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Nuclear DNA C-values are correlated with pollen size at tetraploid but not diploid level and linked to phylogenetic descent in *Streptocarpus* (Gesneriaceae)

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ABSTRACT

Genome size can affect the phenotype of plants by a simple physical effect of the DNA material at the cellular level. Pollen contains the bare necessities to initiate and sustain pollen tube growth and carries the haploid genome. This work investigates the extent to which the nuclear DNA content affects pollen size in an evolutionary context within *Streptocarpus* (Gesneriaceae), by correlating genome size with pollen size of 38 samples representing 36 taxa in a phylogenetic framework. *Streptocarpus* was found to possess an average genome size among diploid species of 0.82 pg (1C). Significant genome downsizing of up to 44.4% was observed among the polyploid species which are exclusively found in Madagascar. The pollen size ranged between 11.27 μm and 25.55 μm at the diploid level, but 1C values were not found to drive pollen size. On the other hand, 1C values in most polyploids showed a strong positive correlation with pollen size, near linear in species of sect. *Parasaintpaulia*. In a phylogenetic context, polyploidy has evolved at least twice in the genus, and contrary to pollen size, genome size was strongly lineage-specific rather than adaptive in *Streptocarpus*. Repeated parallel increases and decreases in genome size (1C, and 1C*x*) during the evolution of the genus were inferred. Overall, in *Streptocarpus* at least, pollen size is a limited predictor of genome size and only partly reflecting ploidy level, but may be of taxonomic value. The study demonstrates that the relationship between pollen size and genome size is not straightforward, and their evolutionary trajectories unlinked.

1. Introduction

Over the last few decades the nuclear DNA C-values have been determined for a large number of plant species (e.g. Bennett and Leitch, 2010). These values can vary by about 2400-fold among angiosperms (e.g. Greilhuber et al., 2006; Pellicer et al., 2010). Much of the variation is assumed to be due to repetitive DNA elements (e.g. Kubis et al., 1998; Gregory, 2001; Meagher and Vassiliadis, 2005), and it was proposed that the nuclear DNA content can affect the phenotype in two ways, by its impact on regulatory processes in the genome (Meagher et al., 2005; Meagher and Vassiliadis, 2005) and by a physical effect of the nuclear DNA content and volume (e.g. Bennett, 1971). The latter can influence the phenotype through effects at the nuclear level such as developmental rates (Hoffmann et al., 2010), cell cycle time and pollen maturation (Bennett, 1972, 1987; Smith and Bennett, 1975; Leitch and Bennett, 2007; Beaulieu et al., 2008; Lomax et al., 2009), guard cell length and epidermal cell area (Snodgrass et al., 2017) and seeds size (Beaulieu et al., 2007).

Intriguing features are the variation of nuclear DNA amounts in homoploid sister species ('C-value enigma', Gregory, 2001) and the decrease of genome sizes[1] (1C*x* values) in polyploids ('genome downsizing', Leitch and Bennett, 2004), phenomena not yet fully understood (Bennett and Leitch, 2005a, 2005b). Polyploidy seems to have played a significant role in plant evolution given the frequency of its occurrence in speciation events (Stebbins, 1940; Wood et al., 2009). Two types of polyploids are distinguished: autopolyploids showing duplication of chromosomes within a species, and allopolyploidy with duplication of chromosomes in interspecific hybrids. The effect of the nature of polyploidy on genome downsizing was reviewed by Eilam et al. (2010), who found a complex situation with typical autopolyploids forming multivalents and having some degree of sterility showed additivity of DNA amounts of the diploid parents, while natural and synthetic diploidized autopolyploids and allopolyploids showed

[1] Greilhuber et al. (2005) was followed in terminology and 'genome size' was used to indicate the amount of DNA of one non-replicated holoploid genome with the chromosome number *n* (1C), the 'monoploid genome size' for the DNA content of one non-replicated monoploid genome with chromosome base number *x* (1C*x*), and the 'nuclear DNA content' to indicate the amount of DNA in the non-reduced nucleus *2n* (2C).

