



Genome size variation in Cactaceae and its relationship with invasiveness and seed traits

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Abstract Because naturalized and invasive plant species have disproportionately smaller genome sizes compared to all angiosperms, genome size has been proposed as a general predictor of invasiveness. The family Cactaceae includes some of the most important invasive species worldwide, and it is one of the plant families with lowest number of genome size estimations. The main goal of this study was to explore possible correlations between genome size and invasiveness in Cactaceae, and between genome size and seed size and weight, traits previously linked with

invasiveness. Propidium iodide flow cytometry was used to estimate the genome size from seeds (mostly) of 256 taxa of Cactaceae (32 of which are known to be invasive, and 13 are considered potentially invasive), and seed size, weight and mass were measured or obtained from previous work. Contrary to expectation, no significant differences were observed in genome size among categories of invasion, suggesting that genome size alone is not a reliable predictor of invasiveness in Cactaceae. Also, no correlations were observed between genome size and the studied seed traits when the whole dataset was considered. At a finer scale, in Opuntioideae, positive significant correlations were observed between genome size and the seed traits studied, and in Cactaceae, the opposite trend was observed. Since Cactaceae species possess low rates of metabolism, selection towards traits linked with genome size may not have been favoured irrespective of the invasive strategy. The large number of genome size estimates reported in this study offers great opportunities for studying the evolution of genome size in this family and for exploring possible correlations between genome size and geographical, environmental and phenotypic traits.

Sara Lopes, Lucie Mota have contributed equally to this work

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Introduction

Human-mediated introductions of species to areas outside their native ranges has been increasing rapidly (Seebens et al. 2021). Only a small fraction of introduced species overcome biotic and abiotic obstacles to survival and reproduction, becoming naturalized, and even fewer turn out to be invasive (Pyšek et al. 2020). Nonetheless, many invasive species cause significant negative environmental, social and/or economic impacts (Vilà et al. 2011; Pyšek et al. 2012; Bacher et al. 2018; Diagne et al. 2020). Management strategies aiming to reduce the negative impacts of invasive species are therefore underway in many parts of the world. The objectives of these initiatives include: (a) prevention (to regulate potential invaders through national and/or international policies and control their introduction at ports of entry); (b) eradication actions (to detect and completely remove invasive species from a region); and (c) long-term management actions (to contain spread and reduce impacts) (Robertson et al. 2020). Among these, prevention actions are the most cost-efficient initiatives (Leung et al. 2002). Evidence-based criteria are needed to identify which alien species have the potential to become invasive if introduced (Kolar and Lodge 2001). Thus, recognizing general patterns that help us predict future invaders is a major challenge in invasion science (Novoa et al. 2020). In that sense, several studies have focused on identifying plant traits associated with invasiveness (Rejmánek and Richardson 1996; Pyšek and Richardson 2008; Küster et al. 2008; Van Kleunen et al. 2010a, b; Mathakutha et al. 2019).

Most of the genetic material of cells is present in the nucleus, and genome size is one of its intrinsic properties. Within different groups of living organisms, cells present massive variations in genome size. In angiosperms alone, genome size varies more than 2400-fold, from 61 Mbp (*Genlisea tuberosa*; Fleischmann et al. 2014) to approximately 150,000 Mbp (*Paris japonica*; Pellicer et al. 2010), with several mechanisms being responsible for this variation. Polyploidization (Comai 2005), hybridization (Baack et al. 2005), higher rates of nucleotide deletion over insertion through illegitimate recombination or through unequal intra-strand homologous recombination, and transposon amplification (Bennetzen et al. 2005) are some of these mechanisms. Research in

recent decades has shown that the amount of nuclear DNA is involved in the scaling of living organisms and that it influences characteristics from cellular to organismal levels – the nucleotypic hypothesis [first proposed by Bennett (1971), reviewed in Greilhuber and Leitch (2013), and further explored by Kempes et al. (2016)]. According to this hypothesis, the DNA may affect the phenotype by expression of its genetic content (as it stores the information necessary for the development and functioning of an organism), but also via nucleotypic effects, including DNA's mass and volume that set absolute limits to both the minimum mass and size of the cell and the minimum time needed for the replication of DNA and for cell division (Bennett 1987a). Considering that in complex multicellular vascular plants the nucleotypic effects are additive, traits such as minimum generation time (Bennett 1972), life cycle (Bennett 1972) and cell cycle time (Francis 2008) are influenced by genome size (Bennett 1987a). Consequently, genome size may be an important ecological determinant of the spatial and temporal patterns of the distribution and abundance of a plant, as it constrains functional traits related to individual growth, reproduction and dispersal [as reviewed in Greilhuber and Leitch (2013) and Roddy et al. (2020)].

Considering the increased genome size estimations obtained in recent decades, several studies have incorporated this trait into the framework of invasiveness prediction. After the initial suggestion that small genomes favour invasiveness (Rejmánek 1996), a growing body of evidence supports this relationship, from cross-species comparative studies at global (e.g., Chen et al. 2010; Pandit et al. 2014; Suda et al. 2015) or regional scales (e.g., Kubešová et al. 2010; Schmidt and Drake 2011; Kuester et al. 2014), to studies at the generic (e.g., Grotkopp et al. 2004; Garcia et al. 2008; Varela-Álvarez et al. 2012) or intraspecific levels (e.g. Lavergne et al. 2010; Meyerson et al. 2016, 2020; Pyšek et al. 2018). Pandit et al. (2014) also found that invasiveness was positively related with ploidy level (and chromosome number) through beneficial effects of heterogenesis, higher rates of cell division and increased phenotypic variation. Therefore, the interaction between genome size and genome duplication, which initially could be regarded as contrasting (as polyploidy leads to an increase in genome size, at least initially), is what underlies the phenotype and

physiology of a plant, and ultimately determines invasion success (Pandit et al. 2014).

