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Evolution of genome size in *Carex* (Cyperaceae) in relation to chromosome number and genomic base composition

Ivana Lipnerová*, Petr Bureš, Lucie Horová and Petr Šmarda

Department of Botany and Zoology, Masaryk University, Kotlářská 2, CZ-61137, Brno, Czech Republic
** For correspondence. E-mail 151317@mail.muni.cz*

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• *Background and Aims* The genus *Carex* exhibits karyological peculiarities related to holocentrism, specifically extremely broad and almost continual variation in chromosome number. However, the effect of these peculiarities on the evolution of the genome (genome size, base composition) remains unknown. While in monocentrics, determining the arithmetic relationship between the chromosome numbers of related species is usually sufficient for the detection of particular modes of karyotype evolution (i.e. polyploidy and dysploidy), in holocentrics where chromosomal fission and fusion occur such detection requires knowledge of the DNA content.
• *Methods* The genome size and GC content were estimated in 157 taxa using flow cytometry. The exact chromosome numbers were known for 96 measured samples and were taken from the available literature for other taxa. All relationships were tested in a phylogenetic framework using the ITS tree of 105 species.
• *Key Results* The 1C genome size varied between 0·24 and 1·64 pg in *Carex secalina* and *C. cuspidata*, respectively. The genomic GC content varied from 34·8 % to 40·6 % from *C. secalina* to *C. firma*. Both genomic parameters were positively correlated. Seven polyploid and two potentially polyploid taxa were detected in the core *Carex* clade. A strong negative correlation between genome size and chromosome number was documented in non-polyploid taxa. Non-polyploid taxa of the core *Carex* clade exhibited a higher rate of genome-size evolution compared with the *Vignea* clade. Three dioecious taxa exhibited larger genomes, larger chromosomes, and a higher GC content than their hermaphrodite relatives.
• *Conclusions* Genomes of *Carex* are relatively small and very GC-poor compared with other angiosperms. We conclude that the evolution of genome and karyotype in *Carex* is promoted by frequent chromosomal fissions/fusions, rare polyploidy and common repetitive DNA proliferation/removal.

Key words: Agmatoploidy, AT/GC ratio, chromosomal fusion and fission, chromosome numbers, DNA content, flow cytometry, GC content, karyotype, phylogeny, polyploidy, symploidy.

INTRODUCTION

The sedge genus *Carex* contains approx. 2000 species, making it one of the most species-rich angiosperm genera (Reznicek, 1990). The sedge family (Cyperaceae) exhibits diffuse centromeres (holocentric or holokinetic chromosomes), pseudomonad origin of pollen (except in the subfamily Mapanioideae; Simpson *et al.*, 2003) and post-reductional meiosis (reviewed in Hipp *et al.*, 2009). Chromosomal fission in most angiosperms exhibiting monocentric chromosomes usually results in deletion of generated acentric fragments, but in *Carex* deleterious effects of such chromosomal rearrangements are presumed to be reduced due to the holocentric structure of chromosomes, which are kinetically active along their entire length rather than just at localized centromeres. Although viability is lower in structural heterozygotes possessing unbroken chromosomes together with homologue fragments (Nordenskiöld, 1963), a newly fragmented (or fused) karyotype could be easily homologized via selfing or backcrossing and that could result in the establishment of a new fully fertile cytotype or chromosomal race, followed by the formation of reproductive barriers, resulting in a new species (Nordenskiöld, 1963; Whitkus, 1988).

Based on this assumption, a high frequency of chromosomal fusion or fission is hypothesized to have occurred during karyotype evolution in the *Carex* species by Heilborn (1924), Luceño and Castroviejo (1991), Escudero *et al.* (2008), Hipp (2007) and Hipp *et al.* (2007, 2009). Probabilistic models of karyotype evolution based on analysis of chromosome number variation in *Carex* favour the gain or loss of an individual chromosome as the most probable step in karyotype evolution, suggesting that fission and fusion play more important roles than polyploidy (Mayrose *et al.*, 2010) or that fission and fusion alone drive karyotype evolution in this genus (Hipp *et al.*, 2009). Whether the extremely broad karyotype variation observed in this genus ($n = 5–47$, 50 or 52–58) (Roalson, 2008; Rotreklová *et al.*, 2011) results purely from such structural rearrangements remains unclear. Another way to distinguish between (*a*) purely numerical mechanisms, such as 'true' (quantitative) aneuploidy or polyploidy, and (*b*) structural mechanisms, such as fusion/fission, could be combining chromosome counting with genome-size measurements.

Although the chromosomal variations observed in other Cyperaceae genera are typically continuous at the infrageneric (Roalson, 2008) or intraspecific levels (Bureš, 1998; Bureš *et al.*, 2004), polyploidy also plays an important role in

karyotype evolution in these genera (Vanzela *et al.*, 2003; da Silva *et al.*, 2010; Zedek *et al.*, 2010). In contrast, polyploidy appears to be much more rare among the *Carex* species (Hipp, 2007; Hipp *et al.*, 2007, 2009; Roalson, 2008). In this genus, intraspecific autopolyploidy has been observed in *Carex siderosticta* based on the presence of tetravalents during meiosis (Tanaka, 1940, 1949). Other evidence of polyploidy in *Carex dolichostachya*, *C. jackiana* and *C. roraimensis* is reviewed in detail by Hipp *et al.* (2009). In monocentrics, a pure arithmetic relationship between chromosome number is usually sufficient to detect polyploidy within a group of related taxa. However, in holocentrics, this empirical pattern could be obscured by chromosomal fission or fusion. Thus, the presence of different chromosome numbers within some subgeneric taxa of *Carex* cannot be conclusively considered as evidence of polyploidy (Roalson, 2008; Rotreklová *et al.*, 2011). Therefore, chromosome counting should be at least combined with DNA content measurement (in the ideal case, both are estimated from the same individual) to evaluate the roles of polyploidy, quantitative aneuploidy and chromosomal fission/fusion in karyotype evolution among holocentrics.

Generally, in this genus, the species with the smallest number of chromosomes, such as *Carex siderosticta*, *C. pachygyna* and *C. ciliatomarginata*, belong to the section *Siderostictae* and form the basal clade, which is a sister clade to the rest of the genus (Roalson, 2008; Waterway *et al.*, 2009). A similar pattern is observed in the holocentric genus *Luzula* from the sister family Juncaceae (Záveská-Drábková and Vlček, 2010). Heilborn (1924) was the first to propose an evolutionary trend from lower to higher chromosome numbers in *Carex*. However, as this hypothesis holds true across a broad phylogenetic scale according to recent probabilistic models of chromosome number evolution in *Carex* sect. *Ovales*, a trend of decreasing chromosome number in advanced taxa is more probable across a finer scale (Hipp, 2007; Mayrose *et al.*, 2010). These authors suggest that descending and ascending karyotype orthoselection contributes to the wide range of chromosome number variations observed in particular phylogenetic lineages.

In addition to polyploidy, agmatoploidy and symploidy, retrotransposon proliferation/removal could also be an important mechanism for karyotype and genome-size evolution in angiosperms (Bennetzen *et al.*, 2005). In Cyperaceae, an important role of retrotransposons has been detected in *Eleocharis*, in which species with larger and fewer chromosomes were more evolved because of a massive proliferation of Ty1-*copia* LTR transposons, whereas numerous small chromosomes were typically observed in 'basal' phylogenetic lineages (Zedek *et al.*, 2010); a similar pattern regarding chromosome size might also be present in *Schoenus* (Bhatti *et al.*, 2007). It could be expected that massive proliferation of a particular motif, such as a retrostransposon, could shift the overall genomic base composition (in the case that the AT/GC ratio of the proliferated motif differs substantially from the base composition of the rest of the genome). Indeed, this trend has been detected using non-phylogenetic analyses in *Eleocharis* and *Carex* (Hralová *et al.*, 2007), but the relationship between genome size and base composition in *Carex* still has not been tested using phylogenetically based correlation methods, which correct for non-independence in comparative analysis and provide reduced variance estimates of regression and correlation coefficients (Felsenstein, 1985; Rohlf, 2006).

In the Angiosperm DNA C-values database (Bennett and Leitch, 2010), there are 49 species of Cyperaceae and 36 entries for the genus *Carex* (approx. 2 % of the total number of species). Most of the *Carex* C-values were derived from the results of a single study (Nishikawa *et al.*, 1984) that was not directly concerned with estimating genome size; therefore, the administrators of the database have performed a recalculation of the *Carex* C-values (Bennett and Leitch, 2010). The remaining C-values in this database were obtained from several studies that typically involved only one or two species of the Cyperaceae family. While the base composition [the genomic AT/GC ratio = (adenine + thymine)/(guanine + cytosine) in the genome] has never been studied in the *Carex* genus or the Cyperaceae family, the related family Poaceae has been shown to exhibit an unusually high GC content compared with other angiosperms (Barow and Meister, 2002; Meister and Barow, 2007; Šmarda *et al.*, 2008). Although the sedge family (Cyperaceae) is included in the Poales clade (APG III; Stevens, 2001 onwards), the holokinetic nature of the chromosomes of its species and generally lower genome sizes markedly distinguish this family from the Poaceae and many other Poales families.

