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Evolution of genome size and genomic GC content in carnivorous holokinetics (Droseraceae)

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• **Background and Aims** Studies in the carnivorous family Lentibulariaceae in the last years resulted in the discovery of the smallest plant genomes and an unusual pattern of genomic GC content evolution. However, scarcity of genomic data in other carnivorous clades still prevents a generalization of the observed patterns. Here the aim was to fill this gap by mapping genome evolution in the second largest carnivorous family, Droseraceae, where this evolution may be affected by chromosomal holokinetism in *Drosera*.
• **Methods** The genome size and genomic GC content of 71 Droseraceae species were measured by flow cytometry. A dated phylogeny was constructed, and the evolution of both genomic parameters and their relationship to species climatic niches were tested using phylogeny-based statistics.
• **Key Results** The 2C genome size of Droseraceae varied between 488 and 10 927 Mbp, and the GC content ranged between 37·1 and 44·7 %. The genome sizes and genomic GC content of carnivorous and holocentric species did not differ from those of their non-carnivorous and monocentric relatives. The genomic GC content positively correlated with genome size and annual temperature fluctuations. The genome size and chromosome numbers were inversely correlated in the Australian clade of *Drosera*.
• **Conclusions** Our results indicate that neither carnivory (nutrient scarcity) nor the holokinetism have a prominent effect on size and DNA base composition of Droseraceae genomes. However, the holokinetic drive seems to affect karyotype evolution in one of the major clades of *Drosera*. Our survey confirmed that the evolution of GC content is tightly connected with the evolution of genome size and also with environmental conditions.

Key words: DNA content, Droseraceae, carnivorous plants, flow cytometry, genome size evolution, GC content, DNA base composition, holocentric chromosomes, holokinetic chromosomes.

INTRODUCTION

Droseraceae consists of three carnivorous genera, two of which are monotypic and equipped with highly specialized snap-traps: *Dionaea muscipula* from the wetlands of North and South Carolina (USA); and *Aldrovanda vesiculosa*, an aquatic species with scattered distribution in Africa, Australia and Eurasia. The third genus, *Drosera* (sundews), includes approx. 250 sticky-leaved species distributed across all the continents except for Antarctica (McPherson, 2010; Gonella *et al*., 2015). Sundews generally grow in wetlands, but some are adapted to seasonal droughts, especially the species from Australia (McPherson, 2008, 2010).

Flowering plants (Angiosperms) exhibit an extremely broad divergence in genome size compared with other Eukaryotes (Bennett, 1972). For instance, the difference between the largest and smallest angiosperm genome is > 2500-fold (Bennett and Leitch, 2012). This variation is considered to be the result of different selective pressures (ecological, physiological, morphological, etc.) on the outcomes of molecular processes (retrotransposon amplification, polyploidy), which vary in their degree across various angiosperm clades (Wendel *et al*., 2013). The smallest angiosperm genomes are known from the carnivorous family Lentibulariaceae (Greilhuber *et al*., 2006; Fleischmann *et al*., 2014; Veleba *et al*., 2014), making these miniature carnivorous species excellent candidates for whole-genome sequencing. Indeed, complete genomic sequences have already been published for *Utricularia gibba* (Ibarra-Laclette *et al*., 2013), *Genlisea aurea* (Leushkin *et al*., 2013), *G. nigrocaulis* and *G. hispidula* (Vu *et al*., 2015). Unlike Lentibulariaceae, the other prominent group of carnivorous plants, Droseraceae, has been analysed only sporadically, and the genome size is known for only nine of approx. 250 existing Droseraceae species (Rothfels and Heimburg, 1968; Veselý *et al*., 2012; Jensen *et al*., 2015). The reported genome sizes (2C = 587 Mbp in *Drosera capensis* to 2C = 5912 Mbp in *Dionaea muscipula*) seem to be generally larger than in Lentibulariaceae (2C = 126 Mbp in *Genlisea aurea* to 2C = 3020 Mbp in *Genlisea hispidula*; Greilhuber *et al*., 2006) but still relatively small compared with genome sizes known in other angiosperms (Bennett and Leitch, 2012). Given the small number of analysed species and other characteristics noted below, it cannot be excluded that this family may still hide species with similarly miniaturized genomes as in the carnivorous family Lentibulariaceae.

