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Uniparentality: advantages for range expansion in diploid and diploid-autopolyploid species

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Uniparental reproduction, the capacity of an individual to produce offspring autonomously, is expected to facilitate range expansion of populations. *Paspalum* spp. reproduce uniparentally by sexual (self-fertility) and asexual (apomixis) reproduction and biparentally by sexual (self-sterility) reproduction. We evaluated the relationship between contrasting reproductive strategies (uni- and biparentality) and their impact on the colonizing ability and geographical range sizes of populations. We determined the cytotype composition of 16 populations of *P. indecorum*, *P. cromyorhizon*, *P. pumilum* and *P. maculosum* and assessed the sexual (self-fertile and self-sterile) and apomictic proportions by cyto-embryological analyses, fertility rates and seed flow cytometry. Data obtained regarding reproductive modes were compared to the distribution range of each cytotype and species. Sexual diploids with moderate degrees of self-fertility and mixed pollination syndromes showed wider distribution ranges than self-sterile diploids. In sexual diploids, increased rates of self-fertility relate to larger distribution areas. In agamic complexes, self-fertility reduces the differences in range sizes between biparental diploids and uniparental tetraploids. In such complexes, the range size of diploid cytotypes explains the range size and dispersal of apomictic tetraploids. Thus, uniparental reproduction via self-fertility and apomixis describes patterns of geographical parthenogenesis in South American species.

ADDITIONAL KEYWORDS: apomixis – cytogeography – diploidy – genetic system – geographical range – mixed pollination syndrome – natural populations – polyploidy – reproductive assurance – self-fertility.

INTRODUCTION

Polyploidy in flowering plants is one of the most significant and spontaneous factors of evolutionary processes, promoting diversification via genome duplication and gene pool fragmentation (Ramsey & Schemske, 1998). Recent estimates suggest that all flowering plants have a polyploid ancestor and share at least two genomic duplication events (Jiao *et al.*, 2011; Soltis & Soltis, 2016; Alix *et al.*, 2017; Wu, Han & Jiao, 2020), whereas only 20–50% of angiosperms are newly formed polyploids (Levin, 2002; Soltis *et al.*, 2009; 2016). Polyploidization is often associated with changes in reproductive systems such as self-fertility (autogamy) or apomixis (asexually formed seeds) (Grant, 1981; Otto, 2007). Self-fertility and apomixis provide advantages for range expansions through uniparental reproduction, i.e. the capacity of a single individual to form a new population distant from the parental population (Pannell, 2015; Pannell *et al.*, 2015; Barrett & Harder, 2017). Biparental reproduction is always associated with allogamy and hence at least two mating partners are needed to produce offspring (Lloyd, 1988). This implies a selective disadvantage

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for conquering new environments whereby often only one pioneering seed is established. Among uniparental plants, those being self-fertile have functional sexual processes (meiosis and fertilization), whereas in apomictic plants sexual mechanisms are not completely functional (Grant, 1989; Eckers *et al.*, 2018). Apomixis involves a bypass of meiosis and the formation of unreduced embryo sacs from a non-reduced megaspore (diplospory) or from a somatic nucellar cell (apospory). In addition, apomixis can be autonomous when pollen is not required for seed formation, or pseudogamous when is required only to trigger endosperm formation. All plants showing pseudogamous apomixis have a compatible pollen–pistil system, and are therefore capable of uniparental reproduction (Quarin, 1999; Hörandl, 2010; Hojsgaard & Hörandl, 2015).

From a genetic viewpoint, uniparentality in sexual plants doubles the probability of an allele being transmitted from mother to offspring, and thus sexual plants exhibiting uniparental reproduction traits avoid the so-called cost of meiosis or gene sharing through reduced rates of genetic mixing (Lloyd, 1988; Eckert, Samis & Dart, 2006; Agrawal & Hartfield, 2016). In apomictic plants, there is a chance to fix heterozygosity and/or advantageous genetic combinations (Vrijenhoek, 1979; 1984; Charlesworth & Willis, 2009; Barrett & Harder, 2017) by uniparentality.

From an ecological viewpoint, uniparental strategies are good for the colonization of new habitats, independently of pollinators and reproductive partners. An evolutionary hypothesis first proposed by Baker (Baker's rule: Baker, 1955, 1967) argued that self-compatible genotypes are more successful than self-sterile or dioecious species after long-distance dispersal, presumably because the independence from foreign pollen sources in selfers, facilitates population establishment and persistence during periods of low density (Rambuda & Johnson, 2004; Eckert *et al.*, 2006). Moreover, selfing populations or species occur more often than outcrossers in geographically and/or ecologically marginal habitats, where outcross pollination may be uncertain (Jain, 1976; Lloyd, 1980; Elle, 2004; Eckert *et al.*, 2006; Grossenbacher *et al.*, 2017). A similar distribution pattern is evident between some apomictic taxa and their sexual relatives, with sexuals occupying a central distribution in the whole species range and apomicts occupying marginal areas, often exhibiting larger ranges at higher latitudes and elevations (Bierzychudek, 1987; Richards, 2003). As for selfers, Baker's law applies equally to apomicts as this strategy assures population establishment in new habitats (Baker, 1967; Hörandl, Cosendai & Temsch, 2008; Pannell *et al.*, 2015; Hojsgaard & Hörandl, 2015). Evidence of this comes from natural distribution of cytotypes in agamic complexes, with apomictic polyploids displaying wider distributional ranges, exceeding those of their sexual conspecific diploid cytotypes. Such a pattern is known as geographical parthenogenesis (Bayer, 1998; Hörandl, 2006; Tilquin & Kokko, 2016).

When the occurrence of uniparental reproduction strategies is associated with different ploidies and/or hybridization, such traits can add genetic complexity and provide genomic novelty for natural selection (Jørgensen *et al.*, 2011; Baniaga *et al.*, 2020). Moreover, since apomixis can freeze genetic variation in distinct clonal lineages, it allows the partition of the ecological niche and a better exploitation of the space resources (Vrijenhoek, 1984; Lynch, 1984). Thus, studying the natural occurrence of ploidies and mating systems provides useful information to understand evolutionary processes involved in the successful occupancy and colonization of new habitats (Stebbins, 1971; Lewis, 1980; Ehrendorfer, 1980; Zozomová-Lihová *et al.*, 2015; Barrett & Harder, 2017; Bougoutaia *et al.*, 2020).

Only a few plant systems enable the study of the effects of uniparental reproduction in nature. *Paspalum* L. is among the richest genera of Poaceae with *c.* 350 species (Rua *et al.*, 2010) showing contrasting genetic systems, allowing the analysis of the consequences of uniparentality and its role in shaping plant distributions. *Paspalum* spp. inhabit ecologically diverse areas throughout America and are responsible for the biodiversity of South American grasslands (Zuloaga & Morrone, 2005). *Paspalum* spp. have monoploid or multiploid populations in nature and express sexuality or apomixis in a strict association with ploidy. Species can be either self-sterile or self-fertile (Ortiz *et al.*, 2013), and they are anemophilous and anemochorous. Ploidy-reproductive trait distributions in natural populations are still poorly understood in *Paspalum*, with a few recent exceptions (Sartor *et al.*, 2011, 2013; Brugnoli *et al.*, 2013, 2014; Karunarathne *et al.*, 2018, 2020). Here we aim to (1) test the association between reproductive pathways (i.e. sexuality and apomixis), mating systems (i.e. self-fertility and self-sterility) and ploidy (diploids and tetraploids) in four *Paspalum* spp., (2) contrast uniparental versus biparental reproductive strategies and distribution ranges and (3) analyse the relative impact of alternative uniparental reproduction strategies (i.e. self-fertility versus apomixis) on distribution ranges. We discuss the data in the context of the genetic systems and colonization advantages of *Paspalum* spp. and provide evidence supporting an active role of uniparental reproduction promoting range expansions.

MATERIAL AND METHODS

Plant material

Four perennial *Paspalum* spp. were used for this study (*P. pumilum* Nees, *P. indecorum* Mez, *P. maculosum* Trin., *P. cromyorhizon* Trin. ex Döll). A brief survey of each species regarding known ploidies and reproductive

modes is presented in the Supporting Information, Table S1. Sixteen populations were collected in north-eastern Argentina in the Mesopotamian region (Table 1, Fig. 1). This region is a subtropical zone, part of the Brazilian Central Plateau, the landscape of which is dominated by the Parana and Uruguay rivers. The region comprises three states (Misiones, Corrientes and Entre Rios) and varies from rainforests in Misiones to fertile pasturelands in Entre Rios. This region comprises the southernmost part of the distribution of *P. pumilum* and *P. maculosum* and the central area of the distribution of *P. indecorum* and *P. cromyorhizon* (see Cytogeographical distribution and range sizes in the Results section). Population sampling was made by collecting rhizome cuttings from single plants in their natural environment. A transect spanning the longest length available across the population was followed to obtain information on the local dispersal of cytotypes. Even representation of individuals within the population was attained by uniform sampling (i.e. the distance between two consecutive individuals remained constant). These samples were taken at least 10 m apart from each other, to avoid sampling the same individual genotype twice. Twenty to 30 individual cuttings were collected per population. The cuttings were grown in pots in a greenhouse for ploidy determination.