The aim of this study was to answer the following questions: (*a*) what is the phylogenetic pattern of genome size and genomic base composition within the genus *Carex*; (*b*) what is the relationship between genome size and chromosome number within particular phylogenetic lineages, and what mode of karyotype evolution does this relationship suggest; and (*c*) is there any relationship between genome size and base composition at a particular phylogenetic scale that might suggest retrotransposon proliferation/removal?

MATERIALS AND METHODS

Sampling, determination and nomenclature

Samples of the genus *Carex* were collected in the field (Eurasian species) or in the botanical gardens (North American species) of Mendel University and Masaryk University, Brno, Czech Republic. Voucher specimens of all sampled plants were deposited in the herbarium at the Department of Botany and Zoology of Masaryk University (BRNU). R. Řepka (Mendel University) revised the identification of all taxa, and A. E. Kozhevnikov (Institute of Biology and Soil Science of the Russian Academy of Science, Vladivostok, Russia) identified the taxa from Russia. One individual per species was sampled in most cases. Soil-free cuttings were temporarily cultivated in pure water in a cultivation room at room temperature under a 16-h day/8-h night regime. The nomenclature applied herein follows Egorova (1999) for Eurasian species and Ball and Reznicek (2002) for North American species.

Genome size and GC content estimation

Herein, the term genome size refers to the 1C-value (Greilhuber *et al.*, 2005). Genome size and GC content were measured in a flow cytometry laboratory at the Department of Botany and Zoology, Masaryk University (Brno, Czech Republic). The measurements were conducted on two flow cytometers (CyFlow ML; Partec GmbH, Münster, Germany) equipped with a 100-mW Cobolt Samba green laser or a high-power UV LED (365 nm). Two different fluorochromes were used in the analyses: intercalating propidium iodide for estimating the absolute DNA content and AT-specific DAPI for calculating GC content. A two-step procedure (Otto, 1990) was used for sample preparation. Briefly, approx. 0·5-cm^2 pieces of young leaves of the sample and the standard were chopped together using a sharp razor blade in a Petri dish containing 1 mL of Otto I buffer (0·1 M citric acid and 0·5 % Tween 20); then an additional 1 mL of Otto I buffer was added. The crude nuclear suspension was filtered through a 50-μm nylon mesh. The filtered suspension was divided into two sample tubes, and either 1 mL of Otto II buffer (0·4 M $Na_2HPO_4{\cdot}12\ H_2O$) supplemented with DAPI or 1 mL of Otto II buffer containing PI was added. The final concentrations of PI and DAPI were 50 and 2·0 μg mL^{-1}, respectively. For each run, we counted 5000 cells. Measurements of all samples were repeated three times on different days, and the results were averaged. As a primary standard, *Oryza sativa* subsp. *japonica* 'Nipponbare' was used (1C = 0·40 pg, GC content = 43·6 %; International Rice Genome Sequencing Project, 2005). As alternative internal standards, we used *Solanum lycopersicum* 'Stupické polní tyčkové rané' [1C = 0·87 pg, GC content = 38·8 %; we employed the values relative to the primary standard rather than using the values of Doležel *et al.* (1992), which were estimated with human leucocytes] and *Carex acutiformis* (1C = 0·41 pg, GC content = 36·6 %; a single plant cultivated in the experimental garden). The reliability of the GC content measurements produced by our flow cytometers was verified using a DNA melting-based method (Šmarda *et al.*, 2012). The GC content was calculated using the equations published in Barow and Meister (2002; eqns 7 and 8) using a mathematical approximation by the *regula falsi* method in an automated Microsoft Excel sheet (URL: http://www.sci.muni.cz/botany/systemgr/download/Festuca/ATGCFlow.xls; Šmarda *et al.*, 2008, 2012). The average coefficient of variance of all peaks in the measurements was 2·80 % for PI staining and 2·19 % for DAPI staining; the average coefficient of variation (CV) of all peaks in the measurements for individual taxa is presented in the Appendix.

Chromosome counts

The chromosome numbers for 94 taxa were taken from our previous study (Rotreklová *et al.*, 2011) and two other unpublished counts undertaken by the same author because of the sample identity (Appendix). The chromosome counts for 55 additional species were taken from Naczi (1999), Stoeva *et al.* (2005) and Roalson (2008) – see Appendix.

Phylogenetic tree

For the purposes of phylogenetic testing, we constructed a phylogenetic tree based on previously published sequences of internal transcribed spacer (ITS) (Starr and Ford, 2009; Ford *et al.*, 2006; Hendrichs *et al.*, 2004*a*, *b*; Starr *et al.*, 2004) available for 107 taxa (incl. two outgroups) in the NCBI GenBank database (Benson *et al.*, 2010; for accession numbers see Supplementary Data Table S2 available online). *Scirpus radicans* and *Eriophorum vaginatum* were selected as outgroups based on the phylogeny of the Cyperaceae family (Muasya *et al.*, 2009). A preliminary alignment was performed in ClustalX (Thompson *et al.*, 1997) using the default settings for slow-accurate alignment. The obtained alignment was manually adjusted using the method of Starr *et al.* (2004) with the program MEGA4 (Tamura *et al.*, 2007). To choose an appropriate model for phylogenetic analysis, we used Modeltest (Posada and Crandall, 1998), which is available online (FindModel; http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). Bayesian tree building was performed with MrBayes 3·1 (Ronquist and Huelsenbeck, 2003) using a Markov Chain Monte Carlo algorithm run of 4000 000 generations with tree sampling every 100th generation. A general time-reversible model incorporating gamma-shaped rate variation with a proportion of invariable sites was used based on the model-selection method implemented in MrModelTest (the difference in Akaike's information criterion from the next-best model = 27·658). The final tree topology was summarized using trees from all partitions after discarding the trees from the first 1000 000 generations. The final topology of the phylogenetic tree was consistent with trees published in previous studies (mentioned above). For the comparison with results of Chung *et al.* (2012), we constructed a merged phylogenetic tree containing taxa from both our and the Chung *et al.* (2012) study using the ITS sequences from NCBI GenBank database [accession numbers follow appendix 2 of Chung *et al.* (2012); where two or more accessions were available for the same taxon we preferred that used in our tree]. The method of alignment and tree building was the same as for our tree. The best-fitting model for tree building was $GTR + I + \Gamma$ (the difference in Akaike's information criteria between selected and second-best model was 15·792). The tree building was performed in MrBayes running Markov Chain Monte Carlo algorithm for 10 000 000 generations with sampling each 1000th generation. The first 2500 000 generations were discarded before the final tree topology was summarized.

Phylogenetic analyses

To analyse evolution of genome size caused with gradual processes (mostly related to chromosomal fissions, fusions and retrotransposon expansions/removals), putative polyploid taxa (*C. baldensis*, *C. buxbaumii*, *C. curvula* and *C. flacca*; Fig. 1) were removed from the subsequent statistical analyses. We used raw data for genome size and GC content, and the data on chromosome number were log transformed as $\log_{10}(2n)$ prior to the statistical analyses. We used phylogenetic independent contrasts (PIC) (Felsenstein, 1985), as

