±2.5	28.5±5.8

Abbreviations, n: number of individuals, plant height with (H; cm) and without flowering culms (Hw; cm), 2nd leaf blade length (LL; cm) and width (LW; cm), 2nd leaf sheath length (ShL; cm), 2nd internode length (NL; cm), inflorescence length (IL; cm).

Table 1. Continued.

Species	Pop	n	BRL	EBR	NR	VL	SL	SW	VP	FP
<i>P. intermedium</i>	PIN2	14	9.5±1.0	110.6±9.8	39.5±2.5	16.5±10.7	2.7±0.2	1.6±0.0	81.2±9.1	114.8±5.6
	PIN4	18	9.0±0.9	145.9±36.4	77.6±9.8	205.6±11.8	3.0±0.1	1.5±0.0	62.0±6.7	77.4±3.5
	PIN7	18	10.9±1.1	135.7±10.9	33.4±5.1	103.3±18.9	2.7±0.1	1.5±0.0	112±12.5	148.2±8.7
	PIN8	18	8.2±1.6	105.7±21.5	45.9±4.8	163.9±29.3	2.5±0.1	1.3±0.1	92.1±12.6	126.8±16.2
	PIN9	18	8.1±1.1	108.5±14.8	71.2±4.7	210.3±19.1	2.4±0.1	1.2±0.1	66.1±6.0	81.7±4.2
<i>P. durifolium</i>	PD3	16	8.6±1.0	90.2±11.9	19.4±3.3	100±14.6	3.0±0.1	1.5±0.1	62.4±6.2	48.5±6.5
	PD4	16	7.7±1.2	84.2±14.6	17.2±3.6	88.6±19.1	3.0±0.1	1.4±0.1	57.4±8.1	74.9±6.7
	PD5	19	7.8±1.1	104.5±18.5	19.5±3.0	89.9±13.5	2.9±0.1	1.4±0.1	60.1±4.2	74.4±4.8
	PD1	19	7.4±1.3	93.3±18.9	17.7±4.0	87.9±16.6	2.9±0.1	1.4±0.1	68.3±6.4	81.9±5.6
	PD2	16	8.2±1.4	98.6±15.6	18.5±3.1	98.6±11.9	2.9±0.1	1.4±0.1	63.6±5.2	76.9±4.3
<i>P. ionanthum</i>	PI3	19	9.3±1.4	69.9±12.3	2.1±0.2	94.5±21.1	4.2±0.3	2±0.0	62.1±8.5	82.4±6.2
	PI4	20	9.5±1.4	70.5±14.4	2.3±0.4	108.0±16.8	4.4±0.3	2.0±0.0	56.6±9.4	75.8±7.4
	PI5	19	10.1±1.4	73.3±11.4	2.2±0.3	116.1±20.9	4.4±0.3	2.0±0.1	77.3±8.8	92.8±8.9
	PI1	19	9.7±1.1	72.3±11.2	2.0±0.1	88.7±3.4	4.2±0.2	2.0±0.0	51.6±10.8	74.0±9.3
	PI2	19	11.0±1.8	82.1±11.9	2.0±0.1	89.3±17.6	4.6±0.2	2.2±0.2	68.3±8.2	83.3±7.6
<i>P. regnellii</i>	PR2	18	10.3±1.2	144.9±23.3	9.4±1.3	164.1±16.2	2.5±0.0	1.5±0.0	133.9±16.0	165.0±2.5
	PR1	20	10.7±1.1	179.8±29.0	10.6±2.2	163.9±16.8	2.5±0.0	1.5±0.0	116.2±8.8	161.2±1.8
	PR3	20	11.0±1.7	164.0±33.2	10.3±2.1	154.9±14.6	2.5±0.0	1.5±0.0	142.3±17.7	169.5±5.7
	PR4	20	11.7±1.6	163.4±25.0	11.0±2.1	157.4±12.9	2.5±0.0	1.5±0.0	144.2±12.7	167.6±4.4
	PR5	19	12.2±1.3	167.9±24.2	11.8±2.9	172.6±11.9	2.5±0.0	1.5±0.0	131.4±16.0	165.4±4.9
<i>P. urvillei</i>	PU1	16	11.4±2.0	139.0±24.0	14.4±3.6	177.6±18.3	2.9±0.1	1.5±0.0	43±10.1	70.1±3.6
	PU3	18	10.5±1.8	145.5±23.1	14.8±2.8	191.0±13.6	2.9±0.1	1.5±0.0	52.8±16	82.2±14.8
	PU4	17	10.4±1.1	133.7±13.8	15.8±1.6	183.0±16.3	2.8±0.1	1.5±0.0	56.6±23.2	85.6±18
	PU5	17	11.1±2.1	139.7±20.6	14.1±1.9	177.2±17.7	2.8±0.1	1.5±0.0	72.1±25	97.4±26.4
	PU2	20	11.8±2.2	144.6±16.9	12.9±2.4	171.2±15.7	2.7±0.2	1.5±0.0	47.8±17.3	79.6±17.5

Abbreviations, n: number of individuals, basal raceme length (BRL; cm), number of spikelets in the basal raceme (EBR), raceme number (NR), flowering culms length with inflorescences (VL; cm), spikelet length (SL; mm) and width (SW; mm), extension of the vegetative period (VP; days) and the flowering period (FP; days).

2.2. Phenotypic variation among populations

We observed a high correlation between vegetative and flowering periods (VP-FP) in *P. intermedium*, *P. durifolium*, *P. ionanthum*, and *P. urvillei* (>0.9; [Supplementary Data, Figure S1](#)). Additionally, we found a high correlation between plant height and the length of the flowering culms with inflorescences (H-VL) in *P. intermedium* and *P. ionanthum*, as well

as between inflorescence length and the basal raceme length (IL-BRL) in *P. regnellii* (>0.9 ; [Supplementary Data, Figure S1](#)). Therefore, in the MANOVA analysis, we removed VP for *P. intermedium*, *P. durifolium*, *P. ionanthum*, and *P. urvillei*, VL for *P. intermedium* and *P. ionanthum*, and BRL for *P. regnellii*.

Paspalum intermedium showed significant differentiation among populations for all traits (Pillai's test, $F = 21.08$, num. D.f. = 52, den. D.f. = 288, $p < 0.001$; [Supplementary Data, Table S2](#)). *Paspalum durifolium* exhibited differentiation among populations (Pillai's test, $F = 22.83$, num. D.f. = 56, den. D.f. = 284, $p < 0.001$) only in FP and LL ([Supplementary Data, Table S2](#)). Pairwise comparisons revealed that the population PD1 differed from PD2, PD4, and PD5 in FP, while PD4 was differentiated from PD1, PD2, and PD5 in LL ([Supplementary Data, Table S3](#)). *Paspalum ionanthum* exhibited differentiation among populations (Pillai's test, $F = 57.88$, num. D.f. = 52, den. D.f. = 328, $p < 0.001$) in FP, H, LL, NL, SL, ShL, and SW ([Supplementary Data, Table S2](#)). *Paspalum regnellii* showed differentiation among populations (Pillai's test, $F = 42.61$, num. D.f. = 48, den. D.f. = 336, $p < 0.001$) in EBR, FP, H, Hw, ShL, VL, and VP ([Supplementary Data, Table S2](#)). *Paspalum urvillei* showed differentiation among populations (Pillai's test, $F = 40.69$, num. D.f. = 52, den. D.f. = 296, $p < 0.001$) in FP, H, Hw, LL, LW, and SL ([Supplementary Data, Table S2](#)). Differentiation among populations for each trait and species is detailed in [Supplementary Data, Table S3](#).

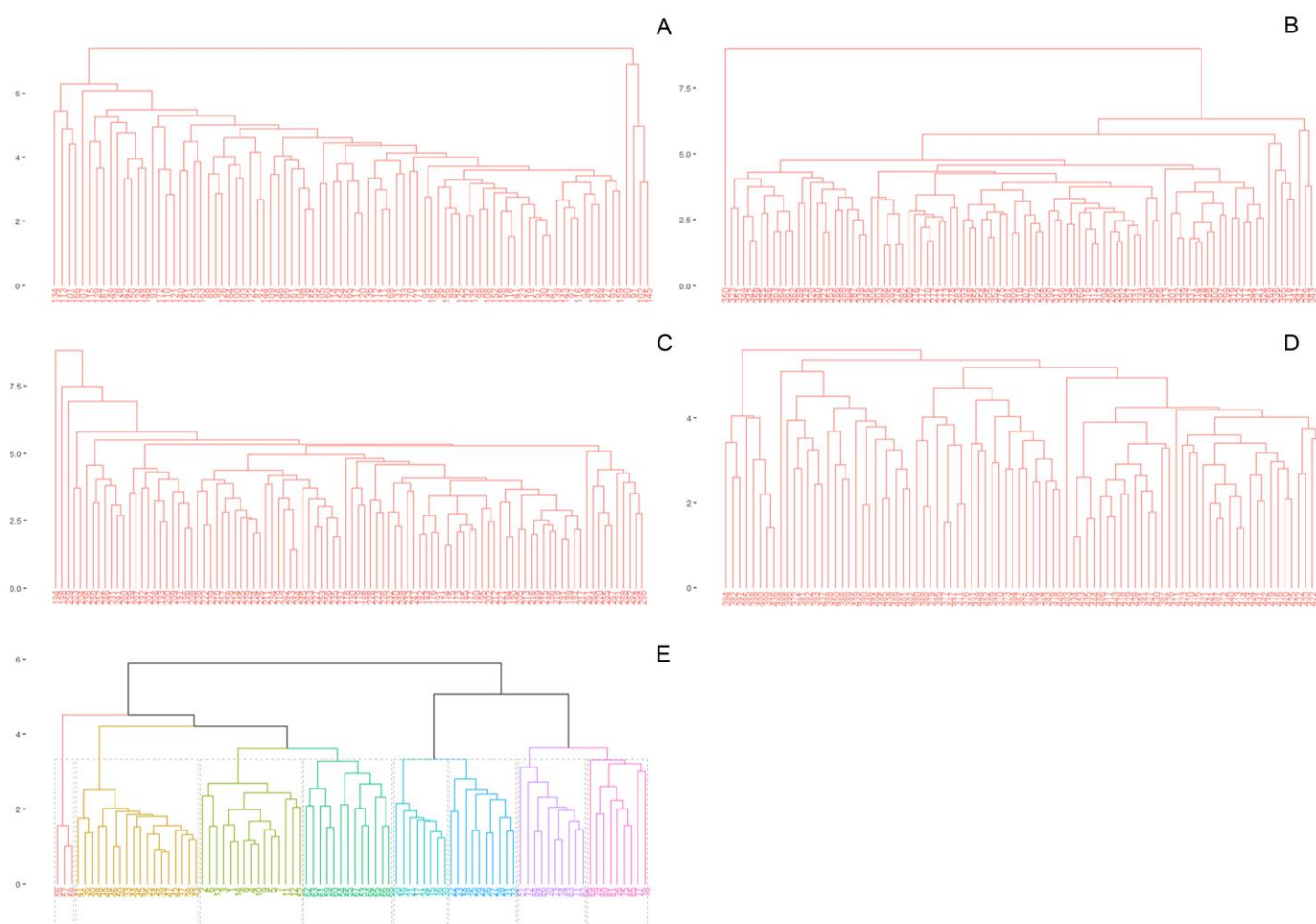


Figure 1. UPGMA trees reflecting optimal number of clusters using phenotypic traits. **A-** *P. durifolium*. **B-** *P. ionanthum*. **C-** *P. regnellii*. **D-** *P. urvillei*. **E-** *P. intermedium*. Clusters within each tree are according to the k-means algorithm and indicated by different colors.

We observed an intermediate correlation between phenotypic variation and geographic distribution in apomictic *P. intermedium* (Mantel statistic $R = 0.41$, $p = 0.001$). A low

correlation between phenotypic variation and geographical distribution was found in *P. ionanthum* (Mantel statistic $R=0.12$, $p=0.015$), *P. regnellii* (Mantel statistic $R=0.14$, $p=0.001$), and *P. urvillei* (Mantel statistic $R=0.17$, $p=0.001$) but no significant correlation was observed in *P. durifolium* (Mantel statistic $R=0.04$, $p=0.141$).

The UPGMA analysis showed that PIN7 of *P. intermedium* clustered separately (Figure 1E). Population PIN4 and PIN9 formed two clusters each, and PIN8 formed four groups, one of them grouped with PIN2 and another with PIN9 (Figure 1E). The k-means clustering ($K=1-10$) indicated eight well-differentiated groups in the apomictic species *P. intermedium* (optimal number of clusters = 8, Figure 1E). In contrast, the four sexual species did not show clear clustering among individuals from the same population (Figure 1A-D). Additionally, the k-means analysis indicated no well-differentiated groups in the sexual species (optimal number of clusters = 1, Figure 1A-D).

The PCA for *P. intermedium* explained 72.9% of the pheno-morphological variability within the first two axes, with a significant contribution of *FP*, *Hw*, *NR*, *IL*, *H*, and *SL* to these axes (Supplementary Data, Figure S2). Along axis 1, population PIN4 was separated towards the positive pole, displaying high values for most of the traits, while populations PIN2 and PIN7 were separated toward the negative pole, showing high values of *FP* (Figure 2A). Over axis 2, populations PIN8 and PIN9 were separated from the other three populations, by showing low values of *BRL*, *EBR*, *SL*, and *SW* (Figure 2A).

