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ORIGINAL ARTICLE

Multiple and independent rearrangements revealed by comparative cytogenetic mapping in the dysploid Leptostachyus group (*Phaseolus* L., Leguminosae)

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Abstract Polyploidy and dysploidy have been reported as the main events in karyotype evolution of plants. In the genus *Phaseolus* L. ($2n = 22$), a small monophyletic group of three species, the Leptostachyus group, presents a dysploid karyotype with $2n = 20$. It was shown in *Phaseolus leptostachyus* that the dysploidy was caused by a nested chromosome fusion (NCF) accompanied by several translocations, suggesting a high rate of karyotype evolution in the group. To verify if this karyotype restructuring was a single event or occurred progressively during the evolution of this group, we analysed *P. macvaughii*, sister to *Phaseolus micranthus* + *P. leptostachyus*. Twenty-four genomic clones of *P. vulgaris* previously mapped on *P. leptostachyus*, in addition to the 5S and 35S rDNA probes, were used for fluorescence in situ hybridization. Only a single rearrangement was common to the two species: the nested chromosome fusion (NCF) involving chromosomes 10 and 11. The translocation of chromosome 2 is not the same found in *P. leptostachyus*, and pericentric inversions in chromosomed 3 and 4 were exclusive of *P. macvaughii*. The other rearrangements observed in *P. leptostachyus* were not shared with this species, suggesting that they occurred after the separation of these lineages. The presence of private rearrangements indicates a progressive accumulation of karyotype changes in the Leptostachyus group instead of an instant genome-wide repatterning.

Key message After a nested chromosome fusion (NCF) and consequent dysploidy, successive and independent rearrangements differentiated the chromosome complements of two species of the Leptostachyus group in a relatively short period of time.

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Keywords BAC-FISH · Dysploidy · Inversion · Karyotype evolution · Nested chromosome fusion · Translocation

Abbreviations

BAC	Bacterial artificial chromosome
Chr	Chromosome
DAPI	4,6-Diamidino-2-phenylindole
FISH	Fluorescence in situ hybridization
NCF	Nested Chromosome Fusion
Mya	Million years ago
rDNA	Ribosomal DNA
Ple	*Phaseolus leptostachyus*
Pma	*Phaseolus macvaughii*
Pvu	*Phaseolus vulgaris*

Introduction

Genome stability is important for species survival and reproduction, but a degree of variability is essential for adaptation to changing environments. Therefore, the evolution of the genomes aims to establish a balance between stability and plasticity through strategies such as increasing or reducing genome size or chromosomes number, as long as no essential genes are lost on the way (Schubert and Vu 2016).

The haploid chromosome number varies widely in plants, from species with $n = 2$, as in *Rhynchospora tenuis* Link (Vanzela et al. 1996), to species with approximately $n = 700$ as in representatives of the genus *Ophioglossum* L. (Khandelwal 1990). Different events may lead to variation in this number; however, only polyploidy and dysploidy seem to be involved in karyotype evolution (Guerra 2000). Polyploidy consists of the multiplication of the entire chromosome complement (Guerra 2008), whereas dysploidy is the increase or reduction of the original haploid number without significant chromatin gain or loss. Dysploidy is usually related to events of fusion (Robertsonian fusion) or centric fission, resulting respectively in descending and ascending dysploidy (Schubert and Lysak 2011). The reduction of chromosome number can also be caused by a nested chromosome fusion (NCF) event, observed in species of Triticeae L. (Luo et al. 2009), *Brachypodium* P. Beauv. (International Brachypodium Initiative 2010; Idziak et al. 2014), and recently detected in *Coffea canephora* Pierre ex A. Froehner. Of the ten fusion events that gave rise to $x = 11$ in coffee, three of them were NCF (Li et al. 2019).

Dysploidy can be detected by analysing chromosome number variation in a given group of species and better interpreted when this information is examined in a phylogenetic context. In Marantaceae R. Brown., dysploidy and also polyploidy seem to be the main factors in chromosome number evolution, which may be associated with species diversification and geographical patterns (Winterfeld et al. 2020). In species of Araceae Juss., chromosome number reductions were predominant, while polyploidization occurred less frequently (Cusimano et al. 2012). The same was observed for a group of high mountain *Artemisia* L. when compared to the rest of the genus (Mas de Xaxars et al. 2015). However, the detection of structural rearrangements involved in the dysploid event requires comparative genomics analyses or comparative genetic or cytogenetic mapping, such as by BAC-FISH technique.

Synteny conservation analyses in crucifers revealed that the main mechanisms behind dysploid events are structural rearrangements such as inversions and translocations (Yogeeswaran et al. 2005; Lysak et al. 2003, 2006). In the model plant *Arabidopsis thaliana* (L.) Heynh., considered as a paleopolyploid, the $n = 5$ was derived from an ancestral karyotype with $n = 8$, found in several other Brassicaceae genera (Yogeeswaran et al. 2005). This strong reduction in chromosome number was promoted by an accelerated rate of rearrangements, mainly inversions and translocations (Lysak et al. 2006). However, since the species of this group have undergone several cycles of polyploidization and diploidization, it is possible that the high rates of chromosome rearrangements may be associated with their polyploid origin.