Here, we explore the relationship between genome size and plant invasiveness within the family Cactaceae. As with other traits, if an association between genome size and invasiveness or other invasive traits exist in this family, genome size could be a relevant trait for predicting invasiveness. The family Cactaceae includes approximately 1400 species, most of which are succulents (Guerrero et al. 2019). Cacti are distinctive elements of arid and semiarid biomes (Hernández-Hernández et al. 2011), and the bulk of their diversity can be found in north-eastern Mexico, southwestern Andean region and south-eastern Brazil (Novoa et al. 2015a). Cactus species were brought to Europe from the Americas in the fifteenth century for agricultural and ornamental purposes (Anderson 2001). Many cacti were subsequently transported to other locations in the horticultural trade which favoured these species because of their drought tolerance and attractiveness (Novoa et al. 2017). Although only about 3% of all Cactaceae species are currently invasive, the family includes some of the most important alien plant species worldwide (Novoa et al. 2015b), some of which have great potential for further expansion. The main hotspots of cactus invasions are Spain, including the Canary Islands, Australia, and South Africa (Novoa et al. 2019). Thorough risk assessments of Cactaceae species have recently been published (Novoa et al. 2015a, 2019). Also, Novoa et al. (2016) explored the potential of barcoding and seed size, mass and appearance to identify invasive cacti.

The origin of this monophyletic family dates back to 30–35 Mya (Arakaki et al. 2011; Hernández-Hernández et al. 2014; Magallón et al. 2015). Its members are divided into four subfamilies: “Pereskioideae”, Maihuenioideae, Opuntioideae and Cactoideae (Guerrero et al. 2019). Phylogenetic analyses showed a paraphyly of subfamily Pereskioideae and the monophyly of the remaining subfamilies, based on molecular data (Hernández-Hernández et al. 2011; Moore et al. 2018; Walker et al. 2018). Within Cactoideae, two clades were recognized: the Cactaceae (distributed in North America) and the “Core Cactoideae” (distributed throughout the American continent and bearing the largest diversity of growth forms) (Hernández-Hernández et al. 2011). Also, both para- and polyphyly were detected along the phylogeny of

the Cactoideae (Hernández-Hernández et al. 2011). In “Core Cactoideae” two large sister clades have been described: “Core Cactoideae I” and “Core Cactoideae II”. Within “Core Cactoideae I”, most species belong to a clade that includes members of the Pachycereeae, Hylocereeae, and Browningieae tribes (the PHB clade). Within “Core Cactoideae II”, a major clade, strongly supported, includes some members of the Browningieae and Cereaeae, and all members of the Trichocereaeae tribe—the BCT clade (Hernández-Hernández et al. 2011). Hybridization (Machado 2008) and polyploidization (Majure et al. 2012) may have played an important role in the evolution of Cactaceae, in particular in the Opuntioideae. In this subfamily there are reports of cases in which allopolyploidy and vegetative propagation were in the origin of new species (e.g., Mayer et al. 2000). In Cactoideae, polyploidy has been detected in *Echinocereus* spp., *Mammillaria* spp. and *Pachycereus pringlei* (Ashman et al. 2013; Gutiérrez-Flores et al. 2018, Hernández-Cruz et al. 2018).

The main aim of this study was to evaluate whether any correlation exists between genome size and invasiveness in 256 taxa of Cactaceae used in the ornamental trade, and thus to evaluate the utility of this trait as a predictor of invasiveness. Plant traits such as seed size, weight and mass were also measured or obtained from previous work to evaluate possible correlations between these traits and genome size. All analysed species are sold as ornamentals (Novoa et al. 2017); 32 taxa are listed as invasive and 13 are considered as potentially invasive (i.e., they belong to genera that present characteristics associated with invasiveness, such as vegetative reproduction and good dispersal mechanisms; Novoa et al. 2015a). Studies with such a large data set for a given family, with invasive, potentially invasive, and non-invasive introduced species at a global scale are very rare and offer a unique opportunity to evaluate the role of genome size in predicting invasiveness. This family is surprisingly under-represented in the Plant DNA C-values Database (Leitch et al. 2020), with only 48 species having recorded genome sizes.

Materials and methods

Plant material

For the present study, a collection of 293 sets of seeds from different genera (250 taxa), plus six living adult plants of six additional taxa were obtained from Imzaadex (Netherlands), Cactus adventures (Spain) and Koehres kaktus (Germany). As there are substantial taxonomical issues within the Cactaceae, taxa from which the seeds were obtained, were previously identified using DNA barcoding by Novoa et al. (2016).

Seeds were conditioned in individual hermetic plastic bags, labelled and stored at room temperature. Using information available in the literature (summarized in Novoa et al. 2016), the status of each taxa (invasive/potentially invasive/non-invasive) was recorded. Living specimens of the six taxa were obtained by regular mail and transplanted to pots (Ø16 cm) filled with regular commercial soil.

Seeds were germinated in plastic cuvettes (4.5 × 4.5 × 4.5 cm) filled with commercial soil or with a mixture of sand and soil in the proportions 1:2, respectively. Immediately after sowing, cuvettes were placed on trays with a thin layer of water to promote germination. Trays were conditioned in a greenhouse at 26 °C with a photoperiod 16/8 h (light/dark). When seedlings emerged, they were transplanted to larger pots (Ø8–10 cm) filled with commercial soil.

Seed parameters

To explore possible relationships between selected seed traits and genome size, seed width, seed length and seed mass were recorded for each analysed taxon or obtained from Novoa et al. (2016). In a few situations, due to the small dimensions of the seeds, seed mass was measured as the average of three to five seeds using a precision digital scale (Radwag AS-110/C/2, RADWAG USA L.L.C, FL, USA). Seed width and length was measured by photographing 1–3 seeds of each taxon together with a scale using a Canon 600D camera coupled to a binocular microscope (Leica M80, Leica Microsystems, Wetzlar, Germany) and performing measurements using Image J (Schneider et al. 2012). When more than one estimate was obtained, seed length and width were represented as the average value of the seeds measured.

Genome size analysis

Genome size estimates were made using flow cytometric (FCM) analyses of nuclei isolated from plant tissues. The primary reference standard selected was *Solanum lycopersicum* (*S.l.*, 2C = 1.96 pg, Doležel et al. 1992), against which other standards were calibrated, namely *Bellis perennis* (*B.p.*, 2C = 3.57 pg, secondary reference standard).

