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Chromosome numbers and genome size data on species of the genus *Petrorhagia* (Caryophyllaceae) from Turkey

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Abstract: Genome size, i.e. the total amount of DNA in an unreplicated somatic nucleus is considered an important character in the biology of organisms, and it is especially relevant for land plants given the extraordinary diversity reported. Despite the continuous growth of data and the efforts aimed at increasing our understanding of plant genome size diversity, more data are needed to have an evenly representation across lineages. With this premise in mind, in this work, we investigated genome size, complemented with chromosome data in eight species of the genus *Petrorhagia* (Caryophyllaceae) from Turkey. Chromosome counts made indicate that the species studied are diploid except for one species, *P. saxifraga,* which is probably a tetraploid, based on previously published estimations of nuclear DNA contents. The presence of both basic chromosome numbers x = 15 and x = 13 is confirmed, the latter being restricted to the species *P. hispidula* and *P. cretica.* The counts carried out for the Turkish endemic species, in *P. hispidula* (2n = 26), *P. pamphylica* (2n = 30) and *P. peroninii* (2n = 30) represent novel data for the genus. Nuclear DNA contents ranged from 1.24 to 2.32 pg/2C (varying 1.87-fold). Based on available data, genome expansions in the genus seem to have occurred through different evolutionary mechanisms, such as polyploidy and differential repetitive DNA activation, evidencing contrasting genomic trajectories between closely related taxa.

Key words: Caryophyllaceae, chromosome number, endemism, flow cytometry, nuclear DNA content

1. Introduction

The genus *Petrorhagia* (Ser.) Link (Caryophyllaceae) comprises 32 species (Strid & Tan 1997), and it is mainly distributed across Europe, West Asia and the Mediterranean basin, being Greece and Turkey considered as important centres of diversification. The systematics of the genus have been under debate for long time, with several taxa being segregated into different genera (e.g., Tunica Scop., *Kohlrauschia* Kunth and Gypsophila L.). However, after several taxonomic revisions, the generic boundaries are currently agreed, and all species have been returned to the genus (see e.g., Ball, 1964; Ball and Heywood, 1964; Coode and Cullen, 1967; Rechinger, 1985; Marhold, 2011). Regarding the presence of Petrorhagia in Turkey, 10 species have been reported to occur in the country, of which four are considered endemic sensu Coode and Cullen (1967). In 1970, the presence of P. syriaca (Boiss.) Mouterde & Greuter was also confirmed by Greuter and Mouterde (1970). Since then, a total of 13 taxa (including species and subspecies) have been recognised based on the checklist of the Flora of Turkey (Aktas, 2012) and other taxonomic treatments (Dönmez et al., 2013).

The species *P. hispidula* (Boiss. & Heldr.) Ball & Heywood, P. lycica (Davis) Ball & Heywood and *P. pamphylica* (Boiss. & Ball) Ball & Heywood are listed as vulnerable, while the species *P. peroninii* (Boiss.) Ball & Heywood is considered at low risk according to the Red Data Book of Turkish plants (Ekim et al., 2000). Despite the interest that some of these species could raise for conservation purposes due to their inclusion on red lists of threatened flora, very little is known regarding the systematics of the genus. Only few representatives of the genus were included in a family-level phylogenetic study of the Caryophyllaceae, which evidenced the potential intricate relationships within the genus and close related genera Greenberg and Donoghue (2011). More recently, Medhani et al. (2018) provided support for the monophyly of *Petrorhagia* based on nuclear markers, though with a relatively small set of species. Some studies focusing on pollen morphology and ecological preferences from Turkish taxa [except for *P. armerioides* (Ser.) P.W.Ball & Heywood and *P. syriaca*]

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were studied by Aktaş et al. (2010a, 2010b). In addition, karyological investigations showed that both diploid and polyploid cytotypes occur in the genus, with chromosome numbers including 2n = 26, 30, and 60 (Böcher et al., 1953; Favarger, 1966; Thomas and Murray, 1983; Çelebioğlu and Favager, 1993; Petrova, 1995; Runemark, 1996; Pavlova and Tosheva, 2004; Diaz Lifante and Parra Martín, 2013; Rice et al., 2014; Peruzzi et al., 2016; Zonneveld, 2019). The Turkish representatives of the genus were also studied by Hilooğlu et al. (2016), who carried out a molecular study using ISSR markers (except for *P. armerioides* and *P. syriaca* and *P. wheeler-hainesii* Rech.f.). The results supported a segregation of the genus in two main genetic clusters, one made up solely by the species *P. lycica*, and an expanded one, comprising the remaining species, some of which would require of further research to confirm their taxonomic status.

