

# Intraspecific ecological niche divergence and reproductive shifts foster cytotype displacement and provide ecological opportunity to polyploids

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- **Background and Aims** Niche divergence between polyploids and their lower ploidy progenitors is one of the primary mechanisms fostering polyploid establishment and adaptive divergence. However, within-species chromosomal and reproductive variability have usually been neglected in community ecology and biodiversity analyses even though they have been recognized to play a role in the adaptive diversification of lineages.
- **Methods** We used *Paspalum intermedium*, a grass species with diverging genetic systems (diploidy vs. autopolyploidy, allogamy vs. autogamy and sexuality vs. apomixis), to recognize the causality of biogeographic patterns, adaptation and ecological flexibility of cytotypes. Chromosome counts and flow cytometry were used to characterize within-species genetic systems diversity. Environmental niche modelling was used to evaluate intraspecific ecological attributes associated with environmental and climatic factors and to assess correlations among ploidy, reproductive modes and ecological conditions ruling species' population dynamics, range expansion, adaptation and evolutionary history.
- **Key Results** Two dominant cytotypes non-randomly distributed along local and regional geographical scales displayed niche differentiation, a directional shift in niche optima and signs of disruptive selection on ploidy-related ecological aptitudes for the exploitation of environmental resources. Ecologically specialized allogamous sexual diploids were found in northern areas associated with higher temperature, humidity and productivity, while generalist autogamous apomictic tetraploids occurred in southern areas, occupying colder and less productive environments. Four localities with a documented shift in ploidy and four mixed populations in a zone of ecological transition revealed an uneven replacement between cytotypes.
- **Conclusions** Polyploidy and contrasting reproductive traits between cytotypes have promoted shifts in niche optima, and increased ecological tolerance and niche divergence. Ecologically specialized diploids maintain cytotype stability in core areas by displacing tetraploids, while broader ecological preferences and a shift from sexuality to apomixis favoured polyploid colonization in peripheral areas where diploids are displaced, and fostered the ecological opportunity for autotetraploids supporting range expansion to open southern habitats.

**Key words:** Apomixis, flow cytometry, geographical parthenogenesis, Grinnellian niche, niche breadth, niche expansion, *Paspalum* L., polyploidy.

## INTRODUCTION

Polyploidy is now considered a widespread and ubiquitous phenomenon in the evolutionary history of species (Otto and Whitton, 2000; Van de Peer *et al.*, 2017). The present advent of new data and methods has propelled a tremendous interest in polyploidy, especially in plant sciences (Madlung, 2013; Soltis *et al.*, 2014). Even though the causality and consequences of polyploidy are better known now than when it was discovered around 100 years ago, we are just starting to understand the complex dynamics of polyploidization and its consequences for the ecological adaptation and evolution of taxa (Ramsey and

Ramsey, 2014). Biogeographical surveys on polyploid species suggest that multiple sets of chromosomes provide polyploids with short- and long-term advantages to better deal with environmental changes, range shifts, persistence across heterogeneous landscapes and colonization of new habitats (e.g. Hegarty and Hiscock, 2008; Parisod *et al.*, 2010). Polyploidization is a major driving force of genetic and ecological change, and a platform for speciation of vascular plants (Husband *et al.*, 2013). Recent studies showed that genome duplications have preceded rapid diversification events in different plant families (e.g. Soltis *et al.*, 2009; Wood *et al.*, 2009). Genome duplication provides genetic redundancy, creates genomic and phenotypic

novelty and influences the ecophysiology and genetic diversity of individuals by shifting heterozygosity levels and allelic combinations, and by introducing epigenetic changes and variation in gene expression. Hence, polyploidization plays a significant role enhancing adaptability to extreme habitats (Osborn *et al.*, 2003; Chen, 2010; Finigan *et al.*, 2012).

Polyploidization events in plants have been recurrently associated with niche divergence and ecological differentiation of cytotypes as an important mechanism for the establishment of new polyploids in nature. This facilitates both the maintenance of intraspecific cytotype diversity as well as sympatric speciation events among closely related taxa (Soltis *et al.*, 2004; Schluter, 2009; Givnish, 2010; Anacker and Strauss, 2014; Glennon *et al.*, 2014; Visger *et al.*, 2016). Thus, polyploidy alters the ecological niche of a species by broadening environmental tolerance and providing ecological and evolutionary flexibility (e.g. Dubcovsky and Dvorak, 2007; Fawcett *et al.*, 2009). Although there are many concepts of ‘niche’ (Soberón and Nakamura, 2009), the Grinnellian niche, which is defined by the sub-set of scenopoetic (non-interactive) environmental conditions under which populations of a species have positive growth rates (Grinnell, 1917; Soberón, 2007), is the one extensively used in recent years. This concept has become popular also because data for niche-defining variables (e.g. topography, average temperature, solar radiation, precipitation, etc.) are progressively becoming available for the entire planet (e.g. Turner *et al.*, 2003). At present, increasing availability of public databases [e.g. the Global Biodiversity Information Facility (GBIF); WorldClim] and information gateways [e.g. Geographic Information Systems (GIS)], and a renewed interest in plant polyploidy allow modern biogeography to use mathematical models [species distribution modelling (SDM)/ecological niche modelling (ENM)] (e.g. Elith and Leathwick, 2009; Soberón, 2010) to better understand how polyploidy and associated features influence niche evolution, habitat suitability and organism distributions.

Natural intraspecific trait variation associated with polyploidy, in particular reproductive modes, is widely known to affect plant physiology, ecology and dispersal abilities. Experimental studies indicate that intraspecific trait variation can have a significant effect on community ecology (Bolnick *et al.*, 2011; Araújo *et al.*, 2013). Intraspecific trait variation is expected to alter population density, niche breadth and the strength of the interaction among phenotypes, affecting the structure of the community, ecological opportunities and adaptive eco-evolutionary dynamics (Bolnick *et al.*, 2011; Wellborn and Langerhans, 2015). The study of traits such as cytotype diversity, dispersal ability, phenology, different reproductive modes and associated environmental signals is essential for understanding local and regional population dynamics (e.g. Castro *et al.*, 2012; Sonnleitner *et al.*, 2016; Visger *et al.*, 2016), provides insights into evolutionary pathways and forces driving species coexistence, ecological opportunity and adaptive diversification (Arrigo and Barker, 2012; Wellborn *et al.*, 2015). For example, reproductive shifts toward self-fertility and apomixis (asexual reproduction via seeds) are frequently linked to polyploid cytotypes (Asker and Jerling, 1992; Robertson *et al.*, 2010) and drastically affect species’ dispersal abilities and distribution patterns. Such features provide reproductive assurance to polyploids by enabling them to overcome density-dependent

reproductive limitations (e.g. minority cytotype disadvantage; Levin, 1975) and gamete incompatibility (Asker and Jerling, 1992; Hojsgaard *et al.*, 2014a), and facilitate ‘founder events’ (e.g. Baker’s Law; Baker 1955). Consequently, polyploids with better colonizing abilities may capitalize on ecological opportunities, achieve wider distributions leading to phenomena such as geographical parthenogenesis (e.g. Hörandl, 2006; Vrijenhoek and Parker, 2009) and enhance diversification abilities via reversals to sex (Hojsgaard *et al.*, 2014b; Hojsgaard and Hörandl, 2015).

Despite the increased interest in the topic, studies examining intraspecific diversity and modelling ecological divergence in diploid–autotetraploid species (e.g. Visger *et al.*, 2016), diploid–allopolyploid species (e.g. Sonnleitner *et al.*, 2016) or other diploid–polyploid associations (e.g. Raabová *et al.*, 2008; Chumová *et al.*, 2017; Paule *et al.*, 2017) are just starting to be feasible as high-resolution climatic data sets are becoming available. More studies carrying comprehensive analyses of intraspecific traits and bioclimatic conditions are needed and essential to better understand the natural forces underlying plant adaptation and distribution in different regions of the world. Here, we utilize the grass species *Paspalum intermedium* Munro ex Morong to decipher natural factors and stressors governing intraspecific trait diversity, cytotype coexistence and their dynamics within and among populations. Niche divergence and changes in phenology and reproductive strategies that may provide a platform for ecological opportunity are also studied to discern ecological consequences of polyploidy.