The collected plants were grown at the experimental field from the Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, Corrientes, Argentina, for reproductive mode analyses and fertility assessment. Vouchers for each sampled population

Table 1. *Paspalum* species: population number (herbaria voucher), population size (*N*), somatic chromosome number (2*n*), collection site and herbaria where the vouchers are deposited

Species	Population	*N*	2*n*	Population collection localities
P. pumilum	H2089[1]	23	20	A. Entre Ríos. Islas del Ibicuy. Médanos, 43 km from Gualeguay on RN 12. S 33.435111; W 59.064528. MNES
	H2118[2]	25	20	A. Ctes. Road from Caa Catí to Mburucuyá. S 27.77579; W 57.65026. MNES
	H2120[3]	24	20	A. Ctes. Route between Caa Catí and Mburucuyá. S 27.83589; W57.75042. MNES
	H2139[4]	21	20	A. Ctes. Between La Cruz and Alvear. S 29.12992; W 56.63878. MNES
P. indecorum	H1701[1]	20	20	A. Mnes. Candelaria. Bonpland. S 27.465667; W 55.429555. MNES
	H1726[2]	20	20, 30*(1)	A. Mnes. Candelaria, road to Santa Ana. S 27.403544; W 55.590077. MNES
	H1870[3]	20	20	A. Mnes. Candelaria, Campo San Juan Reserve. S 27.403619; WO 55.651694. MNES
	H2058[4]	20	20	A. Mnes. Cainguás, 18 km from San Vicente, road to Salto Golondrina. S 27.1285; W 54.489889. MNES
P. maculosum	Hojs484[1]	25	20	A. Ctes. Ituzaingó, RN 118, km 143. S 27.91721; W 57.45810. MNES
	H1728[2]	21	20	A. Ctes. Alvear, RN 14, km 678. S 28.60016; W 56.10051. MNES
	H2145[3]	21	20, 30(1), 40(11)	A. Ctes. Santo Tomé, RP 94. S 28.34389; W 55.93099. MNES
	H2232[4]	20	20, 40(5)	A. Ctes. Alvear. RN14. S 28.67125; W 56.180694. MNES
P. cromyorhizon	H1732[1]	20	20, 40(2)	A. Ctes. Curuzú Cuatiá, RN 127 and Miriñay River. S 29.55815; W 57.50227. MNES
	H1733[2]	20	40	A. Ctes. Paso de los Libres, RP126, between Curuzú Cuatiá and Paso de los Libres, along Miriñay River margin. S 29.84539; W 57.66850. MNES
	H1735[3]	20	40	A. Ctes. Sauce, between Sauce and Perugorría. S 29.95637; W 58.81866. MNES
	H1955[4]	20	40	A. Ctes. Montecaseros, RN14 and Mocoretá stream, right margin. S 30.628889; W 57.983861. MNES

References: H: Honfi AI; Hojs: Hojsgaard DH; *N*: number of individuals per population; A: Argentina; MNES: Universidad Nacional de Misiones Herbarium. (In brackets): plant number with that ploidy within the population. Super-index: indicates the location in Figure 1. * New record for the species.

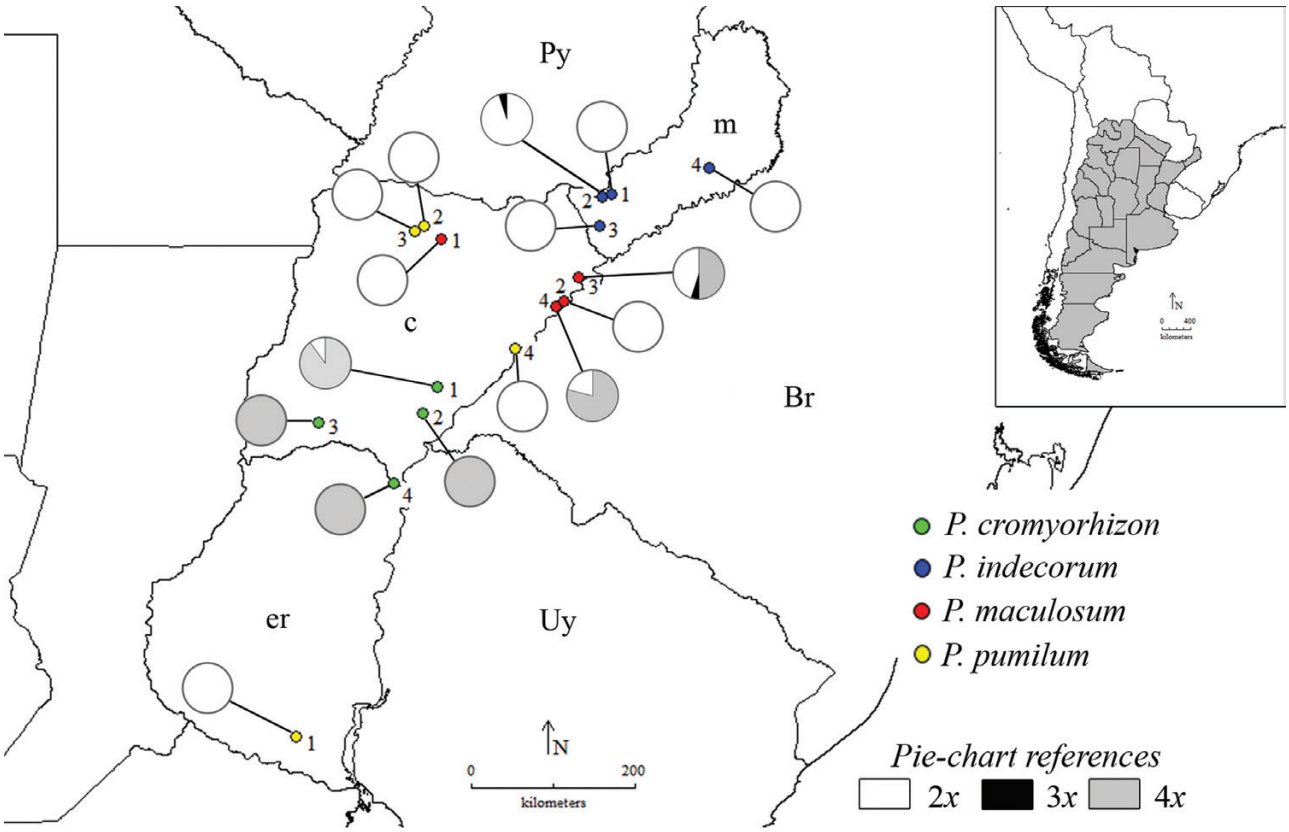

Figure 1. Collection localities and ploidy levels of *Paspalum cromyorhizon*, *P. indecorum*, *P. maculosum*, and *P. pumilum* natural populations. Pie charts show frequencies of cytotypes in each locality (see Table 1). Three localities on Corrientes and one in Misiones (Argentina) were multiploid. For the locality code numbers, see Table 1. References: *m*, Misiones; *c*, Corrientes, *er*, Entre Rios; *Br*, Brazil, *Py*, Paraguay; *Uy*, Uruguay.

were collected in the field and deposited at MNES (Herbario de la Universidad Nacional de Misiones, Instituto de Biología Subtropical, UNaM-CONICET, Misiones, Argentina).

Ploidy determination and cytogeography

Flow cytometry and chromosome analysis were used to determine the ploidy of each individual from the 16 populations. First, mitotic chromosome counts in root tips were determined in at least five plants from each population. Root tips were removed from potted plants, fixed in a saturated solution of α-bromonaphthalene for 3 h at room temperature. Selected root tips were fixed for 12–24 h in 3:1 absolute ethanol:glacial acetic acid and then conserved in 70% ethanol at 4 °C. Most of the pre-treated materials were directly hydrolysed with 1 M HCl at 60 °C for 10 min and stained with basic fuchsine (Schiff Reactive Solution). Root tips were squashed with a drop of 2% aceto-orcein on slides and observed with a LEICA DM LS microscope (Leica, Wetzlar, Germany). These plants were used as the internal standards in the flow cytometry analyses of the ploidy corresponding to their population. The fluorescence intensity of DAPI-stained nuclei was analysed with a Partec PA-II (Partec, Münster, Germany) flow cytometer. The ploidy of each individual was determined using samples of fresh leaf tissue. Briefly, 0.5 cm^2 leaf material was placed in a small Petri dish with a similar amount of tissue from the standard (a plant of the same species in which chromosome number was established by chromosome counts in root tips). After adding extraction buffer (0.5 ml), the tissue was chopped with a sharp razor blade. Following 2 min incubation, samples were filtered through a 50 µm nylon mesh directly into the sample tube, to which 1.5 mL of DAPI (4´,6-diamidino-2-phenylindole) stain solution was added. The mixture was incubated for another 2 min at room temperature and then analysed. The relative fluorescence of at least 3000 particles (nuclei) was measured for each sample using FloMax (Partec, Münster, Germany) and a maximum coefficient of variation (CV) value of 5% was accepted for each sample peak (G_0/G_1 peak). Ploidy was estimated by comparing the DNA histogram peaks in the samples and their internal standard, the ploidy

of which was previously determined by chromosome counting. The plants were measured once, but in case of doubt, measurements were repeated two or more times.

A curated dataset of herbarium specimens was constructed. The general geographical distribution of the species was outlined according to the locations reported in the monograph on *Paspalum* by Zuloaga & Morrone (2005). Additional locations were compiled from previous studies (Supporting Information, Table S2) and our own data from field collection trips. We also used a curated list of GBIF occurrence data available for each species (GBIF.org, 2020) and information from Universidad Nacional de Misiones (MNES) and Instituto de Botánica del Nordeste (CTES) herbaria. We used only georeferenced locations and checked for precision of the coordinates and gross errors following Hijmans & Spooner (2001). In addition to our data concerning chromosome numbers, the few previous counts from the literature were taken into account to outline the specific distribution limits of each cytotype (Supporting Information, Table S2). Repeated locations for the same cytotype were discarded. To estimate range size, known population locations were plotted on a high-resolution map of America. We assigned a circular area with a radius of 50 km to each observation and calculated the total area of all circles per species following Hijmans & Spooner (2001). Areas where circles of a species overlap are only included once. The assumption is that each location represents a group of plants that covers a circular area with a 50-km radius (Hijmans & Spooner, 2001). We used a radius of 50 km grid to strike a balance between the desire for high-resolution and geographical sampling bias (Hijmans & Spooner, 2001). This bias becomes less significant when the circles size increases. We used a circles model because this gives a smoother result than the common convex hull enclosure, particularly for areas with few observations and is less sensitive to the small errors and uncertainties in the locality coordinate data (Hijmans & Spooner, 2001; Wieczorek, Guo & Hijmans, 2004). The area of each circle was summed up to calculate the area in square kilometres using the *raster* package in R software (Hijmans, 2020; R Core Team, 2020) according to the geographical models described in Hijmans & Elith (2017).

Cyto-embryological analysis

The reproductive mode of five randomly selected individuals per population was characterized by a mature embryo sacs analysis. After 2 years of cultivation in the experimental field of FCA-UNNE, spikelets at anthesis from inflorescences of 80 individuals from 16 populations were collected and fixed in FAA 18:1:1 (70% ethanol:glacial acetic acid:formaldehyde) for 24 h, transferred to 70% ethanol and storage at 4 °C. At least 30–35 florets per individual were dissected using a Leica EZ4 stereomicroscope (Leica, Wetzlar, Germany). Then, pistils were cleared using methyl salicylate (Young, Sherwood & Bashaw, 1979) and analysed in a Leica DM2500 microscope (Leica, Wetzlar, Germany) with a differential interference contrast device. *Circa* 150–250 ovules per population were analysed and classified into sexual or apomictic according to the type of embryo sacs (see Reproductive assessment).