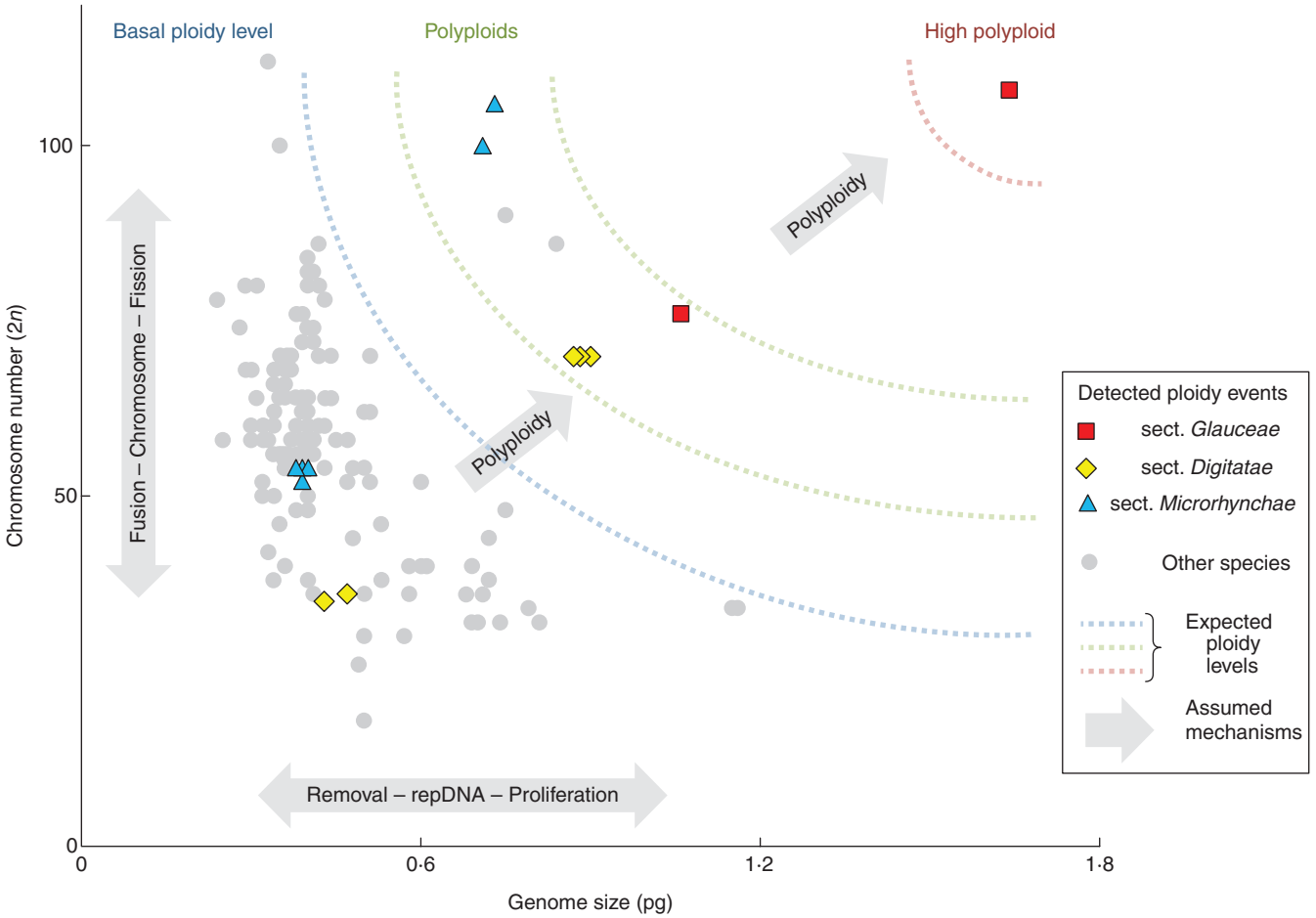


FIG. 1. Distribution of all studied taxa based on genome size and chromosome number in the genus *Carex*. The putative effects of particular mechanisms responsible for the evolution in genome size and chromosome number are indicated with grey arrows. Closely related species, in which co-oriented differences in genome size and chromosome number, and thus where polyploidy is present (particular regression lines are oriented through the origin), are represented with the same symbols (sect. *Digitatae*, sect. *Microrhynchae* and sect. *Glauceae* in yellow diamonds, blue triangles and red squares, respectively). The two grey dots in the polyploid area are *C. baldensis* and *C. curvula* with unknown lower-level ploidy relatives, probably because of their basal and isolated position within the phylogeny of the entire genus (Starr and Ford, 2009; Gehrke *et al.*, 2010). The three main grades (ploidy levels) are separated with dashed lines.

implemented in the Phylocom (Webb *et al.*, 2008) AOTF module (Ackerly, 2006), to examine the relationships between genomic parameters. The differences in genomic parameters between selected groups of taxa were tested with Phylogenetic Generalized Least Squares (PGLS), using the restricted maximum likelihood method and the Brownian motion-based covariance structure. The PGLS was calculated in R program (R Development Core Team, 2012) using the *gls* function of the *nlme* package (Pinheiro *et al.*, 2012) and *corBrownian* function of the *ape* package (Paradis *et al.*, 2004). The significance of phylogenetic signals (Blomberg and Garland, 1992; Blomberg *et al.*, 2003) was tested in Phylocom program with the implemented randomization test always based on 999 tip shuffling randomizations. Standardized PIC values were used as a measure of the evolution rate following the methods of Garland (1992) and Oliver *et al.* (2007), and these values were compared between the groups using standard statistical tests. We also analysed the rate of evolution (Eastman *et al.*, 2011) as implemented in the R package AUTEUR (Eastman *et al.*, 2011) as used by Chung *et al.* (2012) on their *Carex* subg. *Vignea* data. The analysis was performed on the phylogenetic tree with basal ploidy-level species. Three independent runs of 10 000 000 generations (each with default settings) were conducted with visual evaluation of convergence. The first 2500 000 generations of each run were discarded as a burn-in, and the results were pooled across all runs. The statistical significance of the rate differences between the lineages was based on 10 000 comparisons randomly drawn from Monte Carlo sampling iterations as described in Eastman *et al.* (2011) and Chung *et al.* (2012). For direct comparison with the results of Chung *et al.* (2012) the genome sizes and chromosome numbers were taken from the original article (Chung *et al.*, 2012, appendix S1 and S3). The original genome sizes by Chung *et al.* (2012) were recalculated with genome size of standard *Raphanus sativus* 1C = 0·492 pg to be consistent with our genome-size estimates. Afterwards, the datasets were merged (for identical taxa our measurements were preferred). We used analysis of PIC together with analysis of shifts in rate of evolution in AUTEUR on raw data for

genome size and chromosome numbers to examine relationships of these two genomic parameters. The rates of evolution between clades of interests were compared using standardized independent contrasts following the same method as for our data.

RESULTS

Genomic parameters (genome size and GC content) were analysed in 157 taxa of the genus *Carex* (Appendix). The genome size within the genus ranged from 1C = 0·24 pg in *Carex secalina* to 1C = 1·64 pg in *C. cuspidata*. The genomic GC content ranged from 34·8 % in *C. secalina* to 40·6 % in *C. firma.*

At the basal ploidy level (103 taxa), a strong positive correlation was observed between genome size and GC content [correlation of the standardized phylogenetically independent contrasts (Pic*R*) = 0·768, $P < 0·001$]. There was also a negative correlation between genome size and chromosome number (Pic*R* = –0·300, $P = 0·002$). The genome size and chromosome number exhibited strong phylogenetic signals (both $P = 0·001$), and no signal was detected in the GC content ($P = 0·367$).

A comparison of the two main phylogenetic lineages, the core *Carex* clade (Waterway *et al.*, 2009) and *Vignea* clade, revealed that the *Vignea* clade had smaller genome sizes (PGLS $P = 0·026$; Fig. 2). The variation in GC content (PGLS $P = 0·876$) and chromosome number (PGLS $P = 0·488$) did not differ between the two clades. The results of the analyses of genome-size evolution rate using analysis of PIC and AUTEUR were congruent and indicated that the rate of genome-size evolution in the core *Carex* clade was higher than in the *Vignea* clade (comparison of PIC $P = 0·028$; AUTEUR $P = 0·021$; Fig. 3). The location of the shift in the rate of genome-size evolution is at the node dividing the two main subclades of core *Carex* clade as the rate of genome-size evolution in branches leading to the main subclades differed (comparison of PIC $P < 0·001$; AUTEUR $P = 0·018$). The rate of evolution of chromosome number (Fig. 3) and GC content (data not shown) was nearly constant along the whole tree, except for some terminal branches.

Several sections defined by morphology-based classification (*sensu* Egorova, 1999) exhibited a high similarity in terms of their genomic characteristics (Fig. 4), such as *C. muricata* group (sect. *Phaestoglochin*; Appendix), sect. *Ovales*, sect. *Limosae*, sect. *Glaucae* and sect. *Ceratocystis* (= *C. flava* complex; Fig. 4). Other sections showed different patterns, e.g. sect. *Aulocystis* showed high variation in the genomic parameters, containing species with the largest to relatively small genome sizes and GC contents (0·38–1·15 pg, 35·4–40·6 %, respectively; Fig. 4).