*Paspalum* L. is a grass genus with versatile genetic systems (Ortiz *et al.*, 2013). In *P. intermedium* specifically, two cytotypes with contrasting reproductive modes (sexual self-sterile diploids and apomictic self-fertile auto-tetraploids) are found intermingled in sympatry, parapatry or allopatry (Burson and Bennett, 1970; Normann *et al.*, 1989). The centre of diversification of the species is considered to be the sub-tropics of Argentina, where they occur in marshy grasslands in diverse phytogeographic formations along ecological gradients in Argentina, Paraguay, Bolivia and Brazil (Zuloaga *et al.*, 2012). The presence of divergent genetic systems (i.e. allogamy vs. autogamy, sexuality vs. apomixis and diploidy vs. polyploidy) is known to modulate a plant’s genetic diversity and its capacity to respond to environmental constraints that determine the distribution, ecological diversification and evolutionary potential of species and plant communities (Tilman and Lehman, 2001; Pauls *et al.*, 2013; Allan *et al.*, 2015). Hence, *P. intermedium* provides a unique opportunity for studying ecological signals and biological traits governing intraspecific trait variation along spatial and ecological gradients, cytotype coexistence, population dynamics and adaptation at local and regional geographic scales.

In the present study, we (1) evaluate the natural prevalence of *P. intermedium* cytotypes at various spatial scales; (2) evaluate reproductive and phenological shifts; (3) assess climatic and ecological preferences between cytotypes of *P. intermedium*; (4) determine the presence of singular ecological and biological signals driving cytotype distribution and dominance; and (5) provide evidence of niche differentiation between cytotypes and further insights into natural stressors governing the dynamic of cytotype associations, geographic

displacement and range expansions that contribute to local adaptation and ecological opportunity.

## MATERIALS AND METHODS

### *Sampling sites and collection of plant materials*

Plant materials were collected from Eastern Gran Chaco, central and Northern Mesopotamia (core distribution areas of the species), and Northern Pampas and Western Gran Chaco (peripheral distribution of the species) in Argentina (Table 1; Fig. 1; Supplementary Data Table S1) (see Zuloaga *et al.*, 2012). Additional information on cytotype occurrences was gathered from the literature and from material examined at different herbaria (MNES, CTES, BAA, SI, B, GOET, HUH and PE) (acronyms follow Thiers, 2017) (see Supplementary Data Table S2). Sampling was done during two different time periods (November/December and February/March) to avoid seasonal bias on cytotype frequencies and evaluate phenological differentiation between cytotypes. Changes in phenology were evaluated by grouping observations into early (October–December) and late (January–March) flowering followed by testing for independence.

Collection sites were categorized into (1) sites for ploidy determination only (up to three individuals were collected) and (2) sites to evaluate cytotype diversity and dynamics at the population level (on average 30 individuals per population were collected) (Supplementary Data Table 1). For the latter, sampling sites were selected to attain a maximum representation of the distribution range of the species (i.e. North–South and East–West), and include both macro-scale (among populations) and micro-scale (within populations) trends. Overall, samples were collected from 75 localities, out of which 35 were selected for population evaluations (Table 1; Supplementary Data Table S1). A transect spanning the longest length available across the population was followed to obtain information on local dispersal of cytotypes. An even representation of individuals within the population was attained by uniform sampling (i.e. the distance between two consecutive individuals was maintained the same, and varied between 4 and 12 m depending on the spatial

dimensions of each population). Young (i.e. smaller bushes with a diameter <40 cm with no or a few flowering stems) and mature individuals (i.e. taller bushes with a diameter >60 cm with many flowering stems) were distinguished and collected to account for individual turnover and overlapping generations. Several vouchers from all locations were prepared and deposited at different herbaria (CTES, MNES, BAA and SI).

### *Assessment of ploidy and reproductive trait variation*

The ploidy level of each sample was determined by flow cytometry (FC) estimations of relative nuclear DNA contents in comparison with a *P. intermedium* plant with known ploidy ( $2x = 2n = 20$ ). An AT-specific DNA fluorochrome, DAPI (4',6-diamidino-2-phenylindole) was used for FC with a CyFlow® Ploidy Analyser (Sysmex Partec GmbH, Görlitz, Germany). The protocol described by Suda and Trávníček (2006) for dried leaf materials was followed, with modifications (detailed in Supplementary Data Method S1). Histograms with a relative fluorescence intensity of around 5000 nuclei were analysed with CyView™ v. 1.5 data acquisition and data analysis software (Sysmex Partec GmbH, Münster, Germany). A maximum coefficient of variation (CV) value of 5 % was accepted for each sample peak ( $G_0/G_1$  peak). FC ploidy determinations were cross-checked with (1) repetitions of FC measurements in selected samples and (2) chromosome counts in cells at the mitotic division of 17 samples recognized as diploids or tetraploids through FC analyses (following Hojsgaard *et al.*, 2009). Mitotic metaphase cells were observed under a Leica DM5500B microscope (Leica Microsystems GmbH, Wetzlar, Germany) for chromosome counts.

Analysis of reproductive modes was conducted using FC. Open pollinated seeds from three randomly selected individuals per population were collected from a total of 20 *P. intermedium* populations with unknown ploidy (other populations did not bear mature seeds during the fieldwork). After ploidy determination, only three out of 20 were determined as diploid populations. A total of 500 seeds belonging to 15 populations (three diploids and 12 tetraploids) were used to assess variations in reproductive modes at geographic and/or cytotype levels following the methodology of Hojsgaard *et al.* (2014a) with a few modifications (details in Supplementary Data Method S2). The relative fluorescence intensity of around 3000 nuclei was analysed with CyView™, 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 *et al.* (2000) and following considerations for *Paspalum* spp. as in Hojsgaard *et al.* (2013). Sexually derived seeds have a diploid embryo ( $2n$ ; 2C-value) and a triploid endosperm ( $3n$ ; 3C-value), whereas seeds derived from apomixis carry a diploid embryo and a pentaploid endosperm ( $5n$ ; 5C-value) (for details, see Hojsgaard *et al.*, 2013).

### *Cytotype localities, environmental and climatic data*

Since scenopoetic variables (abiotic variables that do not interact with each other) are regarded as being associated with

TABLE 1. Summary of ecoregions, collection sites, number of individuals and ploidy levels of the *P. intermedium* plants analysed

Geographic region	No. of collection sites	Total no. of individuals	Ploidy ( $x = 10$ )
Mesopotamia	Populations	5	119
		17	460
	Solitary individuals	4	5
		6	10
Gran Chaco	Populations	10	301
		10	286
	Solitary individuals	6	6
		6	6
Pampas	Populations	–	–
		1	30
	Solitary individuals	–	–
		1	1
Total	68	1224	