Rationale for the reproductive assessment

In *Paspalum*, each spikelet bears a single perfect floret, and the ovary supports a single ovule, each ovule having one or multiple embryo sacs. Ovules bearing a single embryo sac are typically arranged with an egg apparatus carrying an egg cell and two synergid cells at the micropyle, a large two-nucleate central cell and several antipodal cells at the chalaza. Embryo sacs with this anatomy were considered as meiotic (MES, see Fig. 2A, Supporting Information, Table S3), and such ovules were considered to be sexual. In contrast, some ovules have single or multiple embryo sacs lacking antipodal cells and differing in size and orientation. Embryo sacs with this anatomy were recorded as aposporous (AES, Fig. 2B, Supporting Information, Table S3), and such ovules as apomictic. In addition, ovules with both types of embryo sacs, i.e. ovules with mixed embryo sacs, were described as mixed ovules (MES + AES or MIX, Fig. 2C, Supporting Information, Table S3). Ovules without an embryo sac or with undeveloped, immature embryo sacs at anthesis were also counted (IES, Supporting Information, Table S3). The observed proportion of embryo sacs was estimated according to Hojsgaard, Martínez & Quarin (2013) (Table 2). For the sexual pathway $NM/(NM + NA)$, and for the apomictic pathway $NA/(NM + NA)$, where NM is the observed number of MES in single and mixed ovules ($NM = MES + MIX$) and NA is the observed number of AES in single and mixed ovules ($NA = AES + MIX$). The observed number of IES was not taken into account in the calculation as they do not contribute to any reproductive pathway. A paired *t*-test was performed for the difference among the observed proportion of meiotic and aposporous embryo sacs in R software (Table 2, R Core Team, 2020). When both proportions are equal the expected difference is zero. The maximum potential for sexuality (MPS) and the maximum potential for apomixis (MPA) were estimated according to Quarin (1986), but expressed as proportions. Briefly, $MPS = (MES + MIX)/T$ and $MPA = (AES + MIX)/T$, where T is the total number of analysed ovules including those with IES (Table 2). This is the maximum expected proportion

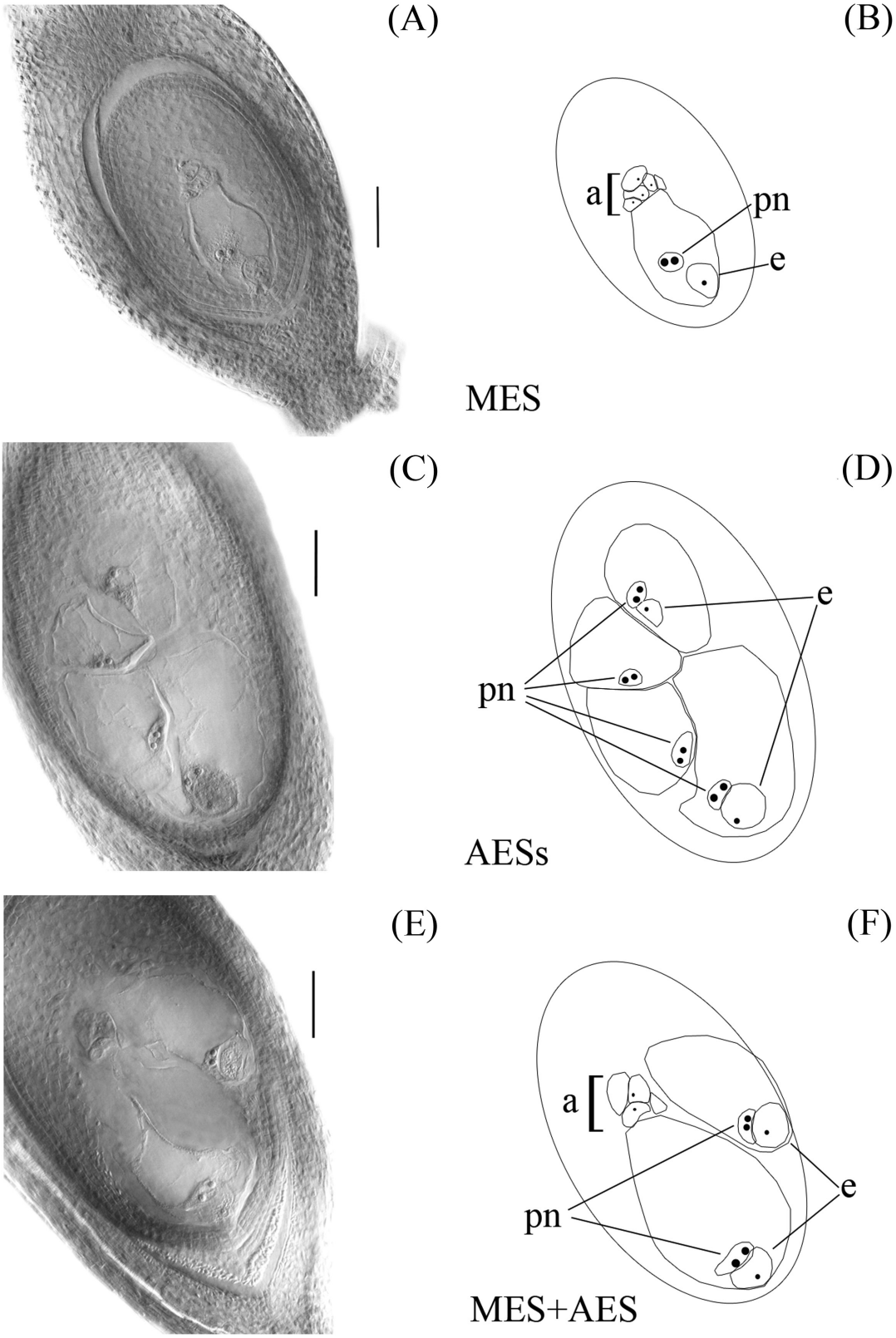

Figure 2. Microscopic images of mature meiotic and apomictic ovules of *Paspalum* and their respective diagrams showing the anatomical differences. A, Diploid meiotic embryo sac of *P. pumilum*. B, Meiotic embryo sac diagram. C, Tetraploid apomictic embryo sacs of *P. cromyorhizon* (AES-aposporous embryo sac). D, Apomictic embryo sacs diagram. E, Multiple

Table 2. Reproductive pathway proportions at the ovule stage for 16 natural populations of four *Paspalum* species, a paired *t*-test values (*t*) for the difference among the observed proportion of meiotic and aposporous embryo sacs, and the sexual (MPS) and apomictic maximum potential (MPA)

Species	Pop	Ploidy	Observed proportions		*t*	*P* value*	MPS	MPA
			Sexual	Apomictic				
P. pumilum	H2089	2*x*	1.00	0.00	142.0	**<0.001**	0.98	0.00
	H2118	2*x*	1.00	0.00	138.0	**<0.001**	0.92	0.00
	H2120	2*x*	1.00	0.00	139.0	**<0.001**	0.89	0.00
	H2139	2*x*	1.00	0.00	142.0	**<0.001**	0.99	0.00
P. indecorum	H1701	2*x*	0.978	0.022	120.4	**<0.001**	0.86	0.02
	H1726	2*x*	0.961	0.039	128.1	**<0.001**	0.97	0.04
		3*x*	0.814	0.186	38.9	**<0.001**	0.84	0.19
	H1870	2*x*	0.903	0.097	95.1	**<0.001**	0.86	0.08
	H2058	2*x*	0.979	0.021	130.3	**<0.001**	0.94	0.02
P. maculosum	Hojs484	2*x*	0.978	0.022	122.4	**<0.001**	0.89	0.02
	H1728	2*x*	1.00	0.00	166.0	**<0.001**	0.89	0.00
	H2145	2*x*	0.675	0.325	9.59	**0.002**	0.76	0.37
		3*x*	0.711	0.289	13.9	**<0.001**	0.92	0.38
		4*x*	0.620	0.380	11.73	**<0.001**	0.90	0.55
	H2232	2*x*	0.735	0.265	55.0	**<0.001**	0.84	0.30
		4*x*	0.674	0.326	27.1	**<0.001**	0.87	0.42
P. cromyorhizon	H1732	2*x*	0.975	0.025	139.5	**<0.001**	0.98	0.02
		4*x*	0.595	0.405	0.46	0.496	0.73	0.50
	H1733	4*x*	0.508	0.492	0.05	0.817	0.95	0.92
	H1735	4*x*	0.496	0.504	0.003	0.953	0.95	0.96
	H1955	4*x*	0.483	0.517	0.24	0.622	0.86	0.92

* Significant values are in bold ($P < 0.05$).

of each reproductive pathway in the following reproductive stage (seed formation).

FCSS ANALYSES

Twenty to 35 seeds per cytotype within each population were used in the flow cytometry of single seed analyses (FCSS) to assess their reproductive origin following the methodology of Karunarathne *et al.* (2018). The relative fluorescence intensity of around 3000 nuclei was analysed with FloMax (Partec, Münster, Germany), and discrete peaks were assigned to embryo and endosperm seed tissues. A maximum CV value of 5% was accepted for each peak. Reproductive pathways were determined according to the rationale by Matzk, Meister & Schubert (2000) and following considerations for *Paspalum* spp. Sexually derived seeds have a relative DNA content of embryo:endosperm ratio 2C:3C, whereas seeds derived from apomixis show an embryo: endosperm ratio of 2C:5C. Thus, seeds with peak ratios (endosperm/embryo) of 1.5 were categorized as sexual, whereas seeds with values of 2.0 or higher were categorized as apomictic. The mean of observed number of apomictic seeds in each species was used as the apomixis degree (AS) in the estimation of the uniparentality coefficient (UC, see next).