It has been hypothesized that selection for small genome sizes may be promoted by nutrient limitation, namely by phosphorus and nitrogen (Leitch and Leitch, 2008), because both are abundant components of nucleic acids (Sterner and Elser, 2002). Carnivory is considered an adaptation to nutrient-poor habitats (Givnish *et al*., 1984), and carnivorous plants could, therefore, act as suitable models to test this hypothesis by

comparing the genome sizes of carnivorous species and closely related non-carnivorous clades. Indeed, the predicted decrease in genome size has been observed together with the evolution/appearance of carnivory in Lentibulariaceae (Veleba *et al*., 2014); however, studies on other carnivorous clades are necessary to generalize this trend.

Possibly, the major peculiarity of Droseraceae compared with other carnivorous lineages (including Lentibulariaceae) is its holokinetic chromosomes, which are typical for *Drosera* species (Rothfels and Heimburg, 1968; Sheikh *et al*., 1995; Kondo and Nontachaiyapoom, 2008; Shirakawa *et al*., 2011*a*, *b*; Zedek *et al*., 2016) with a possible exception of *D. regia* (Shirakawa *et al*., 2011*b*). In contrast to monocentric chromosomes, whose kinetochore formation is restricted to the small areas of the centromeres, holokinetic chromosomes lack primary constrictions and their kinetochores are formed along their poleward surfaces (Bureš *et al*., 2013; Cuacos *et al*., 2015). Holokinetic chromosomes, therefore, tolerate chromosomal fissions or fusions and do not allow more than two crossovers in meiosis (reviewed in Bureš *et al*., 2013; Heckmann and Houben, 2013) which may substantially affect genome and karyotype evolution of their bearers (Escudero *et al*., 2012; Bureš *et al*., 2013; Bureš and Zedek, 2014; Lukhtanov *et al*., 2015; Šíchová *et al*., 2016). One such effect may be a negative correlation between genome size and chromosome number in holokinetic lineages (Nishikawa *et al*., 1984; Roalson *et al*., 2007; Záveská Drábková and Vlček, 2010; Bureš *et al*., 2013; Lipnerová *et al*., 2013; Bureš and Zedek, 2014). Based on the comparison of four holokinetic clades (cyperids, *Drosera*, *Chionographis* and *Myristica*) with their close monocentric relatives, Bureš *et al*. (2013) suggested that holokinetism might be associated with genome size decrease. This association was later confirmed for the cyperid clade with a larger data set and phylogeneticaly corrected analyses by Šmarda *et al*. (2014) who also found a decreased overall genomic percentage of guanine and cytosine (GC content) in this clade. However, the extent to which these trends are general outcomes of holokinetism remains unclear because relevant comparisons of these genomic parameters are lacking in other holokinetic clades.

Thus far, the GC content is known only in two Droseraceae species (*D. menziesii*, 41·3 %; and *D. peltata*, 44·2 %; Veselý *et al*., 2012). In general, the GC content is extremely variable, particularly in bacteria, where it is known to relate to the ecology of particular taxa and lineages (correlated with the thermal optimum and thermal tolerance range; Nishio *et al*., 2003; Foerstener *et al*., 2005; Musto *et al*., 2006; Mann and Phoebe-Chen, 2010). Although the variation in GC content is much narrower in flowering plants (Šmarda and Bureš, 2012), its ecological impact has also been found in monocots, in which a higher GC content was found to be correlated with cold and drought tolerance (Šmarda *et al*., 2014). Droseraceae may serve as a good model for testing some of these predictions on a finer phylogenetic scale, particularly due to the contrasting ecology of Droseraceae species.

In this study, we aim (1) to analyse trends in the genome size and GC content evolution in the family Droseraceae and its close relatives and (2) to test whether the holokinetism in Droseraceae is associated with the predicted effects and patterns in the genome and karyotype evolution, namely (2a) genome downsizing, (2b) decreased GC content and (2c) the existence of a negative correlation between DNA content and chromosome number. Finally, we aim (3) to test the relationship between climatic parameters and GC content on a narrower phylogenetic scale than in our previous analysis across whole monocots (Šmarda *et al*., 2014).

MATERIALS AND METHODS

Most of the samples of Droseraceae were collected from the private collection of Adam Veleba; several samples originated from collections of other carnivorous plant enthusiasts. The related non-carnivorous plants were obtained from the Botanical Garden of the Faculty of Science, Masaryk University in Brno, or collected in the wild. The genomic data of 17 species were taken from the C-value database (Bennett and Leitch, 2012) and several other sources (for a detailed list, see Supplementary Data Table S1).