An event of dysploidy was also observed in a group of *Phaseolus* L., a legume genus that includes common bean (*P. vulgaris* L.) and lima bean (*P. lunatus* L.), as well as three other species of economic importance (Broughton et al. 2003). The genus is exclusively diploid and shows mostly $2n = 22$ (Mercado-Ruaro and Delgado-Salinas 1998), as well as a relative structural karyotype stability (Fonsêca and Pedrosa-Harand 2013; Bonifácio et al. 2012). However, Leptostachyus, a small monophyletic group composed of three species (*P. macvaughii* Delgado, *P. micranthus* Hook. and Arn. and *P. leptostachyus* Benth.) from Mexico and Central America, originated around 2.5 mya, presents a dysploid karyotype with $2n = 20$ (Mercado-Ruaro and Delgado-Salinas 1998; Delgado-Salinas et al. 2006). The results of the comparative cytogenetic mapping between *P. leptostachyus* and *P. vulgaris* revealed that numerous structural rearrangements, including a NCF that gave rise to the dysploid number and several translocations, occurred during the divergence of this lineage, suggesting a high rate of karyotype evolution in the Leptostahyus group (Fonsêca et al. 2016).

To determine if the karyotype repatterning seen in *P. leptostachyus* was a single event or the result of multiple and successive events during evolution of this group, we comparatively mapped *P. macvaughii*, sister to the other two species of the group. If the rearrangements present in *P. leptostachyus* were shared with *P. macvaughii*, they probably occurred before the differentiation of these species. If this were the case, it would suggest a single moment of great genomic restructuring. Alternatively, there was an acceleration of chromosome mutation rate in Leptostachyus group or in *P. leptostachyus* after dysploidy, with a progressive accumulation of rearrangements, which would be, at least in part, exclusive of one or the other species.

Materials and methods

Plant material

Seeds of *P. macvaughii* (G40656) and *P. leptostachyus* (179671), obtained from the germplasm banks of CIAT (Colombia) and Embrapa Genetic Resources and Biotechnology - CENARGEN (Brasília, DF), were germinated in Petri dishes with moistened filter paper. The roots were collected, pretreated in 2 mM 8-hydroxyquinoline for 20 h at 10 °C and fixed in methanol:acetic acid (3:1, v/v). Specimens were maintained on soil in the experimental garden or on vermiculite with modified nutrient solution of Hoagland and Arnon (1950) in the growing room of the Laboratory of Plant Cytogenetics and Evolution at Federal University of Penambuco for seed multiplication.

Mitotic preparations

Root meristems were digested with 2% cellulase (Onozuka) and 20% pectinase (Sigma) solution for 1 h and 30 min at 37 °C in humid chamber. Slides were prepared following a standard squashing technique (Guerra and Souza 2002), or by air drying according to the modified protocol of Carvalho and Saraiva (1993). Briefly, the digested roots were transferred to inclined slides, washed about 5 times with several drops of ice-cold fixative (methanol:acetic acid, 3:1) as the material was chopped, and dried with the aid of a hand pump. Finally, the slides were incubated in 45% acetic acid for 5 min and dried at 37 °C. Slides were stained in 0.1 μg/mL DAPI in 50% glycerol, selected under fluorescence microscopy, destained in ethanol:acetic acid (3:1) for 30 min, followed by absolute ethanol for 1 h, and stored at − 20 °C.

Obtaining and labelling probes

Twenty-three BACs previously mapped cytogenetically in *P. leptostachyus* and other species of the genus (Fonsêca et al. 2010; Bonifácio et al. 2012; Fonsêca and Pedrosa-Harand 2013) were selected for fluorescence in situ hybridization in *P. macvaughii* (Table 1). BAC DNA was extracted by the miniprep technique using the Plasmid Mini Kit (Qiagen). The probes were labelled with Cy3-dUTP (GE) or SpectrumGreen-dUTP (Vysis) by *nick translation* using the Nick Translation Mix kit (Roche). The bacteriophage SJ19.12, a marker for chromosome 10 (Fonsêca et al. 2010), as well as the 5S rDNA (D2, Pedrosa et al. 2002) and 35S rDNA (p*Ta*71, Gerlach and Bedbrook 1979) were also used as probes and labelled with Cy3-dUTP or digoxigenin 11-dUTP (Roche).

Fluorescence in situ hybridization

FISHs were performed according to Fonsêca et al. (2010). The rehybridization of slides was performed according to Heslop-Harrison et al. (1992). The 35S rDNA probe was detected with antidigoxigenin produced in sheep and conjugated to FITC (Roche) and amplified with antisheep IgG produced in donkey and conjugated with FITC (Vector) in 1% BSA in PBS. For probes that generated additional dispersed signals, hybridization was performed using *P. vulgaris* genomic DNA, extracted according to the modified protocol of Weising et al. (2005) and fragmented in boiling water for 50 min (to obtain fragments less than 1 kb) as blocking at different concentrations (20–100×) depending on the BAC probe used.