The PCA for *P. durifolium* explained 40.4% of the distribution of the variability within the first two axes and showed that *NL*, *VL*, and *H* contributed the most to these axes (Supplementary Data, Figure S2). The PCA for *P. ionanthum* explained 39.8% of the distribution of the variability within the first two axes and showed that *BRL*, *IL*, *VP*, and *FP* contributed the most to these axes (Supplementary Data, Figure S2). The PCA for *P. regnellii* explained 37.3% of the distribution of the variability within the first two axes and showed that *VL*, *H*, and *IL* contributed the most to these axes (Supplementary Data, Figure S2). The PCA for *P. urvillei* explained 38.4% of the distribution of the variability within the first two axes and showed that *IL*, *H*, and *NL* contributed the most to these axes (Supplementary Data, Figure S2). As observed in the UPGMA and K-means, there was no distinctive separation among populations of the sexual species (Figure 2B-E). Only *P. ionanthum* showed a separation among populations PI1 and PI5 over the axis Dim2 (Figure 2C).

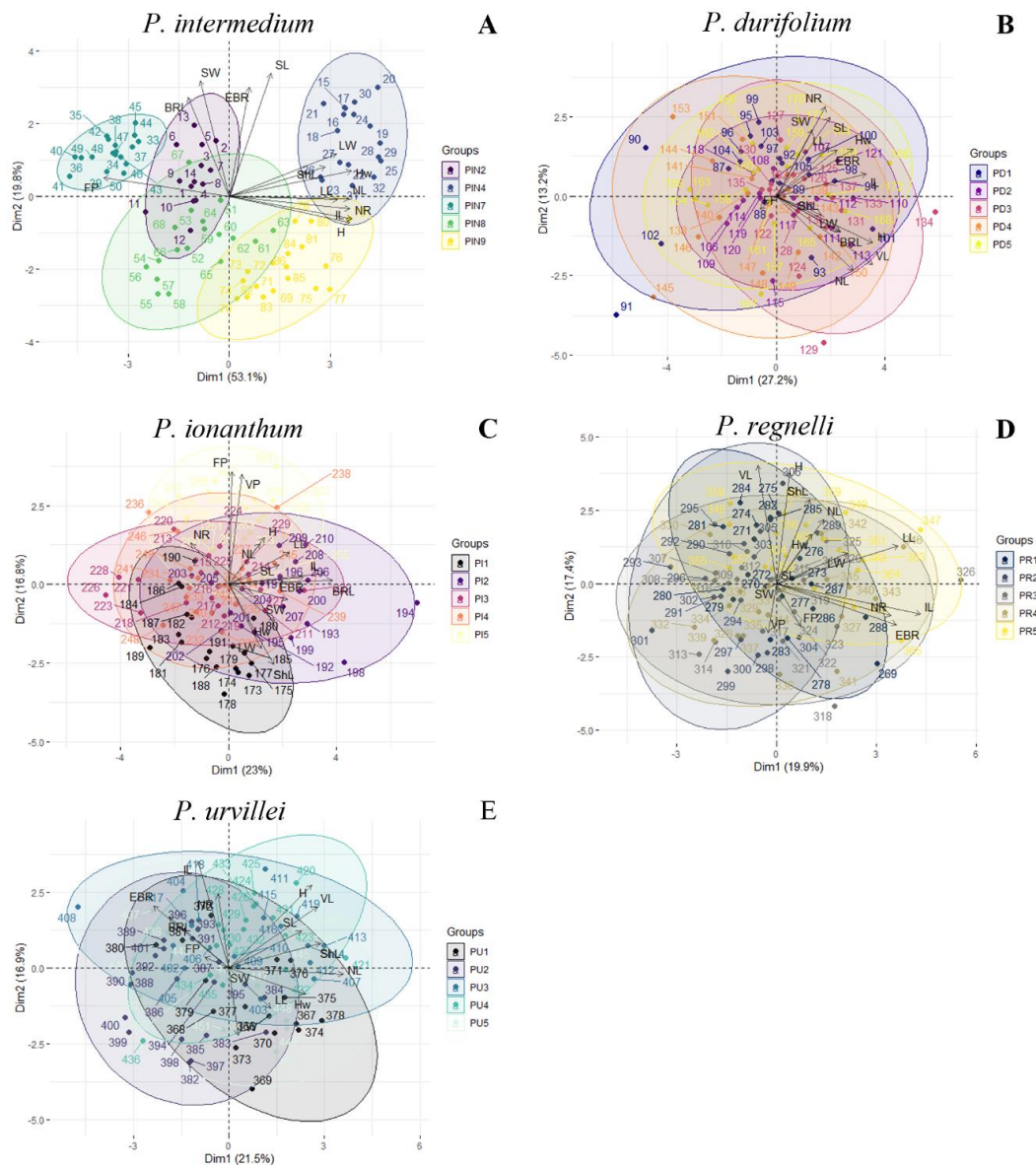


Figure 2. PCA analysis for each species using morpho-phenological traits.

2.3. Molecular and genotypic diversity within species

A total of 124 ISSR fragments for *P. durifolium*, 139 for *P. ionanthum*, 122 for *P. regnellii*, 102 for *P. urvillei*, and 90 for *P. intermedium* were analyzed.

The apomictic populations of *P. intermedium* displayed the lowest values of molecular and genotypic diversity, with each population represented by only one genotype (Table 2). Populations of self-incompatible *P. durifolium* and *P. ionanthum* exhibited high polymorphism and high genotypic diversity indexes (Table 2). One private fragment (PB) was found in population PI4 of *P. ionanthum* (Table 2). In contrast, molecular diversity in self-compatible *P. regnellii* and *P. urvillei* populations was low, especially regarding polymorphism. Genotypic variability was higher in populations of *P. urvillei* than in *P. regnellii*, which showed low numbers of effective genotypes and, consequently, low genotypic diversity values (Table 2). Although unexpected for a sexual species, individuals in *P. regnellii* sharing the same genotype exhibited differences at the morpho-phenological level (Figure 2C).

Table 2. Molecular and genotypic diversity indexes in tetraploid populations of five *Paspalum* species.

Species	Pop	n	Molecular diversity indexes				Genotypic diversity indexes			
			TB	CB	%PL	PB	G	D	E	H
<i>P. intermedium</i>	PIN2	14	81	3	0	1	1	0	1	0
	PIN4	18	75	3	0	3	1	0	1	0
	PIN7	18	78	1	4.4	0	1	0	1	0.022
	PIN8	18	78	1	2.2	0	1	0	1	0.008
	PIN9	19	77	2	1.1	0	1	0	1	0.007
<i>P. durifolium</i>	PD1	19	123	0	77.4	0	19	1	1	1.28
	PD2	16	123	0	80.7	0	16	1	1	1.20
	PD3	16	123	0	79.8	0	16	1	1	1.20
	PD4	16	123	0	75.8	0	16	1	1	1.20
	PD5	20	123	0	79.8	0	20	1	1	1.30
<i>P. ionanthum</i>	PI1	19	131	0	76.3	0	19	1	1	1.27
	PI2	19	132	2	76.3	0	19	1	1	1.27
	PI3	19	134	1	81.3	0	19	1	1	1.27
	PI4	19	134	1	80.6	1	19	1	1	1.27
	PI5	20	131	0	74.8	0	20	1	1	1.30
<i>P. regnellii</i>	PR1	20	120	1	26.2	0	19	0.99	0.96	1.27
	PR2	18	120	0	25.4	0	18	1	1	1.26
	PR3	20	120	0	21.3	0	13	0.91	0.59	1.01
	PR4	20	116	0	14.8	0	8	0.77	0.47	0.72
	PR5	19	115	1	15.6	0	9	0.81	0.48	0.79
<i>P. urvillei</i>	PU1	15	100	0	49.0	0	15	1	1	1.18
	PU2	20	101	0	53.9	0	20	1	1	1.30
	PU3	19	100	0	42.2	0	19	1	1	1.28
	PU4	18	100	0	46.1	0	18	1	1	1.26
	PU5	18	102	0	55.9	0	18	1	1	1.26

Abbreviations. n: number of individuals; TB: total number of bands; CB: No. of common bands (≤50%); %PL: percentage of polymorphic loci, PB: private band number; G: effective number of genotypes; D: Nei's genetic diversity index; E: evenness index; H: Shannon–Wiener index.

2.4. Genetic differentiation and gene flow

Genetic differentiation among populations was the highest in *P. intermedium*, as each population was represented by a unique genotype (Table 3). Differentiation was low in self-incompatible *P. durifolium* and intermediate in *P. ionanthum* (Table 3). A high population structure was observed in self-compatible *P. regnellii* and *P. urvillei* (Table 3). The pairwise F_{ST} values indicated intermediate differentiation for population PIN4 of *P. intermedium* with PIN2, PIN7, and PIN8 (pairwise $F_{ST} > 0.12$, Table 4). Populations of sexual species showed very low genetic differentiation (pairwise $F_{ST} < 0.05$, Table 4).

Table 3. AMOVA test for *Paspalum* species based on ISSR data respectively.

Species	Source	df	SS	MS	Est. Var	Rho _{ST}	p
<i>P. intermedium</i>	Among Pops	4	534.5	133.6	7.7	0.851	0.001
	Within Pops	82	20.2	0.25	0.3		
<i>P. durifolium</i>	Among Pops	4	188.6	47.2	1.7	0.084	0.001
	Within Pops	82	1490.7	18.1	18.2		
<i>P. ionanthum</i>	Among Pops	4	380.7	95.2	4.0	0.18	0.001
	Within Pops	91	1666.8	18.3	18.3		
<i>P. regnellii</i>	Among Pops	4	160.5	40.1	1.8	0.29	0.001

	Within Pops	92	415.8	4.5	4.5		
<i>P. urvillei</i>	Among Pops	4	200.4	50.1	2.3	0.20	0.001
	Within Pops	85	793.8	9.3	9.3		

Abbreviations, df: degree of freedom, SS: square sums, MS: mean of the squares, Est. Var: estimation of variation, Rho_{ST}: analog of F_{ST} for polyploids, *p*: p-value.

Table 4. Pairwise fixation index (AMOVA-derived FST) between populations within each species of *Paspalum* calculated with 999 permutations on ISSR data.

<i>P. intermedium</i>											
Pop	PIN2	PIN4	PIN7	PIN8	PIN9	Pop	PI1	PI2	PI3	PI4	PI5
PIN2	-	***	***	***	***	PI1	-	n.s.	n.s.	n.s.	***
PIN4	0.12	-	***	***	***	PI2	0.04	-	n.s.	n.s.	***
PIN7	0.07	0.13	-	***	***	PI3	0.04	0.03	-	n.s.	***
PIN8	0.07	0.13	0.04	-	***	PI4	0.05	0.04	0.03	-	***
PIN9	0.09	0.09	0.08	0.08	-	PI5	0.05	0.05	0.04	0.04	-
<i>P. durifolium</i>						<i>P. ionanthum</i>					
Pop	PD1	PD2	PD3	PD4	PD5	Pop	PI1	PI2	PI3	PI4	PI5
PD1	-	**	**	**	***	PI1	-	n.s.	n.s.	n.s.	***
PD2	0.02	-	n.s.	n.s.	***	PI2	0.04	-	n.s.	n.s.	***
PD3	0.02	0.02	-	n.s.	***	PI3	0.04	0.03	-	n.s.	***
PD4	0.02	0.01	0.01	-	***	PI4	0.05	0.04	0.03	-	***
PD5	0.02	0.02	0.01	0.02	-	PI5	0.05	0.05	0.04	0.04	-
<i>P. regnellii</i>						<i>P. urvillei</i>					
Pop	PR1	PR2	PR3	PR4	PR5	Pop	PU1	PU2	PU3	PU4	PU5
PR1	-	n.s.	***	***	***	PU1	-	n.s.	n.s.	n.s.	n.s.
PR2	0.02	-	n.s.	***	***	PU2	0.02	-	n.s.	n.s.	n.s.
PR3	0.01	0.01	-	***	***	PU3	0.02	0.03	-	n.s.	n.s.
PR4	0.02	0.01	0.01	-	***	PU4	0.03	0.03	0.03	-	n.s.
PR5	0.03	0.02	0.02	0.02	-	PU5	0.02	0.03	0.03	0.03	-

References, *p*-value: **0.01, ***0.001, n.s. not significant.

The Mantel test demonstrated that genetic variation was geographically correlated in populations of *P. intermedium* ($R^2=0.47$, $p=0.001$), and the isolation by distance test (IBD) inferred eight distance clusters, with six of them showing significant isolation ($p<0.05$, Supplementary Data, Figure S5-A). Genetic variation was correlated with the geographical distribution of populations in self-incompatible *P. ionanthum* ($R^2=0.36$, $p=0.001$) and *P. durifolium* ($R^2=0.13$, $p=0.001$), and self-compatible *P. regnellii* ($R^2=0.34$, $p=0.001$) and *P. urvillei* ($R^2=0.25$, $p=0.001$). The IBD identified seven clusters in *P. durifolium*, with two of them isolated by distance ($p<0.05$, Supplementary Data, Figure S5-B), six clusters in *P. ionanthum* and four of them were isolated ($p<0.05$, Supplementary Data, Figure S5-C), seven clusters in *P. regnellii* with four of them isolated ($p<0.05$, Supplementary Data, Figure S5-D), and eight clusters in *P. urvillei* of which two clusters were isolated ($p<0.05$, Supplementary Data, Figure S5-E).

2.4. Population structure and cluster analysis

Five clusters with high bootstrap (>90%) were identified in the unrooted UPGMA dendrogram for *P. intermedium*, each corresponding to a unique population (Fig. 3A). The k-means algorithm and Structure analysis corroborated these five clusters (Supplementary Data, Figure S3-A) with no genetic admixture among them (Fig. 3B and 3C). This high population differentiation was confirmed by the DAPC, showing a clear separation among populations of *P. intermedium* (Fig. 3D).