The samples for flow cytometry were prepared according to the protocol of Šmarda *et al*. (2008) and measured on two CyFlow flow cytometers (Partec GmbH, Münster, Germany; recently Sysmex) with internal standards whose genome size was derived from comparison with the completely sequenced *Oryza sativa* subsp. *japonica* 'Nipponbare' (International Rice Genome Sequencing Project, 2005; Supplementary Data Table S2). Each sample was processed with two fluorochromes: PI (propidium iodide) and DAPI (4',6-diamidino-2-phenylindole). The intercalating, base-unspecific PI was used to determine the absolute genome size, and the AT-selective DAPI, together with the results from measurements with PI, were used to calculate the genomic GC content. The procedure is detailed in Šmarda *et al*. (2008, 2014); for further details, see the Supplementary Data Methods.

The phylogenetic relationships of the analysed species (listed in Supplementary Data Table S1) were reconstructed based on a concatenated alignment of chloroplast (*rbc*L and *mat*K) and nuclear (ITS) markers (Supplementary Data Methods). The resulting maximum likelihood phylogenetic tree was calibrated using available fossil records and published age estimates (Supplementary Data Methods). Both non-dated and dated phylogenetic trees in Newick format are supplied in Supplementary Data Fig. S1).

The GIS layer of geographic distribution was prepared for each species based on the distribution data of Droseraceae species in the World Checklist of Selected Plant Families, Kew Databases (Govaerts and Cheek, 2014), using the digitized layers of 'TDWG areas of level 3' (*sensu* Brummitt *et al*., 2001). The species concept was revised according to the current literature. For each species, the geographical distribution was transformed to the statistical distributions across each of the 19 bioclimatic variables (19 histograms) from the WorldClim database (Hijmans *et al*., 2005), i e. for each species and a given bioclimatic parameter a histogram was constructed in which the height of each column was given by the area of intersection of the respective bioclimatic GIS (sub-)layer (= sub-range of a given bioclimatic variable) with the GIS layer of geographic distribution of the respective species. Subsequently, the minimum, median and maximum values of the calculated bioclimatic variables were calculated (Supplementary Data Table S3). The precipitation variables were log-transformed prior to all statistical analyses; the temperature variables were used as raw values.

Recent polyploidy events were identified based on a comparison of chromosome numbers taken from the published

literature and the measured genome sizes between closely related species (Supplementary Data Table S1). The analyses of genome size evolution were conducted with monoploid genome size (Cx; i.e. total 2C genome size divided by the ploidy level; Greilhuber *et al*., 2005) instead of the raw measures of DNA content. The monoploid genome size was log10 transformed prior to all statistical analyses; the GC contents and the chromosome numbers were used as raw values.

The statistical tests of the relationships between monoploid genome size, GC content and chromosome numbers were performed using the phylogenetic generalized least-squares method (function 'pgls') using the 'caper' package (v. 0.5.2; Orme *et al*., 2012) in R (v. 3·3; R Core Team, 2013) with λ (branch length transformation) determined by maximum likelihood.

The ancestral states of the monoploid genome size and GC content were reconstructed using the residual maximum likelihood method under the Brownian Motion model (function ace in the R package ape v. 3·5; Paradis *et al*., 2004) and visualized on the phylogenetic tree using the function 'contMap' in the R package 'phytools' v. 0·5-20 (Revell, 2012). Significant changes of the monoploid genome size or GC content in particular nodes were detected by the random tip-value reshuffling algorithm in R (this procedure compares actual node values with values obtained from random reshuffling of the tip values; Šmarda *et al*., 2014) based on 4999 randomizations.

The difference between the monoploid genome size of carnivorous and non-carnivorous species and between the monoploid genome size and the GC content of holokinetic and monocentric species was tested by phylogenetic analysis of variance (ANOVA; function 'aov.phylo', package 'geiger' v. 2.0.6; Harmon *et al*., 2015).

The relationships between the genomic GC content and climatic variables were analysed with a multiple phylogenetic regression approach using the 'pgls' function (package 'caper' in R) and λ (branch length transformation) determined by maximum likelihood. In this analysis, the climatic variables were handled as explanatory variables and were manually forward selected into the final explanatory model of GC content based on the amount of explained variation (in each step, the significant variable with the highest explained variation was included in the model). The α-level for this analysis was 8·33E-4, as the Bonferroni correction was applied to avoid false-positive results.

With respect to particular analysed parameters, analyses were performed with the respective sub-sets of data (Datasets 1–6 in Supplementary Table S1).