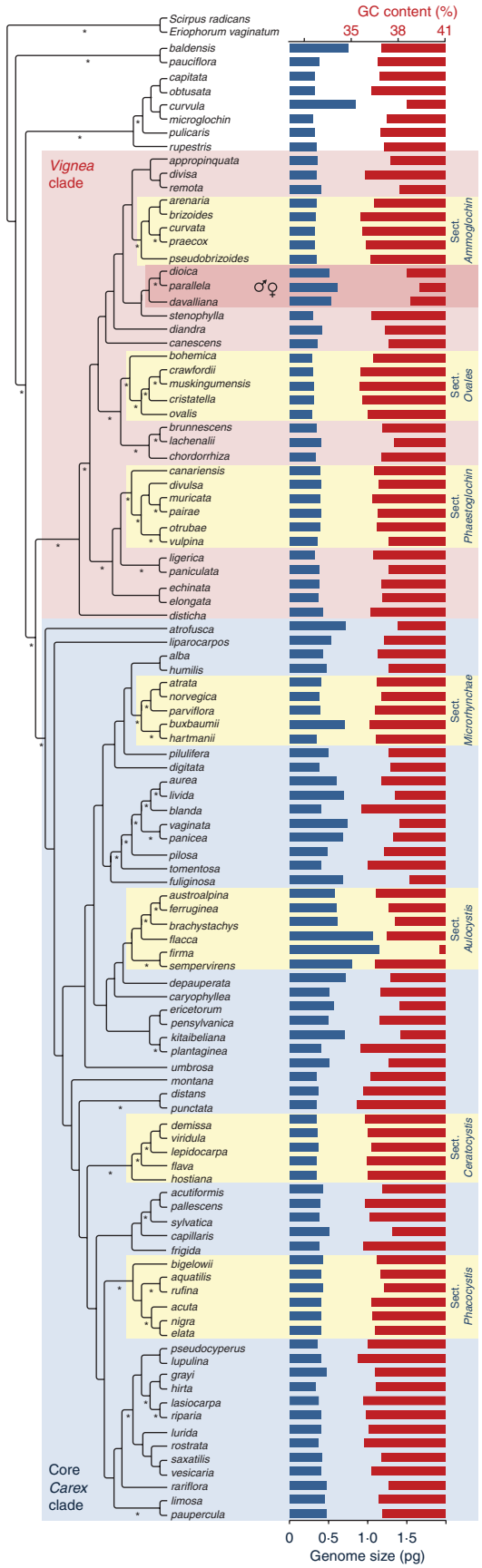
The dioecious species (*C. dioica*, *C. davalliana* and *C. parallela*) have larger genome sizes (1C = 0·5–0·6 pg) compared with their hermaphrodite relatives, i.e. species of the whole clade *Vignea*, where the genome size rarely exceeds 0·45 pg (PGLS $P = 0·001$; Fig. 2 and Appendix). Similarly, the GC content of dioecious species (from 38·5 to 39·3 %) was higher in comparison with the rest of the *Vignea* clade (PGLS $P < 0·001$; Fig. 2 and Appendix), where the GC content ranged from 35·5 to 38·1 %. The results of the analyses of shifts in the rate of evolution revealed a higher rate of genome-size evolution for dioecious species (comparison of PIC $P < 0·001$; AUTEUR $P = 0·029$; Fig. 3) in addition to higher rates of chromosome number evolution (comparison of PIC $P = 0·006$; AUTEUR $P = 0·021$; Fig. 3; this shift was facilitated by decrease in chromosome number) and GC content evolution (comparison of PIC $P < 0·001$; AUTEUR $P = 0·038$; analyses not shown). Both sexes were analysed in *C. dioica* and *C. davalliana*; however, no differences in genome size or GC content were observed (data not shown).

A comparison of related species in some sections showed that the substantial increase in chromosome number (mostly doubling or nearly so) corresponded with an equivalent increase in genome size (Table 1 and Fig. 1), suggesting a polyploid origin of these species, namely *C. callitrichos*, *C. lanceolata* and *C. rhizina* (sect. *Digitatae*), which have chromosome numbers and genome sizes that are 2-fold larger than those of their close relatives *C. humilis* and *C. macroura* (Table 1). Other circumstantial evidence of polyploidy can be observed in sect. *Microrhynchae*, where *C. aterrima*, *C. parviflora*, *C. atrata* and *C. norvegica* all possess genome sizes and chromosome counts that are nearly 2-fold larger than *C. buxbaumii* and *C. adelostoma* (Table 1). A similar pattern was observed in the comparison of *C. flacca* and *C. cuspidata* (section *Glaucae*; Fig. 1); however, in this case, the chromosome number and genome size were only 1·42 and 1·55-fold larger, respectively.

DISCUSSION

Measurement accuracy

Our genome-size estimates were slightly lower (*C. caryophyllea*, *C. humilis*, *C. panicea* and *C. pulicaris*) or higher (*C. flacca*) compared with the data in the Angiosperm DNA C-values database (Bennet and Leitch, 2010), as observed in the five species common to both datasets. Three different groups contributed the data for these five species in the Angiosperm DNA C-values database (Nishikawa *et al.*, 1984; Grime *et al.*, 1985; Mowforth, 1986) using Feulgen densitometry. However, this method suffers from several methodical biases (Greilhuber, 2008), especially when combined with hot hydrolysis, as used for example by Grime *et al.* (1985). Moreover, the relative DNA contents that were estimated by Nishikawa *et al.* (1984) were recalculated to absolute values using an additional standard (*Carex ciliatomarginata*), whose genome size was obtained later using Feulgen densitometry (L. Hanson *et al.*, Royal Botanic Garden, Kew, UK, unpubl. res.). The inaccuracy of the data in the Angiosperm DNA *C*-values database also supports a comparison of our data with the recent analyses of Chung *et al.* (2012). Comparison of genome-size estimates for 13 species included in both studies showed only a minimum difference corresponding to the genome-size estimates of the reference standards. Chung *et al.* (2012) used *Raphanus sativus* 'Saxa' (1C = 0·555 pg DNA; Doležel *et al.*, 1992) as a standard, whose genome size was derived from a comparison with an early human sequence (based on $2C = 7·0$ pg). However, the human genome size was overestimated compared with modern sequencing data (Doležel and Greilhuber, 2010). In our study, we used the sequenced



rice cultivar *Oryza sativa* subsp. *japonica* 'Nipponbare' (International Rice Genome Sequencing Project, 2005) as a 'gold reference standard'. *Oryza sativa* was consequently used for the calculation of genomic parameters for the other two internal standards used in this study (see Methods). Compared with the sequenced cultivar *Oryza sativa* subsp. *japonica* 'Nipponbare', the genome size of *Raphanus sativus* 'Saxa' used by Chung *et al.* (2012) would be 1C = 0·492 pg, which allowed for mutual data recalculation. Compared with the recalculated data, 11 of the 13 species showed a perfect linear correspondence with a coefficient of determination of $R^2 = 0{\cdot}976$. The difference between the genome-size estimates obtained in this study and those of Chung *et al.* (2012) might reflect the different evolutionary histories of North American and European populations and/or intraspecific variations in genome size, which could be geographically conditioned (Šmarda and Bureš, 2010).

The GC content of *Carex firma* clearly exceeds other analysed sedges (Fig. 2; sample collected in the Alps), and its genome size is also very extreme within the *Carex* genus. This estimate was verified using a sample from a different part of its distribution range (the Carpathians) resulting in a very similar estimate: 1C = 1·16 pg (a difference of 0·01 pg, or 1·03 %; see Appendix) and GC content equal to 41·1 % (a difference of 0·52 %). These differences are within an acceptable margin of error for the analysis of genome size (Suda *et al.*, 2003; Suda and Leitch, 2010) and GC content (Šmarda *et al.*, 2012) using flow cytometry.

Genomic parameters in the genus in relation to other angiosperms

Carex species have some of the smallest genome sizes observed in angiosperms (Leitch *et al.*, 2005; Bennet and Leitch, 2010). The minimum genome size for *Carex* is reported in the Angiosperm DNA C-values database for *Carex paxii* (1C = 0·15 pg), and our analysis revealed only a slightly larger minimum genome-size estimate for *Carex secalina* (1C = 0·24 pg). The maximum genome size for this genus is reported in this study for *Carex cuspidata* (1C = 1·64 pg). Sedges exhibit small genome sizes that are similar to other members of the Cyperaceae family and sister family Juncaceae (cf. Bennett and Leitch, 2010), except for the large genomes of some species of *Eleocharis* (Zedek *et al.*, 2010).

Compared with related grasses (Poaceae), *Carex* has a substantially lower GC content [mean of 43·7 % in Poaceae (Meister and Barrow, 2007) vs. 36·7 % in *Carex*]. This GC content is among the lowest detected in angiosperms to date (see Meister and Barrow, 2007; Šmarda and Bureš, 2012).

In *Carex*, the GC content was positively correlated with genome size (Pic$R = 0{\cdot}768$, $P < 0{\cdot}001$). This correlation has

FIG. 2. Variation in genome size (blue) and genomic GC content (red) mapped on a phylogenetic tree of 105 (from 157 studied) species of *Carex* with available ITS sequences in the NCBI GenBank database (for accession numbers, see Supplementary data Table S2). Bayesian consensus tree with all compatible groups from 72 002 trees sampled from posterior probabilities using a GTR + G + Γ model of sequence evolution. The Bayesian posterior probabilities greater than 0·8 are marked with asterisks below the branches. The clade *Vignea*, core *Carex* clade and sections discussed in the text are highlighted in pale red, pale blue and pale yellow, respectively. Dioecious taxa are marked with male/female symbols.