FERTILITY (SEED SET) MEASURES

Fertility was estimated as the percentage of seed production under self- and open-pollinated conditions for two consecutive flowering periods (flowering months October to March, first period: 2016–2017, second period: 2017–2018). Measurements were made in populations established in the FCA-UNNE experimental garden. Each condition was measured in three inflorescences per plant and five plants per population. For self-pollination, inflorescences were bagged before anthesis with sulphite-paper

embryo sacs of both meiotic and apomictic origin coexisting in the same ovule of *P. cromyorhizon* (AES-aposporous embryo sac, MES-meiotic embryo sac). F, A MES+AES or MIX ovule diagram. *References*: *e*, egg cell; *pn*, polar nuclei; *a*, antipodes. Bar scale: 100 µm.

crossing-bags (Baumann Saatzuchtbedarf GmbH, Waldenburg, Germany). For open pollination, inflorescences were bagged once all spikelets were in anthesis. Twenty to 30 days after bagging, filled and empty spikelets (with and without caryopses, respectively) from single inflorescences were sorted out in two groups using a 757 South Dakota Seed Blower (SeedBuro Equipment Company, IL, USA). The total number of filled and empty spikelets was estimated by counting and weighing three sets of 100 spikelets each per inflorescence, and by averaging and extrapolating that value to the total weight of filled and empty spikelets groups per individual (Supporting Information, Table S4). Fertility rates in each pollination condition and flowering period were estimated as the number of filled seeds/ total number of seeds and represented as percentages, being the total number of seeds the sum of empty and filled seeds in each plant (Supporting Information, Table S4). Then a mean percentage was calculated for each population. A three-way analysis of variance (ANOVA) and multiple mean comparisons using the Tukey test was calculated using R software (R Core Team, 2020) (Supporting Information, Table S5). Comparisons were made to identify which condition was predominant in the seed set of each species in each flowering period and to observe whether, under the same environment, populations of separate geographical origins exhibited differences in their seed set. In those populations/ species with more than one ploidy, a two-proportion test was made to compare selfed and open-pollinated seed set between ploidies.

The average seed set obtained in each pollination condition determined the rates of self-fertility (SF) following Daehler (1998) with modifications. The following categories were considered: (1) as non-selfing, from 0 to 5% seed set after self-pollination, but high seed set when open pollinated; (2) as low-selfing, < 20% seed set in self-pollination, but high seed set after open pollination; (3) as moderate-selfing, 20-50% seed set under self-pollination similar to seed set under open pollination and (4) as high-selfing, > 50% self-pollinated seed set comparable to open-pollinated seed set. Previous studies have determined similar rates to define when a species displays a selfing, an outcrossing or a mixed pollination mating system (e.g. Schemske & Lande, 1985; Lloyd & Schoen, 1992; Daehler, 1999; Goodwillie, Kalisz & Eckert, 2005; Rausch & Morgan, 2005; Kalisz *et al.*, 2012; Drummond & Rowland, 2020). They usually calculate the outcrossing rate (*OUT*) to then estimate the selfing rate (*SF* = 1– *OUT*). Thus, a selfing species has a mean *SF* ≥ 80% and some level of outcrossing, an outcrossing species has a mean of *SF* ≤ 20% and *OUT* from 50 to 100%, and a species has a mixed pollination syndrome when both *SF* and *OUT* ranged from 20 to 80% (Lloyd & Schoen, 1992; Goodwillie *et al.*, 2005).

Rates of uniparentality

According to Eckert *et al.* (2006), uniparental reproduction includes self-fertilization and asexuality. The rate of uniparentality represents a relative measure of the degree of uniparental reproduction observed in a species or cytotype. Therefore, it was estimated following the proportion of uniparental reproduction observed in each cytotype. The UC comprised the observed self-fertility and apomixis degree of each cytotype at the seed level. So, $UC = SF + AS$, where *SF* is the mean proportion of seeds obtained in self-pollination assays in each species, and *AS* is the mean observed proportion of apomictic seeds in the FCSS analysis of each species. We considered cytotypes with a UC among 0–0.1 as biparental. Meanwhile, cytotypes with an UC > 0.5 were considered as uniparental. Those cytotypes with an UC between 0.1 and 0.5 were considered as an intermediate state between biparental and uniparental reproduction. So, UC, as a uniparentality rate, is a comparative indicator between species that have offspring of uniparental origin but through different reproductive mechanisms, by self-fertility or apomixis. Although arbitrary, we felt it was instructive for assessing the differences between biparental-uniparental species, as this is the first attempt to combine both sources of uniparentality recognized by the literature (reviewed in Eckert *et al.*, 2006). Usually, asexual reproduction is a neglected mechanism of reproductive assurance, and therefore not considered in this estimation (see Eckert *et al.*, 2006).

RESULTS

Cytogeographical distribution and range sizes

Ploidy was determined for 340 plants (Fig. 3). Populations were either monoploid, i.e. with only one cytotype in the population, or multiploid, i.e. with two or more cytotypes in variable frequencies.

All *P. pumilum* populations collected were diploid (Fig. 1, Table 1). All ploidy reports for this species were diploid and most of them corresponded to the southern range of its geographical distribution, although diploids have also been found in the south-eastern USA (Supporting Information, Table S2). Its distribution comprises both continental and island areas (Dominica and Trinidad and Tobago), from south-eastern USA (Louisiana, Mississippi, Alabama), Costa Rica and the West Indies, to north-eastern Argentina and northern Uruguay (Fig. 4A). The range size of its distribution

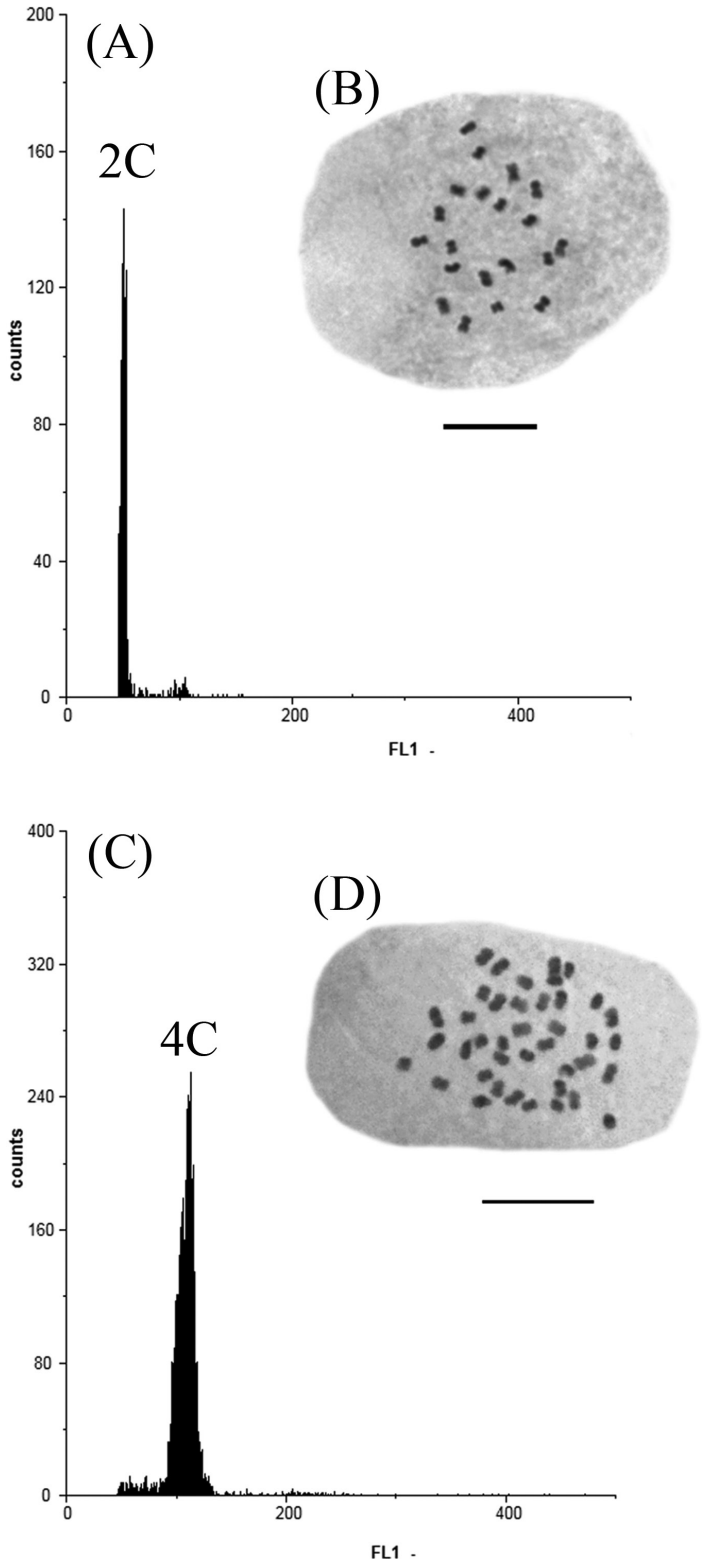

Figure 3. Histograms from cell nuclei of leaves samples showing different ploidy levels in two cytotypes of *Paspalum cromyorhizon* with their corresponding mitotic metaphase. A, Histogram of a diploid plant ($2n = 2x = 20$). B, A mitotic metaphase showing 20 chromosomes. C, Histogram of a tetraploid plant ($2n = 4x = 40$). D, A mitotic metaphase showing 40 chromosomes. Bar scale: 10 µm.