Genome size measurements were obtained using nuclei isolated following the chopping method described by Galbraith et al. (1983), with some modifications. Initial analyses were performed using grown seedlings or adult plants, but these were found to possess a high amount of mucilaginous compounds that hampered the isolation of nuclei in good conditions. As an alternative, root tissues and seeds were tested. Seeds were found to provide histograms of high quality (higher quality than root tissues) and were used to assess genome sizes. Nuclear suspensions were prepared using a single seed, ideally; for taxa with small seeds, more seeds from the same individual were pooled and chopped simultaneously. Seed coats were removed, and seeds were placed in a Petri dish with approximately 50 mg of leaf material of the internal reference standard and 1 ml of WPB Buffer (0.2 M Tris.HCl, 4 mM MgCl₂.6H₂O, 1% Triton X-100, 2 mM EDTA Na₂.2H₂O, 86 mM NaCl, 10 mM metabisulfite, 1% PVP-10), pH adjusted to 7.5 and stored at 4 °C (Loureiro et al. 2007). Chopping intensities were adjusted to provide similar number of sample and standard nuclei. Nuclear suspensions were then filtered through a 50 µm nylon filter into a sample tube and stained with 50 mg/mL propidium iodide (PI, Fluka, Buchs, Germany). Also, 50 mg/mL of RNase (Fluka, Buchs, Germany) were added to destroy RNA and prevent staining of double stranded RNA.

Samples were kept at room temperature and analysed within 5 min in a Cyflow Partec flow cytometer (Partec GmbH, Görlitz, Germany). The equipment presents a 532 nm green solid-state laser, operating at 30 mW. For a given taxon, the amplifier system was set to a constant voltage and gain throughout the whole analysis. Each day, to ensure further sample quality, prior to analysis, the instrument stability and linearity was verified using fluorescent beads (Partec GmbH, Görlitz, Germany). Samples were run when baseline CV values of the fluorescent beads were below 3%.

The results were acquired in the FloMax software (v. 2.4d) as: fluorescence pulse integral in linear scale (FL) histogram; FL vs. time scatterplot (to evaluate fluorescence stability through time); FL vs. fluorescence pulse height scatterplot (to remove duplets); and FL vs. SS in log scale scatterplot (to evaluate the possible effect of secondary metabolites; Loureiro et al. 2021). Polygon regions were defined in the FL vs. SS scatterplot and further applied to the other plots. This enabled us to remove debris and improve the quality of samples (Loureiro et al. 2021). Mean fluorescence values and CV value of the fluorescence of both sample and standard were obtained for at least 1,300 nuclei in each G₀/G₁ peak, whenever possible, given sample flow speed and sample amount. Only samples that presented CV values below 5% were accepted; other samples were discarded and prepared again to achieve better quality. For some taxa the 5% CV value threshold was not obtained due to the high levels of mucilage even after repeated measurements; still, even in those occasions, samples presented CV values below 6%.

For each taxon, up to six estimates of genome size were obtained on different days to account for variation generated by the flow cytometer.

The holoploid genome size in pg (2C; sensu Greilhuber et al. 2005) of each individual was estimated according with the following formula:

$$2C \text{ nuclear DNA content (pg)} \\ = \frac{\text{sample species G}_0/\text{G}_1 \text{ peak FL}}{\text{standard G}_0/\text{G}_1 \text{ peak FL}} \\ \times \text{nuclear DNA content of the standard}$$

Statistical analysis

Descriptive statistics of genome size estimates and seed traits were calculated for each species (mean, standard deviation of the mean, minimum and maximum values). Clades of each taxa followed Hernández-Hernández et al. (2011) classification. Box plots with genome size variation within each category of invasiveness for the whole dataset and at a finer scale, within the Cactoideae subfamily and considering the BCT and PHB clades within the “Core Cactoideae” (sensu Hernández-Hernández et al. 2011), were also obtained. Box plots with genome variation among subfamilies/clades, irrespective of the invasiveness

category and considering invasive and non-invasive species, were also obtained.

Statistical differences in genome size among the three categories of invasiveness and among subfamilies/clades (removing the only representative of “Pereskioideae”) for the whole dataset were assessed using a Generalized Linear Mixed Model (GLMM), with taxa as random factor. Genome size was square root transformed to achieve linear model assumptions. Analyses at a finer scale were only possible for the BCT clade, as for the PHB clade and when considering invasive and non-invasive species for the subfamily comparison, the low number of estimates impeded the analyses.

Linear regressions and correlation analyses between genome size and seed traits, namely seed length, width and mass, were also explored, using the whole data set, and at a finer scale, considering each subfamily/clade, the phylogenetic relationships within the “Core Cactoideae” clade, and each category of invasiveness in the BCT clade (sensu Hernández-Hernández et al. 2011).

All analyses were performed in R software version 3.0.1 (R Core Team 2016), using the packages “ggplot2” for box plots, “car” for Type-III analysis of variance (Fox et al. 2013), “lme4” for generalized linear models and generalized linear mixed models (Bates et al. 2015) and “multcomp” for multiple comparisons after Type-III analysis of variance (Hothorn et al. 2008).

Results

The genome size of 256 Cactaceae taxa was analysed, of which 245 represent new estimations (Supplementary Information 1). This study increases the number of available genome size estimates by 4.9 times.