Genome size, i.e. the total amount of DNA in an unreplicated somatic nucleus (Greilhuber, 2005), is considered an important trait in the biology of organisms, and it is especially relevant for plants given the extraordinary diversity reported to date (Pellicer et al., 2018). The importance of this parameter in the evolution of plants is supported by the multiple implications reported between this trait and many ecological, evolutionary and karyological attributes as well as being influenced by hybridisation and the genomic reorganisations (e.g., Beaulieu et al., 2010; Dyer et al., 2013; Guignard et al., 2016; Pellicer et al., 2021). As stated above, cytogenetic studies in *Petrorhagia* are scarce, and mostly focus on chromosome data. To date, only one species has got its genome size estimated, the species *P. saxifraga*, with 2C-values estimated at 1.30 and 2.24 pg (Vidic et al., 2009, Temsch et al., 2010). The authors also conducted chromosome counts in each accession confirming the presence of both diploid and tetraploid cytotypes (i.e. 2n = 30 and 60, respectively), showing that despite ongoing genome downsizing, polyploidy can be considered a driver of genome size evolution in the genus.

Bearing these precedents in mind and in order to expand our current understanding of genome size diversity in the genus, the present study aimed at (i) generating a dataset of nuclear DNA contents focused on Turkish representatives combined with chromosome data, and (ii) evaluating potential relationships between genome size and changes in chromosome number.

2. Materials and methods

2.1. Plant material

The plants studied here were collected from wild populations in Turkey. Details regarding localities sampled are presented in Table 1 and Figure 1. Taxonomical identifications were based on the Flora of Turkey and East Aegean Islands (Coode and Cullen, 1967). For each species, five individuals were collected (a total of 40 individuals). Seeds and specimen vouchers were deposited at MCBUH (Manisa Celal Bayar University Herbarium).

2.2. 2.2. Chromosome counts

Root-tip meristems were obtained from wild-collected seeds germinated on wet filter paper in Petri dishes at room temperature in the dark. Seedlings were pretreated with 0.002M 8-hydroxyquinoline at room temperature for 3 h. Material was fixed in absolute ethanol and glacial acetic acid (3:1) for 2–4 h at room temperature and stored in the fixative at 4 °C. Samples were hydrolysed in 1N HCl for 2 min at 60 °C, stained with 1% aqueous aceto-orcein for 2 h, and squashed on slides in 45% acetic acid-glycerol (9:1). The best metaphase plates were photographed with a digital camera (AxioCam MRc5 Zeiss) mounted on a Zeiss Axioplan microscope and images were analysed with Axio Vision Ac software version 4.2. Chromosome morphologies and karyograms were constructed based on the determination of centromeric location where possible. Measurements were done using ImageJ software (Shchneider et al., 2012). Calculations included total karyotype length (TKL), long (L) and short (S) chromosome arm length, arm length ratios to calculate centromeric index (CI), intrachromosomal and intercrhomosomal asymmetry indexes (M_{CA} and CV_{CL}) based on Peruzzi and Eroğlu (2013) and Paszko (2006).

