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Chromosome diversity and evolution in Liliaceae

L. Peruzzi[1,*], I. J. Leitch[2] and K. F. Caparelli[1]

[1]*Dipartimento di Biologia, Unità di Botanica Generale e Sistematica, Università di Pisa, via Luca Ghini 5, 56126 Pisa, Italy and*
[2]*Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK*

• *Background and Aims* There is an extensive literature on the diversity of karyotypes found in genera within Liliaceae, but there has been no attempt to analyse these data within a robust phylogenetic framework. In part this has been due to a lack of consensus on which genera comprise Liliaceae and the relationships between them. Recently, however, this changed with the proposal for a relatively broad circumscription of Liliaceae comprising 15 genera and an improved understanding of the evolutionary relationships between them. Thus there is now the opportunity to examine patterns and trends in chromosome evolution across the family as a whole.
• *Methods* Based on an extensive literature survey, karyo-morphometric features for 217 species belonging to all genera in Liliaceae *sensu* the APG (Angiosperm Phylogeny Group) were obtained. Included in the data set were basic chromosome number, ploidy, chromosome total haploid length (THL) and 13 different measures of karyotype asymmetry. In addition, genome size estimates for all species studied were inferred from THLs using a power regression model constructed from the data set. Trends in karyotype evolution were analysed by superimposing the karyological data onto a phylogenetic framework for Liliaceae.
• *Key Results and Conclusions* Combining the large amount of data enabled mean karyotypes to be produced, highlighting marked differences in karyotype structure between the 15 genera. Further differences were noted when various parameters for analysing karyotype asymmetry were assessed. By examining the effects of increasing genome size on karyotype asymmetry, it was shown that in many but not all (e.g. *Fritillaria* and all of Tulipeae) species, the additional DNA was added preferentially to the long arms of the shorter chromosomes rather than being distributed across the whole karyotype. This unequal pattern of DNA addition is novel, contrasting with the equal and proportional patterns of DNA increase previously reported. Overall, the large-scale analyses of karyotype features within a well-supported phylogenetic framework enabled the most likely patterns of chromosome evolution in Liliaceae to be reconstructed, highlighting diverse modes of karyotype evolution, even within this comparatively small monocot family.

Key words: C-value, chromosomes, genome size, karyotype asymmetry, karyotype evolution, Liliaceae, Liliales, polyploidy.

INTRODUCTION

Comparative karyotype analysis of related species has traditionally been used to describe patterns and directions of chromosomal evolution within a group and to infer the evolutionary role that such karyotype changes may have played (e.g. Stebbins, 1971; Stace, 1978; Gonzalez-Aguilera and Fernandez-Peralta, 1984; Watanabe *et al.*, 1995; De Melo Nationiel *et al.*, 1997; Vanzela *et al.*, 1997; Das *et al.*, 1999; Shan *et al.*, 2003). Whereas earlier studies were, to some extent, flawed by the lack of a rigorous phylogenetic framework in which to analyse the data, more recently this problem has been addressed and there are now an increasing number of papers which have analysed karyological data using a well-supported phylogenetic tree. These include the works of Cox *et al.* (1998) on slipper orchids (Orchidaceae), Watanabe *et al.* (1999) on *Brachyscome* (Asteraceae), Venora and collaborators (Venora *et al.*, 2000; Frediani *et al.*, 2005; Caputo *et al.*, 2006; Ruffini Castiglione *et al.*, 2007) on *Vicia* (Fabaceae), Leitch and colleagues on *Nicotiana* (Solanaceae) (Lim *et al.*, 2000, 2004, 2006, 2007), Lysak and collaborators on Brassicaceae (Lysak *et al.*, 2005,

2006, 2007; Lysak and Lexer, 2006; Lysak and Mandakova, 2007) and Pires *et al.* (2006) on Asparagales.