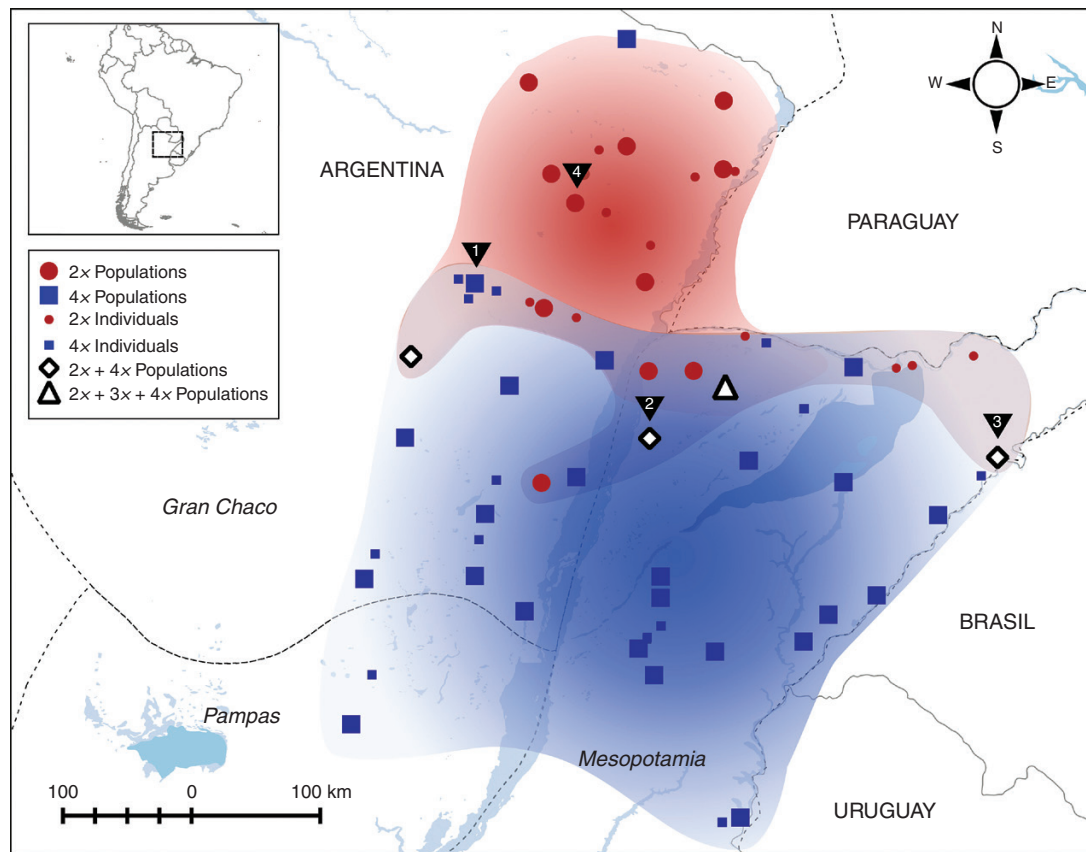


FIG. 1. Map displaying all collection localities of *P. intermedium* and ploidy levels determined in the present study. The North–South cytotype cline is apparent, together with an East–West transition zone where cytotypes occur intermingled in pure and mixed populations. Ploidies at sites of populations are represented by data from at least 30 individual plants. Ploidies at sites of individuals are represented by data from 1–3 individuals. Triangles 1, 2, 3 and 4 indicate populations (geographical sites) representing vis-à-vis ploidy shifts between previous and present records (see the Discussion). Grey lines demarcate country boundaries (block letters) and dotted lines separate ecoregions (italic letters) (*sensu* Cabrera, 1976).

heritable components of the physiology of species (Kearney and Porter, 2009), it was assumed that (1) geographic distribution reflects adaptation and underlying ecological tolerance and (2) the occurrence data assembled here are a non-biased representation of intraspecific diversity and variability. Thus, the absence of any cytotype in a geographic area was considered to be a result of natural processes underlying ecological signals, adaptation and evolutionary mechanisms acting within the species.

Ecological data of 26 bioclimatic variables were downloaded from various open-source databases. Nineteen commonly used bioclimatic variables were retrieved from the WorldClim data set (1950–2000; version 1.4) at 2.5 arc-min resolution (approx. 5 km<sup>2</sup>) (Hijmans *et al.*, 2005; <http://www.worldclim.org>). The elevation data were downloaded from the Shuttle Radar Topography Mission (SRTM; <http://srtm.csi.cgiar.org/>) elevation data set at 30 arc-s (approx. 1 km<sup>2</sup>) resolution. Photosynthetically available radiation (PAR) data were downloaded from the Moderate Resolution Imaging Spectroradiometer (MODIS) database (Myneni *et al.*, 2015; <https://lpdaac.usgs.gov>). The annual mean UV-B radiation data set was downloaded from gIUV (a global UV-B radiation data set for macroecological studies) at 15 arc-min resolution (Beckmann *et al.*, 2014; [www.ufz.de/gIUV](http://www.ufz.de/gIUV)). Cloud cover percentage, vapour pressure and frost day frequency data were downloaded from CGIAR CSI ([www.cgiar-csi.org](http://www.cgiar-csi.org)) at

30 arc-s resolution. Finally, soil type data (soil taxonomy) were downloaded from the SoilGrids database (ISRIC, 2015; [www.soilgrids.org](http://www.soilgrids.org)) in 30 arc-s resolution. Data sets with different resolutions were either aggregated or disaggregated to 2.5 arc-min accordingly using the bilinear method (Hijmans and Van Etten, 2015) to match WorldClim data. R packages ‘sp’ (Bivand *et al.*, 2013) ‘maptools’ (Bivand and Lewin-Koh, 2015) and ‘raster’ (Hijmans *et al.*, 2015) were used in these steps.

For the analysis of past ecological niches of cytotypes, bioclimatic variables for past climatic conditions [Last Glacial Maximum (LGM) – approx. 21 000 years before present (ybp) and Mid-Holocene (MH) – approx. 6000 ybp] were retrieved from WorldClim for two different scenarios (BCC-CSM1-1 and CCSM4; see [www.worldclim.org](http://www.worldclim.org)) at 2.5 arc-min resolution.

#### Environmental niche modeling

Species and cytotype distribution models were constructed using MaxEnt v. 3.3.3k (Phillips *et al.*, 2006). A raster grid stack of all 26 bioclimatic variables for the entire South American continent was generated and the relevant data at each collection point for cytotype distribution analysis were extracted using the R package ‘dismo’ (Hijmans *et al.*, 2016). A multiple logistic

regression was performed to test ecological preferences and associations between ploidy and environmental variables. The equation takes the form of

$$E[Y_i | X_i] = \text{logit}(p_i) = \ln\left(\frac{p_i}{1 - p_i}\right) = \beta_0 + \beta_1.X_{2,i} + \dots + \beta_m.i$$

where *logit*(*p<sub>i</sub>*) is the probability of occurrence of one cytotype, *X<sub>i</sub>* is the value of environmental variables (predictor) and *β* is a regression coefficient. The R-package ‘nnet’ (Venables and Ripley, 2002) was used for the analysis. Principal component analysis (PCA) was performed for the 26 variables using the R-package ‘vegan’ (Oksanen et al., 2016) to determine the main drivers of the niche space and cytotype differentiation. To avoid overfitting the data and minimize niche aggregation of cytotypes, we removed predictor variables exhibiting high pair-wise correlation values (Fisher weighted mean *r* values >0.85) and high collinearity on multiple logistic regression and PCA ordination output. Based on these criteria, 15 bioclimatic and environmental variables were retained and used as predictors to calibrate distribution models in MaxEnt (see Table 2).

For reconstructing past niches, data on PAR, UV-B, cloud cover percentage, vapour pressure, frost day frequency and soil type were not available. Therefore, only eight out of 15 selected bioclimatic predictors together with elevation data (see Table 2) were used for model calibration. Distribution of both cytotypes based on their realized Grinnellian niches was modelled with the present data and simulated into two past climatic periods, the MH and the LGM. The accuracy of past predictions was assessed against predictions for the present data using the area under the model’s receiver operator characteristic (ROC) curve (AUC values), a threshold-independent ROC analysis that measures the performance of models (Hanley and McNeil, 1982).