In the present study, genome size varied 4.83-fold. The lowest genome size we recorded was for *Cylindropuntia spinosior* (Opuntioideae subfamily), an invasive species with genome size of 2.32 pg/2C, and the largest one was obtained for *Espostoa guentheri* (BCT clade within the “Core Cactoideae II”), a non-invasive species, with a genome size of 11.21 pg/2C (Table 1). The genome size values observed in this study fit within the very small (< 2.8 pg/2C, 4.3%), small (2.8–7.0 pg/2C, 82.8%) or medium (7.0–28.0 pg/2C, 12.9%) genome size

Table 1 Summary of the nuclear DNA estimates for 256 Cactaceae taxa analysed in this study considering the three categories of invasiveness (non-invasive, potentially invasive and invasive) and the subfamilies/clades within each invasiveness category (sensu Hernández-Hernández et al. 2011). The

Invasiveness	Mean G.S. (pg)	SD G.S. (pg)	G.S. Min (pg)	G.S. Max (pg)	n
Non-invasive	4.33	1.56	2.42	11.21	211
Cactaeae	3.79	0.58	2.97	7.18	86
“Core Cactoideae I”	3.38	0.90	2.74	6.34	13
“Core Cactoideae II”	4.86	1.92	2.42	11.21	112
Potentially invasive	4.45	1.29	2.37	8.19	13
Opuntioideae	3.51	1.61	2.37	4.65	2
“Core Cactioideae II”	4.63	1.23	3.82	8.19	11
Invasive	5.09	2.18	2.32	9.79	32
“Pereskioideae”	2.42	-	-	-	1
Opuntioideae	4.91	2.09	2.32	9.79	21
“Core Cactioideae I”	6.26	3.53	3.76	8.75	2
“Core Cactioideae II”	5.63	2.21	3.82	8.53	8

average nuclear DNA content (Mean G.S.), the respective standard deviation (SD G.S.), minimum (G.S. Min) and maximum (G.S. Max) values, are all given in picograms (pg). The number of Cactaceae taxa analysed in each category and subfamily/clade (n) is also provided

categories of Leitch et al. (1998). No genome sizes were recorded for the categories representing large or very large genome sizes ($2C \geq 28$ pg). The general quality of the samples was good, with most CV values being under 5%, and, for most samples, using seeds, little debris or secondary metabolites affecting the visualization and scoring of particles were observed (Fig. 1).

Does genome size differ between invasive, non-invasive and potentially invasive species?

Of the 32 invasive species analysed in here, 65.6% belong to the Opuntioideae subfamily, 25.0% to BCT clade within the “Core Cactoideae II” clade, 6.3% to PHB clade within the “Core Cactoideae I” clade, and 3.1% to subfamily “Pereskioideae”.

In invasive species, the smallest genome size recorded was of 2.32 pg/2C for *Cylindropuntia*

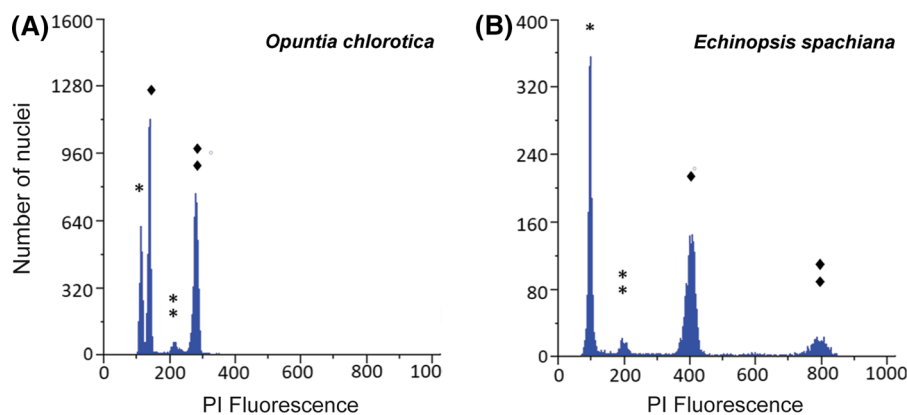


Fig. 1 Illustrative fluorescence histograms of nuclei isolated from *Solanum lycopersicum* as reference standard ($2C = 1.96$ pg, Doležel et al. 1992) and Cactaceae taxa: **a** *Opuntia chlorotica* ($2C = 2.37$ pg), **b** *Echinopsis spachiana*

($2C = 8.13$). In the graphics, the reference standard is denoted by * and ** above the G_0/G_1 and G_2 peaks, respectively. Likewise, sample’s G_0/G_1 and G_2 peaks are marked with ♦ and ♦♦, respectively

spiniosior and the largest was of 9.79 pg/2C for *Tephrocactus articulatus* (both from Opuntioideae subfamily), conferring to invasive species group a 4.23-fold variation in genome size (Fig. 2a, Table 1). Regarding the potentially invasive species group, the smallest genome size was of 2.37 pg/2C for *Opuntia macrorrhiza* (Opuntioideae subfamily) and the largest was for *Echinopsis huascha* (BCT clade within the “Core Cactoideae II”), with 8.19 pg/2C, and so with a variation of 3.46-fold (Fig. 2a, Table 1). For non-invasive species, the smallest genome size was of 2.42 pg/2C for *Parodia warasii* (“Core Cactoideae II”), and the largest was for *Oreocereus leucotrichus*, with 11.21 pg/2C (BCT clade within the “Core Cactoideae II”), accounting for a variation of 4.63-fold in this group alone (Fig. 2a, Table 1).

In non-invasive species, large amounts of genome estimates were between 2.42 pg/2C and 4.5 pg/2C, but there were some estimates between 6 and 8.5 pg/2C. Variation in genome size estimates for the invasive and non-invasive species groups was very similar, but the potentially invasive species group presented the

smallest variation. Members of the Opuntioideae subfamily presented the highest heterogeneity in genome size estimations (Supplementary Information 2).

Statistically, genome size did not differ among the three categories of invasiveness when the whole dataset was used ($F_{2,260} = 5.435$, $P = 0.066$; Fig. 2a), although when the analysis was made considering the phylogenetic relationships within the “Core Cactoideae”, invasive species tend to have higher genome sizes than non-invasive and with potentially invasive having intermediate values ($F_{2,237} = 8.041$, $P = 0.018$; Fig. 2b). When analysing at a finer scale within the BCT clade, again, no differences among categories of invasiveness were observed ($F_{2,109} = 1.299$, $P = 0.522$; Fig. 2c).