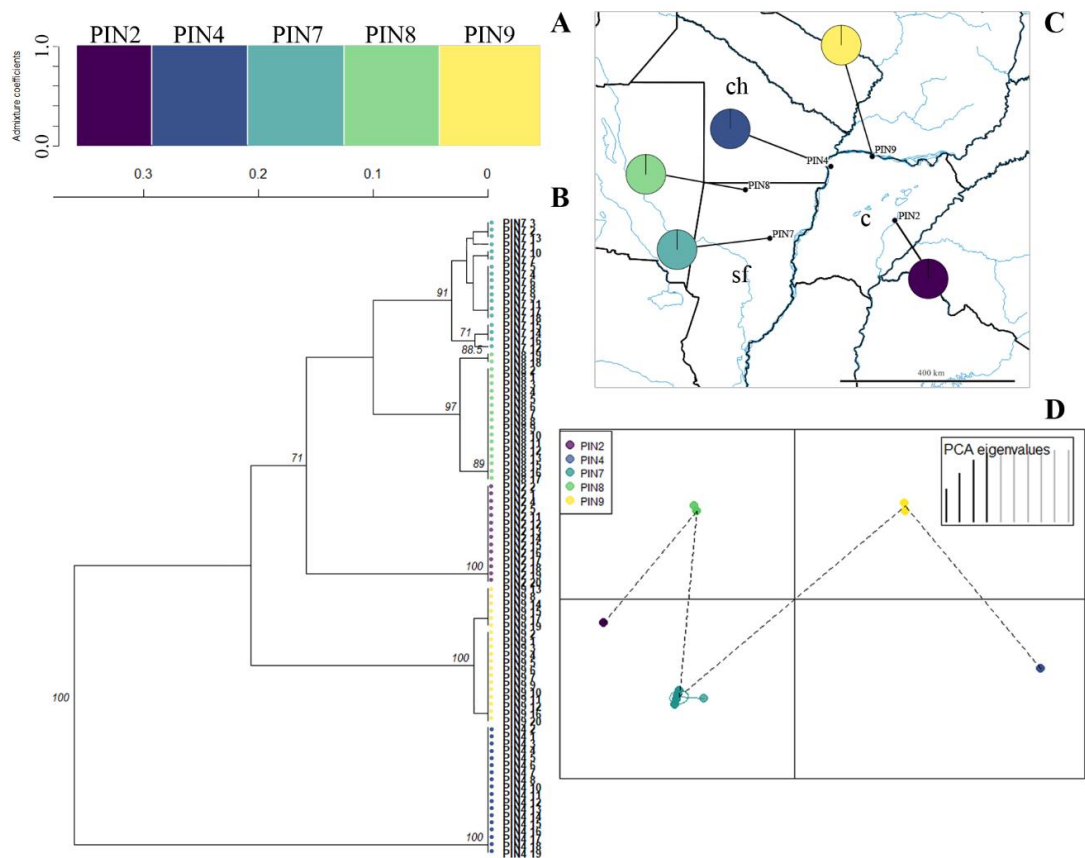


Figure 3. Genetic clusters inferred from ISSR in *Paspalum intermedium* populations. **A.** Bayesian clustering of all individuals (K= 5). Bars represent individuals with the proportion of admixture in different colors. Light grey lines separate populations. **B.** Unrooted UPGMA dendrogram constructed using Nei’s distance with 999 permutations. Only bootstrap values above 70 are shown. Color codes correspond with DAPC legend (see D). **C.** Geographical distribution of *P. intermedium* populations. Color codes in pie charts indicate the genetic composition (admixture coefficients) regarding the Bayesian clustering analysis of each population inferred from ISSRs (see A). **D.** Discriminant analysis of principal coordinates of genetic variation. Lines represent genetic distance of each individual from the centroid of each ellipse, and ellipses represent 95% dispersion of individual genetic variation from each population. Dash lines connect among populations that are more similar. **Abbreviations**, c: Corrientes, ch: Chaco, sf: Santa Fe.

For *P. durifolium*, a single high bootstrap cluster (100%) was recovered, with no preferential clustering among populations (Fig. 4B). The k-means analysis also detected a single cluster for *P. durifolium* (Supplementary Data, Figure S3-B). The structure analysis showed three distinctive clusters for *P. durifolium* (Fig. 4A, C). Populations PD1 and PD2 showed low levels of admixture, while the other three populations showed intermediate levels of admixture and were composed mostly of one genetic cluster (Fig. 4C). According to the DAPC, populations PD1 and PD5 are genetically different over PC1, and populations PD2-3-4 are genetically similar (Fig. 4D).

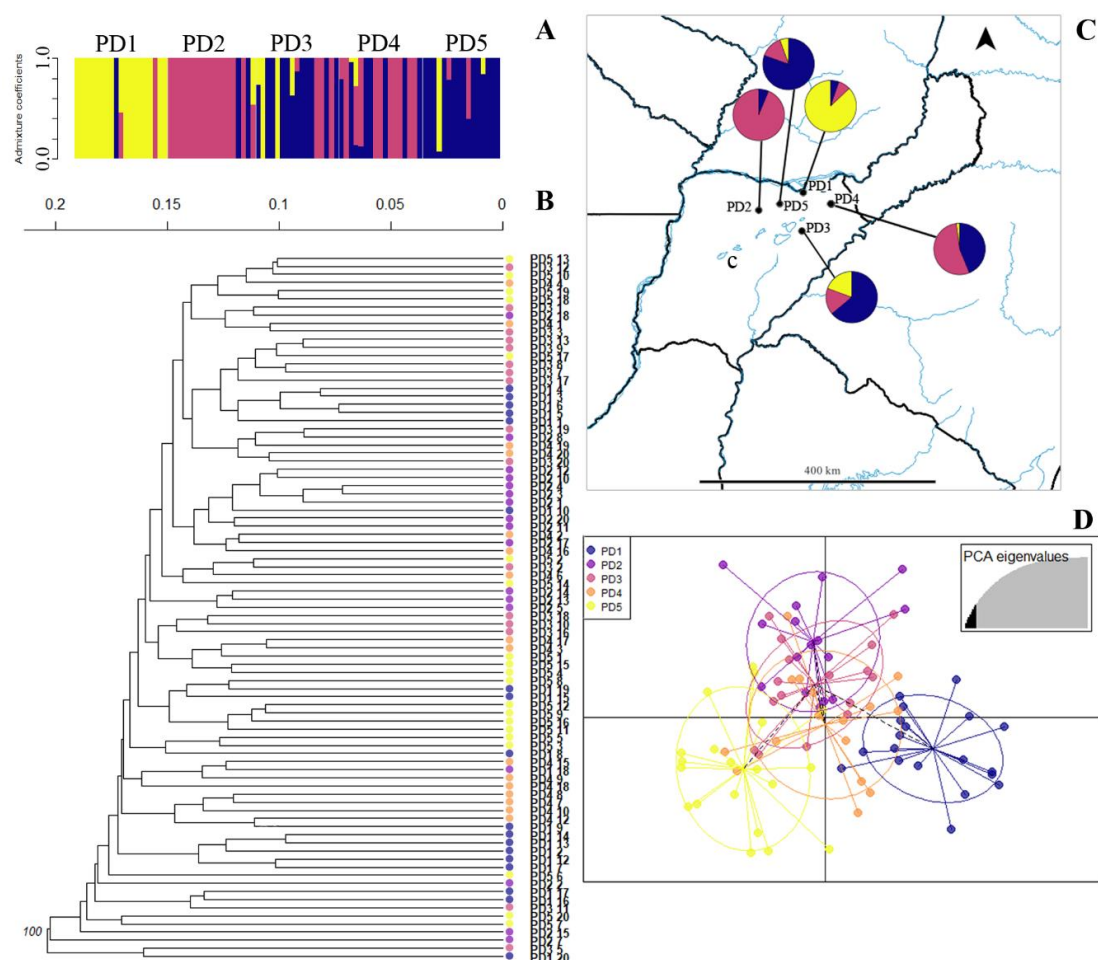


Figure 4. Genetic clusters inferred from ISSR in *Paspalum durifolium* populations. **A.** Bayesian clustering of all individuals (K= 3). Bars represent individuals with the proportion of admixture in different colors. Light grey lines separate populations. **B.** Unrooted UPGMA dendrogram constructed using Nei's distance with 999 permutations. Only bootstrap values above 70 are shown. Color codes correspond with DAPC legend (see D). **C.** Geographical distribution of *P. durifolium* populations. Color codes in pie charts indicate the genetic composition (admixture coefficients) regarding the Bayesian clustering analysis of each population inferred from ISSRs (see A). **D.** Discriminant analysis of principal coordinates of genetic variation. Lines represent genetic distance of each individual from the centroid of each ellipse, and ellipses represent 95% dispersion of individual genetic variation from each population. Dash lines connect among populations that are more similar. **Abbreviations**, c: Corrientes.

There was only one cluster with high bootstrap (100%) for *P. ionanthum*, but individuals showed preferential clustering within their original population (Fig. 5B). The k-means analysis identified four clusters in *P. ionanthum* (Supplementary Data, Figure S3C). The structure analysis in *P. ionanthum* also showed four clusters (Fig. 5A, C) with low levels of admixture except for PI3, which showed intermediate levels of admixture in some individuals (Fig. 5A, C). Populations PI1 and PI5 are genetically different according to the DAPC, which could correspond with their geographical separation as estimated by the Mantel test (Fig. 5D). However geographical distances do not explain the high differentiation among population PI2 and PI3-PI4.

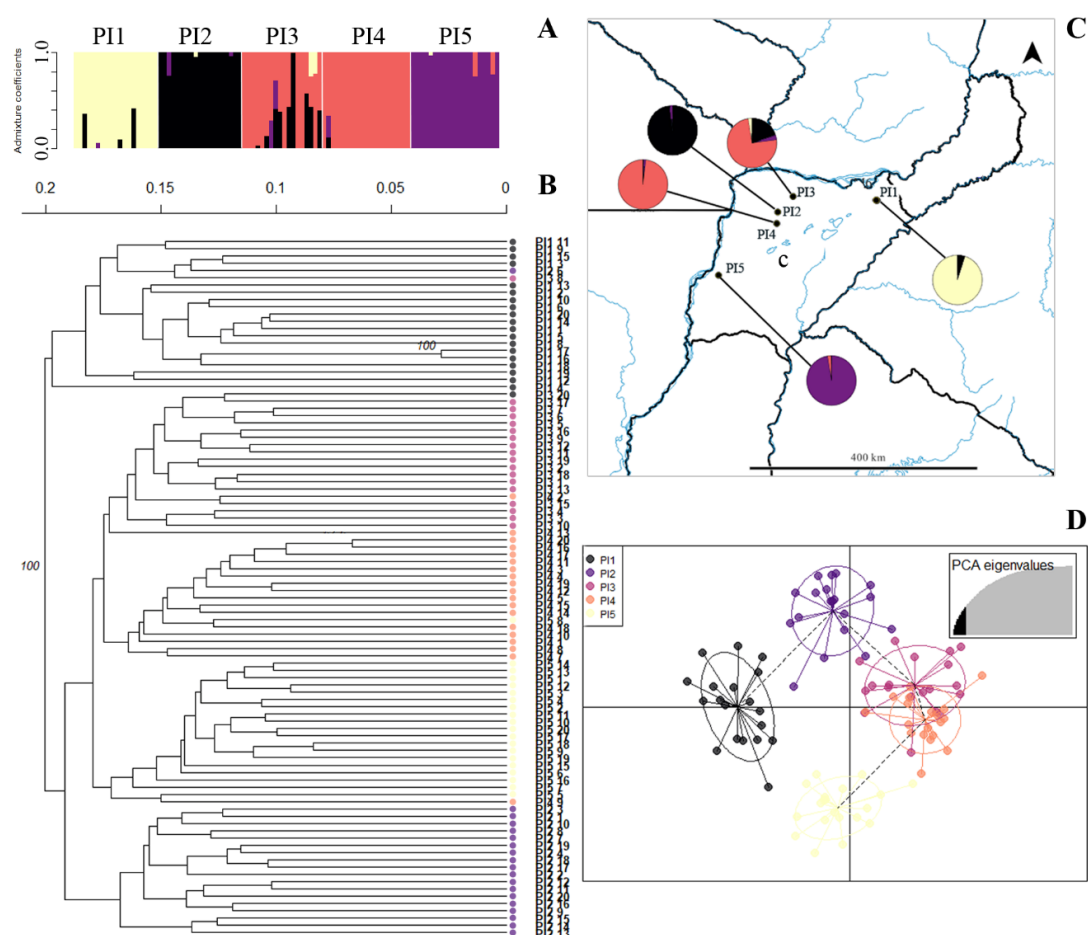


Figure 5. Genetic clusters inferred from ISSR in *Paspalum ionanthum* populations. **A.** Bayesian clustering of all individuals (K= 4). Bars represent individuals with the proportion of admixture in different colors. Light grey lines separate populations. **B.** Unrooted UPGMA dendrogram constructed using Nei's distance with 999 permutations. Only bootstrap values above 70 are shown. Color codes correspond with DAPC legend (see D). **C.** Geographical distribution of *P. ionanthum* populations. Color codes in pie charts indicate the genetic composition (admixture coefficients) regarding the Bayesian clustering analysis of each population inferred from ISSRs (see A). **D.** Discriminant analysis of principal coordinates of genetic variation. Lines represent genetic distance of each individual from the centroid of each ellipse, and ellipses represent 95% dispersion of individual genetic variation from each population. Dash lines connect among populations that are more similar. **Abbreviations:** c: Corrientes.