Analysis of results

Metaphase cells were captured on a Leica DM5500B epifluorescence microscope by DFC345 FX capture system (Leica). The best metaphases were overlaid and adjusted for brightness and contrast in Adobe Photoshop CS6. Chromosomes were identified and numbered according to the orthology with *P. vulgaris* (Fonsêca et al. 2010). Chromosome sizes and approximate positions of markers along chromosomes are only schematically represented.

Results

Phaseolus macvaughii showed $2n = 20$, as previously reported for this species (Mercado-Ruaro and Delgado-Salinas 1998) and also observed for *P. leptostachyus* (Fonsêca et al. 2016). In order to identify the chromosomes and the mechanisms involved in the formation of this dysploid karyotype, which has 20 instead of the 22 chromosomes observed in the rest of the genus, single copy clones for nine of its ten chromosomes were

Table 1 List of clones used as probes for comparative analysis by FISH between *P. macvaughii* (*Pma*) and *P. vulgaris* (*Pvu*), distribution and location of these sequences in both species

Chromosome	Clones	Distribution		Location			
				Arm		Position	
		Pv	*Pma*	*Pv*	*Pma*	*Pv*	*Pma*
*Pvu*1	221F15	Unique*	Unique*	Short	Long	Proximal	Proximal
	257L12	Unique	Unique	Long	Short	Terminal	Terminal
*Pvu*2	17P14	Subtelomeric	Unique	Short	Long	Subtelomeric	Interstitial
	127F19	Unique	Unique	Long	Long (*Pma*1/2)	Interstitial	Interstitial
	255P10	Unique	Unique	Long	Long (*Pma*1/2)	Terminal	Terminal
*Pvu*3	77J14	Unique*	Unique*	Long	Short	Interstitial	Proximal
	91K16	Unique	Unique	Long	Long	Terminal	Terminal
	147K17	Unique	Unique	Short	Short	Interstitial	Interstitial
	174E13	Unique	Unique	Long	Long	Terminal	Terminal
	267H4	Unique*	Unique*	Short	Short	Proximal	Proximal
*Pvu*4	190C15	Unique*	Unique*	Long	Short	Interstitial	Interstitial
	221J10	Unique*	Unique	Short	Long	Terminal	Terminal
*Pvu*6	35S rDNA	Repetitive	Repetitive	Short	Short	Terminal	Terminal
	18B15	Unique	Unique	Long	Long	Terminal	Terminal
*Pvu*7	22I21	Unique	Unique	Long	Long	Interstitial	Interstitial
	33M20	Unique*	Unique*	Long	Long	Terminal	Terminal
	86I17	Subterminal	Unique	Short	Short	Interstitial	Interstitial
*Pvu*8	169G16	Unique*	Unique*	Long	Long	Terminal	Terminal
	177I19	Unique	Unique	Short	Short	Interstitial	Interstitial
*Pvu*9	163I7	Unique	Unique	Long	Long	Interstitial	Interstitial
	224I16	Unique	Unique	Short	Short	Terminal	Terminal
*Pvu*10	5S rDNA	Repetitive	Repetitive	Long	Long	Interstitial	Interstitial
	63H6	Subtelomeric	Unique	Short	Long	Subtelomeric	Subterminal
	SJ19.12	Unique	Unique	Long	Short	Interstitial	Proximal
*Pvu*11	127J2	Unique	Unique	Long	Long	Terminal	Terminal
	179N14	Unique	Unique	Short	Short	Terminal	Terminal

*Dispersed probes showing unique signals after using blocking DNA

cytogenetically mapped and compared to the previous results of *P. leptostachyus*. First, five BACs and one bacteriophage from *P. vulgaris* chromosomes (*Pvu*) 6, 10 and 11 were hybridized to *P. macvaughii* chromosomes. These three chromosomes were involved in the nested chromosome fusion (NCF) that caused the dysploidy event and in the formation of the largest chromosome pair in *P. leptostachyus* (Table 1, Fonsêca et al. 2016). The hybridization with BAC 18B15 (*Pvu*6) labelled a small chromosome pair carrying the unique 35S site, identifying it as orthologous to *Pvu*6 and not involved in the formation of the largest chromosome pair (Fig. 1a). BAC 63H6 (*Pvu*10) evidenced a signal in the subterminal region of the long arm of the largest pair of *P. macvaughii*, the same arm in which the single 5S rDNA site is located (Fig. 1a–c). The other *Pvu*10 (5S rDNA, BAC 63H6 and SJ19.12) and *Pvu*11 (BACs 127J2 and 179N14) probes all hybridized to the largest chromosome pair in *P. macvaughii*, indicating the presence of the same NCF that placed the inverted *Pvu*10 in the centromeric region of *Pvu*11 and led to the formation of the largest chromosome pair (Fig. 1a–c). Thus, a single event caused the descending dysploidy in the ancestral of *P. macvaughii* and *P. leptostachyus*, although chromosome arm sizes vary between these species due to the additional translocation of *Pv*6 to the largest chromosome pair in *P. leptostachyus* only.