2.3. Sequence alignment and analysis

Specimens analysed come from the cultures performed at the glasshouse facilities of the Institut Botànic de Barcelona, from seeds collected in field. Nuclear DNA content estimations were carried out by flow cytometry with propidium iodide following the procedure described in Garnatje et al. (2004). Briefly, young, healthy leaf tissue from each species was placed in a plastic Petri dish and chopped in 1200 µL of LB01 lysis buffer (Dolezel et al. 1989) with a razor blade. The suspension of nuclei was filtered through a nylon mesh with a pore size of 70 µm and stained for 20 min with 36 µL of propidium iodide (60 µg/mL; Sigma-Aldrich Química). Five individuals per species were analysed and two runs per replicate were carried out, using as internal standard, Petunia hybrida Vilm.'PxPc6' (2.85 pg/2C, Marie & Brown 1993) was used. Flow cytometric assessments were conducted at the Centres Científics i Tecnològics of the Universitat de Barcelona using an Epics XL flow cytometer (Coulter Corporation, Hialeah, FL, USA).

2.4. Phylogenetic data mapping

In order to plot and visualize genome size data from a phylogenetic perspective, the UPGMA dendrogram showing the genetic relationships in *Petrorhagia* from Hilooğlu et al. (2016) was replicated. Available genome size data were plotted on the tree using ggplot2 and Phytools packages (Revell, 2012; Wickham, 2016), both available in R (R core Team, 2019).

3. Results and discussion

The list of nuclear DNA contents for each species, complemented with chromosome numbers are shown in Table 2. Detailed information regarding chromosomal and karyotype information is presented in Table 3. Illustrative chromosome pictures and the distribution of genomes sizes reported to date from a phylogenetic perspective (where available) are depicted in Figures 2A and 2B.

3.1. Chromosome numbers in *Petrorhagia*: presence of two base chromosome number

Overall, chromosome numbers obtained in this study are quite stable across the species studied. The counts carried out in *P. hispidula, P. pamphylica* and *P. peroninii* represent new additions to the genus (Figures 2A-ii–2A-iv). All the studied taxa are diploid (but *P. saxifraga,* discussed later), confirming the presence of both basic chromosome numbers x = 15 (of larger incidence, Figure 2A), and x = 13, restricted to the species *P. hispidula* (Figure 2A-ii). Until present, x = 13 had only been reported in the species *P. armerioides, P. cretica* and *P. illyriaca* at diploid level (Favarger, 1966; Petrova, 1995; Runemark, 1996). The results found here in *P. cretica* also indicate the presence of 2n = 30 in this species. Certainly, our chromosome count is the first one from Turkey, and contrasts with the previous report by Favarger (1966). Intraspecific karyotype variation is relatively frequent in plants, sometimes considered an evolutionary driver due to chromosome restructurings (e.g., Gillieseieae, Pellicer et al., 2017), but changes in basic chromosome numbers within species are less frequent and likely derived from production of aneuploid gametes and hybridisation (e.g., *Cardamine pratensis* L., Clapham et al. (1962). Based on this finding, it would be necessary to continue monitoring this species and conduct an expanded sampling to confirm if changes in the basic chromosome number reflect specific chromosomal rearrangements following any geographical patterns, and if so, which could be the taxonomic implications in the long term.

We have also contributed with new counts from Turkey in the species *P. alpina* subsp. *alpina*, *P. dubia* and *P. prolifera* (2n = 30). These species had been studied in the past, and the results presented here agree with previously published reports (Böcher et al., 1953; Thomas and Murray, 1983; Pavlova and Tosheva, 2004; Diaz Lifante and Parra Martín, 2013; Rice et al., 2014; Peruzzi et al., 2016; Zonneveld, 2019). In addition, Böcher et al. (1953), also reported the tetraploid cytotype in *P. prolifera* (2n = 60), thus confirming the incidence of polyploidy in the genus.