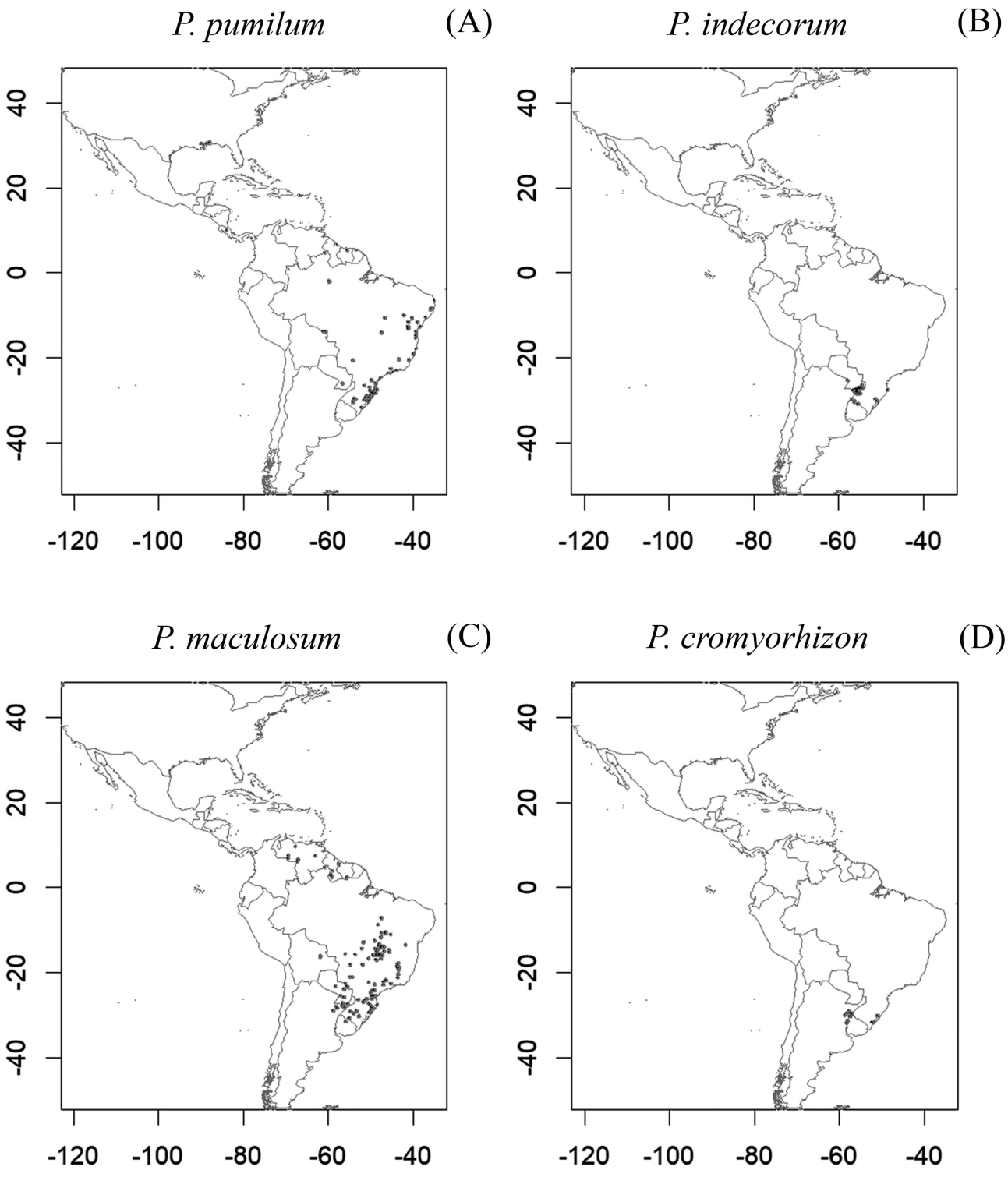

Figure 4. Cyto-geographical distribution based on locations from collected populations, GBIF and those reported in the bibliography for the species. A, *Paspalum pumilum*. B, *P. indecorum*. C, *P. maculosum*. D, *P. cromyorhizon*. In dark green, the circles of 50 km diameter are drawn to illustrate a preliminary distribution of the species.

was estimated to be 456 603.5 km², making it the most widespread diploid species under study.

Three *P.* indecorum populations were diploid (H1701, H1870 and H2058, Table 1). In the H1726 population, apart from diploids, a natural triploid plant was identified (Table 1). All *P. indecorum* populations were collected in Misiones, Argentina (Fig. 1). Biogeographical data indicated that the geographical distribution of *P. indecorum* is narrow, and it ranges from north-eastern Argentina (especially Misiones and northern Corrientes) to southern Brazil (Rio Grande do Sul), with occasional reports in Formosa (Argentina), Florianopolis Island (Santa Catarina, Brazil), Salto (Uruguay) and Guairá (Paraguay) (Fig. 4B). Across the whole geographical area, only diploids had been reported (Supporting Information, Table S2). Our estimation of the size of the distribution area was 115 166 km². This result indicates that the area comprised by *P. indecorum* is approximately one-quarter the size of the range of *P. pumilum*.

Two *P. maculosum* populations were diploid (Hojs484 and H1728), and the other two were multiploid (H2145 and H2232, Table 1). The multiploid population H2145 had a higher proportion of polyploids than diploids, mainly tetraploids (2*x*: 43%, 3*x*: 5%, 4*x*: 52%, Table 1, Fig. 1). Triploids were found intermingled within the population. In contrast, the multiploid population H2232 had a higher proportion of diploids than tetraploids (2*x*: 75%, 4*x*: 25%, Table 1, Fig. 1). All *P. maculosum* populations were collected in Corrientes, Argentina (Fig. 1). The geographical distribution of *P. maculosum* is wide, from northern Venezuela and Guyana to north-eastern Argentina (Fig. 4C). Our estimation of the size of its geographical range was 712 810.6 km². Previous reports with ploidy data are scarce, and the most northerly reports were from diploid and tetraploid individuals collected in Santa Cruz (Bolivia). The most southerly report pertained to tetraploid individuals collected in Rio Grande do Sul (Brazil) (Supporting Information, Table S2). Most chromosome data belong to the centre of origin of *P. maculosum*. The area estimated for the diploid cytotype was 46 022.2 km² (Supporting Information, Fig. S1A), whereas for the tetraploid the area was 1.2 times smaller, estimated at 39 682.5 km² (Supporting Information, Fig. S1B). Both cytotypes seemed to have a joint distribution in the Ñuflo de Chavez province (Santa Cruz, Bolivia; Supporting Information, Table S2), Corrientes and Misiones provinces (Argentina, Table 1, Supporting Information, Table S2) and Rio Grande do Sul State (Brazil, Supporting Information, Table S2). This preliminary projection of known ploidies in the species is 60 453.5 km² wide, but it represents only 8.5% of the global distribution of the species.

Three *P. cromyorhizon* populations were only tetraploid (H1733, H1735 and H1955), and one was a multiploid population (H1732) with diploid and tetraploid individuals (Fig. 3A, B, Table 1). The diploid cytotype had a higher frequency than the tetraploid cytotype in the multiploid population H1732 (2*x*: 90%, 4*x*: 10%, Table 1). All *P. cromyorhizon* populations were collected in Corrientes, the centre of its restricted distribution (Fig. 1). In Argentina, this species occupies periodic flooding areas, with extensive populations surrounding the Uruguay and Miriñay rivers and the Aguapey stream basin in Corrientes and also in Entre Rios (Argentina), in Salto and Soriano (Uruguay) and in Rio Grande do Sul (Brazil) (Fig. 4D). The distribution range of *P. cromyorhizon* is the smallest among the species studied here, at 70 298.4 km². The area estimated for the diploid cytotype was 16 083.8 km² (Supporting Information, Fig. S1C), whereas for the tetraploid it was 46 437.9 km² (Supporting Information, Fig. S1D). The diploid cytotype seems to be confined to a small area, near Miriñay River in Corrientes (Argentina), whereas the tetraploid cytotype are more widely distributed towards the south, in Argentina and Uruguay. The sum of the known-ploidy area for this species is 52 828.7 km², comprising 75.1% of the global area of the species distribution.

Ploidy-dependant variation in reproductive pathways

We noted a general correspondence among the modes of reproduction observed in the cyto-embryological studies (Table 2) and the analysis of relative embryo:endosperm DNA content (Table 3).

Regarding the diploid populations of *P. pumilum,* the MPS values ranged from 0.89 to 0.98 (Table 2), as all analysed ovules bore a single MES (Fig. 2A; Supporting Information, Table S3). The MPA was the lowest value among all species analysed, being 0.00 for all populations (Table 2) due to the lack of ovules with AES or MES + AES. Differences between both reproductive pathways were significant in all populations, and the sexual pathway was prevalent ($P < 0.001$, Table 2). Additionally, all diploid *P. pumilum* populations produced seeds sexually (2C: 3C; Table 3), indicating that the diploid cytotype reproduces only by sexual means.

The MPS values for *P. indecorum* populations ranged from 0.84 to 0.97, as most ovules bore MES (Supporting Information, Table S3). The MPA values ranged from 0.02 to 0.19, with the triploid showing the highest potential for apomictic reproduction (Table 2). All populations had some individuals that showed ovules with MES + AES (Supporting Information, Table S3). Apart from one ovule in a diploid individual of population H1701 with a single AES (0.7%,

Table 3. Reproductive origin of open-pollinated seeds obtained by FCSS analysis

Species	Population	2*n*	*N*	Observed number of seeds with C-values of embryo:(endosperm)*	
				2C:(3C)	2C:(5C)
P. pumilum	H2089	2*x*	25	25	-
	H2118	2*x*	25	25	-
	H2120	2*x*	25	25	-
	H2139	2*x*	25	25	-
P. indecorum	H1701	2*x*	25	25	-
	H1726	2*x*	36	34	2
	H1726	3*x*	20	14	6
	H1870	2*x*	24	24	-
	H2058	2*x*	25	25	-
P. maculosum	Hojs484	2*x*	25	25	-
	H1728	2*x*	24	24	-
	H2145	2*x*	25	25	-
		3*x*	-	na§	na§
		4*x*	25	-	25
	H2232	2*x*	25	25	-
		4*x*	25	-	25
P. cromyorhizon	H1732	2*x*	25	25	-
		4*x*	25	-	25
	H1733	4*x*	26	-	26
	H1735	4*x*	25	-	25
	H1955	4*x*	25	-	25

* Seed originated sexually 2C:(3C) embryo:(endosperm) DNA relative contents, seed originated via apomixis, 2C:(5C) DNA relative contents.
§ No seeds available when FCSS was performed.

Supporting Information, Table S3), only the triploid in population H1726 showed ovules carrying AES (1.0%, Supporting Information, Table S3). This is the first report of AES in diploid and triploid cytotypes for the species. However, the expression of the sexual pathway was significantly higher than the apomictic one in all populations ($P < 0.001$, Table 2). In the FCSS, plants from diploid populations of *P. indecorum* had sexually originated seeds, but those individuals in the multiploid population H1726 also showed a low proportion of seeds originated by apomixis (2C:5C, apospory, Table 3), which was higher in the triploid plant. Altogether, these results showed that diploids in *P. indecorum* reproduce mainly by sexuality, and that the triploid is a facultative apomictic (i.e. a combination of agamospermy and sexuality).