In addition, three other rearrangements were observed on *P. macvaughii* chromosomes when compared to *P. vulgaris*. The first change involved chromosomes 1 and 2. BAC 221F15 (*Pvu*1) hybridized at the proximal region of the long arm, and BAC 257 L12 (*Pvu*1) showed a signal at the end of the short arm of the same chromosome (Table 1, Fig. 1d), whereas in *P. vulgaris* these BACs are in opposite arms, short and long arms, respectively, and in *P. leptostachyus*, these BACs are at different chromosomes. BACs from *Pvu2* hybridized to two chromosomal pairs of *P. macvaughii*. BACs 127F19 and 225P10 hybridized at the end of the long arm of chromosome 1, in the same arm where BAC 221F15 from *Pvu*1 was mapped (Fig. 1e–f), revealing a translocation of the terminal portion of the long arm of *Pvu*2 to the short arm of *Pvu*1 in *P. macvaughii*. To

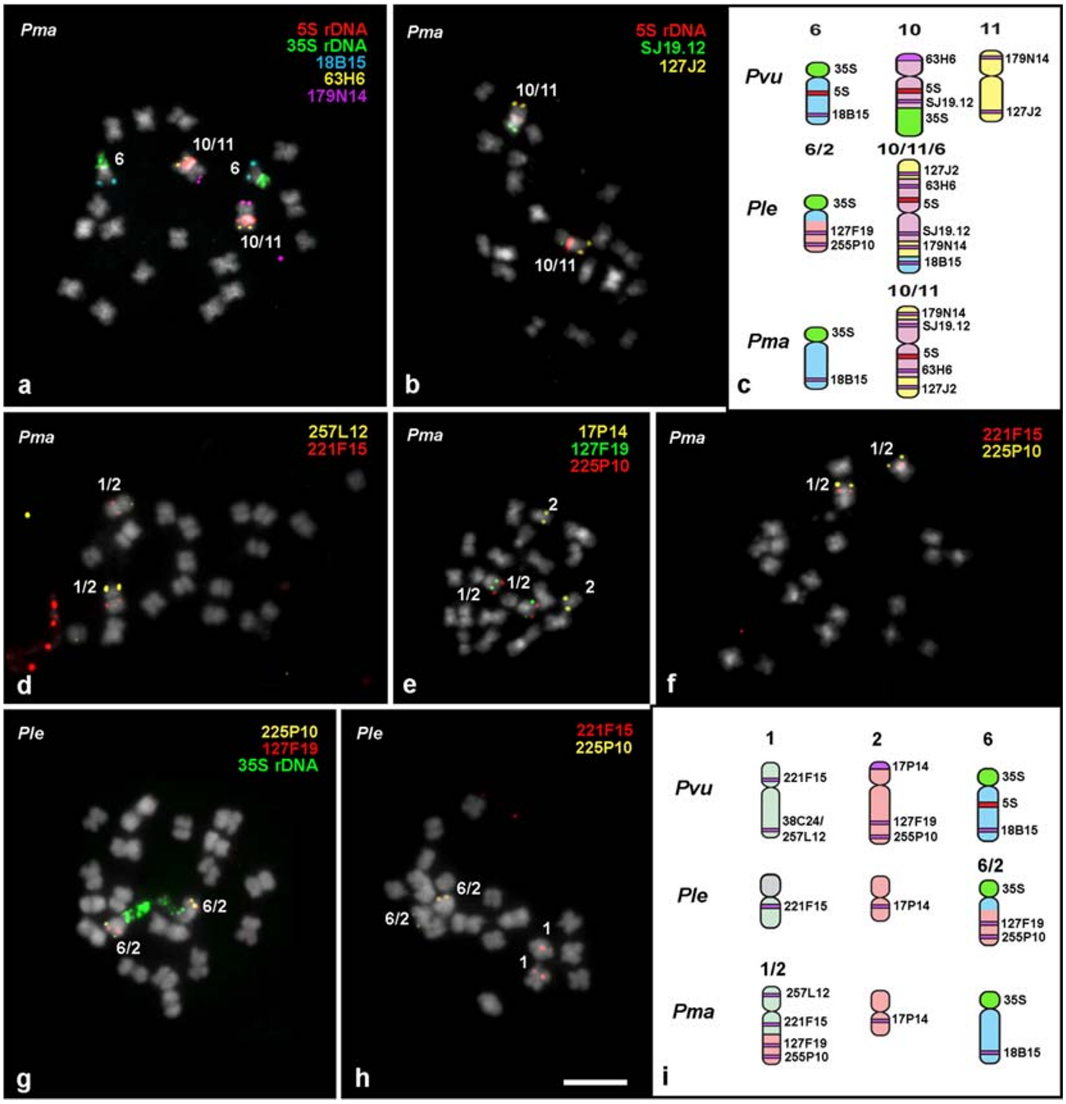

Fig. 1 Fluorescence in situ hybridization in mitotic metaphases of *P. macvaughii* (*Pma*, **a–b**, **d–f**) and *P. leptostachyus* (*Ple*, **g–h**), showing the rearrangements involving chromosomes *Pvu*1, 2, 6, 10 and 11 (schematically represented in **c** and **i**). BACs (**a–b**, **d–f** and **g–h**), bacteriophage (**b**) and rDNA (**a** and **g**) are indicated on the upper side of each cell in the respective colours. Subtelomeric BACs in *Pvu* are between parenthesis. In **a–b**, rearrangements in *Pvu*10 and *Pvu*11, but not *Pvu*6, originated the largest chromosome pair in *P. macvaughii*. In **d–f**, rearrangements involving chromosomes *Pvu*1 and *Pvu*2 in *P. macvaughii*. In **g–h**, rearrangements involving *Pvu*2 and *Pvu*6 in *P. leptostachyus*. Note that 35S rDNA is highly decondensed in (**g**). Chromosomes were counterstained with DAPI and visualized in grey. Bar in (**h**) correspond to 5 μm