*Niche breadth and overlap*

Niche characteristics were extracted using parametric generalized models. Coarse spatial resolution of ecological and geographic properties of the species was used to define Grinnellian niches of cytotypes. Niche breadth and niche shifts were computed as 1.5 s.d. of the Euclidian distance from the centroid of an individual’s cloud for each cytotype and weighted by the Eigenvalues of PCA ordination axes, respectively. The amplitude of cytotype-specific habitat distribution and ecological requirements was considered as a measure of Grinnellian realized specialization (Devictor et al., 2010).

Schoener’s D index was used to assess the overall overlap of the environmental niche space between cytotypes. Therein, the similarity of the niches was summarized from 0 (no similarity) to 1 (complete similarity). The obtained niche overlap was plotted against a randomly simulated niche overlap generated with the assumptions of both niche equivalency and similarity (as described in Broennimann et al., 2012). In order to avoid uninformative data extraction for background environment, environmental data were extracted from random points (500 for diploids and 800 for tetraploids; the number of points was empirically chosen based on the highest AUC values of the SDM) drawn from a circular area around the observed data points. Simulations for niche similarity and equivalency were performed in 1000 replicates each, using the R package ‘ecospat’ (Di Cola et al., 2017).

A new approach was used to visualize density distributions of each cytotype in a collective environmental gradient (CEG). The CEG was computed utilizing all the selected environmental variables (predictors). The data set was transferred into a table with predictors in columns and geographic points in rows. A *z*-transformation was applied to all the predictors (columns) to create a CEG for each cytotype where all variables are collapsed into one single gradient. The transformed values for each locality and cytotype were summed up and used to obtain a ‘collective’ value representing the overall ecological setting for that particular geographical point assuming that all predictors

TABLE 2. Bioclimatic and environmental variables retained for cytotype distribution and niche analysis in *P. intermedium*, its significance values and PCA contributions

Environmental variable	Code	<i>P</i> -value	PC1	Co <sup>2</sup>	PC2	Co <sup>2</sup>	AC
Annual mean temperature (°C)	BIO1	0.0040	−0.9313	0.867404	0.31867	0.101556	8.796792
Isothermality (BIO2/BIO7) (× 100)	BIO3	0.0011	−0.6329	0.400577	0.53660	0.28795	6.974405
Temperature seasonality (s.d. × 100)	BIO4	0.0001	0.96151	0.924507	−0.02525	0.000638	8.07602
Minimum temperature of coldest month (°C)	BIO6	0.0072	−0.9018	0.813358	−0.18677	0.034883	7.5197
Temperature annual range (°C)	BIO7	0.0050	0.6018	0.362187	0.56517	0.31942	7.01954
Mean temperature of wettest quarter (°C)	BIO8	0.0135	−0.2564	0.065749	0.8668	0.751362	9.65046
Mean temperature of driest quarter (°C)	BIO9	0.00013	0.9687	0.938464	0.21164	0.044792	8.73122
Mean temperature of warmest quarter (°C)	BIO10	0.00063	−0.8491	0.721005	0.41351	0.170999	8.35803
Mean temperature of coldest quarter (°C)	BIO11	0.0015	−0.9621	0.925642	0.22777	0.05188	8.70494
Precipitation seasonality (CV)	BIO15	0.0081	0.2617	0.068509	0.91909	0.84474	10.80257
UV-B radiation (J m <sup>−2</sup> d <sup>−1</sup> )	U	0.00177	−0.7011	0.806261	0.67793	0.133702	9.84241
Photosynthetically active radiation (PAR)	P	0.0021	−0.8979	0.49162	0.36565	0.45959	8.65153
Frost day frequency (days per year)	F	0.01733	0.6853	0.874856	0.07113	0.060288	4.16024
Surface vapour pressure (hPa)	S	0.002	−0.9353	0.469697	0.24553	0.00506	8.36329
Elevation	E	0.0231	−0.5258	0.27651	0.22309	0.049773	3.01441

*P*-value, significance values (*α* = 0.05) of the multiple logistic regression analysis on climatic and ecological preferences between diploids and tetraploids; AC, sum of absolute contributions of variables to principal components 1 and 2; PC1 and PC2, eigenvalues of first two axes of the PCA for the ordination of variables demarcating the niche space between the two cytotypes; Co<sup>2</sup>, squared co-ordinates of variables (higher values indicate better representation of variables in the principal components).

contribute to the occurrence of the cytotype at a given location. Collective values were then mapped into the CEG to obtain a kernel density estimation (KDE) (probability density) and to visualize any trend on main ecological preferences relative to each cytotype. For the KDE, a bandwidth of 0.5 s.d. was applied to achieve a moderate smoothing of the resulting density curves (Fig. 2). The CEG was structured into quartile and interquartile points to assess the significance of the data and to better visualize the relative ecological differentiation between cytotypes along the collective environmental gradient (Fig. 2).

### Statistical analyses

Complete spatial randomness (CSR) of all the occurrences was tested with  $K$ -function (also Ripley's  $K$ -function) in the R package 'spatstat' (Baddeley and Turner, 2005), prior to all the statistical analyses and modelling. Furthermore, a  $\chi^2$  dispersion test for spatial point patterns based on quadrat counts (quadrat-test; Baddeley and Turner, 2005) was used to test spatial separation of cytotypes in the sampling area (further details are given in Supplementary Data Fig. S5). All the statistical analyses and mapping were performed in R version 3.3.2 (R Core Team, 2016) unless mentioned otherwise. QGIS [QGIS Development Team. Open Source Geospatial Foundation (2016)] was used for visualization and creating maps.

## RESULTS

### Ploidy level variation, local and regional spatial separation

The ploidy evaluation of a total of 1224 individuals revealed two major cytotypes: diploids ( $2n = 2x = 20$ ;  $N = 431$ ;

35.2 %) and tetraploids ( $2n = 4x = 40$ ;  $N = 793$ ; 64.8 %) (Supplementary Data Fig. S1a, b; Method S1). In addition, one triploid individual ( $2n = 3x = 30$ ) was also recorded (Supplementary Data Table S1). Thirty-one out of the 35 (88.6 %) populations were uniform, consisting of pure diploid or pure tetraploid plants; the remaining four (11.4 %) were mixed-ploidy populations (Hojs456, Hojs470, Hojs481 and Hojs487). Populations consisting of multiple cytotypes are rare in *Paspalum*, and this is the first record for *P. intermedium*. The tetraploid was the most common cytotype, present in 28 (24 pure tetraploid) populations, while the diploid cytotype was found in 15 (11 pure diploid) populations (Fig. 1; Table 1). The rare triploid cytotype was found in a mixed-ploidy ( $2x-4x$ ) population (Fig. 1).

On comparison with previous records by various authors (Supplementary Data Table S2), we observed a shift of ploidy in four localities: three along a contact zone between cytotypes and one in the core distribution area of diploids (details in Figs 1 and 2; Supplementary Data Tables S1 and S2).

The within-population sampling strategy unveiled local-scale distribution patterns of cytotypes in mixed populations (Supplementary Data Fig. S2). While one population (Hojs456) had only five diploid individuals restricted to one end of the population, the rest had various numbers of diploid and tetraploid cytotypes mixed in different patterns along the sampling line (Supplementary Data Fig. S2). A Mann-Kendall rank test for randomness (in the R-package 'randtests'; Caeiro and Mateus, 2014) indicated a non-random distribution in the occurrence of cytotypes along the sampling transects ( $P \leq 0.01$  in all cases), suggesting that the local-scale distribution patterns and turnover followed a certain clustering order. In addition, in population Hojs470, we collected eight young individuals (seven tetraploids and one diploid) widespread among mature individuals (Supplementary Data Fig. S2).