The UPGMA clustering identified only one group with high bootstrap (100%) for *P. regnellii*, but some individuals from the same local populations were clustered together (Fig. 6B), and the k-means recovered three clusters in this species (Supplementary Data, Figure S3D). The structure analysis of *P. regnellii* showed four clusters, which had high levels of admixture in populations PR1 and PR2, an intermediate proportion of admixture in populations PR3 and PR4, and a low proportion of admixture in PR5 (Fig. 6A and C). Southern population PR5 showed a clear differentiation from the other four populations over PC1 in the DAPC (Fig. 6D).

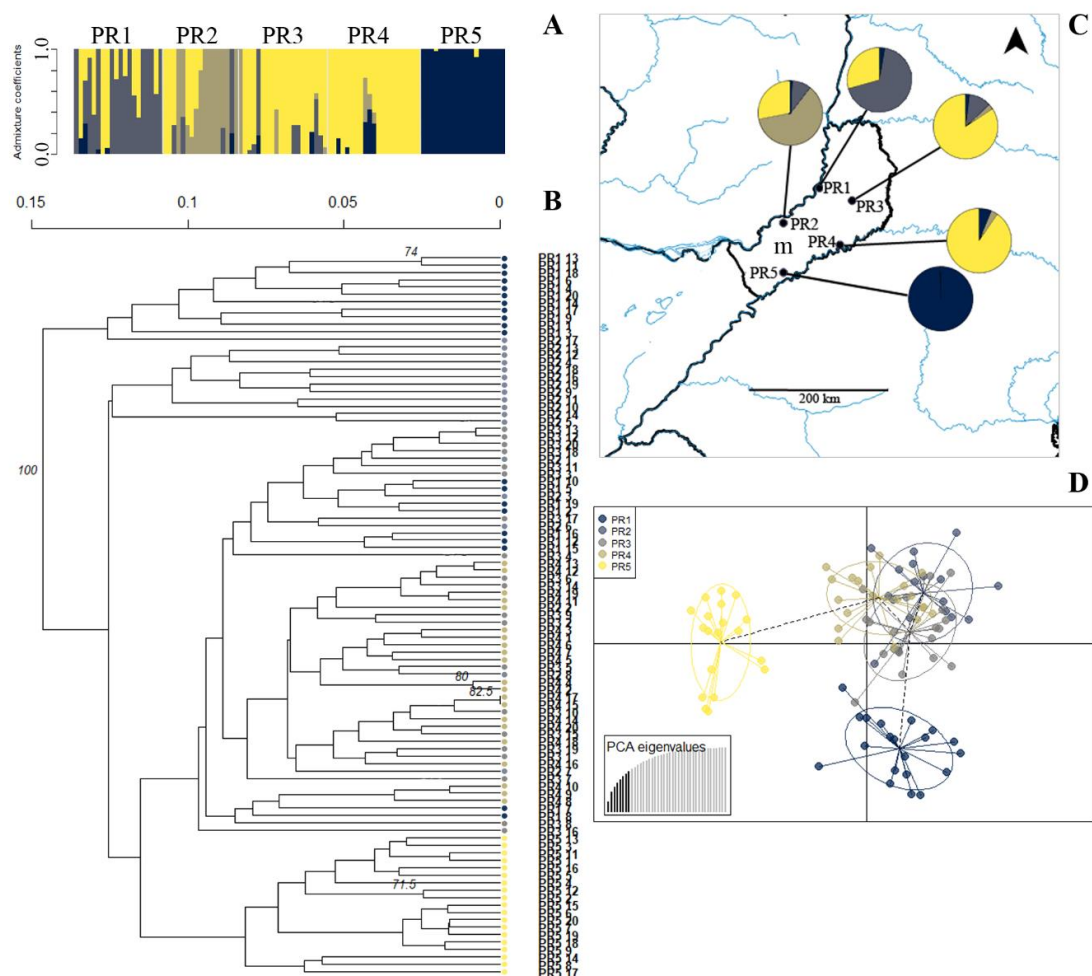


Figure 6. Genetic clusters inferred from ISSR in *Paspalum regnellii* populations. **A.** Bayesian clustering of all individuals (K= 4). Bars represent individuals with the proportion of admixture in different colors. Light grey lines separate populations. **B.** Unrooted UPGMA dendrogram constructed using Nei’s distance with 999 permutations. Only bootstrap values above 70 are shown. Color codes correspond with DAPC legend (see D). **C.** Geographical distribution of *P. regnellii* populations. Color codes in pie charts indicate the genetic composition (admixture coefficients) regarding the Bayesian clustering analysis of each population inferred from ISSRs (see A). **D.** Discriminant analysis of principal coordinates of genetic variation. Lines represent genetic distance of each individual from the centroid of each ellipse, and ellipses represent 95% dispersion of individual genetic variation from each population. Dash lines connect among populations that are more similar. **Abbreviations**, m: Misiones.

As observed in the other three sexual species, only one group with high bootstrap (100%) was observed for *P. urvillei*, with some individuals from the same population clustering together (Fig. 7B), and the k-means analysis detected two clusters in this species (Supplementary Data, Fig. S3E). However, the Bayesian analysis identified four genetic clusters, with PU4 showing a low proportion of admixture, PU2-3-4 showing intermediate admixture coefficients, and PU5 showing a high admixture proportion in some individuals (Fig. 7A, C). In the map, western populations (PU4 and PU5) showed a clear differentiation from the eastern populations (Fig. 7C), and the DAPC showed over PC1, the separation of PU5 from the other four populations (Fig. 7D).

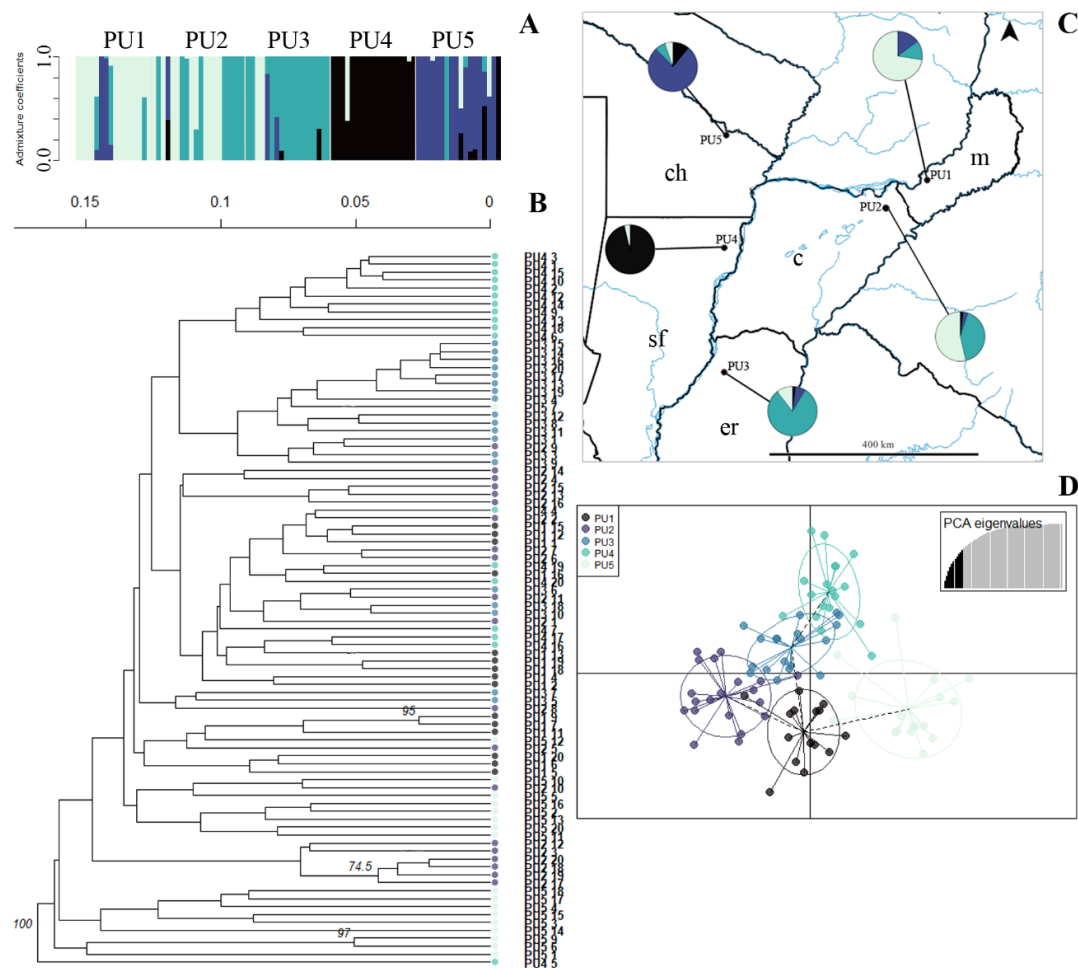


Figure 7. Genetic clusters inferred from ISSR in *Paspalum urvillei* populations. **A.** Bayesian clustering of all individuals (K= 4). Bars represent individuals with the proportion of admixture in different colors. Light grey lines separate populations. **B.** Unrooted UPGMA dendrogram constructed using Nei's distance with 999 permutations. Only bootstrap values above 70 are shown. Color codes correspond with DAPC legend (see D). **C.** Geographical distribution of *P. urvillei* populations. Color codes in pie charts indicate the genetic composition (admixture coefficients) regarding the Bayesian clustering analysis of each population inferred from ISSRs (see A). **D.** Discriminant analysis of principal coordinates of genetic variation. Lines represent genetic distance of each individual from the centroid of each ellipse, and ellipses represent 95% dispersion of individual genetic variation from each population. Dash lines connect among populations that are more similar. **Abbreviations**, c: Corrientes, ch: Chaco, er: Entre Rios, m: Misiones, sf: Santa Fe.

3. Discussion

The reproductive mode is one of the main factors determining the genetic structure of populations [18, 53]. Whether a species is predominantly a selfer, outcrosser, or apomictic significantly influences how genetic diversity is distributed within and between populations. In self-incompatible sexual species, genetic diversity is typically high within populations and low between populations [22-23, 54]. In contrast, self-compatible sexual and apomictic species usually exhibit the highest genetic diversity between populations [22-23, 55]. Thus, understanding the reproductive system is essential for interpreting the genetic diversity, structure, and evolutionary dynamics in allopolyploid species.

3.1. Opposite mating systems led to similar patterns of phenotypic variation but differed in apomictic species

According to Hamrick & Godt [23], obligate allogamous species showed high intra-population variability and low variability between populations. By contrast, species that are self-compatible or reproduce via apomixis exhibit low intrapopulation variability and high interpopulation variability [23, 55]. Our results showed that the four sexual *Paspalum* species included in this study showed similar patterns in the distribution of phenotypic variation, unlike general consensus. Both outcrossing (*P. durifolium* and *P. ionanthum*) and selfing (*P. regnellii* and *P. urvillei*) species had low intrapopulation variation and little to no differentiation among populations. This general low intrapopulation variability in vegetative and reproductive traits is a consequence of cross-pollination in obligated allogamous species [56]. In allogamous populations of *Paspalum indecorum*, cross-pollination reduced interpopulation differentiation and normalized phenotypes within populations [30]. In selfing species, greater differentiation among populations was expected, as usually observed in diploid species [57]. For instance, selfing increases the rate of homozygous genotypes, leading to contrasting phenotypes within populations [23]. These contrasting phenotypes widen the range of phenotypic intrapopulation variation and reduce interpopulation differentiation, as observed in populations of *Paspalum pumilum* [30].

Fragmentation and reduction in population size due to anthropogenic activities have likely contributed to the observed reduction in genetic diversity [58], especially in self-compatible species. Thus, low intrapopulation variation in selfing populations of *P. regnellii* and *P. urvillei* may reflect a recent bottleneck, as they were collected from disturbed environments, such as roadsides or areas impacted by pine plantations. The low levels of phenotypic variation observed may also reflect a low representativeness of the sample sizes analysed for each population (N=14-20) and the constraints in detecting significant differentiation among individuals or populations [59]. However, in *P. urvillei*, high variation in the vegetative period (VP) was observed within populations, which is important for late flowering line selection, a trait usually linked to higher yield and nutritional value in C4 forage plants [60-61]. In wind-pollinated species, variation in flowering time may reflect adaptation to environmental differences, such as photoperiod, temperature, and water availability [62-63]. Hence, this variation could reflect differences in the adaptive responses of plants to their natural environments, as found in common garden experiments [62, 64].

3.2. Local adaptation and phenotypic differentiation among populations

The analysis of interpopulation variance revealed significant differences between populations in several traits. Populations of outcrossers *P. ionanthum* and selfers *P. regnellii* and *P. urvillei* showed low correlations between phenotypic and geographic distances, suggesting that phenotypic variation does not reflect local adaptation to environmental conditions [57, 65-68]. Moreover, phenotypic analyses (PCA) showed overlapping phenotypes among populations and no differentiation in both outcrossing and selfing species. This finding is consistent with the UPGMA analysis, which identified only one phenotypic group for each species, suggesting that wind cross-pollination in outcrossing species (*P. durifolium* and *P. ionanthum*) maintains populations as cohesive phenotypic units [57, 69-70]. In selfing species, increased homozygosity can lead to contrasting phenotypes, thereby widening the range of phenotypic variation within populations and decreasing differentiation between populations [30]. The limited geographic distribution of the collected *P. regnellii* populations in Misiones may also explain the low differentiation, as reduced environmental heterogeneity and potential seed migration between locations could homogenize phenotypic traits [71-72]. In *P. urvillei*, the observed phenotypic homogeneity among populations could reflect an increased rate of cross-pollination, as previously observed in these populations [48]. In species with mixed mating systems, in which reproduction occurs via both selfing and outcrossing [73], pollen dispersal among populations can also contribute to low differentiation.