In *P. maculosum,* the MPS values were similar among populations and ploidies (0.84–0.92, Table 2). In contrast, MPA ranged from 0.00 in diploids to 0.55 in tetraploids (Table 2). Diploids in multiploid populations showed a higher MPA than those in pure diploid populations (Table 2), due to a higher proportion of ovules carrying MES + AES (Supporting Information, Table S3). Diploid populations (Hojs484 and H1728) showed a significant bias towards sexuality when comparing reproductive pathways ($P < 0.001$, Table 2). The 2*x*, 3*x* and 4*x* cytotypes of multiploid populations (H2145 and H2232) also showed significant differences favouring sexuality ($P < 0.001$, Table 2). Nevertheless, diploids in *P. maculosum* produced seeds only through sexuality in the FCSS, whereas tetraploids produced seeds through apomixis (apospory, Table 3). This showed that diploids reproduce sexually, whereas tetraploids reproduce by apomixis.

The MPS values in the multiploid population (H1732) of *P. cromyorhizon* ranged from 0.98 in diploids to 0.73 in tetraploid plants. The diploid cytotype in the H1732 population had the highest number of ovules carrying a single MES observed for the species (95.5%, Supporting Information, Table S3), and the lowest MPA values (0.02; sexual vs. apomictic significant differences $P < 0.001$; Table 2). Among tetraploids, MPA was highest in the pure tetraploid population H1735 (0.96, Table 2). All tetraploid populations showed high values of ovules carrying a single AES (Fig. 2B, 1.9–11.5%, Supporting Information, Table S3) or bearing MES + AES (Fig. 2C, 80.4–92%, Supporting Information, Table S3). The tetraploid counterpart in

the H1732 population and the three pure tetraploid populations showed similar proportions for the apomictic and sexual pathways (Table 2). Besides, diploids in *P. cromyorhizon* produced seeds through only sexuality, whereas tetraploids produced seeds through apomixis (apospory, Table 3). These results indicate that the diploid cytotype in the H1732 population reproduces by sexual means, whereas the tetraploid cytotype in the multiploid population (H1732) and the 4*x* populations (H1733, H1735 and H1955) reproduce by apomixis.

Fertility rates and pollination syndromes

In general, *P. pumilum* populations were highly fertile. Means of population seed set in self-pollination ranged from 30.6 to 91.7% in both flowering periods, indicating a high level of self-fertility in this species (Fig. 5, Supporting Information, Table S4). The mean open-pollination seed production by population ranged from 31.4 to 74.6% (Fig. 5, Supporting Information, Table S4). The ANOVA showed no differences in seed set between pollination conditions (Supporting Information, Table S5), although the production of seeds under open-pollination conditions does not rule out that part of them are of autogamous origin. The ANOVA showed significant differences in seed set among flowering periods and populations (first period: 67.9%, second period: 43.5%, $P < 0.001$, Supporting Information, Table S4S5). Thus, all populations showed a statistically higher seed set during the first flowering period ($P < 0.035$, Table 3).

All *P. indecorum* populations showed a higher seed set in open-pollination trials (Supporting Information, Table S4). The seed set in open pollination was higher in both flowering periods ($P < 0.001$, Supporting Information, Table S4S5). The population mean of seed set under self-pollination ranged from 0.1 to 3.5% considering both flowering periods (Fig. 5, Supporting Information, Table S4), indicating a high self-sterility in this species. All populations had a higher seed set during the first flowering period ($P < 0.043$, Supporting Information, Table S5). All *P. indecorum* populations behave similarly regarding their pollination syndrome

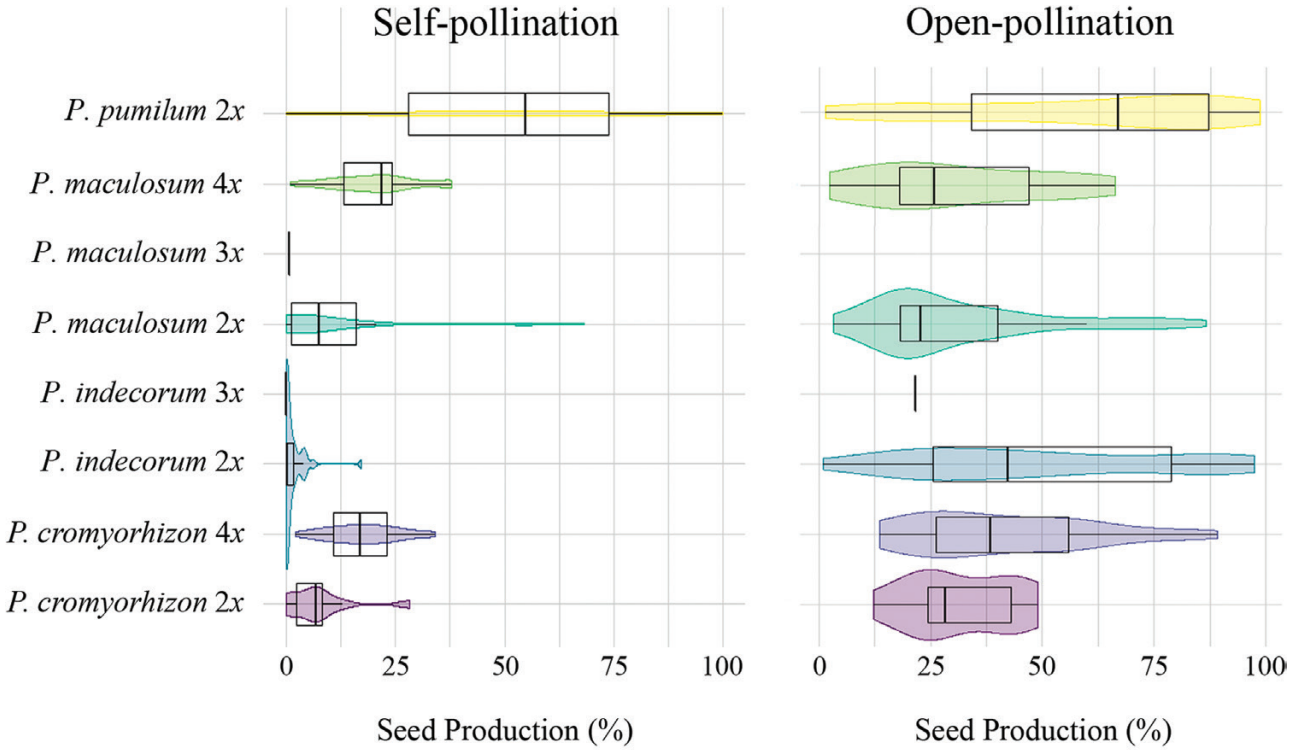

Figure 5. Seed set production in self- and open-pollination conditions (expressed as percentages) of each ploidy level observed per species. Self-pollinated seeds reached high values (>50%) only in 2*x*-*P. pumilum*. Other ploidies-species showed low (e.g. 2*x*-*P. indecorum*, 3*x*-*P. indecorum*, 3*x*-*P. maculosum*) to moderate values (e.g. 2*x*-*P. maculosum*, 4*x*-*P. cromyorhizon*) of selfed seeds. On the other hand, open-pollinated seed had varying values but higher than selfed, with the exception of 2*x*-*P. pumilum* that showed similar seed set rates in both conditions. The violin chart shows the data distribution for each ploidy level per species, including a box-plot chart showing first quartile (Q1), median, third quartile (Q3) and outliers in each condition. The boxes represent 50% of the data.

(Supporting Information, Table S5). In multiploid population H1726, the seed set showed no significant differences between ploidies in open pollination (2*x*: 28.52%, 3*x*: 21.69%, $P > 0.05$, Fig. 5) or in self-pollination (2*x*: 0.92%, 3*x*: 0.12%, $P > 0.05$, Fig. 5).

In *P. maculosum*, seed set in open pollination was higher than by selfing ($P = 0.002$, Supporting Information, Tables S4, S5). However, we report seed formation under self-pollination in diploid cytotypes of *P. maculosum*, with values ranging from 0.6 to 41.2%, indicating a low to intermediate degree of self-fertility (Fig. 5, Supporting Information, Table S4). Triploids and tetraploids from multiploid populations (H2145 and H2232) also showed low to intermediate rates of self-fertility (3*x*: 0.8%, 4*x*: 5.5–27.2%, Fig. 5). No differences were observed among flowering periods in this species (Supporting Information, Table S5). Multiploid population H2232 was unique, showing a higher proportion of seed set under self-pollination than in open pollination during the first flowering period ($P = 0.026$, Supporting Information, Tables S4, S5). The populations showed a dissimilar behaviour only during the first flowering ($P = 0.008$, Supporting Information, Table S5), with the diploid population Hojs484 showing a low seed set (Supporting Information, Table S5). Ploidies within multiploid population H2145 (2*x*–3*x*–4*x*) showed significant differences between them in self-pollination (2*x*: 7.6%, 3*x*: 0.8%, 4*x*: 27.2%, $P < 0.001$) and open pollination (2*x*: 15%, 3*x*: 0%, 4*x*: 40.13%, $P < 0.001$). On the other hand, ploidies in multiploid population H2232 (2*x*–4*x*) showed no differences in either pollination condition (self: 2*x*: 13%, 4*x*: 19.5%; open: 2*x*: 38.3%, 4*x*: 30.6%). When we compared ploidies in general, 2*x* and 4*x* seed set were dissimilar under open pollination during the first flowering period (2*x*: 28.0%, 4*x*: 15.0%, $P = 0.039$). During the second flowering period, we observed significant differences in the seed set under self-pollination conditions (2*x*: 14.6%, 3*x*: 0.8%, 4*x*: 23.8%, $P < 0.001$).

In *P. cromyorhizon*, seed set in open pollination was also higher than by selfing ($P < 0.001$, Supporting Information, Tables S4, S5). Seed set was higher during the first flowering period ($P < 0.001$, Supporting Information, Table S5). Populations showed dissimilar behaviours, with multiploid population H1732 (2*x*–4*x*) showing the lowest values of seed set ($P < 0.001$, Supporting Information, Table S4S5). In the multiploid population H1732, diploids and tetraploids showed no differences among their seed set in either pollination condition (self: 2*x*:7.8%, 4*x*: 14%; open: 2*x*: 31.7%, 4*x*: 34%). When we compare ploidies (2*x* and 4*x*) globally, we observe differences only in self-pollination during the second flowering period, in which, tetraploids produced four times more seed set than diploids (18.3 and 4.7% respectively, $P = 0.005$). Thus, our results showed that the tetraploid cytotype had a higher rate of self-fertility than the diploids, and diploids behaved as outcrossing plants.