verify whether this translocation was a shared rearrangement between the two species in the Leptostachyus group, the BACs 127F19 and 225P10 were hybridized in *P. leptostachyus*. However, these two BACs of *Pvu*2 showed signals on the long arm of chromosome 6, identified by the 35S DNAr site (Fig. 1g), while BAC 221F15 (*Pv*1) showed a signal on another chromosome (Fig. 1h–i). Thus, the translocations involving chromosome *Pv*2 are distinct between *P. macvaughii* (1/2) and *P. leptostachyus* (6/2).

For *Pvu*3, there was a pericentric inversion in *P. macvaughii*, revealed by the hybridization of BAC 77J14 in the proximal region of the short arm of the chromosome, opposite to BAC 91K16 (Fig. 2a–b), instead of both BACs present in the long arm, as in *P. vulgaris* (Table 1). The other BACs of this chromosome showed to be collinear in relation to *P. vulgaris*, while in *P. leptostachyus* BAC 77J14 is in a different chromosome (Fig. 2b–d). For *Pvu*4, BACs 221J10 and 190C15 were in opposite arms when compared to *P. vulgaris*, suggesting a putative pericentric inversion. In *P. leptostachyus* these BACs are at different chromosomes (Fig. 2e-g).

Chromosomes *Pvu*7 and *Pvu*8 did not show any rearrangement in *P. macvaughii*, since BACs 22I21, 33M20 and 86I17, as well as BACs 169G16 and 177I19, respectively, were syntenic in *Pma*7 and *Pma*8 (Table 1, Fig. 3a-d). Thus, it was possible to identify in *P. macvaughii*, in addition to chromosome 6, two other pairs of conserved chromosomes to *P. vulgaris*. *Phaseolus macvaughii* chromosome 9 is metacentric, with the presence of BACs 163I7 and 224I16 in the long and short arm, respectively (Fig. 3e and g). This differs from *P. vulgaris*, which has an acrocentric chromosome 9 carrying a 35S rDNA site on the short arm and both BACs on the long arm, as well as from *P. leptostachyus,* with BACs 163I7 and 224I16 on different chromosomes (Fig. 3f-g). The results of the cytogenetic mapping in *P. macvaughii* are summarized and compared to *P. leptostachyus* and *P. vulgaris* in Fig. 4.