3.3. Apomixis leads to fixation of adapted phenotypes

Apomixis could lead to the fixation of genotypes that are well adapted to their ecological niches [74]. Combined with genetic drift or natural selection, apomixis leads to clonal populations with low evolutionary flexibility but high local specialization [56]. This characteristic of apomictic species is often reflected in low phenotypic variation within populations and high differentiation between populations [55]. Apomictic populations of *P. intermedium* were morphologically uniform within populations but displayed significant differentiation among populations across all phenotypic traits, clustering into eight distinct morphological groups. In contrast to sexual species, apomictic *P. intermedium* showed narrow phenotypic variation, with only one population showing high internode length (NL) variation. Similar patterns of low variation in quantitative traits have been reported in other apomictic species of *Paspalum* [16, 75-76]. The high interpopulation differentiation in *P. intermedium* indicates that each population retains a portion of the phenotypic variation fixed by apomixis, and the significant geographic correlation suggest adaptation to local environments [43]. New adaptive or neutral mutations that arise during rare recombination events (*i.e.* residual sexuality) may be maintained within apomictic populations, leading to the propagation of different clones throughout the distribution range [3, 6, 77-79]. Morpho-phenological analyses like these highlight the importance of doing prior assessments of multiple populations. These studies help designing germplasm conservation programs that preserve all phenotypic clusters, besides helping in the selection of contrasting phenotypes for future breeding programs.

3.4. Contrasting patterns of intrapopulation variation among selfing and outcrossing species

Selfing species typically have smaller effective population sizes and lower recombination rates, leading to reduced genetic diversity, increased linkage disequilibrium, and higher homozygosity compared with outcrossing species [18, 21]. Numerous studies have shown that selfing species have lower genetic diversity and heterozygosity at the population level compared to outcrossing species [25-26, 28, 30, 72, 80].

Our results for outcrossing species (*P. durifolium* and *P. ionanthum*) showed high molecular and genotypic variation within populations, whereas selfing species (*P. regnellii* and *P. urvillei*) displayed lower molecular diversity and genotypic variation. In *P. regnellii*, almost all populations exhibited low numbers of effective genotypes (G), decreasing genotypic diversity. Similar values have been observed in selfing populations of *Hordeum spontaneum* [81] and *Lupinus mutabilis* [82]. In the diploid self-compatible *Paspalum pumilum*, genotype-sharing individuals were attributed to either ramets or shared allelic conditions in the analyzed loci [30]. Because ISSRs are dominant markers, we cannot assume the allelic condition in the individuals with the same multilocus genotype, but this is more plausible because our experimental design avoided collecting ramets. Differences in molecular and genotypic diversity between *P. regnellii* and *P. urvillei* could be due to varying rates of allogamy between these species [48]. As observed in other autogamous species, even low rates of sporadic allogamy can lead to high intrapopulation variation and polymorphism [82-84]. A mixed pollination system can promote greater diversity compared to strict selfing but is generally lower than in outcrossing species [84-85]. The geographic distribution of populations could also play a role in the intrapopulation genetic diversity. Widespread species usually show significantly higher levels of genetic and genotypic diversity than geographically restricted species [18, 22]. Populations of *P. urvillei* were collected in five different Argentinian provinces (see Fig. 7), while populations of *P. regnellii* were restricted to Misiones. The wide geographical distribution of the collected populations combined with the low rates of allogamy-originated seeds of *P. urvillei* could contribute in the differentiation among these selfing species regarding intrapopulation genetic diversity.

3.5. Differences in genetic structure among mating systems

Pollen migration in selfing plants is rare, thus, the lack of gene flow among populations increases genetic differentiation among populations due to genetic drift and fixation of different loci within genetically isolated populations [86–89]. Therefore, selfing species, compared to outcrossing ones, usually show higher interpopulation differentiation, mainly due to the limited gene flow via pollen [86, 89]. In our study, selfing species showed higher values of R_{HOST} than outcrossing species, but *P. ionanthum* showed a significantly high R_{HOST} , even though it is a strictly allogamous species (gametophytic self-incompatibility system) [48]. The genetic diversity observed with molecular markers was geographically correlated in all sexual species, and some of the identified clusters were isolated by distance. Compared with outcrossing species, selfing species always tend to show a stronger spatial genetic structure, due to a lack of gene flow via pollen [29, 89], however, this was not evidenced in the comparison between selfing and outcrossing *Paspalum* species. This could indicate strong isolation among populations intensified by local adaptation [29, 31, 90]. Topography and climate differences could explain the absence of pollen movement and restricted seed dispersal in these *Paspalum* species. Topographic barriers, such as the Paraná River, Iberá Wetlands, land-use land-cover changes, and climatic differences, like precipitation, could further contribute to genetic differentiation among populations as observed in our DAPC and Bayesian analysis of genetic clusters.

3.6. Population structure in apomictic species showed no admixture

The evolutionary success of an agamic species depends on the possibility of eventually acquiring genetic variability to adapt to long-term environmental fluctuations. However, genetic uniformity is expected within populations of strictly asexual species [10–12]. In *P. intermedium*, low molecular and genotypic variation was observed within populations, with all populations being essentially uniclonal. These findings align with observations in other apomictic *Paspalum* species, where intrapopulation diversity was minimal [16, 42–43]. Low genotypic diversity is typical of apomictic taxa, as the absence of meiotic recombination restricts the generation of new genetic variants. The limited intrapopulation diversity in *P. intermedium* likely reflects the absence of gene flow and fixation of independent mutations within populations [43]. Some populations exhibited private genetic bands, which could be due to random mutations fixed by genetic drift [8, 91–92]. Furthermore, independent genotypes could prove advantageous in each local population, consequently fixing a portion of the whole genetic variation [89, 93–96], as suggested by the uniclonal populations of *P. intermedium*. Additionally, habitats with contrasting environments may favor the fixation of locally adapted genotypes, promoting high interpopulation diversity [13–14, 43, 97–98]. Population structure analyses revealed that apomictic populations exhibited higher differentiation between populations than sexual species, with almost all genetic variation found among populations (85%). These findings align with previous studies on other apomictic taxa, such as *Limonium dufourii* [99] and *Ranunculus carpaticola* [95]. High levels of clonal clustering are common in apomictic species due to limited dispersal ability [11, 13, 100–101], as was observed by the results of our cluster analysis, showing that individuals within local populations of *P. intermedium* clustered together. These results agree with the observation by Karunarathne & Hojsgaard [43] of recurrent autopolyploidization events in genetically distinct diploid populations, consequently fixing dissimilar portions of the diploid's gene pool. Moreover, there is an apparent lack of seed migration among populations of this apomictic species, rendering clusters without admixture unlike sexual species, as observed in the Bayesian analysis and isolation by distance (IBD) tests. Geographical barriers, like the Parana River or Iberá Wetlands, could enhance even more the differentiation among populations hindering seed exchange among populations [31, 33–34, 69].

4. Materials and Methods

4.1. Plant Material

Twenty-five populations of five tetraploid species were collected from northeastern Argentina (Table 5). Ploidy levels and reproductive modes were previously described in Schedler et al. [48]. Sampling was conducted from November to March during the years 2013 to 2015. Natural distribution of each species according to Zuloaga & Morrone [102] was considered for population sampling. The sampling sites covered the distribution range of these species in northeastern Argentina. Within the natural area of each species, only populations with a linear size ≥ 200 m that were monoploid and tetraploid were considered for this work (see Schedler et al. [48]). We selected populations that were at least 50 km apart. A uniform sampling strategy was employed, ensuring even representation within each population by maintaining a distance of 10 m between consecutive individuals. Five populations, with approximately 20 plants per population, were collected for each species. Besides, habitat type was recorded for each species as undisturbed habitats (monotypic or transition grassland), and disturbed habitat (when the species was on road edges or forestation margins) (see Table 5). Only plants that survived two years post-transplant in the field were used in the phenotypic and genetic analysis (N, Table 5). Rhizome cuttings of each plant were cultivated in pots within a greenhouse. Once rooted, the plants were transferred to the experimental field of FCA-UNNE in Corrientes, Argentina. Herbarium vouchers for each sampled population were collected and deposited at the CTES and MNES Herbaria.

Table 5. *Paspalum* species, population identification (pop), number of plants (N), reproductive modes and mating systems (rm) and coordinates of each collection site.

Species	Pop	N	rm	Location and habitat type
<i>P. durifolium</i>	PD1	19	S* ss	C, 27.627033°S, 56.747883°W. U.
	PD2	18	S ss	C, 27.931683°S, 57.5166° W. U.
	PD3	17	S* ss	C, 28.290583° S, 56.770316° W. D.
	PD4	18	S* ss	C, 27.825216° S, 56.2549° W. U.
	PD5	20	S* ss	C, 27.560766° S, 57.153066° W. U.
<i>P. ionanthum</i>	PI1	20	S* ss	C, 27.821083° S, 56.27213° W. D.
	PI2	20	S* ss	C, 28.237416° S, 58.0542° W. U.
	PI3	16	S ss	C, 27.753416° S, 57.76096° W. U.
	PI4	20	S ss	C, 28.033733° S, 58.0362° W. U.
	PI5	20	S ss	C, 29.1651° S, 59.096583° W. D.
<i>P. regnellii</i>	PR1	20	S sf	M, 26.54153° S, 54.7251° W. D.
	PR2	19	S sf	M, 27.052833° S, 55.25066° W. U.
	PR3	20	S sf	M, 26.7271° S, 54.247416° W. D.
	PR4	20	S sf	M, 27.374017° S, 54.42505° W. U.
	PR5	19	S sf	M, 27.77713° S, 55.24996° W. U.
<i>P. urvillei</i>	PU1	17	S sf	M, 27.276883° S, 55.46225° W. D.
	PU2	20	S sf	C, 27.821083° S, 56.272133° W. D.
	PU3	20	S sf	ER, 31.02038° S, 59.420366° W. D.
	PU4	20	S sf	SF, 28.59555° S, 59.41675° W. D.
	PU5	18	S sf	Ch, 26.40195° S, 59.37876° W. D.
<i>P. intermedium</i>	PIN2	14	A ap ps sf	C, 28.87166° S, 57.26405° W. D.
	PIN4	18	A ap ps sf	C, 27.615016° S, 58.75398° W. U.
	PIN7	18	A ap ps sf	SF, 29.29074° S, 60.17697° W. U.
	PIN8	18	A ap ps sf	SF, 28.15866° S, 60.74791° W. D.
	PIN9	19	A ap ps sf	C, 27.38394° S, 57.78926° W. U.

‡ data from Schedler et al. [48] except for *P. intermedium*. **Abbreviations** S: Sexuality; ss: self-sterile; sf: self-fertile; A: Apomixis; ap: apospory; ps: pseudogamy. *A low proportion of aposporic embryo sac were observed in these populations, but none apomictic seeds [48] Argentina's provinces: C:

Corrientes, Ch: Chaco, ER: Entre Rios, M: Misiones, SF: Santa Fe. **Habitat types: D: disturbed habitats, U: undisturbed habitats.**

4.2. Morpho phenological traits

Morphological variation was assessed using 13 phenotypic traits, while phenological variation was estimated using two traits. Measurements were made during the flowering peak of each species, and all populations were cultivated under the same environment. The traits measured included plant height with (H; cm) and without flowering culms (Hw; cm), 2nd leaf blade length (LL; cm) and width (LW; cm), 2nd leaf sheath length (ShL; cm), 2nd internode length (NL; cm), inflorescence length (IL; cm), basal raceme length (BRL; cm), number of spikelets in the basal raceme (EBR), raceme number (NR), flowering culms length with inflorescences (VL; cm), spikelet length (SL; mm) and width (SW; mm). The phenological traits measured were the extension of the vegetative period (VP; days) and the flowering period (FP; days).

4.3. Statistical analyses of morpho phenological traits

To assess the variation within populations, we calculated the mean, standard deviation (s.d.), and coefficient of variation (C.V.) for each trait within each population (Table 2; Supplementary Data, Table S1) using the *rstatix* package in R [103]. We established an upper limit of 30% C.V. to define homogeneity within populations (indicating low dispersion around the mean). Values exceeding 30% were considered indicative of heterogeneity among individuals within the same population (Supplementary Data, Table S1).

We evaluated variability within and between populations using MANOVA with Pillai's statistic, testing univariate and multivariate assumptions for normal distribution using the *rstatix* package in R. Non-normal variables were transformed using a logarithmic function. Variables that remained non-normal after transformation were excluded from the MANOVA. A Pearson's correlation test was conducted to assess the relationships between variables, and only those with a pairwise Pearson coefficient >-0.9 and <0.9 were considered (Supplementary Data, Figure S1), as MANOVA is sensitive to correlated variables over 0.9 or -0.9. We used a Kruskal-Wallis test to identify which morpho-phenological traits showed significant differences among populations (Supplementary Data, Table S2). A post-hoc test using Games-Howell's statistic with Tukey's p-adjustment was performed to identify specific population differences (Supplementary Data, Table S3).

To identify clustering patterns, we constructed a phenogram using Euclidean distances via the UPGMA method. We also applied k-means clustering to determine the optimal number of clusters, using a K range from 1 to 10 with 1000 bootstrapping iterations, the *factoextra* and *stats* packages in R. The significance of each morpho-phenological trait in the discrimination of populations/individuals was determined using eigenvalues from a PCA (Supplementary Data, Figure S2). The correlation between phenotypic data, geographical distribution, and genetic data was analyzed using the Mantel test with 1000 permutations from the *vegan* package in R. Graphical representations were generated using the *ggbiplot*, *ggplot2*, and *ggfortify* packages in R.