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genome downsizing. While the ancestor of angiosperms appears to have had small genomes (Soltis et al., 2003; Leitch et al., 2005), the genome size was found not to be necessarily a useful marker at higher taxonomic levels, but may be of value at the species level (e.g. Greilhuber, 1979; Zonneveld et al., 2005).

Pollen size varies considerably in plants, from about 13 to 130 μm in diameter (e.g. Knight et al., 2010), by five orders of magnitude in volume among angiosperms, and can vary greatly even within genera (Wodehouse, 1935; Muller, 1979). Pollen has important biological functions in sexual reproduction including transfer of genetic material via pollinators, and providing energy for pollen germination and pollen tube growth. The mode of pollen transport does not appear to be a main factor in the evolution of pollen size for animal pollinated angiosperms (Harder, 1998). On the other hand, pollen size per se has been found to present some taxonomic value in some plant families such as Malvaceae at the genus level (El Naggar and Sawady, 2008), or at species level in *Salsola* (Chenopodiaceae) (Toderich et al., 2010).

A positive correlation between pollen grain size and nuclear DNA content may perhaps be most obvious due to its physical effect, because of the reduction in function of pollen, carrying only the haploid genome and contains the bare necessities to initiate and sustain growth of the pollen tube. A direct correlation between nuclear DNA content and pollen volume was found across grasses (Bennett, 1987). The study by Knight et al. (2010) across the plant kingdom found a positive relationship between pollen size and C-values for a split between gymnosperms and angiosperms but only weak correlations for a few larger angiosperm lineages. In comparisons among congeners they discovered a tendency of larger pollen with increasing genome size. Although both studies did not account for ploidy level variation. Very few studies have systematically investigated the link between pollen size and genome size in relation to phylogenetic relationships, and none has been carried out at all at the species level within a genus.

While for about half of the angiosperm families genome size estimates are available, few studies have involved Gesneriaceae. In this family, to date species of only half a dozen genera out of ca. 148 in the family (sensu Weber et al., 2013) have been investigated; the European *Haberlea* (Zonneveld et al., 2005; Petrova et al., 2014) and *Ramonda* (Siljak-Yakovlev et al., 2008), the New World *Sinningia* (Zaitlin and Pierce, 2010), and the Old World *Primulina* (Kang et al., 2014), *Saintpaulia* (Loureiro et al., 2007) and *Streptocarpus* (Hanson et al., 2001). Recent molecular systematic work on subtribe Streptocarpinae of Gesneriaceae (sensu Weber et al., 2013) subsumed all taxa from Africa, Madagascar and Comoro Islands into a single genus *Streptocarpus*, including *Saintpaulia* and now contains 176 species (Nishii et al., 2015). For this genus to date, the nuclear DNA content of only two species are published, for *Streptocarpus cyaneus* (Hanson et al., 2001), and one for the hitherto *Saintpaulia ionantha*, now *Streptocarpus ionanthus* (Loureiro et al., 2007).

Most species in *Streptocarpus* s.l. are diploid with a basic chromosome number of either $x = 15$ or 16 (Möller and Pullan, 2015onwards). Polyploidy, which might affect pollen size most apparently, is reported for only a few Madagascan species, ranging from tetraploidy to octoploidy (Milne, 1975; Jong and Möller, 2000; Briggs, 2004). The origin of the polyploids by auto- or allopolyploidy has not been addressed so far, but Milne (1975) reported bivalent formation for the hexaploid *Streptocarpus variabilis* and the octoploid *Streptocarpus hildebrandtii*. One way to detect allopolyploidy in phylogenetic studies is to compare phylogenetic trees constructed from different genomes (e.g. Hegarty and Hiscock, 2004; Lundberg et al., 2009).