Also, statistically significant differences were observed among subfamilies/clades ($F_{3,259} = 28.559$, $P < 0.001$) with Cactaceae and the “Core Cactoideae I” having significantly lower genome sizes than the “Core Cactoideae II” ($P < 0.05$), and with

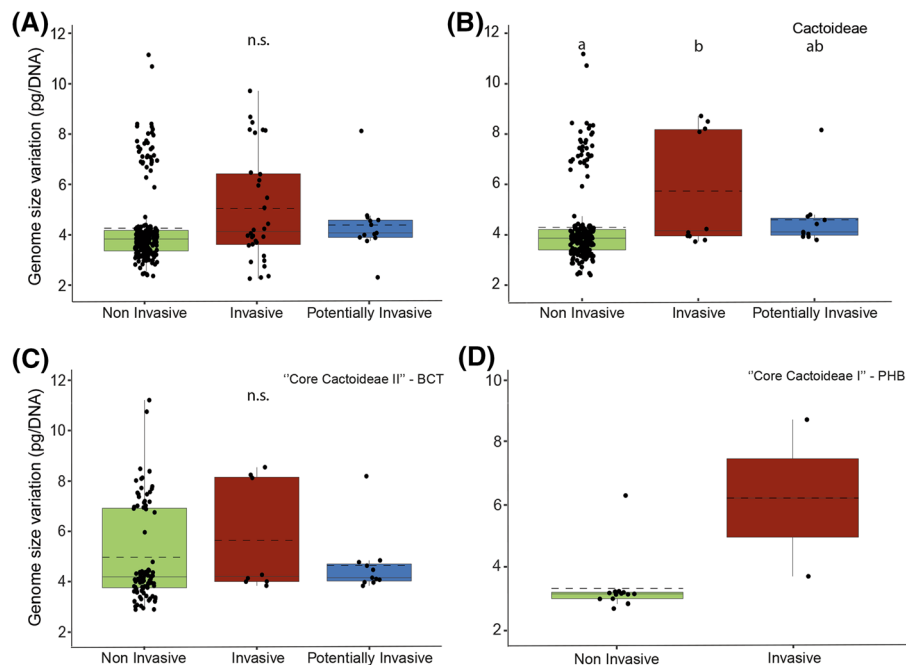


Fig. 2 Genome size variation within invasive, non-invasive and potentially invasive species using the whole dataset (a), and within the Cactoideae (b), the BCT clade (C; “Core Cactoideae II” sensu Hernández-Hernández et al. 2011) and the PHB clade (D; “Core Cactoideae I”). Boxes extend from the 25% and 75% percentiles and whiskers extends from the hinge to the largest

value no further than 1.5 * the inter-quartile range. Medians are depicted as a horizontal line within the box and the mean as dashed line. Different letters represent statistically significant differences at $P < 0.05$; n.s. represents the absence of statistically significant differences at $P > 0.05$. Statistical analyses were not performed in D, due to the low number of estimates

Opuntioideae having intermediate values (Supplementary Information 2).

For the three categories of invasiveness, the genome size values of each species were more frequent between 0 and 6 pg/2C (non-invasive—84.7%; invasive – 66.7%; potentially invasive—92.3%), being the highest percentage observed for the genome size categories [3–4] and [4–5] of genome size (non-invasive: 51.8% [3–4] and 27% [4–5]; invasive: 24.2% [3–4] and 21.2% [4–5]; potentially invasive: 61.5% [4–5] and 23.1% [3–4]) (Fig. 3). Regarding the higher values of genome size ([10–11] and [11–12]), only non-invasive species possess values in this range (0.5% on both [10–11] and [11–12]). Genome sizes of category [9–10] occurred only in invasive species (3%) (Fig. 3).

How much of the seed traits are explained by genome size?

Considering the whole dataset, non-significant relationships were found between seed length and genome size within each category of invasiveness: non-invasive ($R = 0.02$; $P = 0.774$), invasive ($R = 0.09$; $P = 0.709$) and potentially invasive species ($R = -0.37$; $P = 0.213$) (Fig. 4). The absence of significant correlations was also observed between genome size and seed width (non-invasive: $R = -0.0546$; $P = 0.432$; invasive: $R = 0.05$, $P = 0.850$; potentially invasive: $R = 0.35$, $P = 0.244$) (Fig. 4) and between genome size and seed mass (non-invasive: $R = 0.05$;

$P = 0.439$; invasive: $R = 0.17$; $P = 0.481$; potentially invasive: $R = 0.32$; $P = 0.281$) (Fig. 4).

The analysis at a finer scale at the subfamily/clade level without considering invasiveness categories (due to a reduced number of estimates for invasive and potentially invasive species) revealed that genome size was significantly positively correlated with seed length and width within subfamily Opuntioideae ($R = 0.59$, $P = 0.034$ and $R = 0.67$, $P = 0.012$, respectively), and negatively correlated with Cactaeae within subfamily Cactoideae ($R = 0.22$, $P = 0.044$ and $R = 0.24$, $P = 0.024$, respectively); no significant correlations were obtained for the “Core Cactoideae” ($R = 0.09$, $P = 0.274$ and $R = 0.03$, $P = 0.741$, respectively) (Fig. 5a–b). For seed mass, although the same trend was observed within subfamilies/clade, no significant correlations were found (Fig. 5c).

At the BCT and PHB clades level and within each category of invasiveness in the BCT clade, no significant correlations were found between genome size and any of the seed traits (Supplementary Information 3 and 4).

Discussion

A large body of literature supporting correlations between genome size and invasiveness has appeared in the last two decades (e.g., Grotkopp et al. 2004; Garcia et al. 2008; Chen et al. 2010; Kubešová et al. 2010; Lavergne et al. 2010; Schmidt and Drake 2011; Varela-Álvarez et al. 2012; Kuester et al. 2014; Pandit

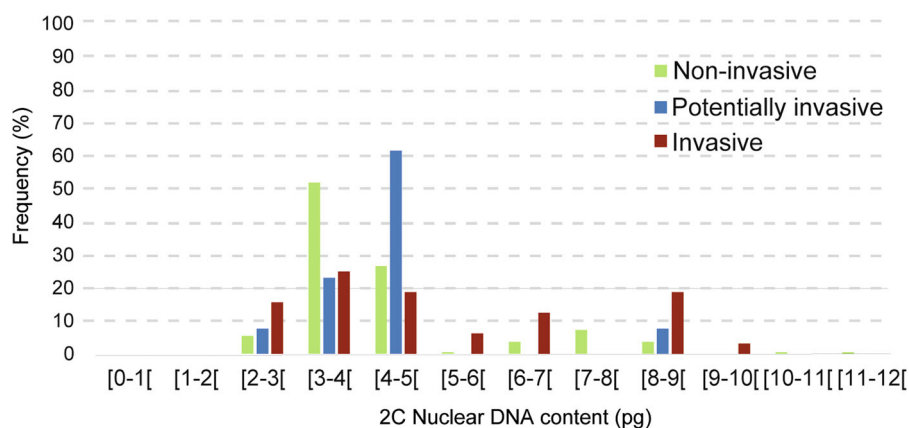


Fig. 3 Frequencies of distribution of genome size values according with the invasiveness categories: non-invasive species (black bars, $n = 211$), invasive species (dark grey, $n = 32$) and potentially invasive species (light grey, $n = 13$)