4.4. DNA isolation and ISSR protocol

Total genomic DNA was extracted from fresh young leaves of plants cultivated in pots, following the protocol described by Brugnoli et al. [16]. DNA quality was confirmed by assessing DNA integrity and verifying the absence of RNA contamination. Each DNA sample was standardized to 20 ng μL^{-1} for use in PCR amplification.

Sixteen ISSR primers were used for PCR amplification. The primers analyzed were: (AC)8T, (AC)8G, (AG)8T, (AG)8C, (AG)8GC, (AGAC)4GC, (ATG)5GA, (CA)8G, CAG(CA)7, (CT)8G, (CTC)6AC, (GA)8C, (GA)8T, (GA)8TC, (GAG)7AC, (TC)8A. PCR reactions were conducted in 25 μL final volume containing 20 ng of template DNA, 2.5 μL 10X reaction buffer, 1.5 μL of 25 mM MgCl_2 , 1.5 μL of 5 μM primer, 1.25 μL of 2 mM dNTPs,

0.2 µl of Taq DNA polymerase (5 U µl⁻¹), and H₂O. DNA amplifications were performed in a T100 thermal cycler (Bio-Rad, US) using the following thermal cycle: an initial denaturing step at 94 °C for 5 min; 40 cycles of 94 °C for 1 min, primer annealing at a specific temperature for 45 s (Supplementary Data, Table S4), 72 °C for 2 min, and a final extension at 72 °C for 5 min. PCR products were separated by electrophoresis in 2% agarose gels in 1X TAE buffer (1M Tris, 0.5M EDTA, acetic acid, pH 8), at 60 V for 3.5 h, and stained with ethidium bromide (1 µg ml⁻¹). The molecular profiles were visualized under UV light, photographed, and analyzed with the GelDoc-It Imaging System.

4.5. Molecular diversity within populations

Molecular diversity within each population was calculated using the total number of bands (TB), the number of locally common bands occurring in ≤ 50% populations (CB), the percentage of polymorphic loci (PL%), and the number of private bands (PB) per population, using GenAlEx 6 [104]. Genotypic variation was assessed using the number of effective genotypes (G), Nei's diversity index (D), evenness index (E), and Shannon diversity index (H) under an independent mutation model with five mutational steps (M = 5) using GENOTYPE/GENODIVE [see 105].

4.6. Differentiation between populations

Population structure for each species was estimated using AMOVA with significance testing based on 999 permutations, using the *adeigenet*, *poppr*, and *mmmod* packages in R [106–108]. We calculated genetic divergence using the R_{HST} value, a multiallelic analog of F_{ST} for dominant markers and polyploids, and estimated gene flow using pairwise- F_{ST} with Nei's distance among populations via the *hierfstat* package in R [109].

Pairwise genetic distances among populations were estimated using Nei's Distance with 999 permutations using the *poppr* package in R. A clustering analysis was performed, and a UPGMA dendrogram was constructed using the package *poppr* in R. The effective number of clusters was analyzed using k-means clustering with the *adeigenet* package in R (Supplementary Data, Figure S3). For the structure analysis, the *LEA* package was employed [110], which estimates admixture coefficients using sparse non-negative matrix factorization algorithms and provides STRUCTURE-like outputs. We used a loci-prior model with 20,000 iterations for a K range of 1 to 10 ($K = [1–10]$), with 50 replicates each (Supplementary Data, Figure S4). Identified clusters were then plotted into a map using the packages *LEA* and *mapplots* in R. Discriminant analysis of principal coordinates (DAPC) was conducted to visualize the distribution of genetic diversity between populations using the *adeigenet* and *ade4* packages in R [111]. A Mantel test with 999 permutations was used to determine the geographical isolation among genetic clusters using the *vegan* package, and significant isolated clusters were identified using the *mpmcorrelogram* package in R [112] (Supplementary Data, Figure S5).

5. Conclusions

Our phenotypic analyses of four sexual *Paspalum* species, two outcrossing (*P. duri-folium* and *P. ionanthum*) and two selfing (*P. regnellii* and *P. urvillei*), revealed low intra-population phenotypic variability and no significant differentiation among populations for both groups. In selfing species, homozygosity increases, leading to reduced intrapopulation variation and greater differentiation among populations. Conversely, allogamous species benefit from cross-pollination, maintaining phenotypic uniformity across populations. Despite expectations of greater differentiation among selfing species, overlapping phenotypic ranges were observed in both mating systems, suggesting that gene flow via pollen or seeds plays a significant role in these populations. In contrast, the apomictic species *P. intermedium* showed low intrapopulation variation but high differentiation between populations, indicating a fixation of well-adapted genotypes through genetic drift and local adaptation, leading to distinct morphological clustering.

Outcrossing species like *P. durifolium* and *P. ionanthum* exhibited high molecular and genotypic variation within populations while selfing species such as *P. regnellii* and *P. urvillei* displayed comparatively lower genetic diversity. Differences in polymorphism and genotypic diversity among selfing species could be attributed to varying rates of allogamy and geographic distribution. Selfing species generally exhibit greater population differentiation due to limited pollen migration, increasing genetic drift and the fixation of alleles in isolated populations. Indeed, higher values of genetic differentiation were observed in selfing species compared to outcrossing ones. However, *P. ionanthum*, despite being strictly outcrossing, exhibited notable genetic differentiation among populations. This highlights the role of geographic range in genetic diversity, as isolation by distance was evident in both selfing and outcrossing species, indicating the importance of pollen and seed dispersal in shaping population structure.

In apomictic populations of *P. intermedium*, low molecular and genotypic variation led to mostly uniclinal populations with minimal polymorphism compared to the selfing and outcrossing species. This lack of gene flow and limited sexual reproduction resulted in a highly structured genetic landscape, with significant differentiation among populations. The presence of distinct dominant genotypes across populations suggested local adaptation to specific environmental conditions. Apomictic species like *P. intermedium* experience high population differentiation and limited genetic exchange, leading to a strong spatial genetic structure. These findings underscore the complexities of genetic diversity in apomictic species, emphasizing the influence of local adaptation and restricted gene flow.

While these findings provide valuable insights, future studies with larger sample sizes, broader geographical scopes, and additional molecular markers are necessary to validate these patterns and better understand the interplay between reproductive modes and genetic diversity in polyploid species of *Paspalum*. Furthermore, understanding the interplay between reproductive modes and genetic diversity can reduce time and money costs in conservation and breeding strategies for *Paspalum* spp.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Coefficients of Variation of each phenotypic trait (in percentages); Table S2: Kruskal-Wallis Test; Table S3: Games-Howell post hoc test. Only traits that showed differentiation among populations are presented; Table S4: Annealing temperature of ISSR primers; Figure S1: Correlation among phenotypic variables in each species; Figure S2: Contribution of each phenotypic variable to PCA; Figure S3: The effective number of cluster obtained using a k-means approach with the package *adeget* in R; Figure S4: The effective number of cluster obtained using for the STRUCTURE-like approach with the package *LEA* in R; Figure S5: Identification of cluster isolated by distance using the *mpmcorrelogram* package in R.

Author Contributions: Conceptualization, A.V.R., M.S., E.J.M. and A.I.H.; data curation, software, validation, visualization and formal analysis, A.V.R. and M.S.; methodology, A.V.R., M.S., A.L.Z. and E.A.B.; writing—original draft preparation, A.V.R.; writing—review and editing, A.V.R., A.I.H., E.J.M., M.S., E.A.B., A.L.Z., D.H.H. and C.A.A.; supervision, E.J.M. and A.I.H.; resources, D.H.H., C.A.A., E.J.M. and A.I.H.; project administration, D.H.H., E.J.M. and A.I.H.; funding acquisition, D.H.H., E.J.M. and A.I.H. All authors have read and agreed to the published version of the manuscript. A.V.R. and M.S. contributed equally.

Funding: This research was financed by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 2012-2014-112-201101-00469), Agencia Nacional de Promoción Científica y Técnica (ANPCYT, PICT-2012-261; PICT 2017-4203 RAICES; PICT 2020 SERIE A 3783), ANPCYT-UNNE (PICTO-OTNA 2011-080), Bilateral Cooperation Programme Level II (MINCYT-CONICET-DFG, RD 20150202-0167), and Universidad Nacional del Nordeste (UNNE, PI A003-2011). These data were part of the doctoral research of M.S. under the direction of E.J.M. and A.I.H. M.S. received a fellowship from ANPCYT and CONICET. E.J.M., A.I.H., E.A.B., A.L.Z., C.A.A., and A.V.R. belong to the scientific staff in CONICET.

Data Availability Statement: All the data presented in this study are available in the article, in Supplementary Files and binary (ISSR) and phenotypic arrays are uploaded in XXX.

Acknowledgments: We are grateful to Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste (FCA-UNNE, Argentina) for the given space in the experimental field. We would also like to thank members of Genética y Mejoramiento de Especies Forrajeras group at Instituto de Botánica del Nordeste and students from FCA-UNNE for their help with the maintenance of live plant populations in the experimental field. We also thank the anonymous referees that led to valuable improvements in the text.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Acuña, C.A.; Martínez, E.J.; Zilli, A.L.; Brugnoli, E.A.; Espinoza, F.; Marcón, F.; Urbani, M.H.; Quarín, C.L. Reproductive systems in *Paspalum*: Relevance for germplasm collection and conservation, breeding techniques, and adoption of released cultivars. *Front. Plant Sci.* **2019**, *10*, 1377. <https://doi.org/10.3389/fpls.2019.01377>
2. Ortiz, J.P.A.; Pupilli, F.; Acuña, C.A.; Leblanc, O.; Pessino, S.C. How to become an apomixis model: The multi-faceted case of *Paspalum*. *Genes* **2020**, *11*(9), 974. <https://doi.org/10.3390/genes11090974>
3. Asker, S.E.; Jerling, L. *Apomixis in plants*. CRC Press, Boca Raton, **1992**.
4. Holsinger, K.E. Reproductive systems and evolution in vascular plants. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 7037-7042. <https://doi.org/10.1073/pnas.97.13.7037>
5. Wright, S. The genetic structure of populations. *Nature* **1950**, *166*, 247-250. <https://doi.org/10.1038/166247a0>
6. Müller, H.J. The relation of recombination to mutational advance. *Mutat. Res.* **1964**, *1*(1), 2-9. [https://doi.org/10.1016/0027-5107\(64\)90047-8](https://doi.org/10.1016/0027-5107(64)90047-8)
7. Nogler, G.A. Gametophytic apomixis. In *Embryology of Angiosperms*, Johri, B.M. Ed. Springer-Verlag, Berlin, **1984**, pp. 475-518. https://doi.org/10.1007/978-3-642-69302-1_10
8. Hörandl, E.; Paun, O. Patterns and sources of genetic diversity in apomictic plants: implications for evolutionary potentials. In *Apomixis: Evolutions, mechanisms and perspectives*, Hörandl, E.; Grossniklaus, U.; van Dijk, P.J.; Sharbel, T.F. Eds., ARG Gantner Verlag, Rugell, Liechtenstein, Germany, **2007**, pp. 170-194.
9. Lynch, M. Destabilizing Hybridization, General-Purpose Genotypes and Geographic Parthenogenesis. *Q. Rev. Biol.* **1984**, *59*, 257-290. <https://doi.org/10.1086/413902>
10. Menken, S.B.J.; Morita, T. Uniclinal Population Structure in the Pentaploid Obligate Agamosperm *Taraxacum albidum* Dahlst. *Plant Species Biol.* **1989**, *4*, 29-36. <https://doi.org/10.1111/j.1442-1984.1989.tb00045.x>
11. Štorchová, H.; Chrtěk, J.; Bartish, I.V.; Tetera, M.; Kirschner, J.; Štěpánek, J. Genetic variation in agamospermous taxa of *Hieracium* sect. *Alpina* (Compositae) in the Tatry Mts. (Slovakia). *Plant Syst. Evol.* **2002**, *235*, 1-17. <https://doi.org/10.1007/s00606-002-0219-8>
12. Niissalo, M.A.; Leong-Škorničková, J.; Šída, O.; Khew, G.S. Population genomics reveal apomixis in a novel system: uniclinal female populations dominate the tropical forest herb family, Hanguanaceae (Commelinales). *ApB Plants* **2020**, *12*(6), plaa053. <https://doi.org/10.1093/aobpla/plaa053>
13. Houliston, G.J.; Chapman, H.M. Reproductive strategy and population variability in the facultative apomict *Hieracium pilosella* (Asteraceae). *Am. J. Bot.* **2004**, *91*, 37-44. <https://doi.org/10.3732/ajb.91.1.37>
14. Lo, E.Y.Y.; Stefanovic, S.; Dickinson, T.A. Population genetic structure of diploid sexual and polyploid apomictic hawthorns (*Crataegus*; Rosaceae) in the Pacific Northwest. *Mol. Ecol.* **2009**, *18*, 1145-1160. <https://doi.org/10.1111/j.1365-294X.2009.04091.x>
15. Majeský, L.; Vašut, R.J.; Kitner, M.; Trávníček, B. The pattern of genetic variability in apomictic clones of *Taraxacum officinale* indicates the alternation of asexual and sexual histories of apomicts. *PLoS One* **2012**, *7*(8), e41868. <https://doi.org/10.1371/journal.pone.0041868>
16. Brugnoli, E.A.; Urbani, M.H.; Quarín, C.L.; Zilli, A.L.; Martínez, E.J.; Acuña, C.A. Diversity in apomictic populations of *Paspalum simplex* Morong. *Crop Sci.* **2014**, *54*, 1656-1664. <https://doi.org/10.2135/cropsci2013.11.0780>
17. Toenniessen, G.H. Feeding the world in the 21st century: Plant breeding, biotechnology, and the potential role of apomixis. In *The Flowering of Apomixis: From Mechanisms to Genetic Engineering*; Savidan, Y.; Carman, J.G.; Dresselhaus, T.; Eds., CIMMYT: Mexico DF, Mexico; IRD: Marseille, France; European Commission OC VI (FAIR): Brussels, Belgium, **2001**, pp. 1-7.
18. Loveless, M.D.; Hamrick, J.L. Ecological determinants of genetic structure in plant populations. *Annu. Rev. Ecol. Syst.* **1984**, *15*, 65-95. <https://doi.org/10.1146/annurev.es.15.110184.000433>
19. Foxe, J.P.; Slotte, T.; Stahl, E.A.; Neuffer, B.; Hurka, H.; Wrieth, S.I. Recent speciation associated with the evolution of selfing in *Capsella*. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 5241-5245. <https://doi.org/10.1073/pnas.0807679106>
20. Nordborg, M. Linkage disequilibrium, gene trees and selfing: an ancestral recombination graph with partial self-fertilization. *Genetics* **2000**, *154*, 923-929. <https://doi.org/10.1093/genetics/154.2.923>
21. Glémin, S.; Bazin, E.; Charlesworth, D. Impact of mating systems on patterns of sequence polymorphism in flowering plants. *Proc. Biol. Sci.* **2006**, *273*, 3011-3019. <https://doi.org/10.1098/rspb.2006.3657>
22. Hamrick, J.L.; Godt, M.J. Allozyme diversity in plant species. In *Plant population genetics, breeding, and genetic resources*, Brown, A.H.D.; Clegg, M.T.; Kahler, A.L.; Weir, B.S. Eds. Sinauer Associates Inc Publishers, Sunderland, Massachusetts, **1989**. 43-63.
23. Hamrick, J.L.; Godt, M.J.W. Conservation genetics of endemic plant species. In *Conservation genetics. Case histories from nature*, Avise, J.C.; Hamrick J.L. Eds. Chapman and Hall, New York, **1996**, pp. 281-304. https://doi.org/10.1007/978-1-4757-2504-9_9
24. Nybom, H.; Bartish, I.V. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspec. Plant Ecol. Evol. Syst.* **2000**, *3*, 93-114. <https://doi.org/10.1078/1433-8319-00006>
25. Eschmann-Grupe, G.; Neuffer, B.; Hurka, H. Extent and structure of genetic variation in two colonizing *Diplotaxis* species (Brassicaceae) with contrasting breeding systems. *Plant Syst. Evol.* **2004**, *244*, 31-43. <https://doi.org/10.1007/s00606-003-0077-z>
26. Nybom, H. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Mol. Ecol.* **2004**, *13*, 1143-1155. <https://doi.org/10.1111/j.1365-294X.2004.02141.x>
27. Koelling, V.A.; Hamrick, J.L.; Mauricio, R. Genetic diversity and structure in two species of *Leavenworthia* with self-incompatible and self-compatible populations. *Heredity* **2010**, *106*, 310-318. <https://doi.org/10.1038/hdy.2010.59>