Table 4. Uniparentality rates for each cytotype in four *Paspalum* species

Species	2*n*	SF	AS	UC
P. pumilum	2*x*	0.53	0.0	0.53
P. indecorum	2*x*	0.02	0.01	0.03
	3*x*	0.00	0.3	0.30
P. maculosum	2*x*	0.14	0.0	0.14
	3*x*	0.01	*nd*	0.01
	4*x*	0.21	1.0	1.21
P. cromyorhizon	2*x*	0.08	0.0	0.08
	4*x*	0.18	1.0	1.18

References: 2*n*, ploidy level; SF, mean proportion of self-fertility seed set; AS, mean proportion of apomictic seed set; UC, uniparentality rate (SF + AS).

Uniparentality rates

With the self-fertility and apomixis rates assessed at the seed level, we classified the species in a biparental-uniparental scale (Table 4). First, the diploid cytotype in *P. pumilum* was sexual, with high seed set under self-pollination and having a UC > 0.5; it was therefore classified as a uniparental species (Table 4). Second, the diploid cytotype of *P. indecorum* was sexual and self-sterile, with a UC close to zero, and it was classified as biparental. In contrast, the triploid cytotype of *P. indecorum* was facultatively apomictic but self-sterile, showing an intermediate rate of uniparentality (Table 4). Third, sexual diploids in *P. maculosum* showed intermediate rates of self-fertility, having an intermediate uniparentality rate (Table 4). Triploids in *P. maculosum* were classified as biparental, as they showed no sign of apomictic or selfed seeds, and tetraploids were classified as uniparental, with the highest UC, as they reproduced by apomixis and showed intermediate proportions of selfed seed set (Table 4). Finally, diploids in *P. cromyorhizon* were scored as biparental, whereas tetraploids showed a high uniparental rate (Table 4).

DISCUSSION

Uniparentality is a feature of plants that multiply without mating partners, usually considered an advantage during colonization of new areas. Selfing, apomixis and vegetative multiplication are alternative types of uniparentality, and at least the first two have a relevant quantitative effect on plant fertility and, consequently, on the establishment or maintenance of a

new population. In this study, we analysed alternative reproductive modes, including biparental and uniparental types, and associated geographical ranges in four *Paspalum* spp. to show a clear trend between reproduction types from biparental to uniparental and the range of distribution of natural populations. The discussion that follows enlightens the reader on the evolutionary processes.

Selfing is associated with wider geographical distributions

Unlike self-fertile (uniparental) species, self-sterile ones (biparental) cannot produce a new generation without a reproductive partner, and this is expected to impose disadvantages for colonization of new areas. The independence from foreign pollen sources makes selfers more successful in new habitat colonization and increases chances of establishing a new population relative to self-sterile or dioecious species (Baker's Rule: Baker, 1955; Levin, 2010). Also, selfing plays a major role in the newly colonized area, where the lack of pollinators or compatible reproductive partners produces a pollen limitation affecting seed production (Burd, 1994; Larson & Barrett, 2000; Ashman *et al.*, 2004; Rambuda & Johnson, 2004; Eckert *et al.*, 2006).

To investigate the effects of biparental vs. uniparental reproduction on distribution patterns we studied the occurrence and genetic system of *P. pumilum* and *P. indecorum*. In our reproductive assessment of these diploid species, *P. pumilum* was self-fertile, exhibiting autogamous behaviour and displaying high seed set under self-pollination. In contrast, diploids of *P. indecorum* were highly self-sterile, showing low values (to null) of selfed seeds and thus an obligate allogamous behaviour. Despite the relatively low number of reports on the ploidy and reproductive biology of these species, the diploid cytotype of *P. pumilum* has consistently been found to reproduce by self-fertility, suggesting that this may be the situation throughout its distribution in the Americas. In contrast, reports on the distribution of the self-sterile diploid of *P. indecorum* are restricted to a small region in subtropical South America, where it can be considered endemic. Similar cases of narrow-wide patterns of geographical distribution associated with biparental-uniparental strategies are observed in other *Paspalum* spp. For example, sexual diploid self-sterile species like *P. bertonii* Hack., *P. chaseanum* Parodi or *P. equitans* Mez, have a limited distribution in north-eastern Argentina and bordering areas in Paraguay, Uruguay and Brazil (Zuloaga & Morrone, 2005; Ortiz *et al.*, 2013), whereas self-fertile species like *P. repens* P.J.Bergius occur from the southern USA and Mexico to northern Argentina (Chase, 1929; Burson, 1997; Zuloaga & Morrone, 2005; Ortiz *et al.*, 2013). Likewise, several examples in other plant species show a similar association between self-fertility and wider distribution, giving support to Baker's law (Rambuda & Johnson, 2004; Van Kleunen & Jonhson, 2007a, b; Van Kleunen *et al.*, 2008).

Species geographical expansion by long-distance dispersal events is strongly affected by the number of populations exporting seeds, i.e. propagule pressure (Baker, 1955, 1967, 1974; Levin, 2000; Peniston, Barfield & Holt, 2019). Although self-incompatible species need repeated dispersal events to the same area before establishing a new population, in self-compatible species a single event would be enough to establish a new population. Under self-fertility, each offspring is considered as a potential founder of a new dispersion cycle, which promotes changes in the distribution margins. In fact, self-fertility is a feature present in species considered as invasive or pioneers (Barrett, 1996; Bernardello *et al.*, 2001; Rambuda & Johnson, 2004; Van Kleunen *et al.*, 2008; Hao *et al.*, 2011). This explains the wider geographical distribution of sexual diploids with higher rates of self-fertility, like *P. pumilum*. Furthermore, in peripheral areas of the distribution of a species, self-fertility serves as a passive mechanism of reproductive isolation (Levin, 2000, 2010), favouring ecotype differentiation under environmental heterogeneity. Thus, self-fertility would facilitate population isolation, conducive to divergence and endemism by vicariance or founder effect speciation. Notably, isolated and endemic diploid species, which are closely related to *P. pumilum*, have been recently described, for example, the new endemic *P. chilense* Catanzaro & G.H.Rua ($2n = 2x = 20$) from southern Chile (Catanzaro *et al.*, 2015) and *P. giullietieae* Pimenta, G.H.Rua & R.P.Oliveira (Pimenta *et al.*, 2013) from eastern Brazil, both based on specimens previously identified as *P. pumilum*.

Influence of apomixis in range distribution

The co-existence of cytotypes and alternative modes of reproduction are important factors that define the pattern of geographical distribution in a species. In *Paspalum*, ploidy is strongly correlated with reproduction mode. Diploids always produce sexual seeds even though the formation of unreduced female gametes or apomictic seeds has been observed at low frequencies in some species (Hojsgaard *et al.*, 2008; Siena *et al.*, 2008; Delgado *et al.*, 2016). Polyploids from conspecific diploids always reproduce through facultative apomixis, whereby a plant might produce few sexual seeds among most asexual seeds. *Paspalum maculosum* and *P. cromyorhizon* were no exception, with diploids being sexual and tetraploids showing apomixis (pollination syndromes discussed next),

providing us an example for comparing among sexual and apomictic cytotypes regarding their distribution.

Like selfing, apomixis is an efficient strategy to expand species distribution beyond their range. Apomixis works fixing genotypes. Genotypes adapted to the local environment are provided with the advantage of reproductive assurance (by apomixis) to rapidly colonize the available habitats, thus expanding the distribution of the species (Hojsgaard & Hörandl, 2015, 2019). Moreover, apomixis provides an extra advantage by skipping the minority cytotype disadvantages compared to sexuality, and allows a single neopolyploid individual to establish a small population embedded in the parental population (Levin, 1975; Hojsgaard, 2018).

In nature, the sexual self-sterile diploid cytotype of *P. cromyorhizon* was scarce and geographically restricted, whereas the apomictic tetraploid cytotype showing a uniparental rate of 1.18 was common and widely distributed, extending beyond the range of the sexual diploids. The current and previous data on cytotype distribution, the relative abundance and larger distribution of the apomictic cytotype in *P. cromyorhizon* suggest that it possesses a better colonizing ability. Frequently, polyploids undergo mating system shifts increasing either self-compatibility or selfing rate, facilitating higher rates of establishment in new habitats relative to diploids (Husband *et al.*, 2008). This shift to self-compatibility in pseudogamous apomicts ensures endosperm formation through self-fertility. The combination of self-fertility and apomixis observed in the tetraploids of *P. cromyorhizon* provides substantial reproductive assurance and ensures its persistence in nature and its pioneering capacities.

Besides our previous comments on the advantages of self-fertility during dispersal events, apomixis enhances the establishment of new polyploid populations (Hojsgaard, 2018; Hojsgaard & Hörandl, 2019) and promotes geographical displacement between cytotypes (Karunarathne *et al.*, 2018) and ecological differentiation from the parental populations (Karunarathne *et al.*, 2020). In the case of *P. cromyorhizon*, historical collections of diploids from areas that currently have only tetraploid cytotypes indicate that apomictic polyploids might be outperforming sexual diploids. Such competition between cytotypes often explains observed patterns of geographical distribution of cytotypes in agamic complexes. The geographical distribution of *Paspalum notatum* Flüggé and *P. simplex* Morong also features widely distributed polyploid cytotypes towards the south and north of America and restricted diploid populations in northern and central Argentina (D´Aurelio *et al.*, 2004; Pozzobon & Valls, 1997; Urbani *et al.*, 2002; Brugnoli *et al.*, 2014).

The concept of geographical parthenogenesis is used to describe those agamic complexes in which apomictic cytotypes had a wider distribution compared to the sexuals (Bierzychudek, 1985; Kearney, 2005; Hörandl, 2006; Hörandl *et al.*, 2008; Tilquin & Kokko, 2016). Several taxa, including *Antennaria* Gaertner, *Rubus* L., *Taraxacum* F.H.Wigg. and *Ranunculus* L. are well-known examples of geographical parthenogenesis (Bayer, 1990; Van Dijk, 2003; Hörandl & Paun, 2007). In most of these agamic complexes, the apomictic cytotypes are widely distributed and sexual cytotypes are usually restricted to warm southern areas of their distribution (Bayer & Stebbins, 1987; den Nijs *et al.*, 1990; Hörandl, 2006). Nevertheless, there are complexes, including *Pilosella officinarum* F.W.Schultz & Sch.Bip. in which sexual cytotypes have a wide centred distribution and apomictic cytotypes range towards the south and north of the species distribution (Mráz *et al.*, 2008).