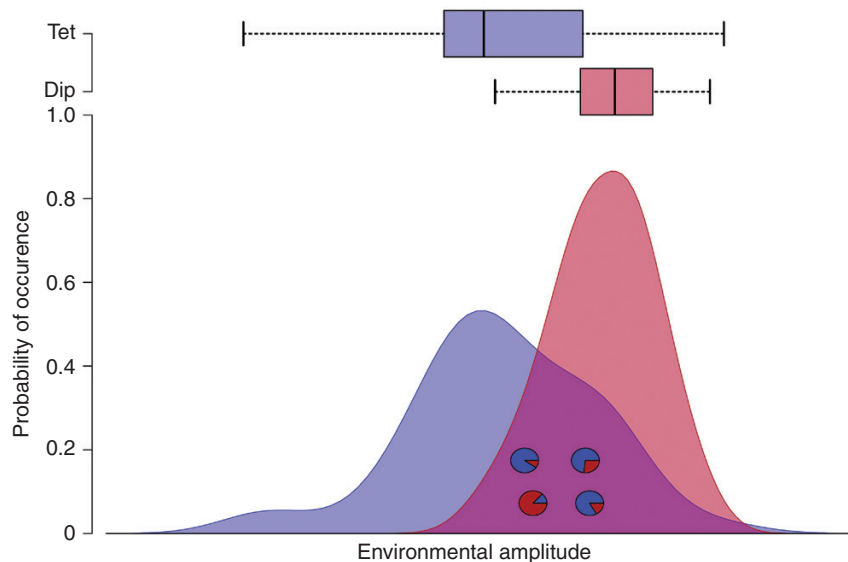


FIG. 2. Niche breadth of diploid (red) and tetraploid (blue) *P. intermedium* cytotypes depicted as probability density function for occurrences along the collective environmental gradient of the ecological requirements of species. Boxplot-ranked sets of data (quartiles and interquartile range) further illustrate ecological differentiation between cytotypes. The ecological gradient of species coalesces the observed environmental heterogeneity after pooling ( $z$ -transformed) data of environmental variables showing significant differences ( $P < 0.05$ ). Pie charts indicate the relative position of mixed-ploidy populations within the environmental gradient ( $x$ -axis) (upper left, Hojs487; lower left, Hojs481; upper right, Hojs470; lower right, Hojs456), and slices represent percentages of each cytotype (red = diploids, blue = tetraploids).



The spatial randomness test for the recorded occurrences of the two cytotypes with Ripley's  $K$ -function showed deviations of  $\lambda K(r)$  (the expected vs. observed number of points per unit area) from the Poisson (theoretical) distribution (paired-end  $t$ -test  $P < 0.001$ ) (Supplementary Data Fig. S3a). This indicates a non-random distribution of ploidy levels among all geographically dispersed data points. In addition, a Pearson  $\chi^2$  goodness-of-fit test using quadrat counts showed a deviation ( $\chi^2 = 161.14$ ,  $P = 0.0001$ ; Supplementary Data Fig. S3b) in the observed distribution compared with the null distribution, thus confirming a clustering of cytotypes along the observed North–South spatial separation and East–West contact zone of sympatric and parapatric occurrences (mixed-ploidy populations were considered both diploid and tetraploid in the analysis; triploids were not considered).

#### Cytotype reproduction modes and ploidy as a proxy for reproductive biology

A total of 500 seeds originating from 45 individuals (100 seeds from nine diploids and 400 seeds from 36 tetraploids) were analysed to assess reproductive modes of *P. intermedium* (Table 3). Single-seed histograms produced two types of peak configurations, corresponding to different embryo to endosperm DNA content ratios: peak configurations 2C:3C correspond to sexual seeds, carrying a diploid embryo and a triploid endosperm; peak configurations 2C:5C correspond to clonal seeds, carrying a parthenogenetic diploid embryo and a pentaploid endosperm (see details in Supplementary Data Method S2; Fig. S1c). In diploid plants, only seeds with a 2C:3C peak configuration were observed, and therefore diploids are considered as obligate sexuals (all seeds were produced after syngamy of meiotic gametes). Tetraploid plants presented a moderately low proportion of sexual seeds (<30%; Table 3) and a larger amount of clonal seeds (>70%; Table 3). Hence, tetraploids are considered as facultative apomicts.

Since ploidy levels in *P. intermedium* (Norrman et al., 1989; this study) as well as in other *Paspalum* spp. and grasses (e.g. Galdeano et al., 2016) are tightly connected to divergent reproductive syndromes, our reproductive screenings validate the use of ploidy as *a priori* information and a proxy for reproductive biology in *P. intermedium*.

#### Phenological shift and intraspecific ecological differentiation of cytotypes

In our analysis of phenology, we recorded a total of 38 sites in flowering during the early and late season trips and added 33 records of materials with known ploidy from herbaria. Three

out of four mixed-ploidy populations were flowering; however, ploidy levels of individuals were unknown during collection. The probability of blooming incidence per ploidy indicates that diploids tend to flower early in the season ( $n = 24$ ;  $P = 0.0074$ ; d.f. = 1) while the probability of finding tetraploids with flowering stems was the same for both the early and the late summer periods ( $n = 47$ ;  $P = 0.2108$ ; d.f. = 1).

Generalized linear models with multiple error distribution (logistic regression) detected 15 environmental variables having significant differences between cytotypes ( $P \leq 0.0147$  in all cases; Table 2), indicating unique ecological and climatic preferences. Thus, most extreme cytotype differences were found for Bio1, annual mean temperature ( $P = 0.00019$ ); Bio6, minimum temperature in the coldest month ( $P = 0.00037$ ); and Bio15, precipitation seasonality ( $P = 0.0114$ ) (Supplementary Data Figs S4 and S5). PAR and UV-B radiation (mean value for diploids, PAR =  $0.532 \pm 0.009$  J m<sup>-2</sup>, UVB =  $415 \pm 79$  J m<sup>-2</sup>; tetraploids, PAR =  $0.511 \pm 0.01$  J m<sup>-2</sup>, UVB =  $402 \pm 92$  J m<sup>-2</sup>; Supplementary Data Fig. S4) also showed strong association with differences in climatic preferences between cytotypes ( $P = 0.000138$  and  $0.00026$ , respectively). The elevation, however, did not show a strong correlation as the species distribution range is restricted to a topographically flat area ( $58\text{--}156 \pm 18.2$  m for 2x;  $35\text{--}93 \pm 12.9$  m for 4x; Table 2; Supplementary Data Figs S4 and S5).

Scenopoetic variables gathered from all localities defined the realized niche of species and displayed divergent differences between cytotypes (Supplementary Data Fig. S4), with tetraploids occupying broader environmental ranges than diploids. The diploid range was fully enclosed within the tetraploid range in eight out of 13 variables, and tetraploids included the core of the ecological preferences of diploids (i.e. interquartile ranges) in 12 out of 13 variables (Figs 2 and 3). In the PCA, two principal components explained the majority of environmental variation observed for the *P. intermedium* data set (see Supplementary Data Fig. S5). PC1 (represented 44.1% of the variation) was explained by temperature-related variables, vapour pressure, PAR, UV-B radiation, frost day frequency and soil type, and defined the Euclidean space of diploids. PC2 (represented 31.8% of the variation) was best explained by precipitation-related variables (Table 2; Supplementary Data Fig. S5). The PCA revealed a shift in the Euclidean space between cytotypes along the PC1 axis and a large overlap along the PC2 axis, with tetraploids having a greater niche breadth (Figs 2 and 3). The differentiation of niche optima in Fig. 3 is defined as the Euclidean distance between centroids of ellipses weighted by the inertia of the first two axes after decomposition of inertia (six axes). The observed niche overlap of diploids and tetraploids (Schoener's  $D = 0.25$ ) is significantly lower ( $P = 0.0099$ ) than the simulated overlap

TABLE 3. Reproductive mode variation between cytotypes of *P. intermedium* in the study area

Ploidy	$n$ (pop.)	$n$ (ind.)	Seeds	PI $\pm$ s.d.	Rep. path	Proportion $\pm$ s.d.
Diploid	3	9	100	$1.45 \pm 0.039$	Sex.	$100 \pm 0.0\%$
Tetraploid	12	36	400	$1.49 \pm 0.089$	Sex.	$27.9 \pm 7.0\%$
				$2.40 \pm 0.071$	Apo.	$72.1 \pm 9.7$

$n$  (pop.), number of populations;  $n$  (ind.), number of individuals; Sex., sexuality (cross- and self-fertility); Apo., pseudogamous apomixis; PI, peak index or endosperm:embryo peak ratio in flow cytometry analyses; Rep. path, reproductive pathway.