The pollen of *Streptocarpus* species have been studied in detail by Weigend and Edwards (1996), who reported all 128 species examined as possessing single pollen grains, except for *Streptocarpus daviesii* for which tetrads were reported earlier (Hilliard and Burtt, 1971). The size range found among the species was 7 × 7 to 24 × 16 μm in polar and equatorial diameters respectively, a roughly three-fold variation in dimensions across the taxa. The presence of a range of ploidy levels in *Streptocarpus* and the publication of a recent comprehensive phylogeny for the genus (Nishii et al., 2015), make the genus an ideal object to investigate pollen size evolution in relation to genome size variation including the effects of polyploidization in a phylogenetic context.

In the present study the nuclear DNA content for 36 taxa of *Streptocarpus* was determined by flow cytometry, and the genome size (1C values, *n*) correlated with pollen size to investigate in detail to what extend it is determined by its DNA content, particularly where polyploidy is involved. Thus, all naturally occurring polyploid species in *Streptocarpus* and an artificially created allotetraploid of known recent hybrid origin were included. The artificial tetraploid was useful for observations on the effect of polyploidy on pollen size and as a benchmark for estimating the levels of genome downsizing of the natural polyploids (Leitch and Bennett, 2004). It was also attempted to shed light onto the nature of the natural polyploid *Streptocarpus* species by comparing nuclear and chloroplast phylogenies. The monoploid genome size (1C*x*), genome size (1C) and pollen size data were analyzed on a dated phylogenetic tree to investigate the evolutionary trajectories of these characteristics and their interplay over evolutionary time. The study may also provide information on the taxonomic value of pollen size and nuclear DNA levels for the genus.

2. Materials and methods

2.1. Plant material

Thirty-eight samples covering 34 species, two subspecies, and one artificial hybrid of *Streptocarpus* were included (Table A1), representing eight of the 12 sections established by Nishii et al. (2015). Seventeen of the species were from subgen. *Streptocarpus*, 21 samples from subgen. *Streptocarpella*. Twelve of the species of subgen. *Streptocarpus* came from Africa, five from Madagascar, and 11 of subgen. *Streptocarpella* came from Africa, and 10 samples from Madagascar.

Species of subgen. *Streptocarpus* have a basic number of $x = 16$ chromosomes, while those of subgen. *Streptocarpella* have $x = 15$ (Möller and Pullan, 2015onwards). Four of the Madagascan species of *Streptocarpus* included were polyploids, two in subgen. *Streptocarpella* (*Streptocarpus beampingaratrensis* subsp. *antambolorum* with $2n = 4x = 60$, *Streptocarpus andohahelensis* with $2n = 6x = 90$) and two in subgen. *Streptocarpus* (*S. variabilis* with $2n = 6x = 96$, *S. hildebrandtii* with $2n = 8x = 128$) (Milne, 1975; Jong and Möller, 2000; Briggs, 2004). The Madagascan *Streptocarpus perrieri* was reported with $2n = 4x = 64$, for a count from the basal meristem in the seedling cotyledon (Jong and Möller, 2000). However, the material used here was determined from root tips as $2n = 2x = 32$. The artificial fertile allotetraploid hybrid (with $2n = 4x = 60$), represented a cross between *Streptocarpus vestitus* × *Streptocarpus muscosus*, both of sect. *Hova*.

2.2. Flow cytometry

Sample preparation was based on methods adapted from Costich et al. (1991). Healthy leaves were collected and kept cold and moist. They were first rinsed with distilled water and blotted dry, then 100 mg (for some samples up to 250 mg) of leaf blade placed in a Petri dish on ice, and 1 ml of solution A added [14.5 ml $MgSO_4$ buffer (0.246 g 10 mM $MgSO_4 \cdot 7H_2O$, 0.37 g 50 mM KCl, 0.12 g 5 mM Hepes, pH 8.0), 15 mg dithiothreitol (Sigma D-0632), 300 μl propidium iodide (5 mg/ml, Calbiochem 537,059), 375 μl Triton X-100 (10% w/v)]. To each sample 50 mg leaf material of an internal standard (*Pisum*