On the other hand, cytotypes can overlap niches, and diploids and polyploids co-exist in certain areas (Visger *et al.*, 2016; Mairal *et al.*, 2018; Duchoslav *et al.*, 2020). Alternatively, cytotypes may show no clear environmental differentiation complex cytogeographical pattern (Duchoslav, Fialová & Jandová, 2016; Duchoslav *et al.*, 2020; Spoelhof, Soltis & Soltis, 2017; Morgan *et al.*, 2020, and references therein). As in *P. cromyorhizon,* the area of the apomictic tetraploids in *P. maculosum* surpasses the sexual diploids (with a mixed pollination system, discussed next) at the south of the species distribution, where ploidy and modes of reproduction are well-studied. However, when comparing the cytotypes area towards the north of the distribution no clear differentiation was noted, probably due to the lack of ploidy and reproductive pathway reports in this area of the distribution of *P. maculosum*. This lack of a clear divergent pattern could relate to the size of the area occupied by the sexual diploids that being influenced by the mixed pollination syndrome (discussed next). In agamic complexes, such as *P. maculosum* or *P. cromyorhizon*, the range size of one cytotype influences the distribution of the other sympatric cytotypes and the degree of overlap among them (Visger *et al.*, 2016; Mairal *et al.*, 2018; Duchoslav *et al.*, 2020).

As observed in *Paspalum intermedium* Munro (Karunarathne *et al.*, 2018, 2020), polyploidy is probably a trait increasing the ecological tolerance and provides access to more variable environments, which, combined with apomixis and the loss of self-sterility, gives *P. maculosum* and *P. cromyorhizon* the potentiality to colonize and exploit them. Whether the observed patterns and colonizing abilities in tetraploids of *P. cromyorhizon* and *P. maculosum* follow a particular ecological genetic model for

the exploitation of resources, like the frozen niche variation or the general-purpose genotype models (Vrijenhoek, 1984), which might help us to explain our observations, it remains to be tested.

Mixed pollination syndromes dissolve geographical parthenogenesis patterns in agamic complexes

As stated before, in *P. maculosum* the observed differences in the geographical distribution of cytotypes are less pronounced than in other cases. Evidence in support of this hypothesis comes from comparing distribution of cytotypes in *P. cromyorhizon* and *P. maculosum*. Although uniparental apomictic tetraploids of *P. cromyorhizon* occupy larger areas compared to its conspecific biparental diploids, such a pattern in *P. maculosum* is absent. In this comparison, a moderate reproductive assurance given by a rate of uniparentality of 0.14 in diploids of *P. maculosum* probably favours their colonization abilities.

Monoploid and multiploid populations of *P. maculosum* exhibit a mixed pollination syndrome at the diploid level. A mixed pollination system is considered either as a transitory stage between outcrossing to selfing or as a stable reproductive system (Arista *et al.*, 2017, and references therein). To our knowledge, mixed pollination syndromes such as the one reported here for *P. maculosum* were previously unknown in *Paspalum*. Hence, in diploid *P. maculosum*, seed propagules derived from outcrossed and selfed events and this 'double-chance' seed set protects the diploid area against conspecific tetraploid competitors. A mixed pollination syndrome at the diploid level can promote both maintenance and expansion of the species range. Although self-sterility allows for exploiting genetic variability and reducing the consequences of inbreeding, self-fertility supports pioneering individuals to get established in new geographical areas. Moreover, since the diploids co-exist in some areas with tetraploids, the production of self-fertilized seeds reduces the frequency of $2x$–$4x$ matings and limits the negative consequences of interploidy crosses on plant fitness.

In the species range of *P. maculosum*, there is a contest regarding intraspecific uniparentality, in which diploids derived their advantage in colonization from self-fertility and tetraploids from apomixis strategies (see previous). Apomixis was present at the ovule stage in diploid *P. maculosum*, but was absent at the seed stage. So, uniparentality in *P. maculosum* results in a successful agamic complex in which the sexual diploids still retain a large area of distribution blurring the geographical divergence from the apomictic tetraploids. Probably, self-fertility in diploids plus apomixis and self-fertility in tetraploids act synergistically to expand the range of the species as a whole.

The uniparental contest hypothesis for the diploid-autopolyploid co-existence

Currently, only a few studies have considered the role of fertility in the performance of diploids in multiploid populations or contact zones (Rivero-Guerra, 2008; Kolář *et al.*, 2017) or regarding the success of neopolyploid establishment and diploid parent persistence (Norrmann & Keeler, 2003; Castro *et al.*, 2011; Castro *et al.*, 2020). However, most studies focused only on neopolyploid traits, such as origin, dispersal, establishment and evolutionary potential (McIntyre, 2012; Hülber *et al.*, 2015, Liu *et al.*, 2015; Duchoslav *et al.*, 2016; Kolář *et al.*, 2017; Gaynor *et al.*, 2018; Morgan *et al.*, 2020). Moreover, reproductive or geographical isolation of polyploids from the complex is considered to protect the polyploid cytotype during demographic establishment when it is infrequent (minority cytotype). When diploids (or other parental cytotypes of neopolyploids) are considered, studies focus on how co-existence is maintained in multiploid population (or contact zones), addressing the rates of unreduced gametes production, crossability rates within and among cytotypes and chromosomal, phenological, behavioural and mechanical changes among cytotypes (Norrmann & Keeler, 2003; Rivero-Guerra, 2008; Castro *et al.*, 2011; Kolář *et al.*, 2017; Castro *et al.*, 2020).

Most studies address the hypothesis that polyploids will evolve to occupy wider or more extreme environments than their diploid progenitors (Husband, Baldwin & Suda, 2013), but a differentiation of the ecological niche is not a requisite for autopolyploid establishment (Gaynor *et al.*, 2018). On the basis of our results concerning the autopolyploid establishment process, at least two hypothetical scenarios could be described after the polyploid cytotype overcame minority disadvantage. In the first, neopolyploids co-exist in a multiploid population intending to replace the $2x$ progenitor in the same range, and the fertility of each cytotype originates a counteracting propagule pressure. As a result of this interaction, the frequencies of each ploidy in a multiploid population vary over time according to the fertility displayed in each cytotype. As they compete for a successful propagule input, cytotypes achieve stable co-existence with minor changes in their frequencies within multiploid populations. Thus, uniparentality via self-fertility favours competition towards diploids, and uniparentality via apomixis promotes competition towards polyploids, as seen in the agamic complexes of

P. maculosum and *P. cromyorhizon*. In the alternative scenario, a multiploid population results in a monoploid state as the outcome of cytotype competition. Hence, the 'tug of war' ends up in a similar (*P. maculosum*) or shifted (*P. cromyorhizon*) distribution of polyploids regarding the distribution of their conspecific diploids.

Finally, uniparentality enables the local co-existence of multiple ploidies, allowing the colonization of new areas and decreasing exclusion of minority cytotypes. Future analyses of genetic variability from each population and the role of stochastic events should give a deeper insight into these population colonization histories and the role of uniparentality mediated by selfing and apomixis in them.

CONCLUSIONS

Detailed descriptions of genetic systems and distribution are now available for many species. This study reveals a contrast in geographical distribution patterns among uniparental and biparental reproductive strategies in close association with ploidy for four *Paspalum* spp. We observed that uniparental strategies (self-fertility and apomixis) enlarge the geographical distribution of the cytotypes with these strategies, and in some multiploid species, the contest among self-fertility and apomixis can blur the geographical differentiation among cytotypes allowing them to co-exist. This is our first attempt to estimate uniparentality rates combining observed self-fertility and apomixis rates in *Paspalum* spp. Nevertheless, to improve our future estimations of uniparental reproduction rates and their consequences in natural populations we will need to focus on the combination of both uniparental strategies.

To advance our general understanding of evolution in biparental and uniparental taxa, future studies regarding the distribution of genetic diversity (within and among populations) and the study of ecological tolerance and niche breadth in these species can contribute in several ways. First, differences in the genetic systems raise many hypotheses as to how cytotypic and genetic diversity arises and are maintained in natural populations, and how polyploidy, apomixis and self-fertility contribute, relative to genic diversity, to evolutionary divergence in plants. Second, the precise role of the mating system (combined with agamospermy) in shaping niche breadth of plant species remains unclear, and different studies have provided contrasting results. These investigations may shed light on the contribution of the mating system to the divergence of a taxonomically diverse genus through differential adaptation to abiotic/biotic environmental components.

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AUTHOR CONTRIBUTIONS

EJM, DHH and AIH planned and designed the study. All authors contributed to plant collection efforts. AVR conducted laboratory and field work, performed statistical analyses and interpreted data. The first manuscript draft was written by AVR. All authors provided feedback on the manuscript and have agreed to the submitted version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DATA AVAILABILITY

The data underlying this article are available in the article and in its online supplementary material.

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SUPPORTING INFORMATION

Additional Supporting Information can be found in the online version of this article at the publisher's website.

Figure S1. Geographical distribution of diploids and tetraploids of *Paspalum maculosum* and *P. cromyorhizon*, based on locations from collected populations and those reported in the bibliography (see Supplementary Table 1). A, Diploid *P. maculosum*. B, Tetraploid *P. maculosum*. C, Diploid *P. cromyorhizon*. D, Tetraploid *P. cromyorhizon*. In dark green, the circles 50 km in diameter depict the potential distribution of the cytotypes.

Table S1. Previous reports on cytology and reproductive modes (*S*, sexual; *Ap*, aposporous apomictic; *App*, aposporous potential, i.e. with occasional ovules with an aposporous sac beside the sexual sac; *ss*, self-sterile; *sf*, self-fertile) on single plant studies of selected *Paspalum* spp.

Table S2. Bibliography of collection sites with known ploidy (*x*) of the four studied *Paspalum* spp.

Table S3. Cyto-embryological analyses of 16 natural populations of four *Paspalum* spp. Number (*N*) and percentages (%) of ovules bearing four different embryo sac types. *MES*: meiotic embryo sac, *AES*: aposporous embryo sac; *MES* + *AES*: ovules bearing meiotic and aposporous embryo sacs, *IES*: immature, absent or undeveloped embryo sacs

Table S4. Comparisons of seed set during two flowering periods under self- and open-pollination conditions and total values (%) for both conditions in 16 natural populations of four *Paspalum* spp.

Table S5. Three-way ANOVA of seed set under two pollination conditions (self- and open pollination) and during two flowering periods (first and second) in four populations