In general, *Petrorhagia* chromosomes observed here were quite small, with total karyotype lengths ranging just from 7.70 to 24.01 μm. Unfortunately, the reduced size and condensation status of some of the chromosomal plates did not allow us to present an in-depth description of the karyotypes in *P. peroninni* and *P. pamphylica.* Overall, the values obtained for the centromeric indexes indicate that karyotypes are relatively symmetrical (CI: 0.44–0.45), which is also reflected by the karyotype formulas, where many of chromosomes are classified as metacentric. Available karyotype information in previous studies in the genus is scarce, probably due to the difficulties of obtaining high resolution metaphase plates due to the small-sized chromosomes. Our results agree with previous data in *P. prolifera* by Thomas and Murray (1983), which evidenced the presence of mostly metacentric chromosomes in the karyotype of the species, and chromosomal sizes that fall within the range of observed values in this study. In addition, they report the presence of one pair of telocentric chromosomes in *P. velutina* (Guss.) P.W.Ball & Heywood (Syn. *P. dubia* (Raf.) G.López & Romo), another representative of the genus present in Turkey. This suggest that, despite the relatively presence of symmetrical karyotypes reported in our study, chromosomal rearrangements might result in changes in the overall symmetry.

3.2. Small genomes are prevalent in the genus *Petrorhagia*

Nuclear DNA contents estimated in this study are listed in Table 2. The values obtained showed a range of variation of about 1.87-fold. The smallest genome, with just 1.24 pg/2C was obtained in *P. hispidula* (2n = 2x = 26) and the largest one (2.32 pg/2C) in *P. peroninii* (2n = 2x = 30). Genome size data reported here have been measured for the first time in all studied taxa, with the exception of *P. saxifraga* (Vidic et al., 2009; Temsch et al., 2010). This species was first studied by Vidic et al. (2009), which reported a tetraploid cytotype from serpentine soils with a 2n = 60 and a genome size of 2.24 pg/2C. One year after publication of this result, Temsch et al. (2010) revisited the study plots and found a different cytotype, diploid, which was chromosomally confirmed, with a genome size of 1.30 pg/2C. Both cytotypes had been also reported in previous studies (Favarger, 1966; Çelebioğlu and Favarger, 1993; Rice et al., 2014; Zonneveld, 2019) evidencing that polyploidy might be relatively frequent in this species. Based on the abovementioned evidences, it seems plausible to attribute a tetraploid cytotype to the accession studied here, given that our genome size estimate is close to Vidic's et al. (2009) tetraploid sample (i.e. 2.10 pg/2C). Such scenario contrasts with the genome size found in *P. peroninii,* for which we obtained a diploid chromosome number, but whose nuclear DNA content is close to that of the likely tetraploid *P. saxifraga* (i.e. 2.32 pg/2C). Genome size doubling is frequently associated to whole genome duplications and polyploidy in plants, and results in an (almost) duplication of the DNA content (Pellicer et al., 2018). However, it is not surprising to find examples in which nearly genome size duplications occur between species with the same chromosome number, such as for

Table 1. Provenance of the taxa studied.

Taxon	Location, collector and data
P. alpina subsp. *alpina*	Turkey: Tokat: Erbaa, Osman village, towards the Erbaa, roadside, beneath the hornbean-pinus mixture forest, 1100m, 23.VII.2003, K.A 1230.
P. cretica	Turkey: Manisa: Demirci, Simav road, meadow, 1000m, 13.VI.2003, K.A 1126.
P. dubia	Turkey: Denizli: Babadağ mountain, around the village, 870m, above the hillside, 24.VI.2003, K.A 1140.
P. hispidula (E)	Turkey: Konya: Between Akşehir and Yalvaç, Sultan mountain, before the come to Akşehir, 20km, roadside, dry hillside, 1550m, 11.VIII.2003, K.A 1270.
P. pamphylica (E)	Turkey: Antalya: Lara road, near the Dedeman otel, in front of the Fasilis residence, above the clif, stones and hillstones, 95m, 27.VI.2003, K.A 1190
P. peroninii (E)	Turkey: Antalya: Alanya, between Mahmutlar and Gözüküçüklü village, 3.km, roadside, dry meadow, 24.IX.2003, K.A 1280.
P. prolifera	Turkey: Edirne: between Keşan and Enez, near Enez, 18.km, Çeribaşı fork in a road, meadow, 20.VI.2003, K.A 1150.
P. saxifraga	Turkey: Ordu: Akkuş, entering the city, around the picnic area, 1325m, 22.VII.2003, K.A 1220.