(mean = 0.65) under niche equivalency (Supplementary Data Fig. S6a). On the other hand, the niche similarity test indicated that the observed environmental space similarity is higher than expected on a random basis ( $P = 0.297$ ) (Supplementary Data Fig. S6b). These reject the null hypothesis that diploids and tetraploids occupy equal climatic niche spaces, and recognize that both cytotypes occupy habitats with slightly dissimilar climatic regimes and environmental resources.

The collective environmental gradient further shows this tendency by visualizing the probability density of cytotypes along a continuous gradient representing all ecological settings of the species. Diploids are symmetrically centred and display inferior ecological amplitude compared with tetraploids (Fig. 2). In contrast, tetraploids can grow in a wider range of environmental conditions, exhibiting a broader yet lower probability density along the gradient with a median value and main ecological preferences shifted away from those of diploids (Fig. 2). Overall, tetraploids display wider ecological amplitude and are ‘generalists’, while diploids are ‘specialists’. Environmental niche differentiation of *P. intermedium* cytotypes followed a latitudinal gradient where tetraploids grow under more extreme environmental conditions (e.g. lower temperatures and light radiation) and therefore can cope well with seasonal changes in southern areas.

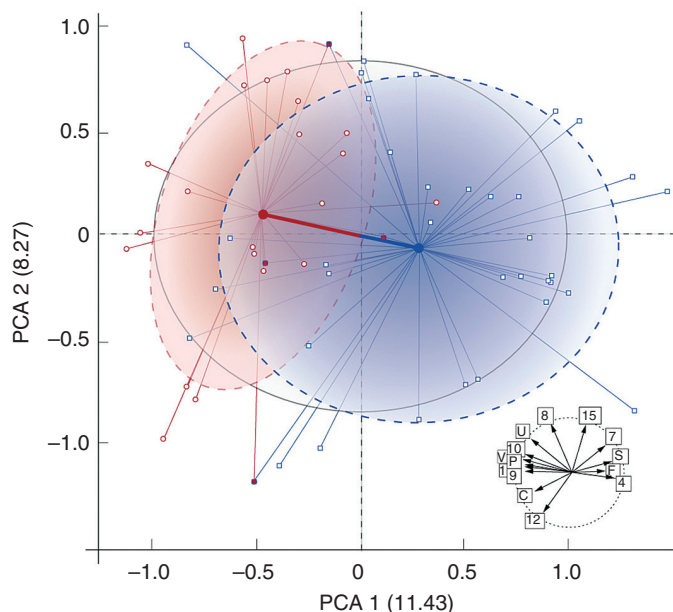


Fig. 3. Multidimensional analysis of the ecological niche of *P. intermedium* and the shift in niche optima between cytotypes. The specialization of cytotypes and differentiation of niche optima are reflected by the multidimensional volume (here represented in a 2-D space) represented by the spatial distribution of points (collection sites, each indicating a particular environmental set-up), and the distance between centroids of ellipses. Main environmental variables used in the ordination are shown in the correlation circle ( $r = 1$ ; codes follow Table 2). Red circles symbolize diploids, blue squares tetraploids and red-filled blue squares heteroploid sites. Eigenvalues for inertia of the first two axes are given in parentheses. Red and blue arrows indicate the direction of the shift in niche optima for diploid and tetraploid cytotypes. The grey-shaded ellipse represents the niche space of the species. Dotted ellipses indicate diploid (red) and tetraploid (blue) niches.

#### Ecological niche modelling and past distribution

Model simulations produced high AUC scores (0.83 and 0.81 for diploids and tetraploids, respectively) and thereby highly accurate predictions of climatic niche spaces which reflect the realized range of distribution of each cytotype. Prediction scores  $>0.65$  were considered strong signals for habitat suitability of the cytotypes (see Fig. 4). MaxEnt predictions show that the environmental niche spaces of both cytotypes nearly reflect their realized niches in the sampling area, with a few exceptions. For example, the climatic niche of diploids is marginally expanded toward the North-east of their realized distribution (Fig. 4; Supplementary Data Table S2), and the climatic niche of tetraploids is expanded toward the North along a stretch of the Paraguay River, reaching Bolivia and Brazil (Fig. 4). Likewise, a surprisingly large area predicting a niche overlap around the core distribution of diploids is not realized for the distribution range of tetraploids. The complete absence of tetraploids in this region suggests a zone of cytotype exclusion (Figs 1 and 4).

Projections of past environmental niche space of *P. intermedium* for both CCSM4 and BCC-CSM1-1 past climatic scenarios showed a temporo-spatial range shift in estimated spatial distributions of cytotypes. Both ecological models indicated the absence of suitable habitats for either cytotype in our current sampling area during the LGM (25 000 ybp), and the presence of suitable climatic conditions for diploids in northern Bolivia and central and southern parts of Brazil (Supplementary Data Fig. S7). Towards the MH (approx. 6000 ybp), changes in environmental conditions in the South American continent moved habitat suitability of diploids towards southern parts of Brazil, Paraguay and northern Argentina, thus expanding its distribution area, and priming the conditions for a successful establishment of tetraploids as projected for northern Argentina and southern Paraguay (Supplementary Data Fig. S7).

## DISCUSSION

Knowledge on plant distributions and intraspecific trait variability is central to underpin ecological and physical factors affecting evolutionary dynamics and history of species. Studies on cytotype distributions and associated environmental and reproductive traits provide valuable insights into diploid–polyploid dynamics and factors responsible for contraction–expansion cycles (e.g. Cosendai and Hörandl, 2010; Caperta et al., 2016; Sonnleitner et al., 2016). The present macro- and micro-scale study on *P. intermedium* is an attempt to recognize environmental factors and biological traits affecting cytotype coexistence, population dynamics and ecological adaptation outlining early events endowing polyploidization and speciation in plants.

#### Cytotype composition and distribution patterns

The distribution range of *P. intermedium* is centred in north and eastern Argentina with records spanning to Southern Brazil, eastern Paraguay and northern Uruguay (Zuloaga et al., 2012). The study revealed a North–South spatial segregation of the two cytotypes with a narrow East–West overlapping