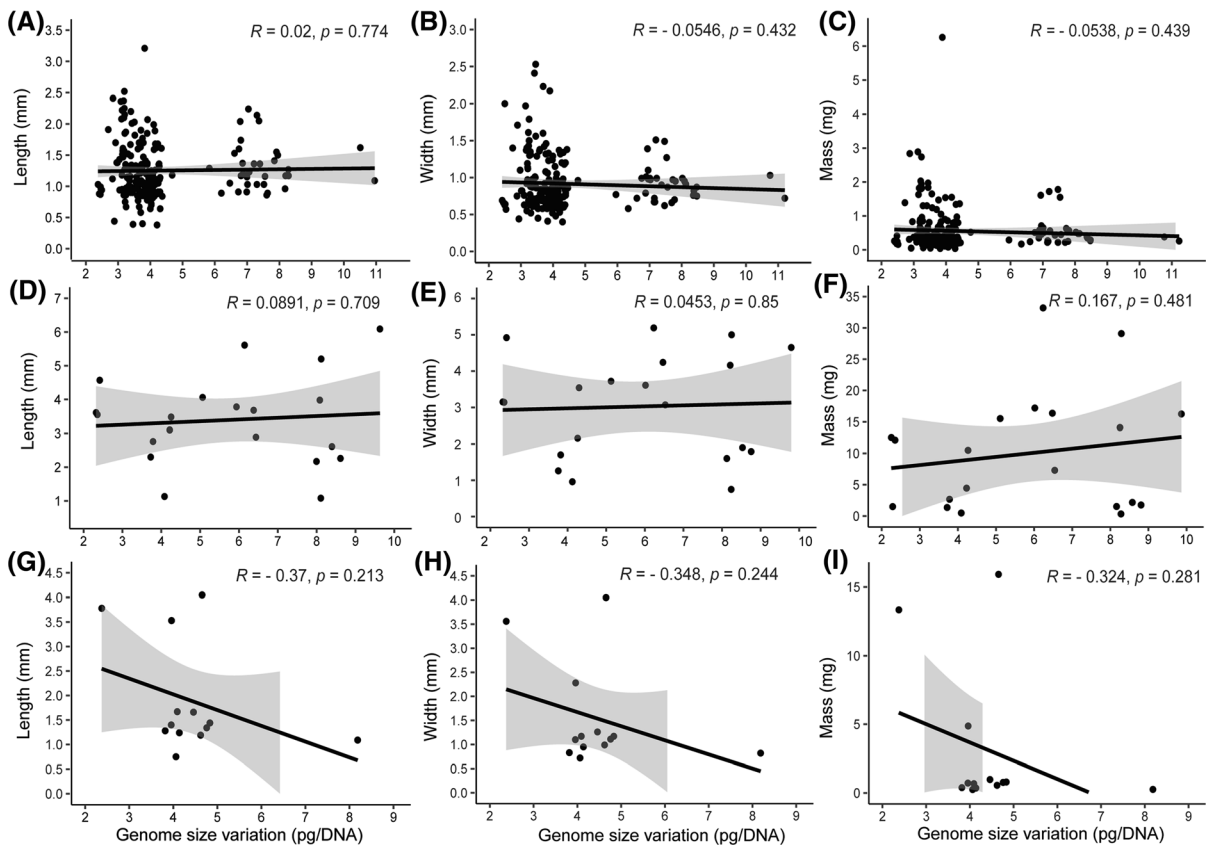


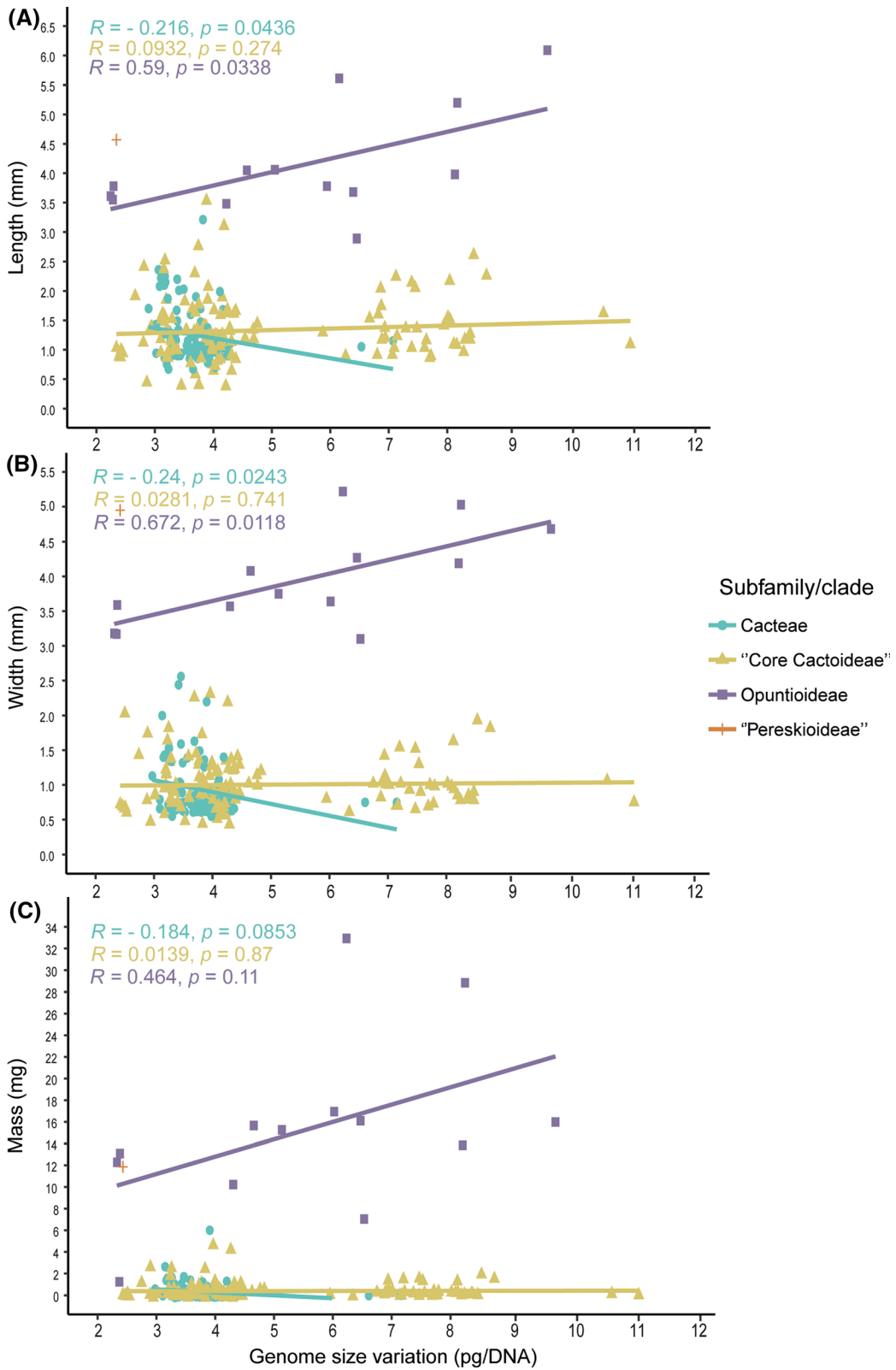
Fig. 4 Linear regressions between genome size and seed traits for each category of invasiveness: **a–c**—non-invasive (A—seed length; B—seed width; C—seed mass); **e–g**—invasive (E—

seed length; F—seed width; G—seed mass); H–J—potentially invasive (H—seed length; I—seed width; J—seed mass). R and P values are provided in each graph

et al. 2014; Meyerson et al. 2016; Pyšek et al. 2018). Strong evidence now also exists for correlations between genome size and other traits influencing invasiveness, such as minimum generation time (Bennett 1972; Leitch and Bennett 2007), seed characteristics (Grotkopp et al. 2004; Beaulieu et al. 2007a), relative growth rate of seedlings (Grotkopp and Rejmánek 2007), life cycle strategy (Bennett 1972), specific leaf area (Morgan and Westoby 2005; Beaulieu et al. 2007b), leaf water content (Meyerson et al. 2016), stomatal size, density nutrient and water consumption (Beaulieu et al. 2008; Simonin and Roddy 2018), stem height and number (Meyerson et al. 2016), herbivore-defence traits (Meyerson et al. 2016), phenology (Kubešová et al. 2010), competitiveness (Pyšek et al. 2018), and plasticity (Meyerson et al. 2020). If a correlation between the genome size of Cactaceae and invasiveness or other invasive traits were demonstrated, genome size could be an

informative trait to be used for predicting invasiveness in this important family.

Several studies performed so far suggested that naturalized and invasive plant species have notably smaller genome sizes relative to all angiosperms (Kubešová et al. 2010; Suda et al. 2015). Suda and co-authors (2015) reviewed the recognized relationships between genome size and plant traits known to influence invasiveness based on the “large genome constraint” hypothesis proposed by Knight et al. (2005). They concluded that species with small genomes are able to present a wider array of trait states than species with large genomes, which might confer a better adaptability to variable habits, an important determinant of invasiveness. A small genome may facilitate shorter life cycles and faster rates of cell division, translating to earlier germination, faster plant growth and development, and expression of traits such as both higher photosynthetic



◀ **Fig. 5** Linear regressions between genome size and seed traits within the subfamilies/clades of Cactaceae (sensu Hernández-Hernández et al. 2011): A—seed length; B—seed width; C—seed mass. In each graph, R and P values are provided for each subfamily/clade