E: Endemic for Turkey, K.A: Kamuran Aktaş.

Table 2. Nuclear DNA content and chromosome numbers of the studied taxa. [1]Somatic chromosome number (a; indicate new counts). [2]Nuclear DNA content [2C value (standard deviation)]. [3]Monoploid genome size. [4]Monoploid genome size in Mbp; 1pg = 978 Mbp (Doležel et al., 2003).

Taxon	Chromosome number[1]	Genome size 2C-values (pg)[2]	Genome size 1Cx-value (pg)[3]	Genome size 1Cx-value (Mbp)[4]
P. alpina subsp. *alpina*	2n = 30	-	-	-
P. cretica	2n = 30	1.61 ± 0.10	0.81	792.18
P. dubia	2n = 30	1.65 ± 0.02	0.83	811.74
P. hispidula	2n = 26[a]	1.24 ± 0.01	0.62	606.36
P. pamphylica	2n = 30[a]	1.66 ± 0.03	0.83	811.74
P. peroninii	2n = 30[a]	2.32 ± 0.22	1.16	1134.48
P. prolifera	2n = 30	1.37 ± 0.04	0.69	674.82
P. saxifraga	2n = 30, 60	2.10 ± 0.04	1.05	1026.90

Table 3. Karyotype features of studied Petrorhagia species. [1]TKL: Total karyotype length. [2]M^{CA}: Mean centromeric asymmetry (Peruzzi and Eroğlu, 2013). [3]CV^{CL}: Coefficient of variation of chromosomal length (Paszko, 2006).

Species	2n	TKL[1] (µm)	Short arm (mean ± SD) (µm)	Long arm (mean ± SD) (µm)	Centromeric index	MCA[2]	CVCL[3]	Karyotype formula
P. dubia	30	15.168	0.231 ± 0.033	0.275 ± 0.043	0.458 ± 0.033	8.45	13.5	28M+2SM
P. hispidula	26	29.729	0.502 ± 0.098	0.641 ± 0.101	0.447 ± 0.438	12.31	15.0	20M+6SM
P. pamphylica	30	17.001	-	-	-	-	-	-
P. prolifera	30	24.017	0.362 ± 0.063	0.439 ± 0.064	0.451 ± 0.048	9.77	12.8	26M+4SM
P. peroninii	30	7.720	-	-	-	-	-	-

Figure 1. Wild populations of the studied Petrorhagia taxa in Turkey. Map image downloaded from https://commons.wikimedia.org/w/index.php?curid=10750328 .