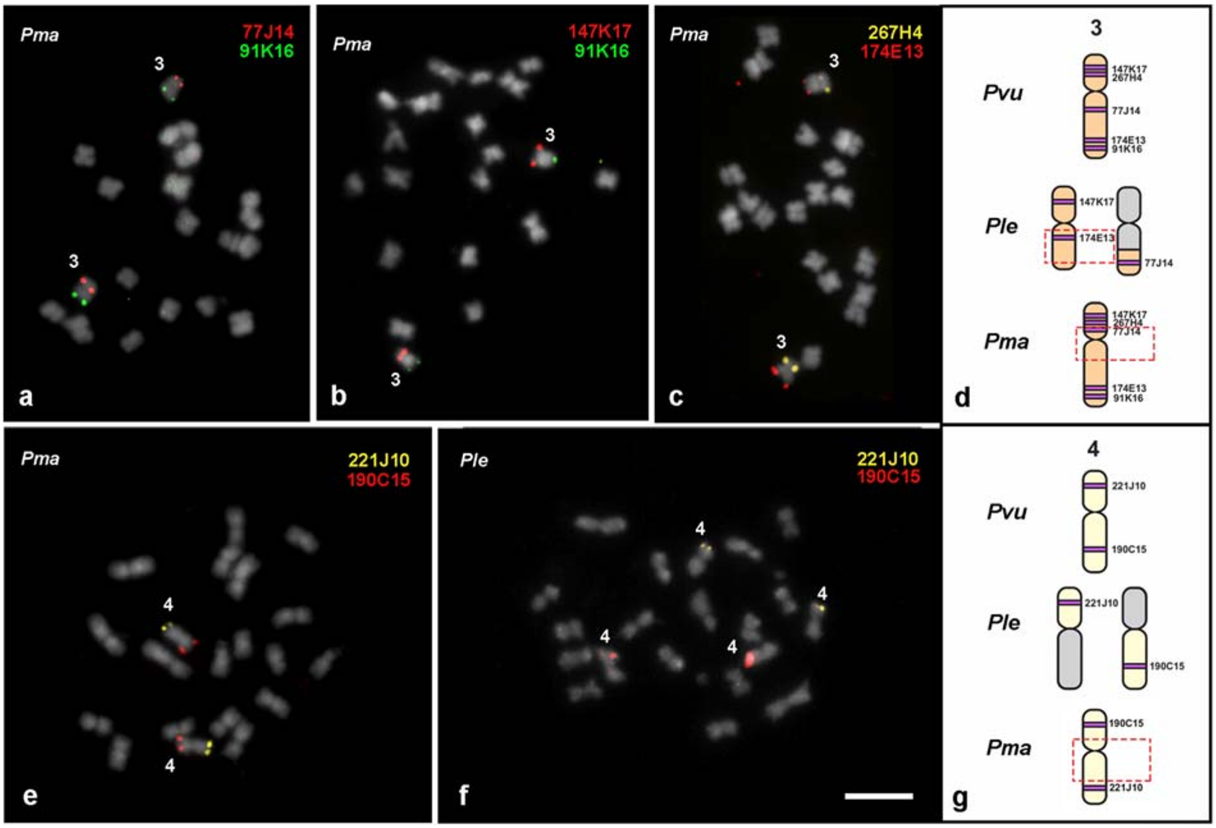

Fig. 2 Fluorescence in situ hybridization in mitotic metaphases of *P. macvaughii* (*Pma*, **a**–**c**, **e**) and *P. leptostachyus* (*Ple*, **f**), showing rearrangements involving chromosomes *Pvu*3 (**a**–**c**) and *Pvu*4 (**e**–**f**), schematically represented in **d** and **g**, respectively. The BACs used are indicated on the upper side of each cell in the respective colours. Chromosomes were counterstained with DAPI and visualized in grey. Red boxes on chromosomes indicate putative inversion events. Bar in (**f**) correspond to 5 µm