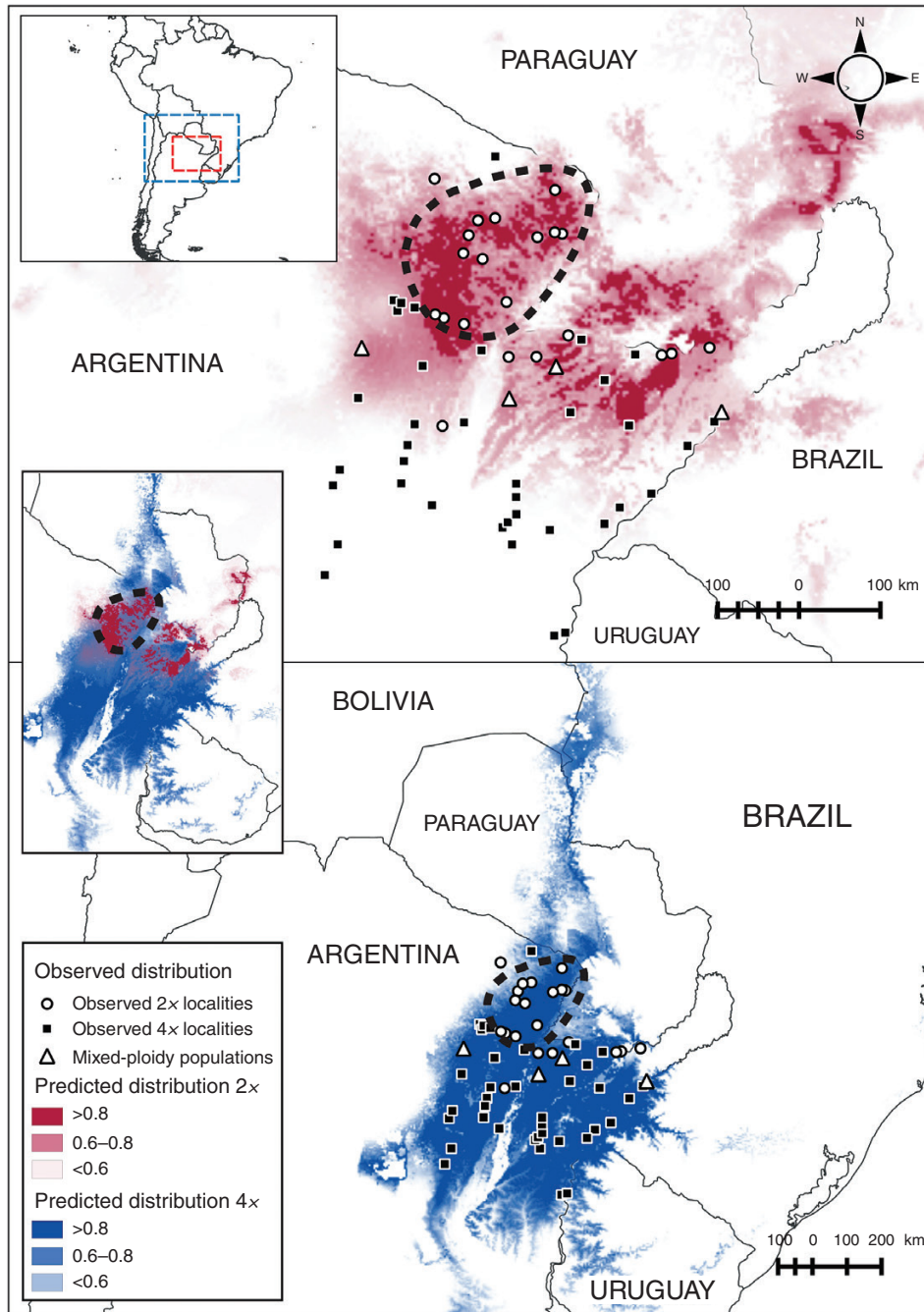


FIG. 4. The output of species distribution modelling using MaxEnt. The map shows the realized and potential distribution ranges of diploid (red) and tetraploid (blue) cytotypes of *P. intermedium* in the study area. The realized and the potential habitat suitability (the probability of occurrence inferred from the model output values of the AUC) shows similar predicted distributions for diploids, but not for tetraploids. The area inside the dotted line represents a zone where ecological conditions meet the requirements for the coexistence of diploids and tetraploids, yet it is only occupied by diploids.

zone in the centre. Despite the fact that tetraploids are evolutionarily younger than diploids (see below), it has become the most common cytotype, occupying two-thirds of the species' geographic range.

Odd polyploids are known to be infrequent in nature; however, they provide crucial information on fundamental mechanisms of polyploid formation and establishment (e.g. Ramsey and Schemske, 1998; Husband, 2004). Previous and present

findings of rare triploid cytotypes of *P. intermedium* do not provide much information, but rather an opportunity to study the role of triploids in polyploid formation and population dynamics that need to be addressed in a set of new experiments. The presence of one tetraploid population in the far North-west of the species' distribution may represent a case of polyploidization in the northern periphery of diploids, a glimpse of the progression of past cytotype displacement

(further details below), a consequence of anthropogenic activities (extensive human intervention in the area is evident through agricultural activities) or a combination of such factors.

#### *Ecological specialization and niche differentiation between cytotypes*

In *P. intermedium*, the realized niche specialization based on environmental parameters and background similarity tests, niche breadth and cytotype densities showed significant differentiation on ecological requirements between cytotypes, indicating that diploids are adapted to a narrow range of ecological settings compared with tetraploids. By having a broader and transgressive niche breadth, tetraploids enclose the whole range of climatic preferences of diploids; a situation observed in other polyploid complexes as well (e.g. *Claytonia perfoliata* complex, McIntyre, 2012; Tolmeia, Visger et al., 2016), and expected in autopolyploids such as *P. intermedium*, recurrently originated from a diploid's gene pool. However, unlike in the *C. perfoliata* complex (McIntyre, 2012) and other autopolyploid systems such as *Allium oleraceum* (Duchoslav et al., 2016), in *P. intermedium* we observe a significant separation of ecological optima between cytotypes, suggesting a segregation of ploidy-related ecophysiological aptitudes for the exploitation of environmental resources after polyploidization. Recently established polyploids usually display intermediate ecological preferences compared with diploids and established polyploids (e.g. Maherali et al., 2009; Levin, 2011). Similarly, tetraploids from mixed populations in *P. intermedium* (presumably the product of recent polyploidization events and therefore younger than those occurring in southern areas) show ecological preferences intermediate to those observed in diploids and general tetraploids, which further reinforces our observations of cytotype dynamics. Different studies of niche shifts in autopolyploids support the common hypothesis that polyploids evolve to occupy wider or more extreme ranges than their progenitors (reviewed in Spoelhof et al., 2017). Accordingly, the directional and opposed shift in niche optima observed between *P. intermedium* cytotypes, the bimodal distribution of cytotypes and skewness observed for tetraploids along the collective environmental gradient suggest the action of past disruptive selection on established tetraploids favouring a divergent departure between the ecological preferences of cytotypes. Niche differentiation is a primary mechanism to avoid competitive exclusion by diploid progenitors and foster polyploid establishment (e.g. Levin, 2003; Ramsey, 2011). Thus, establishment and persistence of *P. intermedium* polyploids apparently took place at the expense of becoming (sub-) adapted to a broader range of ecological conditions and less competitive in areas where multidimensional space meets the niche optimum of diploids. The question remaining is whether the observed broader ecological tolerance is due to an effect of polyploidy (e.g. Kearney, 2005) or caused by the fact that asexuality is probably freezing a range of genotypes among autopolyploid clones (carrying a sub-set of genes from the diploid ancestors) adapted to local narrow niches (Vrijenhoek, 1979). While a shift from sexuality to apomixis can partition the use of resources in polyploids, it may not necessarily affect the niche dynamics (Dellinger et al.,

2016). Nevertheless, achieving reproductive assurance through asexuality certainly shields polyploids from environmental stress (Freeling, 2017; see next section) and confer higher colonizing ability to tetraploids (Hörandl, 2006; Hojsgaard and Hörandl, 2015). Molecular genetic analyses will benefit us to better understand the origin of tetraploids, the competitive dynamics between cytotypes within mixed populations, and the effect of asexuality and environmental stressors on partitioning genetic diversity and resource use.

#### *Population dynamics, ploidy shifts and ecological displacement between cytotypes*

The North–South distribution pattern of *P. intermedium* cytotypes is defined by a divergence in cytotype-specific ecological preferences. In core distribution areas, seasonal environmental variables foster cytotype stability. Dispersion of cytotypes following a seasonal–latitudinal gradient as observed in *P. intermedium* is not rare in nature (Španiel et al., 2008; Trávníček et al., 2011; Zozomová-Lihová et al., 2015). Diploid–tetraploid coexistence is possible by different pre- and post-zygotic isolation barriers (Husband and Sabara, 2003) or by character displacement and ecological differentiation (Beans, 2014). Despite being rarely found in nature, niche displacement (i.e. when the niche of a cytotype is affected by the presence of another cytotype) through a shift in niche optima or breadth plays an important role in enabling closely related species to coexist. For example, Sonnleitner et al. (2016) found that contact zones in *Senecio carniolicus* were stabilized and reinforced by ecological differentiation of cytotypes as a result of habitat displacement. Unlike in other species, in *P. intermedium* the uneven replacement and local and regional spatial separation of cytotypes, the biased recruitment of young polyploid individuals in heteroploid populations and the discrepancy between predicted and observed distributions in tetraploids suggest a pattern of unstable temporal coexistence and directional turnover during which one cytotype is locally displaced reliant on ecological specialization and local environmental conditions.