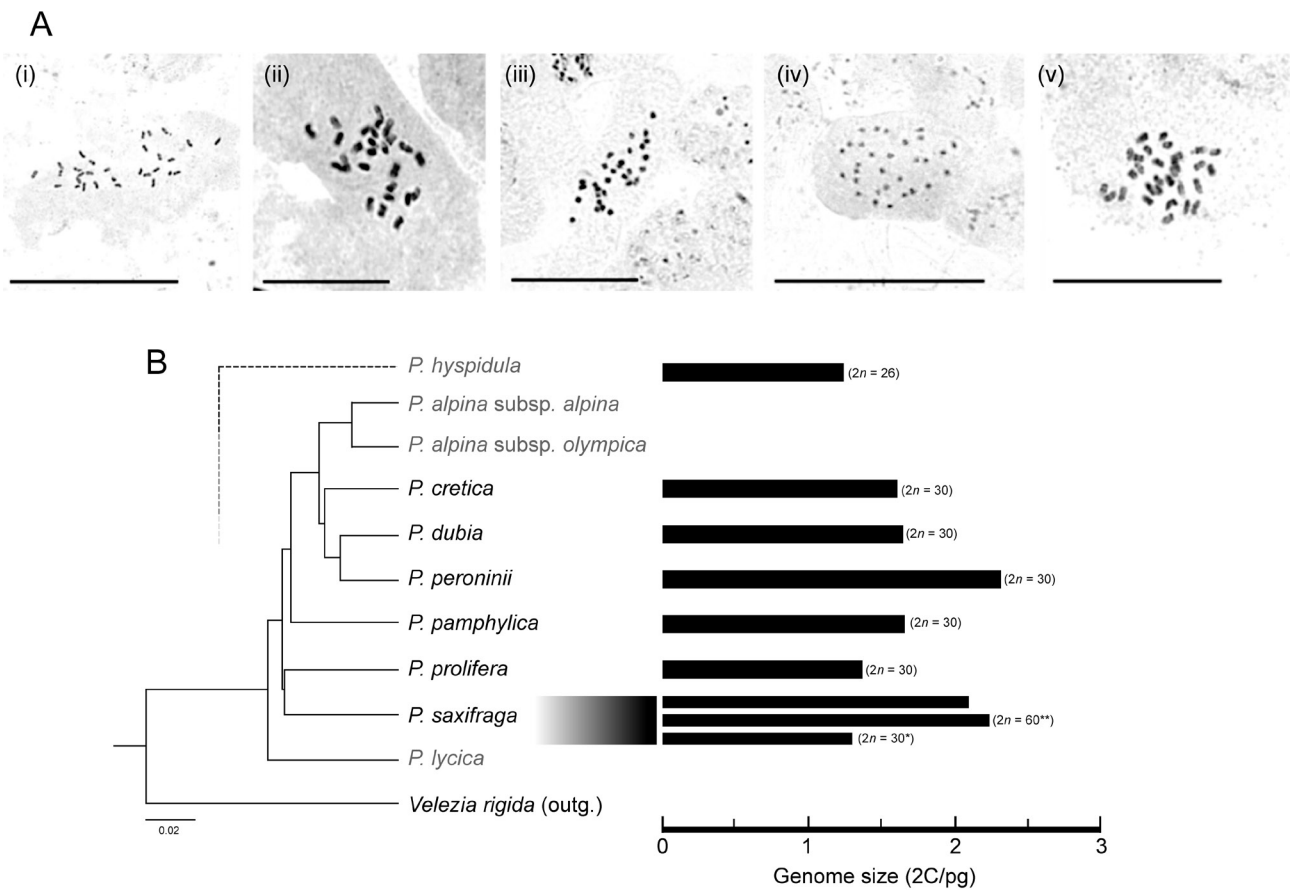

Figure 2. Chromosome and genome size data in *Petrorhagia*. A. Illustrative chromosome numbers in (i) *P. dubia,* (ii) *P. hispidula,* (iii) *P. pamphylica,* (iv) *P. peroninii,* (v) *P. prolifera.* Scale bars are 10 µm. B. Genome size data available (2C pg) mapped onto the dendrogram reconstructed from Hilooğlu et al. (2016). Note that published 2C-values for *P. saxifraga* are indicated with asterisks [*Vidic et al. (2009) and **Temsch et al. (2010)].

example in *Heloniopsis* A.Gray (Pellicer et al., 2021b) or *Oryza* L. (Piegu et al., 2006). Activation and differential dynamics of repetitive elements (mainly retroelements) are responsible for changes in genome size in plants, and bursts of amplification could underpin significant genome expansions such as the abovementioned. Whether this is the case in *P. peroninii* it is yet to be confirmed, but available genome size and chromosome data suggest that genomic dynamism in this species could have been driven by the accumulation of repetitive DNA in the genome, resulting in an almost duplication of the DNA content. Exploring, however, the genomes of this species with high throughput sequencing technologies will be necessary to confirm such point in the future.

4. Conclusion

New chromosome counts and genome size data have been generated in this study, providing further insights into the evolution of the genus. Results indicate that, although relatively stable chromosome numbers are prevalent, changes in base chromosome number and polyploidy are found evidencing certain level of chromosomal restructurings in the genus. Despite polyploidy being one of the main drivers of genome size change in plants, it is absent in the species *P. peroninii,* which has the largest genome found in the genus, hence suggesting that the activity of repetitive DNA might be underpinning such genome expansion, given its confirmed diploid chromosome number. Integrating both cytogenetic and future genomic approaches will be crucial to confirm such trend.

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