rates and specific leaf area (Knight et al. 2005; Knight and Beaulieu 2008; Simon and Roddy 2018; Roddy et al. 2020). Small genomes may be advantageous when compared with large genomes in situations where faster growth can, at least partially, compensate for short growing seasons or unfavourable environmental conditions (Bennett 1987b). Suda et al. (2015) also suggested that some of the constraints of large genomes [e.g., obligate perennials, Bennett (1972); absence of very small seeds, Beaulieu et al. (2007a), lower water-use efficiency, Beaulieu et al. (2008)], do not seem compatible with the traits of a successful invader. In all, many of these traits are related with metabolism, rates of development and growth, and seed characters.

Contrary to general expectations, our results suggest that genome size alone is not a reliable predictor of invasiveness in Cactaceae as, overall, no differences in genome size were observed between invasive and non-invasive species (indeed, genome sizes were greater, rather than lower, in invasive species within Cactoideae). The same finding has been reported for some other plant groups (e.g., *Acacia* spp., Gallagher et al. 2011; 99 invasive plant species from several families, Moura et al. 2020). The review of Suda et al. (2015) also highlighted that some of the traits affected by genome size may potentially have conflicting roles in plant invasiveness, making it difficult to predict invasion potential based on this factor alone. We add to this discussion the consideration that traits associated with invasiveness are not equally relevant at all stages of the invasion process. Regarding genome size, Kubešová et al. (2010) suggested that small genomes may play a role in naturalization but are less important during the transition from naturalized to invasive species. Multiple factors determine invasiveness, and the link between species traits, including genome size, and plant invasiveness is highly complex and context specific (Richardson and Pyšek 2006; Novoa et al. 2020; Pyšek et al. 2020). For example, in Australian *Acacia* species, small genomes did not seem to impose any functional constraints, with non-significant

differences observed between invasive and non-invasive species (Gallagher et al. 2011). In that case, plant height coupled with the native distribution range, were the most important traits correlated with invasion success.