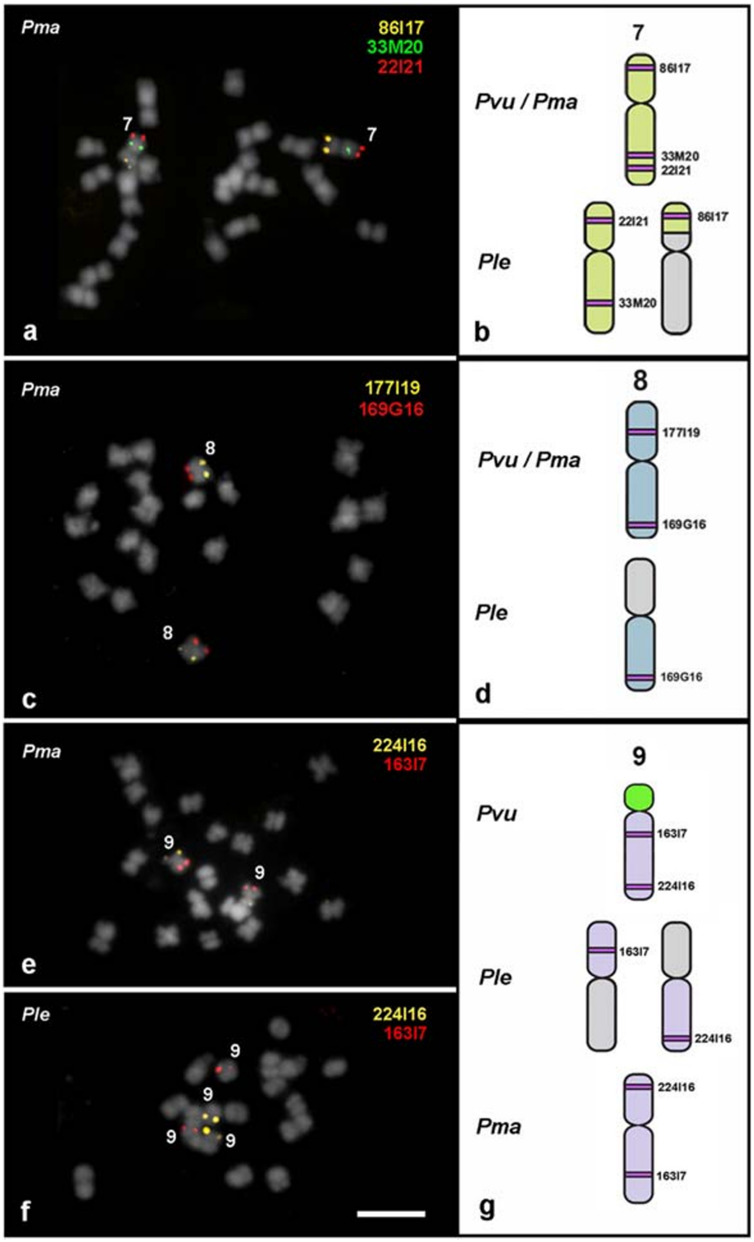

Fig. 3 Fluorescence in situ hybridization in mitotic metaphases of *P. macvaughii* (*Pma*, **a**, **c**, **e**) and *P. leptostachyus* (*Ple*, partial, **f**) showing conservation of synteny for chromosomes *Pvu*7 (**a**–**b**), *Pvu*8 (**c**–**d**), and *Pvu*9 (**e**, **g**) in *P. macvaughii* when compared to *P. vulgaris.* Difference between *Pma*9 and *Pvu*9 is attributed to rearrangement in *P. vulgaris*, but *Ple*9 shows synteny break (**f**). The BACs used are indicated on the upper side of each cell in the respective colours. Chromosomes were counterstained with DAPI and visualized in grey. Bar in **f** correspond to 5 µm

Discussion

In this work, nine of the ten chromosome pairs of *P. macvaughii* could be mapped and compared to *P. vulgaris* (Fonsêca et al. 2010) and *P. leptostachyus* (Fonsêca et al. 2016 and present work). We demonstrated that the descending dysploidy that originated the karyotype with $2n = 20$ in the Leptostachyus group was a single event resulting from the centric insertion of all or a large part of *Pvu*10 in *Pvu*11 (Fonsêca et al. 2016). However, unlike in *P. leptostachyus* (Fonsêca et al. 2016), chromosome 6 was not involved in the formation of the largest pair in *P. macvaughii*, and, therefore, the dysploidy in the group is associated to a single NCF (Fig. 4). In the cotton tribe (Gossypieae), a dysploidy in the clade that includes *Kokia* Lewton and *Gossypioides* Skovst. ex J.B.Hutch. ($n = 12$) occurred after divergence of this branch from *Gossypium* ($n = 13$). This reduction of one chromosome pair resulted from several structural rearrangements involving three

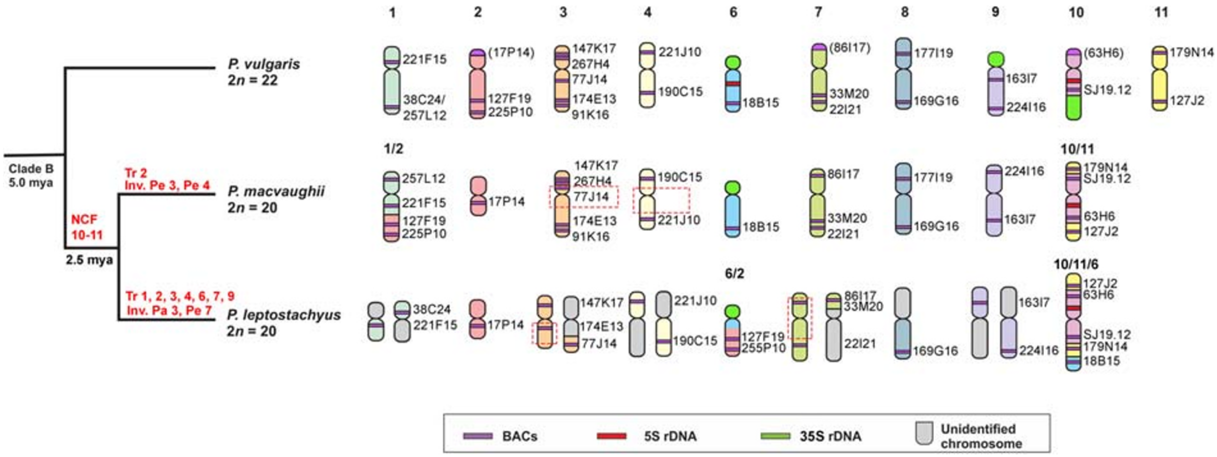

Fig. 4 Schematic representation of *P. macvaughii* chromosomes compared to *P. vulgaris* (Fonsêca et al. 2010) and *P. leptostachyus* (Fonsêca et al. 2016 and present data). Subtelomeric BACs in *Pvu* are between parenthesis. Chromosomal rearrangements are represented above each branch in red by the abbreviations: NCF (nested chromosome fusion), Inv (inversion), Pe (pericentric), Pa (paracentric) and Tr (translocation). The numbers indicate the chromosome pairs involved. Red boxes on chromosomes indicate putative inversion events. Phylogenetic relationships between species according to Delgado-Salinas et al. (2006)

chromosome pairs (Udall et al. 2019). A scenario of multiple rearrangements is also proposed to explain the dysploid reduction observed in tribe Boechereae Al-Shehbaz, Beilstein and E.A. Kellogg, from Brassicaceae ($n = 8 \rightarrow n = 7$; Mandáková et al. 2020).