RESULTS

Variation of genomic parameters in Droseraceae and related clades

The genomes of the 71 analysed Droseraceae species (Table 1; 66 newly reported here) were relatively small, with medians of 1252 Mbp for 2C and 509 Mbp for Cx. The smallest genome was found in *Drosera hamiltonii* (2C = 488 Mbp, Cx = 244 Mbp), while the absolute largest was detected in the tetraploid *D. ordensis* (2C = 10 927 Mbp, Cx = 2732 Mbp) and the largest monoploid genome size in *D. micrantha* (2C = 7489 Mbp, Cx = 3745 Mbp). The genomes of 42 species (Table 1; 17 newly reported here) of related families (Drosophyllaceae, Nepenthaceae, Ancistrocladaceae, Dioncophyllaceae, Plumbaginaceae, Polygonaceae and Tamaricaceae) varied from the smallest, 2C = 669 Mbp, Cx = 335 Mbp in *Plumbago auriculata* (Plumbaginaceae), to 2C = 20 833, Cx = 10 416 Mbp in carnivorous *Drosophyllum lusitanicum* (Drosophyllaceae).

The GC content variation in Droseraceae (Table 1) was 7·6 %, with the lowest value found in *Drosera prolifera* (37·1 %) and the highest in *D. oreopodion* (44·7 %). The values of species of related clades varied between 36·3 % (*Nepenthes pervillei*) and 45·1 % (*Rumex acetosa*).

Phylogeny of Droseraceae and related clades

The Caryophyllales diversified at the turn of the lower and upper Cretaceous (Supplementary Data Fig. S2). The carnivorous Caryophyllales (families Droseraceae, Nepenthaceae, Dioncophyllaceae, Ancistrocladaceae and Drosophyllaceae) form a monophyletic clade in which Ancistrocladaceae and Dioncophyllaceae were ancestrally carnivorous (Heubl *et al*., 2006). They diverged from the Frankeniaceae + Tamaricaceae clade 93·31 Mya. The Polygonaceae + Plumbaginaceae clade diverged from the carnivorous Caryophyllales + (Frankeniaceae + Tamaricaceae) clade 98·69 Mya.

Within the carnivorous Caryophyllales, the crown node is 74·48 Ma old, marking the minimum age of carnivory in the Caryophyllales. The individual carnivorous genera evolved during the Palaeogene. The estimated crown age of the Droseraceae is 54·67 Ma (Fig. 1; Supplementary Data Fig. S2). Within Droseraceae, the two genera of snap-traps (*Aldrovanda* and *Dionaea*) split at least 45·09 Mya. There are two basal species of *Drosera*, i.e. *D. arcturi* and *D. regia*, which diverged from the rest of the genus 54·11 and 52·21 Mya, respectively. The remaining species of *Drosera* form two main clades that split 46·47 Mya. The first clade comprises the subgenera *Stelogyne*, *Theocalyx* and *Drosera* (*D. sessilifolia–D. trinervia* clade; hereafter referred to as the 'Cosmopolitan clade' because its members occur on all the continents except for Antarctica), and the second clade includes the subgenera *Bryastrum*, *Lasiocephala*, *Ergaleium* and *Phycopsis* (*D. binata–D. omissa* clade; hereafter referred to as the 'Australian clade' because most of its members are restricted to Australia and adjacent areas).

Genome size evolution in Droseraceae

The reconstructed evolution of the monoploid genome size shows opposite trends in the two main clades of the genus *Drosera* (Fig. 1). The genomes of the species from the Cosmopolitan clade show a reduction tendency, and multiple significant downsizing events have been detected in several nodes of this clade (Fig. 1). In contrast, the genomes of species from the Australian clade (particularly in the subgenera *Bryastrum* and *Lasiocephala*) exhibit a tendency for genome growth with multiple significant upsizing events detected (Fig. 1). The genome size in the rest of the Australian clade (i.e. the subgenera *Ergaleium* and *Phycopsis*) is relatively stable.

No difference was detected in a phylogeny-based comparison between monoploid genome sizes of carnivorous and non-

TABLE 1. *Results of genome size and genomic DNA base composition (GC content) measurements*