Roddy et al. (2020) suggested patterns at the community-scale in genome size along gradients in productivity, i.e., an influence of the environmental conditions of each habitat on the species community based on the rates of metabolism. Therefore, in habitats that can handle high rates of metabolism, species with small genomes are favoured, whereas in habitats with water and nutrient limitation, high rates of metabolism may not always be advantageous. This may reduce the selection towards species with small genome sizes (Roddy et al. 2020). Indeed, Cactaceae are adapted to arid and semiarid habitats which are characterized by low productivity (Anderson 2001). Furthermore, they are succulent, have very low growth rates, and exhibit Crassulacean Acid Metabolism (CAM) photosynthesis (Guerrero et al. 2019). Roddy et al. (2020) tested the hypothesis that, if metabolism is one selection agent on genome size, then selection towards small genomes may be weak among CAM species. They observed that even after considering for shared phylogenetic history, genome sizes were significantly greater among CAM than among C3 species. In our dataset, genome sizes were heterogeneous among taxa, with values ranging from very small sizes (a few) to intermediate values; this was observed in invasive and non-invasive species. This may be due to the fact that rather than favouring species with small genome sizes and thus high rates of metabolism, harsh and less productive habitats allow for variable growing strategies (and metabolic rates), potentially leading to more diverse genome sizes at the community level (Roddy et al. 2020).

Another issue to consider is that the relationship between genome size and invasiveness might be masked by the interplay with ploidy and hybridization. Pandit et al. (2014) found that, through beneficial effects of heterogenesis, increased rates of metabolism and higher phenotypic variation, polyploidy increases the likelihood of a species to be invasive. Similarly, polyploids have also been hypothesized to be better adapted to extreme environments (Brochmann et al. 2004). Indeed, the subfamily Opuntioideae is well known for both hybridization and polyploidization (Guerrero et al. 2019); some reports suggest that

successful invaders that originated after allopolyploidization are able to occupy habitats different from those of the progenitor taxa (e.g., Mayer et al. 2000). All species from this subfamily in our dataset were invasive or potentially invasive; although the mean genome size was lower than that observed in Cactoideae, this subfamily had the highest heterogeneity in genome size (Supplementary Information 2B). Also, as evident in Fig. 2 and Supplementary Information 1, irrespective of the invasiveness category and subfamily/clade, a discontinuity of genome sizes is observed, with a cluster of genome size estimations around 3.8 pg/2C and a second cluster with approximately 8.0 pg/2C, which might be an indication of genome duplications. Furthermore, the only taxon of subfamily “Pereskioideae”, an early diverging lineage of Cactaceae (Edwards et al. 2005), presented one of the smallest genome sizes of the taxa analysed in this study ($2C = 2.42$ pg) and among the estimations available for the family. This suggests that genome upsizing through polyploidy and hybridization may have had an important role in the evolution of Cactaceae. However, it should be borne in mind that, because angiosperms diploidized “quickly” (i.e., in the first million years; Lynch and Conery 2000; Qiao et al. 2019) and downsized their genomes after whole-genome duplications (Leitch and Bennett 2004), evaluating the relative effects of ploidy versus genome size on invasiveness, and in Cactaceae in particular, can be complex.

Previous studies that have explored correlations between seed characteristics, namely seed size and weight, and invasiveness, showed that plants could benefit from different strategies as successful invaders, and successful invasive species can have either small or large seeds (Pyšek and Richardson 2008). While seedlings of species with larger and heavier seeds are usually stronger and contain more nutritional resources, and therefore have a lower probability of mortality (Daws et al. 2007), once the alien species is established, small seeds are beneficial for long-distance dispersal, favouring invasive spread (Moodley et al. 2013). In Cactaceae, Novoa et al. (2016) found that already-invasive species have significantly larger and heavier seeds than non-invasive species, and the same was true for potentially invasive taxa. In our case, no significant correlations were observed between genome size and the seed traits that we studied among invasiveness categories. Therefore, as

observed for *Acacia* (Gallagher et al. 2011), in Cactaceae the non-significant relationship of genome size with invasiveness might be explained by the lack or mixed relationship with invasiveness determinants. For example, if the seed traits studied have been shown to be related with invasiveness (as demonstrated by Novoa et al. 2016), they are not correlated with genome size, or this correlation differs among clades. Indeed, irrespective of the invasiveness strategy, a significant positive relationship between genome size and seed length and width was observed in subfamily Opuntioideae, similarly to what has been previously observed (Knight and Ackerly 2002; Grotkopp et al. 2004; Knight et al. 2005). By opposition, in Cactaceae, a significant negative relationship between genome size and seed length and width was observed, suggesting that other factors and evolutionary mechanisms may have operated in this tribe. In Cactaceae, invasive species disperse mainly vegetatively and tend to be spread over significantly larger areas in the native range than non-invasive species. Moreover, no invasive cactus species are of conservation concern in their native range, suggesting that the same characteristics that permit some cactus taxa to be widespread in their native distribution contribute to their capacity to overcome abiotic filters and establish with success in new regions (Novoa et al. 2015b).

This study greatly increases the number of available genome size estimates for Cactaceae species. Until now, estimates were available for 48 taxa only, of which 33 were obtained with the most reliable method, i.e., propidium iodide flow cytometry. Of these, we re-estimated the genome size of seven taxa and our results are highly comparable—the small differences being attributed to the use of different tissues (seeds in our case versus leaves in most of the existing estimations), standards, instruments and laboratories (Doležel et al. 1998). The availability of such a large dataset of genome size estimations opens new avenues to study genome size evolution in this family, for example to disentangle the role played by polyploidy (as in Loureiro et al. 2013; Vitales et al. 2019), and to explore possible correlations between genome size and geographical (e.g., altitude, latitude, see Knight et al. 2015 for a review), environmental (e.g., precipitation, temperature, as in Carta and Peruzzi 2016), and phenotypic (e.g., cell size and density, as in Roddy et al. 2020) traits.

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Availability of data and material Raw data is available in Supplementary Information.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent for publication All authors have contributed significantly to the work and agree with the content of the submission.

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