Despite the collinearity of sequences along chromosome *Ple/Pma* 10/11, all BACs located in the long arm in *P. macvaughii* are in the short arm of *P. leptostachyus*, and vice versa. It is possible that the additional translocation of part of chromosome 6 to chromosome 10/11 of *P. leptostachyus* (Fonsêca et al. 2016) resulted in a slight change in the length of the chromosome arms, transforming the short arm of the largest ancestral pair, conserved in *P. macvaughii*, into the long arm in *P. leptostachyus.* Additionally, it is possible that quantitative changes in the pericentromeric heterochromatin of this chromosome, after the separation of both species, also contributed to this change in arm ratio. Differences in centromere position for chromosomes 6, 8 and 10 were observed between *Vigna aconitifolia* (Jacq.) Marechal. and *V. unguiculata* (L.) Walp. without detected changes in collinearity and may be related to variation in the 35S rDNA block size or other repetitive sequences (Oliveira et al. 2020).

Chromosome 6 has a 35S rDNA site in *P. macvaughii*, as all previously analysed *Phaseolus* species (Pedrosa-Harand et al. 2006; Bonifácio et al. 2012; Fonsêca and Pedrosa-Harand 2013; Fonsêca et al. 2016), reinforcing this terminal 35S site on the short arm as a plesiomorphic character. Similarly, the 5S rDNA site was conserved in chromosome 10, which correspond to the largest chromosome pair (10/11) in *P. macvaughii* and *P. leptostachyus* (Fonsêca et al. 2016). However, the two species of Leptostachyus group share with *P. lunatus* a putative pericentric inversion on chromosome 10 that placed the 5S rDNA site at the short arm (Bonifácio et al. 2012). This event probably occurred before the separation of the Leptostachyus and Lunatus groups and, thus, is not related to the dysploidy or to the other rearrangements in Leptostachyus.

Excepted for the NCF that gave rise to the dysploid karyotype, none of the detected rearrangements in *P. macvaughii* and *P. leptostachyus* was shared within this group. Chromosome 2 was involved in translocations both in *P. macvaughii* and *P. leptostachyus*, but while in *P. macvaughii* the translocation was with chromosome 1, in *P. leptostachyus*, it was with chromosome 6 (Fonsêca et al. 2016). Furthermore, *P. leptostachyus* showed exclusive translocations involving at least chromosomes 1, 2, 3, 4, 6, 7 and 9 (Fonsêca et al. 2016). Similarly, while *P. macvaughii* chromosome 3 showed a pericentric inversion, *Ple*3 showed a translocation and a paracentric inversion (Fonsêca et al. 2016; Fig. 4). Therefore, multiple, independent events occurred after the dysploidy and the separation of the two species. In *Ricotia* L. (Brassicaceae), species with $n = 14$ were the result of independent events of dysploidy, and part of one $n = 14$ group went through further rearrangements resulting in $n = 13$ (Mandáková et al. 2018).

In this study, we demonstrated that a single NCF gave rise to the $2n = 20$ karyotype in the ancestral of the Leptostachyus group. After this event, further species-specific rearrangements occurred in each lineage (Fig. 4). All these events occurred in the last 2.5 million years, during or after species separation (Delgado-Salinas et al. 2006). Chromosome rearrangements may contribute to species isolation, as observed in *Drosophila* (Fuller et al. 2019), *Lepidoptera* (de Vos et al. 2020) and rodents (Capilla et al. 2016). In plants, rearrangements also contributed to speciation in wild emmer wheat (Wang et al. 2020) and reproductive isolation in *Carex* L. (Cyperaceae; Escudero et al. 2016). They are also believed to constitute key evolutionary innovation underlying the diversification of Boechereae (Mandáková et al. 2020). The presence of exclusive rearrangements for the two species after the NCF suggests not a single moment of major genomic restructuring, but a high rate of karyotype evolution, with successive and independent rearrangements, in a relatively short period since the origin of the group. The investigation of the third species, *P. micranthus*, may reveal further rearrangements, shedding light to the chromosome evolution after the dysploidy event in this group. This future work may benefit from the recently developed oligonucleotide painting probes for chromosomes 2 and 3 of *P. vulgaris* (Martins, Lívia do Vale et al., unpublished results). In the absence of a diploidization process, since polyploidy did not occur in the genus *Phaseolus* (Schmutz et al. 2014), the cause for this accelerated rate of chromosome evolution in the Leptostachyus group remains unknown.

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Authors' contribution MEF: performed experiments, organized the figures, and drafted the manuscript. AF: analysed and discussed the data. APH: designed and supervised the research and corrected the manuscript. All authors read, discussed, and approved the final version of the manuscript.

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Compliance with ethical standards

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Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All authors approved the final version of the manuscript.

Code availability Not applicable.

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