Species	2C (Mbp)	GC (%)	Ploidy level*	Cx (Mbp)
Droseraceae				
Aldrovanda vesiculosa	938	42·8	2	469
Dionaea muscipula	5705	43·9	2	2853
Drosera aberrans	987	41·9	2	494
D. adelae	594	37·6	2	297
D. admirabilis	792	39·7	–	–
D. afra	613	39·6	–	–
D. aliciae	1949	40·0	8	244
D. allantostigma	2858	43·5	2	1429
D. anglica	4715	44·2	4	1179
D. arcturi	1050	39·7	2	525
D. auriculata	846	42·3	2	423
D. barbigera	4215	43·2	2	2108
D. binata	1465	41·5	2	733
D. binata var. *multifida*	1519	41·4	–	–
D. burmannii	504	38·7	2	252
D. capensis	789	39·0	4	197
D. cistiflora	671	41·8	4	168
D. collinsiae	905	40·1	4	226
D. cuneifolia	702	40·3	4	176
D. dilatatopetiolaris	4868	42·8	2	2434
D. erythrorhiza	1687	42·7	–	–
D. falconeri	5253	43·0	2	2627
D. filiformis	4877	43·5	2	2439
D. filiformis var. *tracyi*	5930	42·8	–	–
D. gigantea	1060	40·2	2	530
D. grantsaui	1069	39·9	–	–
D. graomogolensis	1629	40·4	4	407
D. hamiltonii	488	40·1	2	244
D. helodes	3586	43·0	2	1793
D. hilaris	738	40·9	4	185
D. indica	1307	40·9	2	654
D. intermedia	2516	42·5	2	1258
D. kaieteurensis	2695	41·7	–	–
D. lanata	854	39·0	2	427
D. latifolia	1102	40·9	4	276
D. leucoblasta	4121	42·1	2	2061
D. menziesii	967	40·8	2	484
D. meristocaulis	2969	38·9	2	1485
D. micrantha	7489	44·4	2	3745
D. modesta	1158	40·7	–	–
D. monantha	776	40·3	–	–
D. natalensis	1040	40·8	4	260
D. neocaledonica	1136	38·1	4	284
D. nidiformis	1027	40·8	–	–
D. oblanceolata	1933	40·1	–	–
D. omissa	2170	42·2	2	1085
D. ordensis	10 927	44·2	4	2732
D. oreopodion	3292	44·7	–	–
D. peltata	829	43·0	2	415
D. petiolaris	4707	42·2	2	2354
D. prolifera	502	37·1	2	251
D. pulchella	1862	43·4	2	931
D. pygmaea	1252	41·6	2	626
D. ramentacea	1361	40·8	–	–
D. regia	835	40·2	2	418
D. roraimae	2683	41·9	–	–
D. roseana	3513	43·3	2	1757
D. rotundifolia	2331	44·5	2	1166
D. sessilifolia	497	38·4	2	249
D. sewelliae	3863	40·4	2	1932
D. schizandra	1186	40·1	2	593
D. spatulata	586	38·5	2	293
D. spiralis	1259	40·4	4	315
D. tomentosa	1105	40·0	4	276
D. trinervia	573	40·1	4	143
D. ultramafica	2325	40·6	–	–
D. venusta	1054	39·8	–	–
D. verrucata	4653	43·5	2	2327
D. viridis	2316	42·9	–	–
D. whittakeri	946	41·2	–	–
D. zonaria	889	41·5	–	–
Dioncophyllaceae				
Triphyophyllum peltatum	1167	40·2	2	584
Drosophyllaceae				
Drosophyllum lusitanicum	20 833	41·0	2	10 417
Plumbaginaceae				
Armeria alpina	7600	41·0	2	3800
A. vulgaris	8663	42·7	2	4332
Ceratostigma plumbaginoides	743	39·7	2	372
Plumbago auriculata	669	38·8	2	335
Polygonaceae				
Bistorta major	5354	42·2	4	1339
Fallopia dumetorum	1324	40·5	2	662
Muehlenbeckia complexa	1414	39·9	2	707
Oxyria digyna	1909	41·3	2	955
Persicaria amphibia	2732	39·7	4	683
P. hydropiper	1300	41·0	2	650
P. lapathifolia	1458	43·8	2	729
P. maculosa	3015	40·8	4	754
P. mitis	3071	40·3	4	768
Polygonum arenastrum	1445	44·9	4	361
Reynoutria japonica	8279	40·5	8	1035
Rumex acetosa	6104	45·1	2	3052
R. alpinus	868	44·0	2	434
R. arifolius	5912	44·1	2	2956
R. conglomeratus	1370	44·5	2	685
R. crispus	3948	40·8	4	987
R. maritimus	1962	40·3	4	491
R. patientia	4305	41·2	6	718
Tamaricaceae				
Myricaria germanica	2872	40·8	2	1436
Tamarix tetrandra	2823	37·0	2	1412

*For sources of chromosome number data, see Supplementary Data Table S1.

carnivorous species ($P = 0{\cdot}680$; Supplementary Table S1: Dataset 1) or between holokinetic and monocentric species ($P = 0{\cdot}600$; Supplementary Table S1: Dataset 1). Within the holokinetic species of *Drosera*, a weak negative correlation was observed between the Cx genome size and the monoploid chromosome number ('pgls' $\lambda = 1$, $P = 0{\cdot}08$; Fig. 2; Supplementary Table S1: Dataset 4). This negative correlation was apparent in the Australian clade ('pgls' $\lambda = 0$, $P < 0{\cdot}001$; Supplementary Table S1: Dataset 5), while it was absent in the Cosmopolitan clade ('pgls' $\lambda = 1$, $P = 0{\cdot}813$; Supplementary Table S1: Dataset 6) when both clades were analysed separately (Fig. 2).