28. Reisch, C.; Bernhardt-Römermann, M. The impact of study design and life history traits on genetic variation of plants determined with AFLPs. *Plant Ecol.* **2014**, *215*, 1493–1511. <https://doi.org/10.1007/s11258-014-0409-9>
29. Huang, R.; Chu, Q.H.; Lu, G.H.; Wang, Y.Q. Comparative studies on population genetic structure of two closely related selfing and outcrossing *Zingiber* species in Hainan Island. *Sci. Rep.* **2019**, *9*, 1–11. <https://doi.org/10.1038/s41598-019-54526-y>
30. Reutemann, A.V.; Honfi, A.I.; Karunarathne, P.; Eckers, F.; Hojsgaard, D.H.; Martínez, E.J. Comparative analysis of molecular and morphological diversity in two diploid *Paspalum* species (Poaceae) with contrasting mating systems. *Plant Reprod.* **2024**, *37*(1), 15–32. <https://doi.org/10.1007/s00497-023-00478-3>
31. Temunović, M.; Franjić, J.; Satović, Z.; Grgurev, M.; Frascaria-Lacoste, N.; Fernández-Majarrés, J.F. Environmental heterogeneity explains the genetic structure of continental and mediterranean populations of *Fraxinus angustifolia* Vahl. *Plos One* **2012**, *7*(8), e42764. <https://doi.org/10.1371/journal.pone.0042764>
32. Xu, B.; Sun, G.; Wang, X.; Lu, J.; Wang, I.J.; Wang, Z. Population genetic structure is shaped by historical, geographic, and environmental factors in the leguminous shrub *Caragana microphylla* on the Inner Mongolia Plateau of China. *BMC Plant Biol.* **2017**, *17*, 200. <https://doi.org/10.1186/s12870-017-1147-7>
33. Wang, I.J.; Bradburd, G.S. Isolation by environment. *Mol. Ecol.* **2014**, *23*, 5649–5662. <https://doi.org/10.1111/mec.12938>
34. Sexton, J.P.; Hangartner, S.B.; Hoffmann, A.A. Genetic isolation by environment or distance: which pattern of gene flow is most common? *Evolution* **2014**, *68*, 1–15. <https://doi.org/10.1111/evo.12258>
35. Mijangos, J.L.; Pacioni, C.; Spencer, P.B.; Craig, M.D. Contribution of genetics to ecological restoration. *Mol. Ecol.* **2015**, *24*(1), 22–37. <https://doi.org/10.1111/mec.12995>
36. Allen, W.J.; Bufford, J.L.; Barnes, A.D.; Barratt, B.I.; Deslippe, J.R.; Dickie, I.A.; Goldson, S.L.; Howlett, B.G.; Hulme, P.E.; Lavrel, S.; O'Brien, S.A.; Waller, L.P.; Tylanakis, J.M. A network perspective for sustainable agroecosystems. *Trends Plant Sci.* **2022**, *27*(8), 769–780. <https://doi.org/10.1016/j.tplants.2022.04.002>
37. Quarin, C.L. The nature of apomixis and its origin in Panicoid grasses. *Apomixis Newsl.* **1992**, *5*, 8–15.
38. Zuloaga, F. O.; Morrone, O.; Davidse, G.; Filgueiras, T. S.; Peterson, P. M.; Soreng, R. J.; Judziewicz, E. J. *Catalogue of New World grasses (Poaceae): III. subfamilies Panicoideae, Aristidoideae, Arundinoideae, and Danthonioideae. Contr US Natl Herb*, **2003**, *46*, 1–662.
39. Ortiz, J.P.A.; Quarin, C.L.; Pessino, S.C.; Acuna, C.A.; Martínez, E.J.; Espinoza, F.; Hojsgaard, D.H.; Sartor, M.E.; Cáceres, M.E.; Pupilli, F. Harnessing apomictic reproduction in grasses: what we have learned from *Paspalum*. *Ann. Bot.* **2013**, *112*(5), 767–787. <https://doi.org/10.1093/aob/mct152>
40. D'Aurelio, L.D.; Espinoza, F.; Quarin, C.L.; Pessino, S.C. Genetic diversity in sexual diploid and apomictic tetraploid populations of *Paspalum notatum* situated in sympatry or allopatry. *Plant Syst. Evol.* **2004**, *244*, 189–199. <https://doi.org/10.1007/s00606-003-0070-6>
41. Brugnoli, E.A.; Urbani, M.H.; Quarin, C.L.; Martínez, E.J.; Acuña, C.A. Diversity in diploid, tetraploid, and mixed diploid-tetraploid populations of *Paspalum simplex*. *Crop Sci.* **2013**, *53*(4), 1509–1516. <https://doi.org/10.2135/cropsci2012.08.0497>
42. Sartor, M.E.; Rebozzio, R.N.; Quarin, C.L.; Espinoza, F. Patterns of genetic diversity in natural populations of *Paspalum* agamic complexes. *Plant Syst. Evol.* **2013**, *299*, 1295–1306. <https://doi.org/10.1007/s00606-013-0797-7>
43. Karunarathne, P.; Hojsgaard, D. Single independent autopolyploidization events from distinct diploid gene pools and residual sexuality support range expansion of locally adapted tetraploid genotypes in a South American grass. *Front. Genet.* **2021**, *12*, 736088. <https://doi.org/10.3389/fgene.2021.736088>
44. Burson, B.L.; Bennett, H.W. Cytology and reproduction of three *Paspalum* species. *J. Hered.* **1970**, *61*, 129–132. <https://doi.org/10.1093/oxfordjournals.jhered.a108056>
45. Quarin, C.L. A tetraploid cytotype of *Paspalum durifolium*: Cytology, reproductive behavior and its relationship to diploid *P. intermedium*. *Hereditas* **1994**, *121*, 115–118. <https://doi.org/10.1111/j.1601-5223.1994.00115.x>
46. Burson, B.L. Cytology of *Paspalum chacoense* and *P. durifolium* and their relationship to *P. dilatatum*. *Bot. Gaz.* **1985**, *146*, 124–129. <https://doi.org/10.1086/337506>
47. Martínez, E.J.; Quarin, C.L.; Hayward, M.D. Genetic control of apospory in apomictic *Paspalum* species. *Cytologia* **1999**, *64*, 425–433. <https://doi.org/10.1508/cytologia.64.425>
48. Schedler, M.; Reutemann, A.V.; Hojsgaard, D.H.; Zilli, A.L.; Brugnoli, E.A.; Galdeano, F.; Acuña, C.A.; Honfi, A.I.; Martínez, E.J. Alternative evolutionary pathways in *Paspalum* involving allotetraploidy, sexuality, and varied mating systems. *Genes* **2023**, *14*(6), 1137. <https://doi.org/10.3390/genes14061137>
49. Norrmann, G.A. Citología y modo de reproducción en dos especies de *Paspalum* (Gramineae). *Bonplandia* **1981**, *17*, 149–158
50. Brown, W.V.; Emery, H.P. Apomixis in the Gramineae: Panicoideae. *Am. J. Bot.* **1958**, *45*, 253–263. <https://doi.org/10.1002/j.1537-2197.1958.tb13125.x>
51. Bashaw, E.C.; Hovin, A.W.; Holt, E.C. Apomixis, its evolutionary significance and utilization in plant breeding. In *Proceedings of the 11th International Grasslands Congress*, Norman, M.J.T., Ed. Surfers Paradise: Australia University of Queensland Press, **1970**, pp 245–248.
52. Karunarathne, P.; Reutemann, A.V.; Schedler, M.; Glücksberg, A.; Martínez, E.J.; Honfi, A.I.; Hojsgaard, D.H. Sexual modulation in a polyploid grass: a reproductive contest between environmentally inducible sexual and genetically dominant apomictic pathways. *Sci. Rep.* **2020**, *10*, 1–14. <https://doi.org/10.1038/s41598-020-64982-6>
53. Jain, S.K. Population structure and the effects of breeding system. In *Crop genetic re-sources for today and tomorrow*, Frankel, O.H.; Hawkes, J.G. Eds., Cambridge University Press, Cambridge, **1975**, pp 15–36.