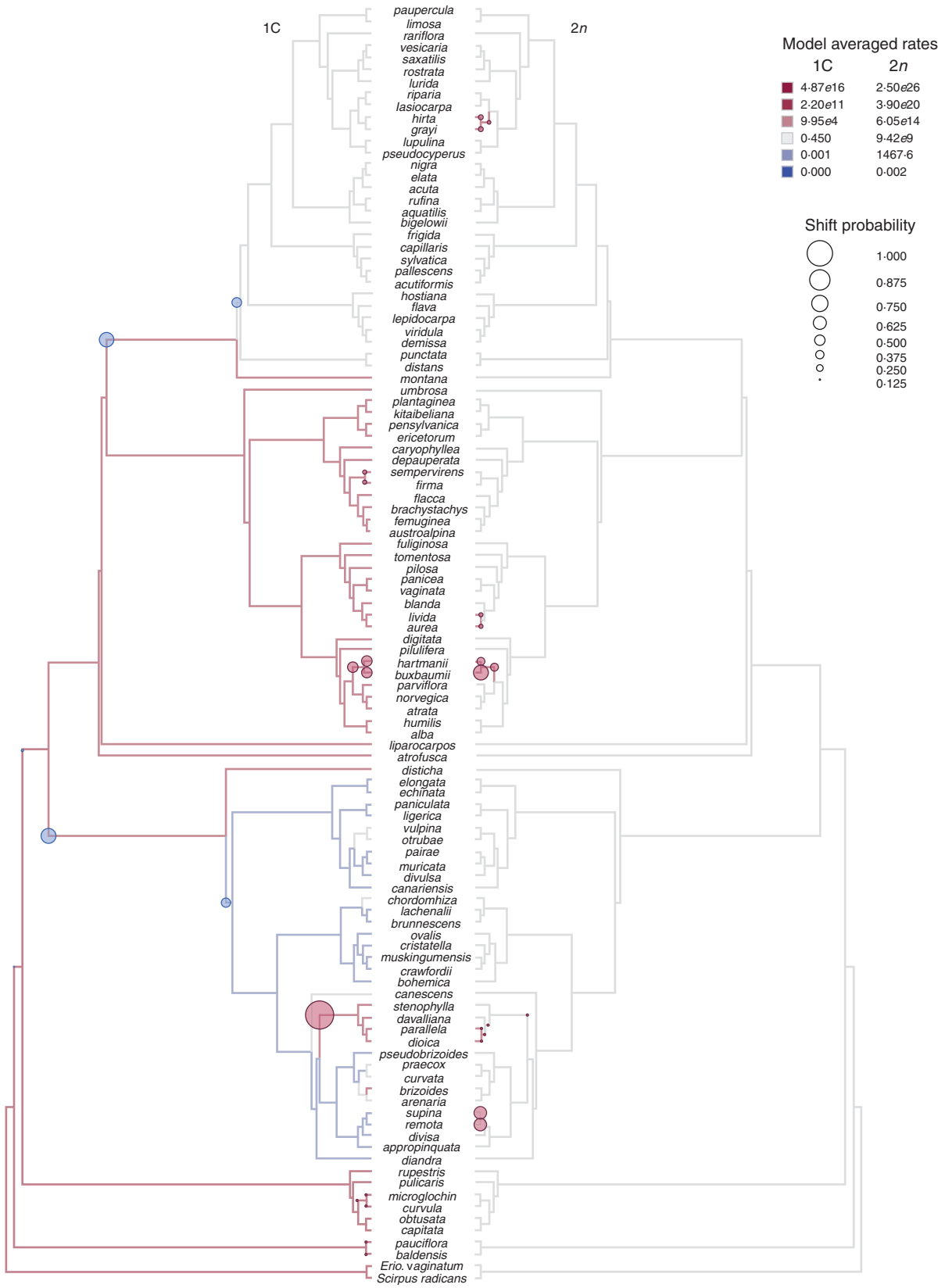


FIG. 3. Reversible-jump MCMC analysis of shifts in the rates of evolution of genome size (1C) and chromosome number (2*n*). Model-averaged rates of evolution of 1C and 2*n* are averaged over three pooled rjMCMC runs of 10 000 000 generations each, sampled every 1000th generation after discarding the first 2500 000 generations of each run. The relative sizes of the circles represent the frequency at which the shift in the rate of evolution occurred at that node in the sample from the posterior probability distribution of evolutionary rate shifts.

been documented at a low taxonomic level (Bureš *et al.*, 2007; Šmarda *et al.*, 2008); however, this positive trend has not been confirmed among seed plants (Barow and Meister, 2002). The absence of a monotonous correlation across angiosperms (in contrast to evidence obtained at a finer phylogenetic scale) suggests a particular mechanism of genome-size evolution (e.g. proliferation or removal of a particular repetitive motif differing in GC content from the rest of the genome) among closely related taxa but not among seed plants. Unimodal relationships between GC content and the entire genome size spectrum of angiosperms have been suggested (Veselý *et al.*, 2012), i.e. a positive correlation for taxa with small genomes, no correlation for medium-sized genomes and a negative correlation for taxa with extremely large genomes, such as geophytic plants. The positive correlation detected in our study for sedges with small genomes is consistent with this predicted trend.

Evolutionary rates of genome size

The core *Carex* clade exhibits higher rate of genome-size evolution compared with the *Vignea* clade. This higher rate remains evident when polyploid taxa (occurring in the core *Carex* clade and among taxa sister to *Carex* and *Vignea* clades) are included in the analysis (AUTEUR $P = 0{\cdot}029$; analysis not shown). Chung *et al.* (2012) observed a uniform rate of genome-size evolution across the *Vignea* clade and an increased rate in chromosome number evolution in the eastern North American subclade of the *Carex* sect. *Ovales*, which belongs to the *Vignea* clade. In our dataset (mostly European species), no increased rate of chromosome number evolution was observed for *Carex* sect. *Ovales*, even among the data for the *Vignea* clade as was found by Chung *et al.* (2012) (mostly American and Asian taxa of *Vignea* clade). When the datasets of both studies were analysed together (175 taxa without polyploids), a significant shift in the rate of chromosome number evolution was observed in the eastern North American taxa of *Carex* sect. *Ovales* (phylocom $P = 0{\cdot}016$, AUTEUR $P = 0{\cdot}032$). When considering the same merged dataset, the evolutionary rate of genome size is significantly reduced in the section *Ovales* (phylocom $P = 0{\cdot}002$, AUTEUR $P = 0{\cdot}015$), which suggests that this section exhibited the lowest evolutionary rate of genome size compared with the rest of the genus (2·8-fold lower when average rates of *Carex* sect. *Ovales* and the rest of genus are compared; Fig. 5).

The increase in genome size observed in the three dioecious *Carex* species (*Carex davalliana*, *C. dioica*, and *C. parallela*) corresponded to observations in the genus *Asparagus* (Štajner *et al.*, 2002), and the significance of this increase was consistent with an accelerated rate in genome size. This increase could be mediated through transposon accumulation during the evolution of sex chromosomes in this 'dioecious' clade, as observed in the dioecious species of *Silene* (Charlesworth, 1991; Vyskot and Hobza, 2004). Indeed, an unusually large pair of chromosomes has been detected in the karyotype of the dioecious species *Carex davalliana* in this study and in studies by Rotreklová *et al.* (2011), Luceño (1992) and Heilborn (1937); the chromosomes were not examined in the other two dioecious species in our previous study, and there

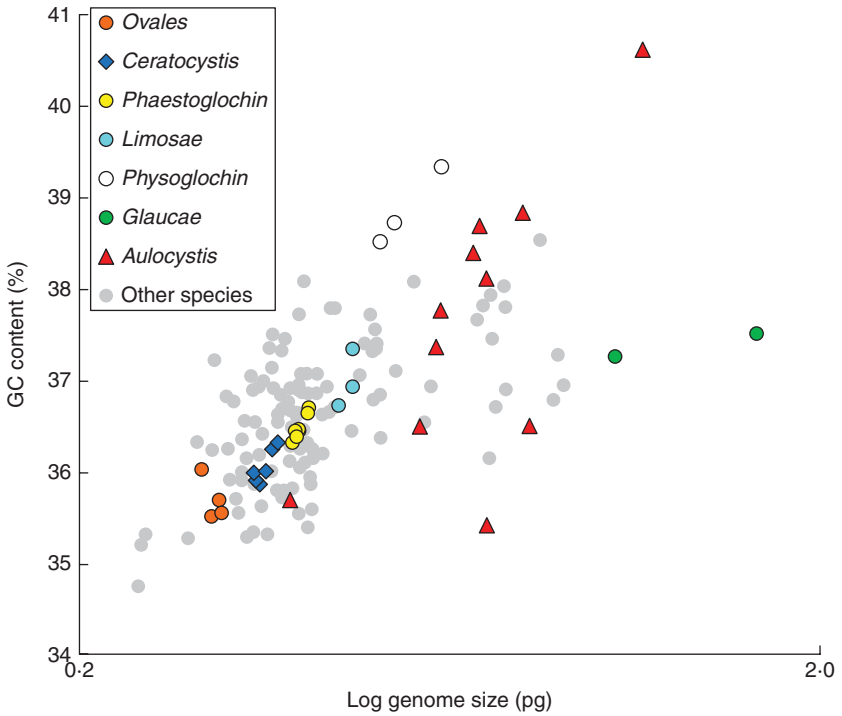


Fig. 4. Genome size (1C) and genomic GC content in selected sections of the genus *Carex*. These genomic characteristics separate some phylogenetic lineages as sections, e.g. *Phaestoglochin*, *Ovales*, *Limosae*, *Glaucae*, *Physoglochin* and *Ceratocystis*. The positive correlation observed in section *Aulocystis* suggests the proliferation or selective removal of GC-rich sequences. The grey circles represent other species in the genus. See the text for further details.

is no evidence among the published literature of the presence of larger chromosome pairs in their karyotypes (see Heilborn, 1924, fig. 1). The potential activity of transposons in the dioecious species of *Carex* could also be supported from data showing a shift in the GC content in these species (Fig. 2; see the discussion on the modes of karyotype evolution in the section *Aulocystis*). When the somatic DNA amount/somatic chromosome number ratio is used as a proxy of average chromosome size (average chromatid size), the differences between the size of the chromosomes of dioecious species and their hermaphroditic relatives (the rest of *Vignea* clade) contrasts even more (PGLS $P < 0{\cdot}001$, Fig. 6).