Genomic GC content evolution in Droseraceae

Several reductions in the GC content were observed in the Cosmopolitan clade, with the exception of four temperate species (*Drosera anglica*, *D. filiformis*, *D. intermedia* and *D. rotundifolia*), where the GC content increased (Fig. 1). A

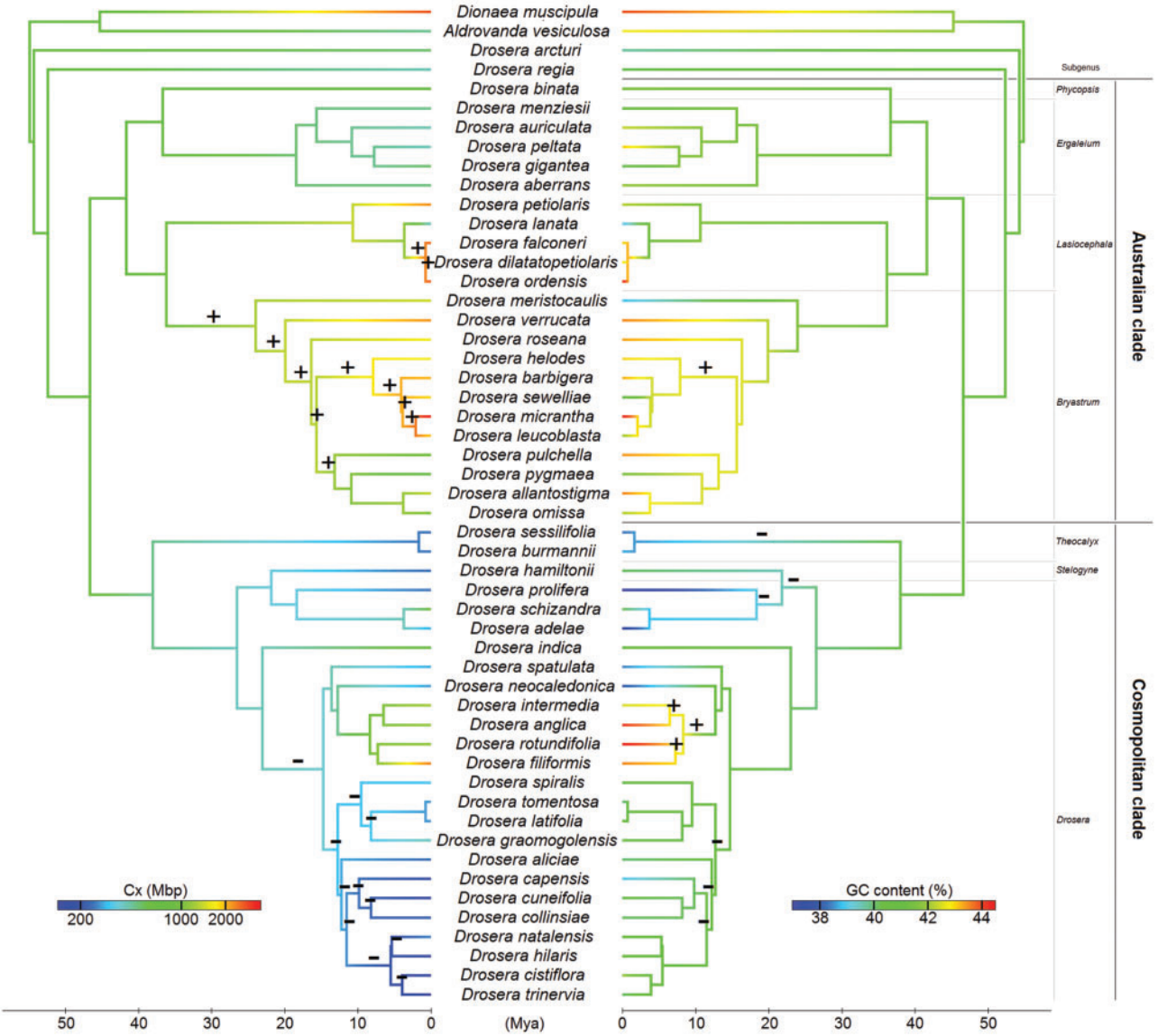

FIG. 1. Ancestral state reconstruction of monoploid genome size (left) and GC content (right) in Droseraceae. Significant increases and decreases ($P < 0{\cdot}05$) of monoploid genome size or GC content are marked with '+' and '–' signs above the branches leading to particular nodes.