The model prediction for the distribution of diploids is not significantly different from the observed distribution (Fig. 4). However, the prediction for tetraploids indicates that polyploids should coexist along with diploids in its main distribution zone; a situation that has not been realized according to our field observations. This suggests the presence of a wide area of tetraploid exclusion. In addition, the current observation that only a pure diploid population is found in an area in the core zone of diploids where one tetraploid was collected 30 years ago (Normann et al., 1989), and that diploids are being replaced by tetraploids in peripheral areas of its distribution suggests that tetraploids may only overcome ecological competition in the marginal zones of diploids with greater environmental heterogeneity. Thus, tetraploids may fail to become locally established in areas where optimal niche requirements of diploids are successfully met. Reciprocal transplantation experiments would certainly provide more accurate conclusions on this observation.

By definition, boundaries of distribution in plants represent zones of ecological transition, i.e. areas where environmental conditions do not satisfy the main ecological preferences of a

particular species or group (Grant, 1981). Even when plants exhibit plasticity to environmental conditions, their performance at niche edges may decline due to the effects of biotic and abiotic factors on their reproductive success (Vergeer and Kunin, 2013). Cytotypes occupying habitats in areas of ecological transition, irrespective of the mode of coexistence (i.e. sympatry or parapatry), are prone to ecophysiological subadaptation. Changes in reproductive strategies (e.g. allogamy–autogamy, sexuality–apomixis) and ploidy levels are known to improve local and regional performance (Hörandl, 2006). Mixed populations of *P. intermedium* appear in a region of spatial niche overlap between cytotypes, a zone of ecological transition between diploid–tetraploid niche optima (Fig. 3) where competition is expected to be stronger and driven not only by ecological differences (as the ecological requirements of neither cytotype were fully met) and spatial segregation of cytotypes but also by reproductive changes. In fact, two out of four mixed populations (Hojs456 and Hojs470) found in the transition area where ecological conditions resembled more those of the niche optima of diploids (interquartile range; Fig. 2) harboured a significantly higher number of tetraploids. Similarly, one of the other two populations (Hojs481) located in a transition area where conditions resembled more those of the niche optima of tetraploids was dominated by diploids, indicating that the reproductive mode might have an effect, even if temporary, on local cytotype success. Apomixis is known to shelter the polyploid from introgressive hybridizations, particularly heteroploid hybridizations (Hörandl and Tensch, 2009). The observed incongruity between ecological conditions, niche preferences and population composition mentioned above, together with the documented ploidy shifts, suggests the existence of a temporal succession of polyploid establishment–diploid displacement events. In this case, whenever a tetraploid is successfully established in the peripheral areas of diploids, the new heteroploid population will eventually reach a situation of asymmetric turnover between cytotypes which will most probably drive diploids to a local extinction.

In *P. intermedium*, a shift to apomixis not only shelters the polyploid from introgression of diploids (only one triploid among 122 individuals in mixed populations), thus reducing fitness loss by infertile hybrids and avoiding minority cytotype disadvantages (Levin, 1975), but also facilitates the multiplication of superior genotypes better adapted to local environmental conditions. In marginal areas where both cytotypes co-occur in sympatry, a generalist strategy with broader ecological tolerance and a capacity clonally to propagate rare and highly adapted genotypes may enhance the relative fitness of polyploids and their chances to displace diploids locally. Our observation of seven out of eight young *P. intermedium* plants sampled in a population with mixed ploidy being tetraploids and the non-random distribution of cytotypes within mixed populations supports the interpretation of non-random turnover and local displacement between cytotypes.

#### *Reconstruction of past migrations, and evolutionary history of polyploid cytotypes*

Reconstruction of past climatic niches indicated that only diploids of *P. intermedium* may have existed in northern Bolivia,

and central and south Brazil during the LGM around 21 000 ybp, in an area in southern Amazonia that was colder and drier than now, occupied by grasslands and savanna (e.g. Behling, 2002). During late Quaternary, neither tropical climates nor vegetation were stable and, as climate started to warm up, deglaciation (14 000 to 8000 ybp) transformed global vegetation distributions, even in tropical zones (Comes and Kadereit, 1998; Williams, 2009). Toward the MH (6000 ybp), the forest cover expanded and thermophilous taxa moved to higher altitudes and latitudes, reshuffling distributions of species with dramatic changes in some cases. For example, *Picea* suffered a biogeographic shift of around 2000 km northward from the central eastern USA (Williams, 2009). Similar regional to continental shifts in distributions took place in different species in South America (e.g. *Araucaria* forest; Behling, 2002), which may have affected the distribution of diploid *P. intermedium*. The present climatic niche modelling showed a shift of diploid occurrence during the Holocene, from central-east Brazil to southern areas in northern Argentina and Paraguay, perhaps a consequence of species' migration to track adaptive peaks as the fitness landscape changed (Supplementary Data Fig. S7). The presence of fossil impressions of *P. intermedium* spikelets found in Gran Chaco region (northern Argentina) suggests that the species lived in the area around MH (Contreras et al., 2015), which agrees with model reconstructions of past vegetation types and distribution in South America (Cerling et al., 1997; Piovano et al., 2009).

Plant taxa primarily respond to climate variations via local changes in abundance and, consequently, climate change shapes vegetation dynamics in the long run (Williams, 2009). In *P. intermedium*, climatic and fitness landscape changes seem to have prompted geographic shifts to new environments. The question remains of whether diploids and tetraploids coexisted in those areas adapted to similar climatic niches and diverged later, or whether tetraploids directly occupied vacant niches unfavourable for diploids while moving south. In either case, niche divergence facilitated the spatial segregation and establishment of both cytotypes. Since apomixis and selfing are known to provide superior colonization abilities in peripheral areas via uniparental reproduction (Baker's Law; Baker, 1955), niche availability and segregating ecophysiological and reproductive traits may have delivered the appropriate background for polyploids to expand into southern habitats that are inaccessible to diploids where primary production and productivity measures are lower (Alcaraz-Segura et al., 2013). Broader ecophysiological tolerance of tetraploids and their habitat-associated characteristics featured by the Mesopotamian water system that drains toward Parana delta to the Atlantic Ocean certainly favoured tetraploid dispersal, which eventually shaped the currently observed North–South distribution pattern.

#### SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Table S1: details of collection sites, collection codes, number of individuals and ploidy of *P. intermedium* materials collected in the present study. Table S2: collection sites of *P. intermedium* plant materials with a known ploidy level from previous studies. Figure S1:



flow cytometry histograms from different tissues and individuals of *P. intermedium*. Figure S2: composition and placement of individual cytotypes within mixed-ploidy populations. Figure S3: spatial analysis of cytotype distribution of *P. intermedium*. Figure S4: boxplots depicting *P. intermedium* cytotype ecological preferences and niche differentiation for most significant bioclimatic and environmental variables. Figure S5: ordination scaling plot for environmental variables and their dimensional contribution to the distribution of diploid and tetraploid *P. intermedium* cytotypes. Figure S6: histograms displaying the distribution of 100 randomly simulated niche overlap scores. Figure S7: reconstruction of past environmental niches showing suitable habitats available for diploid and tetraploid cytotypes of *P. intermedium*. Method S1: flow cytometry ploidy estimations. Method S2: flow cytometric seed screening.

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