Modes of karyotype evolution

Overall, the relationship between genome size and chromosome number suggests the distribution of all measured taxa into three main groups that are separated by substantial gaps related to differences in the degree of polyploidy. Here, the three groups are termed as basal ploidy groups (= non-polyploids), polyploids and high polyploids (Fig. 1). The polyploids include species of the sect. *Microrhynchae* and sect. *Digitae* (Fig. 1 and Table 1). In sect. *Digitatae*, where polyploidy could be deduced from the study of Tanaka (1948), polyploidy was observed between two species formerly considered as conspecific (*C. rhizina* and *C. macroura* from the *C. pediformis* complex). The polyploid *C. flacca* and high polyploid *C. cuspidata* have been long considered to be subspecies of *C. flacca.* As the genome size of both taxa is the largest of the remaining *Carex* species, these species may represent putative tetraploid (*C. flacca*) and hexaploid species (*C. cuspidata*). Altogether, nine polyploid or high polyploid species (*Carex adelostoma*, *C. baldensis*, *C. buxbaumii*, *C. callitrichos*, *C. curvula*, *C. cuspidata*, *C. flacca*, *C. lanceolata* and *C. rhizina*) represent a surprisingly high portion (5·7 %) of studied taxa; Hipp *et al.* (2009) reported only four undoubtedly documented polyploid taxa in the entire genus (*Carex dolichostachya*, *C. jackiana*, *C. roraimensis*, and *C. siderosticta*). As none of the nine polyploid species detected in our study belong to the clade *Vignea* and Chung *et al.* (2012) did not identify any polyploid taxa among the 87 North American taxa of the *Vignea* clade, we conclude that the occurrence of polyploidy is much rarer in this clade compared with the core *Carex* clade. *C. roraimensis* was the only polyploid identified in the *Vignea* clade, but this was based on inference from chromosome counts alone, and that from a single individual (Hipp *et al.*, 2006). The two polyploid sections in our data, sect. *Microrhynchae* and sect. *Digitatae*, form one clade in the phylogenetic tree (Fig. 2). Thus, based on the published data [our study and that of Chung *et al.* (2012) together comprising 231 taxa and approx. 12 % of the species diversity of the genus], we conclude that the occurrence of polyploidy is rare in the genus and phylogenetically clustered.

In monocentric taxa, a positive correlation between the somatic DNA amount and somatic chromosome number is expected at a fine phylogenetic scale, which is used for the detection of polyploidy using flow cytometry (Suda *et al.*, 2007). In contrast, a negative non-phylogenetic 2C/2*n* correlation was suggested for various holocentric taxa, including *Carex* (Nishikawa *et al.,* 1984), Cyperaceae and Juncaceae (Roalson *et al.,* 2006) as well as all holocentric plants (Bureš *et al.*, 2013). Although Chung *et al.* (2012) did not observe a negative correlation in the relatively recently derived section *Ovales* (crown age approx. 4·33 million years; Escudero *et al.*, 2012) they reported a weak but significant negative correlation in the rest of the *Vignea* clade (crown age approx. 24·0 million years) when section *Ovales* was excluded. Therefore, these authors suggested that an analysis of the 2C/2*n* relationship on a broader phylogenetic scale in *Carex* was required. When considering our raw DNA content

TABLE 1. *Chromosome numbers and genome sizes of sedges from two sections, suggesting the presence of polyploidy*

	2*n*	1C (pg)
Sect. *Digitatae*		
C. humilis	35	0·47
C. macroura	35	0·43
Polyploids:		
C. callitrichos	70	0·90
C. lanceolata	70	0·87
C. rhizina	70	0·88
Sect. *Microrhynchae*		
C. aterrima	52	0·39
C. parviflora	54	0·39
C. atrata	54	0·40
C. norvegica	54	0·38
Polyploids:		
C. buxbaumii	100	0·72
C. adelostoma	106	0·73

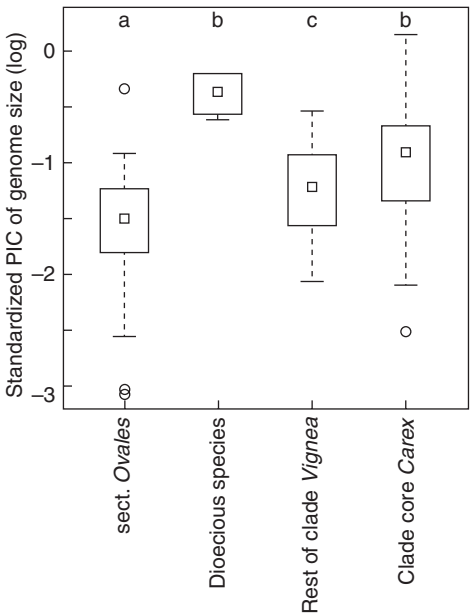


FIG. 5. Box and whisker plot showing differences in the rates of genome-size evolution. Dioecious species in addition to the taxa of the core *Carex* clade exhibit the highest rates of genome-size evolution, whereas the taxa of the *Carex* sect. *Ovales* exhibit the lowest rate of evolution. Letters indicate the results of one-way ANOVA ($F_{1,3} = 14{\cdot}86$, $P < 0{\cdot}001$) and Tukey's *post-hoc* analysis on standardized phylogenetic independent contrasts (PIC). The standardized PIC values of the genome size were log transformed.

data, the previously suggested negative correlation between genome size and chromosome number was naturally obscured by polyploidy (see above and Fig. 1). When considering 'basal ploids', the negative correlation was evident in our data (Pic$R = -0{\cdot}300$, $P = 0{\cdot}002$) across the entire genus *Carex*. Moreover, this negative phylogenetic correlation remains significant when our sample set (without polyploids) is analysed in combination with the dataset of Chung *et al.* (2012;

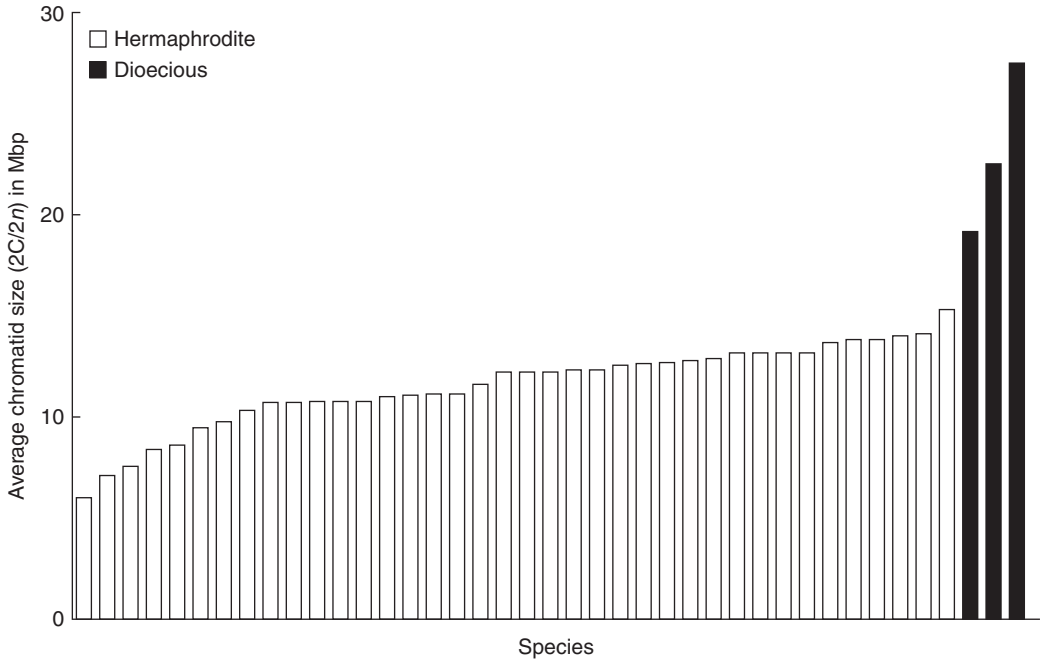


FIG. 6. Comparison of chromosome size of the hermaphrodite and dioecious species of the clade *Vignea*. Dioecious species possess significantly larger chromosomes than their hermaphrodite relatives (PGLS $P < 0{\cdot}001$).