single GC increase was also detected in the Australian clade, subgenera *Bryastrum* (Fig. 1). No difference was found in the GC content between the holokinetic *Drosera* species and the closely related monocentric species ($P = 0{\cdot}975$; Supplementary Table S1: Dataset 2).

The GC content variation of Droseraceae in the summary explanatory model is best explained by the genome size (log-transformed 2C), which is positively correlated with the GC content ($P = 4{\cdot}06$E-8; explained residual variation = 45·57 %; Supplementary Table S1: Dataset 3). Removing the effect of genome size in the model, the GC content further increases with an increasing annual range of temperature (median temperature annual range Bioclim variable; $P = 4{\cdot}41$E-5, explained residual variation = 21·5 %; Supplementary Table S1: Dataset 2). After removing the effect of genome size and median annual temperature range, no other variable was able to explain the significant portion of the remaining residual variation in CG contents.

DISCUSSION

The genomes of the carnivorous species of the Caryophyllales have a 'standard' size which is comparable with its non-carnivorous relatives. Indeed, they are far from being truly miniature as in the carnivorous family Lentibulariaceae, whose genomes are strikingly smaller than the genomes of their non-carnivorous relatives (Veleba *et al.*, 2014). The family Lentibulariaceae represents a unique lineage with unusually structured genomes (Ibarra-Laclette *et al.*, 2013; Leushkin *et al.*, 2013) and overall morphology (absent roots and leaves in

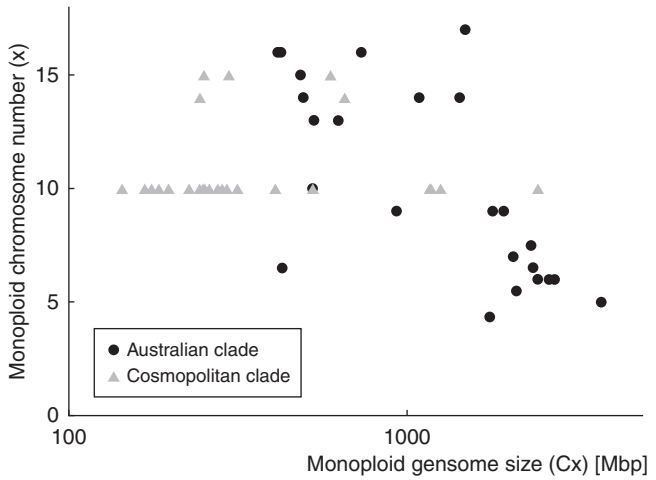

FIG. 2. Relationship between monoploid genome sizes (Cx) and basic (monoploid) chromosome numbers (x) in holokinetic species of *Drosera*. Note a negative correlation between both parameters in the Australian clade ($P < 0{\cdot}001$), which probably resulted from the holokinetic drive.

Utricularia and *Genlisea*), while the morphological constitution of carnivorous Caryophyllales species is similar to a typical plant body. This questions whether genome downsizing in Lentibulariaceae is a direct consequence of carnivory and eventual nutrient starvation, or rather associated with some peculiar molecular properties of Lentibulariaceae (Jobson and Albert, 2002; Ibarra-Laclette *et al*., 2011*a*, *b*), or connected with their extreme body reduction.

Holokinetism has been suggested to be associated with genome size and GC content decrease (Bureš *et al*., 2013; Šmarda *et al*., 2014). In the present study, we have not confirmed lower genome size previously reported in *Drosera* (Bureš *et al*., 2013). This is most probably because we tested it phylogenetically this time. Similarly, we have not detected a decrease in the GC content associated with the evolution of holokinetism in *Drosera*. This suggests that genome downsizing and GC content decrease need not to be a direct consequence of holokinetism.