54. Hamrick, J.L.; Nason, J.D. Consequences of dispersal in plants. In *Population dynamics in ecological space and time*, Rhodes, O.E.; Chesser, R.K.; Smith, M.H. Eds., The University of Chicago Press, Chicago, **1996**, pp 203-235. 811
55. Ellstrand, N.C.; Roose, M.L. Patterns of genotypic diversity in clonal plant species. *Am. J. Bot.* **1987**, *74*(1), 123-131. 812
- <https://doi.org/10.1002/j.1537-2197.1987.tb08586.x> 813
56. Stebbins, G.L. *Variation and Evolution in plants*. Columbia University Press, New York. **1950**. <https://doi.org/10.7312/steb94536> 814
57. Charlesworth, D.; Charlesworth, B. Quantitative genetics in plants: the effect of the breeding system on genetic variability. *Evolution* **1995**, *49*(5), 911-920. <https://doi.org/10.2307/2410413> 815
58. Aguilar, R.; Quesada, M.; Ashworth, L.; Herrerias-Diego, Y.; Lobo, J. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. *Mol. Ecol.* **2008**, *17*, 5177-5188. 816
- <https://doi.org/10.1111/j.1365-294X.2008.03971.x> 817
59. Hoban, S.; Schlarbaum, S. Optimal sampling of seeds from plant populations for ex-situ conservation of genetic biodiversity, considering realistic population structure. *Biol Cons*, **2014**, *177*, 90-99. 818
60. Coleman, S.W.; Moore, J.E.; Wilson, J.W. Quality and utilization. In *Warm season grasses*, Moser, L.E.; Burson, B.L.; Sollenberger, L.E. Eds. ASA, CSSA, SSSA, Madison, WI, **2004**, pp 267-308. <https://doi.org/10.2134/agronmonogr45.c8> 819
61. Mullet, J.E. High-biomass C4 grasses Filling the yield gap. *Plant Sci.* **2017**, *261*, 10-17. 820
- <https://doi.org/10.1016/j.plantsci.2017.05.003> 821
62. Rathcke, B.; Lacey, E.P. Phenological patterns of terrestrial plants. *Annu. Rev. Ecol. Syst.* **1985**, *16*, 179-214. 822
- <https://doi.org/10.1146/annurev.es.16.110185.001143> 823
63. Mozdzer, T.J.; Caplan, J.S.; Hager, R.N.; Proffitt, C.E.; Meyerson, L.A. Contrasting trait responses to latitudinal climate variation in two lineages of an invasive grass. *Biol. Invas.* **2016**, *18*, 2649-2660. <https://doi.org/10.1007/s10530-016-1218-y> 824
64. Primack, R.B. Variation in the phenology of natural populations of montane shrubs in New Zealand. *J. Ecol.* **1980**, *68*(3), 849-862. <https://doi.org/10.2307/2259460> 825
65. Takebayashi, N.; Morrell, P.L. Is self-fertilization an evolutionary dead end? Revisiting an old hypothesis with genetic theories and a macroevolutionary approach. *Am. J. Bot.* **2001**, *88*(7), 1143-1150. <https://doi.org/10.2307/3558325> 826
66. Merilä, J.; Crnokrak, P. Comparison of genetic differentiation at marker loci and quantitative traits. *J. Evol. Biol.* **2001**, *14*, 892-903. <https://doi.org/10.1046/j.1420-9101.2001.00348.x> 827
67. Wright, S.I.; Kalisz, S.; Slotte, T. Evolutionary consequences of self-fertilization in plants. *Proc. Royal. Soc. B* **2013**, *280*, 20130133. <https://doi.org/10.1098/rspb.2013.0133> 828
68. Chung, M.Y.; Merilä, J.; Li, J.; Mao, K.; López-Pujol, J.; Tsumura, Y.; Chung, M.G. Neutral and adaptive genetic diversity in plants: An overview. *Front. Ecol. Evol.* **2023**, *11*, 1116814. <https://doi.org/10.3389/fevo.2023.1116814> 829
69. Lenormand, T. Gene flow and the limits to natural selection. *Trends Ecol. Evol.* **2002**, *17*(4), 183-189. [https://doi.org/10.1016/S0169-5347\(02\)02497-7](https://doi.org/10.1016/S0169-5347(02)02497-7) 830
70. Dufresne, F.; Stift, M.; Vergilino, R.; Mable, B.K. Recent progress and challenges in population genetics of polyploid organisms: an overview of current state-of-the-art molecular and statistical tools. *Mol. Ecol.* **2014**, *23*(1), 40-69. <https://doi.org/10.1111/mec.12581> 831
71. Baker, H.G. Support for Baker's law-as a rule. *Evolution* **1967**, *21*(4), 853-856. <https://doi.org/10.2307/2406780> 832
72. Ingvarsson, P. A metapopulation perspective on genetic diversity and differentiation in partially self-fertilizing plants. *Evolution* **2002**, *56*(12), 2368-2373. <https://doi.org/10.1111/j.0014-3820.2002.tb00162.x> 833
73. Goodwillie, C.; Kalisz, S.; Eckert, C.G. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annu. Rev. Ecol. Evol. Syst.* **2005**, *36*(1), 47-79. <https://doi.org/10.1146/annurev.ecol-sys.36.091704.175539> 834
74. Hörandl, E. The complex causality of geographical parthenogenesis. *New Phytol.* **2006**, *171*, 525-538. <https://doi.org/10.1111/j.1469-8137.2006.01769.x> 835
75. García, M.V.; Balatti, P.A.; Arturi, M.J. Genetic variability in natural populations of *Paspalum dilatatum* Poir. analyzed by means of morphological traits and molecular markers. *Genet. Resour. Crop Evol.* **2007**, *54*, 935-946. <https://doi.org/10.1007/s10722-006-9147-8> 836
76. Reis, C.A.D.O.D.; Dall'Agnol, M.; Nabinger, C.; Schifino-Wittmann, M.T. Morphological variation in *Paspalum nicorae* Parodi accessions, a promising forage. *Sci. Agric.* **2010**, *67*, 143-150. <https://doi.org/10.1590/S0103-90162010000200003> 837
77. Lynch, M.; Bürger, R.; Butcher, D.; Gabriel, W. The mutational meltdown in asexual populations. *J. Hered.* **1993**, *84*(5), 339-44. <https://doi.org/10.1093/oxfordjournals.jhered.a111354> 838
78. Hojsgaard, D.; Hörandl, E. A little bit of sex matters for genome evolution in asexual plants. *Front. Plant Sci.* **2015**, *6*, 82. <https://doi.org/10.3389/fpls.2015.00082> 839
79. Hodač, L.; Klatt, S.; Hojsgaard, D.; Sharbel, T.F.; Hörandl, E. A little bit of sex prevents mutation accumulation even in apomictic polyploid plants. *BMC Evol. Biol.* **2019**, *19*, 1-11. <https://doi.org/10.1186/s12862-019-1495-z> 840
80. Pettengill, J.B.; Briscoe Runquist, R.D.; Moeller, D.A. Mating system divergence affects the distribution of sequence diversity within and among populations of recently diverged subspecies of *Clarkia xantiana* (Onagraceae). *Am. J. Bot.* **2016**, *103*, 1-11. <https://doi.org/10.3732/ajb.1500147> 841
81. Dawson, I.K.; Chalmers, K.J.; Waugh, R.; Powell, W. Detection and analysis of genetic variation in *Hordeum spontaneum* populations from Israel using RAPD markers. *Mol. Ecol.* **1993**, *2*, 151-159. <https://doi.org/10.1111/j.1365-294X.1993.tb00104.x> 842

82. Chirinos-Arias, M.C.; Jiménez, L.E.; Vilca-Machaca, L.S. Análisis de la Variabilidad Genética entre treinta accesiones de tarwi (*Lupinus mutabilis* Sweet) usando marcadores moleculares ISSR. *Sci. Agropecu.* **2015**, *6*, 17–30. <https://doi.org/10.17268/sci.agropecu.2015.01.02>
83. Vidal, R.; González, A.; Gutiérrez, L.; Umaña, R.; Speranza, P. Distribución de la diversidad genética y sistema reproductivo de *Stipa neesiana* Trin. et. Rupr. *Agrocienc. Urug.* **2011**, *15*, 1–12. <https://doi.org/10.31285/AGRO.15.605>
84. Blambert, L.; Mallet, B.; Humeau, L.; Paillet, T. Reproductive patterns, genetic diversity and inbreeding depression in two closely related *Jumellea* species with contrasting patterns of commonness and distribution. *Ann. Bot.* **2015**, *118*, 93–103. <https://doi.org/10.1093/aob/mcw014>
85. Wang, J.L.; Zhao, N.X.; Gai, Y.B.; Lin, F.; Ren, A.Z.; Ruan, W.B.; Chen, L. RAPD analysis of genetic diversity and population genetic structure of *Stipa krylovii* Reshov. Inner Mongolia steppe. *Russ. J. Genet.* **2006**, *42*, 468–475. <https://doi.org/10.1134/S1022795406050024>
86. Wright, S. Isolation by distance under diverse systems of mating. *Genetics* **1946**, *31*, 39–59. <https://doi.org/10.1093/genetics/31.1.39>
87. Allard, R.W.; Jain, S.K.; Workman, P.L. The genetics of inbreeding populations. In *Advances in Genetics* Caspari, E.W. Ed., NY Academic, New York, **1968**, pp 55–131. [https://doi.org/10.1016/S0065-2660\(08\)60425-3](https://doi.org/10.1016/S0065-2660(08)60425-3)
88. Slatkin, M. The rate of spread of an advantageous allele in a subdivided population. In *Population Genetics and Ecology*, Karlin, S.; Nevo, E. Eds., NY Academic, New York, **1976**, pp 767–780. <https://doi.org/10.1016/B978-0-12-398560-6.50037-7>
89. Duminil, J.; Hardy, O.J.; Petit, R.J. Plant traits correlated with generation time directly affect inbreeding depression and mating system and indirectly genetic structure. *BMC Evol. Biol.* **2009**, *9*, 177. <https://doi.org/10.1186/1471-2148-9-177>
90. Charlesworth, D. Effects of inbreeding on the genetic diversity of populations. *Philos. Trans. R. Soc. Lond.* **2003**, *358*, 1051–1070. <https://doi.org/10.1098/rstb.2003.1296>
91. Gornall, R.J. Population genetic structure in agamosperous plants. In *Molecular systematic and plant evolution*, Hollingsworth, P.M.; Bateman, R.M.; Gornall, R.J.; Eds. Taylor & Francis, London, **1999**, pp 118–138. <https://doi.org/10.1201/9781439833278.ch7>
92. Paun, O.; Greilhuber, J.; Temsch, E.M.; Hörandl, E. Patterns, sources and ecological implications of clonal diversity in apomictic *Ranunculus carpaticola* (*Ranunculus auricomus* complex, Ranunculaceae). *Mol. Ecol.* **2006a**, *15*, 897–910. <https://doi.org/10.1111/j.1365-294X.2006.02800.x>
93. Widén, B.; Cronberg, N.; Widén, M. Genotypic diversity, molecular markers and spatial distribution of genets in clonal plants, a literature survey. *Folia Geobot.* **1994**, *29*, 245–63. <https://doi.org/10.1007/BF02803799>
94. Richards, A.J. Apomixis in flowering plants: an overview. *Philos. Trans. R. Soc. B* **2003**, *358*, 1085–1093. <https://doi.org/10.1098/rstb.2003.1294>
95. Paun, O.; Stuessy, T.F.; Hörandl, E. The role of hybridization, polyploidization and glaciation in the origin and evolution of the apomictic *Ranunculus cassubicus* complex. *New Phytol.* **2006b**, *171*, 223–236. <https://doi.org/10.1111/j.1469-8137.2006.01738.x>
96. Gutiérrez-Ozuna, R.; Eguiarte, L.E.; Molina-Freaner, F. Genotypic diversity among pasture and roadside populations of the invasive buffelgrass (*Pennisetum ciliare* L.) in north-western Mexico. *J. Arid Environ.* **2009**, *73*, 26–32. <https://doi.org/10.1016/j.jaridenv.2008.09.007>
97. Cosendai, A.C.; Wagner, J.; Ladinig, U.; Rosche, C.; Hörandl, E. Geographical Parthenogenesis and Population Genetic Structure in the alpine Species *Ranunculus kuepferi* (Ranunculaceae). *Heredity* **2013**, *110*, 560–569. <https://doi.org/10.1038/hdy.2013.1>
98. Bogunić, F.; Siljak-Yakovlev, S.; Mahmutović-Dizdarević, I.; Hajrudinović-Bogunić, A.; Bourge, M.; Brown, S.C.; Muratović, E. Genome size, cytotype diversity and reproductive mode variation of *Cotoneaster integerrimus* (Rosaceae) from the Balkans. *Plants* **2021**, *10*, 2798. <https://doi.org/10.3390/plants10122798>
99. Palacios, C.; Kresovich, S.; Gonzalez-Candelas, F. A population genetic study of the endangered plant species *Limonium dufourii* (Plumbaginaceae) based on amplified fragment length polymorphism (AFLP). *Mol. Ecol.* **1999**, *8*, 645–657. <https://doi.org/10.1046/j.1365-294X.1999.t01-1-00597.x>
100. Levin, D.A.; Kerster, H.W. Neighborhood structure in plants under diverse reproductive methods. *Am. Nat.* **1971**, *105*, 345–54. <https://doi.org/10.1086/282729>
101. Moura, Y.A.; Alves-Pereira, A.; da Silva, C.C.; Souza, L.M.; de Souza, A.P.; Koehler, S. Secondary origin, hybridization and sexual reproduction in a diploid-tetraploid contact zone of the facultatively apomictic orchid *Zygopetalum mackayi*. *Plant Biol.* **2020**, *22*, 939–948. <https://doi.org/10.1111/plb.13148>
102. Zuloaga F.O.; Morrone O. *Revisión de las especies de Paspalum para América del Sur austral: (Argentina, Bolivia, sur del Brasil, Chile, Paraguay y Uruguay)*. St. Louis, Missouri, USA: Missouri Botanical Garden, **2005**, pp. 297.
103. R Core Team. **2024**. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
104. Peakall, R.O.D.; Smouse, P.E. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **2006**, *6*(1), 288–295. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
105. Meirmans, P.G.; Van Tienderen, P.H. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Mol. Ecol. Notes* **2004**, *4*(4), 792–794. <https://doi.org/10.1111/j.1471-8286.2004.00770.x>
106. Jombart, T. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **2008**, *24*, 1403–1405. <https://doi.org/10.1093/bioinformatics/btn129>
107. Winter, D.J. MMOD: an R library for the calculation of population differentiation statistics. *Mol. Ecol. Resour.* **2012**, *12*, 1158–1160. <https://doi.org/10.1111/j.1755-0998.2012.03174.x>

-
108. Kamvar, Z.N.; Tabima, J.F.; Grünwald, N.J. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* **2014**, *2*, e281. <https://doi.org/10.7717/peerj.281>
109. Goudet, J. Hierfstat, a package for R to compute and test variance components and F-statistics. *Mol. Ecol. Notes*. **2005**, *5*, 184–186. <https://doi.org/10.1111/j.1471-8286.2004.00828.x>
110. Frichot, E.; Francois, O. LEA: an R package for Landscape and Ecological Association studies. *Methods Ecol. Evol.* **2015**, *6*(8), 925–929. <https://doi.org/10.1111/2041-210X.12382>
111. Thioulouse, J.; Dray, S.; Dufour, A.; Siberchicot, A.; Jombart, T.; Pavoine, S. Multivariate Analysis of Ecological Data with ade4. *Springer*, **2018**. <https://doi.org/10.1007/978-1-4939-8850-1>
112. Matesanz, S.; Gimeno, T.E.; de la Cruz, M.; Escudero, A.; Valladares, F. Competition may explain the fine-scale spatial patterns and genetic structure of two co-occurring plant congeners. *J. Ecol.* **2011**, *99*, 838–848. <https://doi.org/10.1111/j.1365-2745.2011.01812.x>

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.