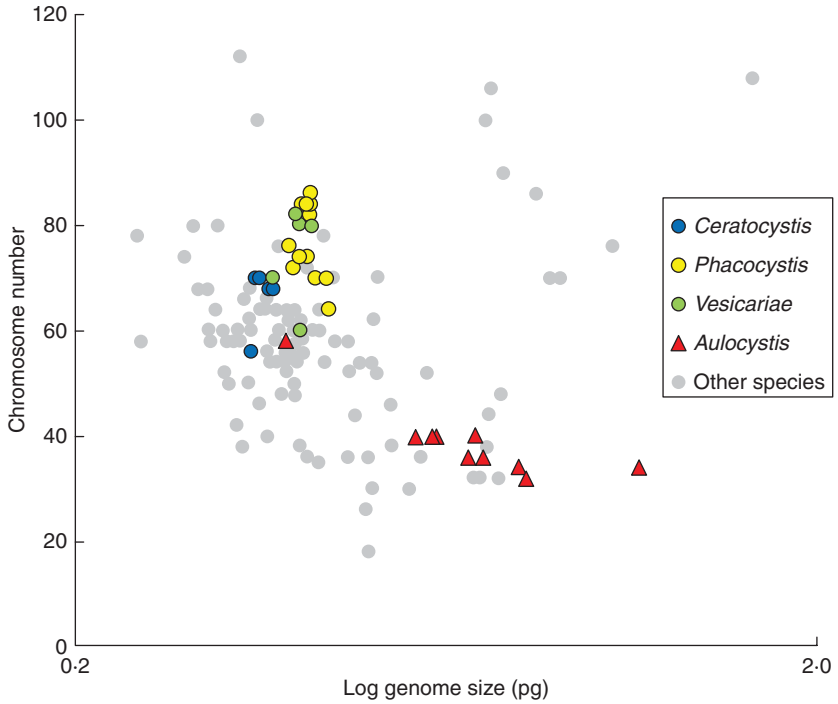


FIG. 7. Genome size (1C) and chromosome number in the genus *Carex*. Sections *Ceratocystis*, *Phacocystis* and *Vesicariae* exhibit a typical pattern of chromosomal fusion and fission. The pattern of section *Aulocystis* suggests genome expansion through the proliferation of repetitive sequences. The grey circles represent other species in the genus.

Pic$R = -0{\cdot}228$, $P = 0{\cdot}002$). The negative correlation between genome size and chromosome number strongly suggests that repetitive DNA removals/proliferations and chromosomal rearrangements (fusions/fissions) play important roles in the karyotype evolution of *Carex* (Fig. 1). This empirical conclusion is consistent with the probabilistic analyses of chromosome-number evolution suggesting prevalence of chromosomal rearrangements in chromosomal evolution of the genus (Hipp, 2007; Mayrose *et al.*, 2010). An alternative explanation for the negative correlation between genome size and chromosome number, as described by Roalson *et al.* (2006), suggests that an increase in the number of chromosomes (fission events) could be associated with a continual reduction of genome size mediated by an as-yet unrecognized mechanism that is related to the loss of broken chromosome ends prior to the restoration of newly formed telomere regions, which results in an increase in the number of chromosomes accompanied by a small decrease in genome size.

In the sections *Ceratocystis*, *Phacocystis* and *Vesicariae* (Fig. 7), changes in chromosome number seem to be coupled with no or minor changes in DNA content (Appendix), which suggests a dominant role of chromosomal fusion and fission in the karyotype evolution of these sections. In section *Ceratocystis* (*C. flava* complex), quantitative aneuploidy has been previously suggested as the prevalent mode of chromosomal evolution, which was based on the study of chromosome number and their meiotic behaviours (Schmid, 1982). Accepting quantitative aneuploidy as the reason for chromosome number change, this change should be proportional to the respective changes in genome size. As this is not the case with our data, we assume that quantitative aneuploidy plays a negligible role in karyotype evolution of this section. The well-resolved phylogeny available for section *Ceratocystis* allowed us to hypothesize a putative role for fusion and fission in karyotype evolution of this group. The cladogenesis is regularly associated with an increase in chromosome number (keeping genome size more or less constant), suggesting a prevalence of chromosomal fission over chromosomal fusion ($2n = 68–70$ for advanced *C. lepidocarpa*, *C. demissa* and *C. viridula*, whereas $2n = 56$ in basal *C. hostiana* and *C. flava*). This finding should be considered with care because of the incomplete species coverage in recent phylogenies of this section.

A pattern of karyotype and genome-size evolution different from the previous case was observed in the section *Aulocystis*. Here, the increase in genome size was accompanied by an increase in GC content (Fig. 4) and a slight decrease in chromosome number (Fig. 7). This pattern might be explained by a combination of (*a*) the above-mentioned chromosomal fusion/fission responsible for slight changes in chromosome numbers and (*b*) changes in genome size through the proliferation/removal of GC-rich repetitive elements. The latter process may be analogous to that observed in the Cyperaceae genus *Eleocharis*, where the massive amplification of Ty1-*copia*-like elements induces a significant chromosome enlargement in advanced taxa (Zedek *et al.*, 2010). The activity of some GC-rich retrotransposons may also provide a likely explanation for the positive correlation between genome size and GC content observed in *Carex*. Alternatively, if the negative correlation between chromosome number and genome size mediated through the loss of broken chromosome ends proves true (Roalson *et al.*, 2006), then the positive correlation between genome size and GC content could be explained as a consequence of the following: if putative chromosomal 'fragile sites' consist of large, expanded CCG minisatellites (which are suggested breakpoints in human chromosomes; Sutherland *et al.*, 1998), then the subsequent removal of these 'fragile sizes' (as suggested by Roalson *et al.*, 2006) would result in a correlation between the genome size and GC content.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: list of the locations of the sampled taxa. Table S2: list of the species and accession numbers from the NCBI GenBank database for phylogenetic analyses. Figure S1: phylogenetic tree with Bayesian posterior probabilities.

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APPENDIX

Estimates of genome size (1C), the average coefficient of variation (CV) of measurements of the genome size (CV PI), genomic GC content, the average CV of measurement of the GC content (CV DAPI) and number of chromosomes in the genus *Carex*. The chromosome numbers were adopted from the literature. Unknown numbers are marked as NA. Infrageneric classification is as in Egorova (1999).

Taxon	1C (pg)	CV PI (%)	GC content (%)	CV DAPI (%)	2*n*	Section
Subgenus *Carex*						
C. lurida	0·40	2·63	36·10	2·29	60*	*Vesicariae*
C. saxatilis subsp. *saxatilis*	0·42	3·82	36·85	1·77	80*	
C. saxatilis subsp. *laxa*	0·40	3·59	36·89	2·02	80†	
C. rostrata subsp. *rostrata*	0·37	2·11	35·80	1·91	70*	
C. rostrata subsp. *dichroa*	0·38	4·11	36·78	2·94	NA	
C. vesicaria	0·40	3·60	36·27	2·10	82†	
C. melanostachya	0·38	3·57	35·80	2·48	54*	*Tumidae*
C. riparia	0·41	1·99	35·95	2·06	72*	
C. antoniensis	0·37	4·43	36·55	2·37	NA	*Pseudocyperae*
C. pseudocyperus	0·36	1·96	36·01	2·18	66*	
C. grayi	0·47	2·44	36·46	2·24	52*	*Lupulinae*
C. lupulina	0·41	3·53	35·41	2·84	56†	
C. hirta	0·33	2·54	36·58	2·02	112†	*Carex*
C. lasiocarpa	0·38	3·53	35·69	2·01	56†	
C. sordida	0·35	4·49	35·62	2·05	100*	
C. limosa	0·45	1·86	36·73	2·06	58*	*Limosae*
C. paupercula	0·47	2·69	36·94	1·83	58†	
C. rariflora	0·47	3·08	37·34	1·77	52†	
C. koraginensis subsp. *koraginensis*	0·51	3·45	36·38	2·17	NA	*Scitae*
C. koraginensis subsp. *krascheninnikovii*	0·43	3·72	36·61	2·12	64*	
C. pallescens	0·39	2·89	35·82	2·05	64*	*Porocystis*
C. acutiformis	0·43	2·49	36·93	1·90	78†	*Paludosae*
C. lepidocarpa	0·37	2·09	36·26	2·34	68*	*Ceratocystis*
C. demissa	0·35	3·02	35·88	2·10	70*	
C. flava	0·35	2·03	35·93	1·94	56*	
C. hostiana	0·34	2·02	36·00	2·30	56*	
C. jemtlandica	0·37	3·31	36·33	1·85	68*	
C. viridula	0·36	2·16	36·02	2·10	70†	
C. strigosa	0·34	2·07	35·30	2·13	66*	*Silvaticae*
C. sylvatica	0·38	2·33	36·12	2·59	58*	
C. cuspidata	1·64	2·51	37·51	2·11	108†	*Glaucae*
C. flacca	1·06	1·92	37·27	1·64	76*	
C. austroalpina	0·58	3·18	36·50	1·71	40†	*Aulocystis*
C. brachystachys	0·61	2·66	37·76	1·84	40†	
C. bulgarica	0·79	4·50	38·84	2·59	34†	
C. ferruginea	0·60	2·66	37·36	2·00	40*	
C. firma (Alps)	1·15	2·97	40·61	1·67	34†	
C. firma (Carpathians)	1·16	3·14	41·13	1·98	34†	
C. frigida	0·38	2·56	35·70	2·29	58*	
C. fuliginosa	0·69	2·18	38·70	2·21	40†	
C. kitaibeliana	0·71	2·81	38·13	3·06	36†	
C. macrolepis	0·71	2·83	35·42	1·58	36†	
C. mucronata	0·68	2·58	38·41	2·16	36*	
C. sempervirens	0·81	2·55	36·51	1·39	32*	
C. atrofusca	0·72	3·33	37·93	1·55	38†	*Chartoteuchium*
C. capillaris	0·50	2·31	37·55	2·27	54†	*Chlorostachyae*
C. tenuiformis	0·36	4·08	37·15	2·18	40†	
C. curvula	0·84	2·31	38·54	2·18	86†	*Curvulae*
C. distans	0·37	1·87	35·74	2·92	70*	*Spirostachyae*
C. punctata	0·34	2·99	35·34	2·54	68†	
C. acuta	0·41	2·05	36·24	2·27	82*	*Phacocystis*
C. aquatilis subsp. *aquatilis*	0·41	3·13	36·83	1·90	74*	
C. aquatilis subsp. *stans*	0·39	3·60	36·26	2·01	76†	
C. bigelowii subsp. *dacica*	0·42	2·25	36·64	2·38	70*	
C. bigelowii subsp. *rigidioides*	0·44	3·70	37·79	2·25	70*	
C. buekii	0·44	2·37	36·71	2·14	64‡	
C. cespitosa	0·39	2·61	36·94	2·13	72*	
C. elata	0·40	2·50	36·46	1·98	74*	
C. kamtschatica	0·41	3·50	37·07	1·95	NA	
C. nigra	0·40	2·49	36·33	2·29	84*	
C. rufina	0·42	2·99	37·06	1·79	86*	
C. alba	0·43	2·36	36·65	2·06	54*	*Digitatae*
C. callitrichos	0·90	3·21	36·95	2·34	70*	
C. digitata	0·38	1·84	37·45	1·86	48*	