Aside from positive or no correlation between genome size and chromosome number (Zedek *et al*., 2010; Chung *et al*., 2012; Escudero *et al*., 2015), a negative correlation is commonly detected in holokinetic lineages (Nishikawa *et al*., 1984; Roalson *et al*., 2007; Bureš *et al*., 2013; Lipnerová *et al*., 2013; Záveská Drábková and Vlček, 2010; Bureš and Zedek, 2014). It has been hypothesized that this negative correlation is promoted by the holokinetic drive, which is based on a size-dependent competition between homologous chromosomes in asymmetric meiosis (Bureš and Zedek, 2014). Indeed, we have observed such a negative correlation in the Australian clade of *Drosera*, where the holokinetic drive seems therefore to have shaped the karyotype evolution. There are species with a few large chromosomes (e.g. *Drosera micrantha*, 2C = 7489 Mbp, $2n = 10$, mean chromosome size, 2C/$2n$ = 749 Mbp) as well as species with many small chromosomes (e.g. *Drosera peltata*, 2C = 829 Mbp, $2n = 32$, mean chromosome size, 2C/$2n$ = 26 Mbp), which results in the above-mentioned negative correlation (Fig. 2). The presence of holokinetic chromosomes does not automatically indicate the presence of the holokinetic drive (Bureš and Zedek, 2014). Likewise, the relatively stable chromosome counts and small differences in genome size among species in the Cosmopolitan clade indicate that the holokinetic drive plays no or only a negligible role there. Alternatively, it is possible that the holokinetic drive and the carnivory-driven selection for small genomes have opposite effects on genome size in Droseraceae. If the holokinetic drive prefers larger chromosomes, which may indeed be the case in the Australian clade of *Drosera* (Table 1; Fig. 1), the carnivory-driven selection for small genomes may be counteracted by genome size enlargement due to the holokinetic drive. Such opposition of the two evolutionary forces may have obscured any differences in genome size between carnivorous and non-carnivorous as well as holokinetic and monocentric species.

It should be noted that a recent study doubted the occurrence of holokinetic chromosomes in *Drosera aliciae*, *D. binata* and *D. rotundifolia* based on the chromosomal staining by a supposedly universal mitotic centromere marker H2AThr120ph (Demidov *et al*., 2014). However, in *D. rotundifolia*, chromosomal fragments induced by gamma irradiation are regularly inherited by daughter cells during mitosis (Shirakawa *et al*., 2011*a*) which is strong evidence for chromosomal holokinetism; *D. aliciae* and *D. binata* have not been studied this way. It is therefore possible that H2AThr120ph is not a universal mitotic centromere marker or at least not able definitely to distinguish between monocentric and holokinetic chromosomes. On the other hand, there is a hypothetical possibility that some species may be monocentric in mitosis but holokinetic in meiosis (Zedek and Bureš, 2016) which might be the case for *D. aliciae* and *D. binata*.

Both the genome size and GC content are perhaps often driven by the same process, such as the proliferation or removal of GC-rich or GC-poor transposable elements (Šmarda and Bureš, 2012), causing a commonly detected positive correlation of GC content with genome size in genera with relatively small genomes (Bureš *et al*., 2007). However, the GC content also seems to have an adaptive role (Šmarda *et al*., 2014), reflecting differences in the physical properties of GC and AT base pairs, such as the higher stacking interaction in GC base pairs and consequently a higher thermal stability of GC-rich DNA (Biro, 2008; Šmarda and Bureš, 2012). This trend has also been confirmed in monocots where higher GC contents are favoured in cold and dry climates (Šmarda *et al*., 2014).

A similar pattern has also been found in Droseraceae, where species with higher GC content are mostly found in areas with stronger annual temperature fluctuations, typical of temperate and Mediterranean regions. As an example may serve northern temperate sundews (*Drosera anglica*, *D. rotundifolia*, *D. intermedia* and *D. filiformis*) or *Drosera* subgenera *Bryastrum* from the Mediterranean climate of West Australia (McPherson, 2008, 2010), all possessing relatively high GC contents (Table 1; Fig. 1). In contrast, low GC contents can be expected in areas with low temperature fluctuations, typically in the tropical regions. Examples include the species of the 'rainforest sundews' (*Drosera adelae*, *D. prolifera* and *D. schizandra*) from northern Queensland, in Australia (McPherson, 2008, 2010), or *Drosera meristocaulis* from the Neblina massif on the Brazilian–Venezuelan border (Rivadavia *et al*., 2012).

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.com and consist of the following. Supplementary Methods: detailed description of flow cytometry measurements, sequencing and phylogenetic tree construction. Figure S1: the phylogenetic tree with posterior values. Figure S2: the phylogenetic tree with node ages. Table S1: detailed information about accession numbers used for phylogenetic tree construction and genomic parameters of all analysed species. Table S2: results of flow cytometry measurements. Table S3: genomic and BioClim variables.

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