Continued

APPENDIX *Continued*

Taxon	1C (pg)	CV PI (%)	GC content (%)	CV DAPI (%)	2*n*	Section
C. humilis	0·47	2·56	37·37	1·81	36*	
C. lanceolata	0·87	3·88	36·79	2·37	70*	
C. ornithopoda	0·36	2·21	36·92	2·24	54†	
C. macroura	0·43	2·54	36·21	2·05	35*	
C. rhizina	0·88	3·31	37·29	1·86	70*	
C. quadriflora	0·35	3·33	36·99	2·16	46*	
C. ussuriensis	0·42	3·50	36·18	2·32	NA	
C. aurea	0·60	3·21	36·94	2·29	52*	*Paniceae*
C. falcata	0·75	3·56	37·80	1·89	48†	
C. livida	0·70	3·12	37·81	1·45	32*	
C. panicea	0·69	2·56	37·66	1·69	32*	
C. vaginata	0·74	3·18	38·03	1·79	32*	
C. campylorhina	0·39	3·31	36·64	2·02	58*	*Depauperatae*
C. depauperata	0·72	3·08	37·46	3·11	44*	
C. michelii	0·39	2·04	36·31	2·02	62†	
C. pilosa	0·48	2·02	37·06	2·13	44†	
C. xiphium	0·40	4·44	37·07	1·94	56*	
C. ericetorum	0·57	2·54	38·08	2·11	30†	*Acrocystis*
C. fritschii	0·50	2·09	37·38	2·10	30*	
C. montana	0·34	1·87	36·15	2·21	38*	
C. pensylvanica	0·50	3·76	36·78	2·79	36*	
C. pilulifera	0·50	2·22	37·32	1·79	18*	
C. riloensis	0·49	2·18	37·72	2·62	26*	
C. tomentosa	0·40	2·68	36·05	2·07	48*	
C. adelostoma	0·73	3·26	36·71	1·68	106*	*Microrhynchae*
C. aterrima	0·39	2·55	36·30	2·54	52*	
C. atrata	0·40	2·36	36·59	2·35	54*	
C. buxbaumii	0·71	2·68	36·15	1·97	100*	
C. hartmanii	0·34	3·49	36·54	2·30	68‡	
C. norvegica	0·38	2·49	36·90	1·91	54*	
C. parviflora	0·39	2·19	36·48	2·12	54*	
C. hallerana	0·48	3·07	37·39	1·70	54*	*Hallerianae*
C. microcarpa	0·58	2·17	36·55	2·61	36†	*Rhynchocystis*
C. pendula	0·37	2·64	36·63	2·17	58*	
C. caryophyllea	0·51	2·81	36·84	1·70	62*	*Mitratae*
C. depressa subsp. *transsilvanica*	0·51	2·58	36·84	2·86	70†	
C. umbrosa	0·50	3·39	37·36	2·00	62*	
C. hordeistichos	0·25	2·26	35·33	2·76	58*	*Secalinae*
C. secalina	0·24	2·29	34·75	2·44	NA	
C. supina	0·40	1·77	36·46	2·14	38†	*Lamprochlaenae*
C. liparicarpos	0·53	3·55	37·10	1·65	38†	
C. blanda	0·41	2·99	35·59	2·43	36*	*Laxiflorae*
C. plantaginea	0·40	3·43	35·56	2·45	50*	*Careyanae*
C. platyphylla	0·36	3·38	35·32	2·54	70‡	
Subgenus *Vignea*						
C. leiorhyncha	0·24	3·92	35·22	2·33	78†	*Phleoideae*
C. otrubae	0·39	2·38	36·59	2·24	60*	*Vulpinae*
C. vulpina	0·36	2·79	37·35	2·35	68†	
C. remota	0·40	2·38	38·08	1·86	62*	*Remotae*
C. remotiuscula	0·33	4·44	36·56	2·46	NA	
C. appropinquata	0·36	3·01	37·50	2·27	64†	*Heleoglochin*
C. canariensis	0·39	2·12	36·39	3·00	58*	
C. diandra	0·42	1·73	37·09	2·07	60*	
C. hansenii	0·38	4·22	36·85	2·81	64*	
C. paniculata	0·38	3·18	37·33	2·36	60*	
C. arenaria	0·35	3·53	36·42	2·01	64†	*Ammoglochin*
C. brizoides	0·33	2·68	35·57	2·11	58*	
C. curvata	0·32	1·94	35·71	2·15	58*	
C. ligerica	0·33	3·86	36·37	2·09	58†	
C. praecox	0·32	1·74	35·91	2·43	58*	
C. pseudobrizoides	0·35	2·75	36·18	2·18	56*	
C. disticha	0·43	2·77	36·20	2·04	60*	*Holarrhenae*
C. divisa	0·34	3·49	35·87	2·85	60†	*Divisae*
C. chordorrhiza	0·34	2·43	36·90	1·93	62*	
C. stenophylla	0·30	2·57	36·25	2·43	60*	*Boernera*
C. echinata	0·38	2·15	36·85	2·08	58*	*Stellulatea*

Continued

APPENDIX *Continued*

Taxon	1C (pg)	CV PI (%)	GC content (%)	CV DAPI (%)	2*n*	Section
C. contigua	0·39	2·14	36·40	2·55	58†	*Phaestoglochin*
C. divulsa	0·41	2·96	36·70	2·51	58†	
C. chabertii	0·39	1·95	36·46	1·85	54‡	
C. leersiana	0·39	2·13	36·42	2·67	58†	
C. muricata	0·39	1·82	36·34	2·49	58†	
C. pairae	0·41	2·00	36·64	1·93	58†	
C. elongata	0·37	2·66	36·92	2·08	56*	*Elongatae*
C. brunnescens	0·35	2·26	36·93	2·11	56*	*Canescentes*
C. canescens	0·36	2·67	37·35	2·09	56*	
C. lachenalii	0·40	2·76	37·73	1·95	64*	
C. crawfordii	0·30	3·05	35·52	2·69	68*	*Ovales*
C. cristatella	0·31	3·54	35·69	2·69	64*	
C. muskingumensis	0·31	2·58	35·55	2·67	80*	
C. ovalis	0·29	3·00	36·02	2·29	68*	
C. bohemica	0·29	1·78	36·32	2·32	80*	*Cyperoideae*
C. argunensis	0·33	3·99	35·92	2·33	42*	*Petratae*
C. enervis	0·33	3·96	35·99	2·43	60*	*Enerves*
C. micropoda	0·44	3·79	37·79	2·42	70†	*Callistachys*
C. davalliana	0·53	2·01	38·72	2·03	46*	*Physoglochin*
C. dioica	0·51	1·78	38·52	2·10	52†	
C. parallela	0·62	3·00	39·33	2·25	44†	
Other species						
C. baldensis	0·75	2·98	36·89	1·82	90†	*Baldenses*
C. pulicaris	0·32	2·18	36·83	2·40	60†	*Psyllophora*
C. capitata	0·32	2·55	36·78	1·92	50†	*Capituligerae*
C. obtusata	0·32	3·07	36·26	2·32	52*	*Petraeae*
C. rupestris	0·34	2·95	37·05	1·88	50†	
C. microglochin	0·30	3·34	37·23	2·22	58†	*Leucoglochlin*
C. pauciflora	0·38	1·78	36·68	2·32	76†	
C. distachya	0·28	2·09	35·27	3·88	74†	Not defined

* Adopted from Rotreklová *et al.* (2011), based on the same samples.
† Adopted from Roalson (2008).
‡ *Carex hartmanii* and *C. chabertii* counted by O. Rotreklová (unpubl. res.); *C. buekii* adopted from Stoeva *et al.* (2005); *C. platyphylla* adopted from Naczi (1999).