The karyotype is the phenotypic aspect of the chromosome complement as seen at mitotic metaphase. A description of the karyotype typically includes the chromosome number [the product of two variables, basic number (x) and ploidy, resulting in chromosome number ($2n$)], the absolute and/or relative length of chromosomes (reflecting genome size), the position of primary and secondary constrictions (Levan *et al.*, 1964), the distribution of material with different staining properties (Stebbins, 1971; Stace, 2000; Levin, 2002; Lysak and Lexer, 2006) and the degree of symmetry. A symmetrical karyotype is characterized by mainly metacentric and sub-metacentric chromosomes of approximately equal size. Changes to an asymmetric karyotype can arise by shifts in centromere position towards the telomere (intrachromosomal) and/or by the addition or deletion of chromatin from some but not all chromosomes, leading to differences in size between the largest and smallest chromosomes (interchromosomal). These processes are not usually correlated, though there are some reports that they are in some groups (Stebbins, 1971).

The chromosomes of Liliaceae have long attracted attention, given their diversity in size, number and structure (e.g. Sato,

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* For correspondence: E-mail lperuzzi@biologia.unipi.it

1943; Cave, 1970; Sen, 1975; Tamura, 1995). However, attempts to infer patterns of chromosome evolution by comparative karyotype analysis have been hampered by the lack of a rigorous phylogenetic framework together with a lack of agreement over the genera comprising Liliaceae. Examples of some of these contrasting circumscriptions are outlined in Table 1. There is now a general consensus of the genera comprising subfamily Lilioideae [*Amana*, *Cardiocrinum*, *Erythronium*, *Fritillaria*, *Gagea* (including *Lloydia*), *Lilium* (including *Nomocharis*), *Notholirion* and *Tulipa*] and the phylogenetic relationships between them (Fay and Chase, 2000; Patterson and Givnish, 2002; Fay *et al.*, 2006). Similarly, genera comprising subfamily Medeoloideae (*Clintonia* and *Medeola*) and their relationships are well supported (Fay *et al.*, 2006). However, the placement of *Tricyrtis*, *Calochortus* and the clade comprising *Prosartes*, *Scoliopus* and *Streptopus* (Streptopeae) has been more problematic (Patterson and Givnish, 2002; Rønsted *et al.*, 2005; Fay *et al.*, 2006). Nevertheless, by taking a broad circumscription of Liliaceae, as used by Chase *et al.* (2000), Fay and Chase (2000) and the Angiosperm Phylogeny Group (APG II, 2003), the opportunity to examine the wealth of cytogenetic data available for the 15 genera comprising Liliaceae *sensu* APG II (2003) within a phylogenetic context now exists. Figure 1 summarizes the current understanding of these phylogenetic relationships.

Despite phylogenetic issues noted above, some previous karyotype analyses have been informative. For example, Tamura (1995, 1998*a*, *b*) highlighted differences in karyotype structure between the small and variable number of chromosomes in genera such as *Calochortus*, *Prosartes*, *Scoliopus*, *Streptopus* and *Tricyrtis*, and the larger and more stable chromosome numbers found in subfamily Lilioideae (e.g. *Lilium*, *Fritillaria*, *Tulipa*). Tamura (1995. 1998*a*, *b*) also noted clear differences in karyotype structure between genera in subfamily Medeoloideae ($x = 7$) and Lilioideae ($x = 12$) and the distinct karyotype shared by all representatives of tribe Lilieae, comprising two longer metacentric chromosome pairs and ten shorter telocentric pairs.

More recently, research in Liliaceae *sensu* APG II (2003) revealed considerable variability in genome size, broadly paralleling chromosome heterogeneity (Leitch *et al.*, 2007). Large genomes (mean $1C = 35.9$ pg) characterized subfamilies Lilioideae and Medeloloideae, whereas smaller genomes (mean $1C = 5.6$ pg) characterized the remaining genera (Leitch *et al.*, 2007). In their analysis, genome size data were compiled from the Plant DNA C-values database (Bennett and Leitch, 2005*b*) and Zonneveld *et al.* (2005), together with new genome size estimates for 23 species of Liliaceae to give a total data set of 78 species. Despite containing some of the largest genomes so far reported in the angiosperms, a phylogenetic analysis revealed that the ancestral genome size reconstructed for the family was just $1C = 6.67$ pg, similar to that reported for *Smilax* in Smilacaceae (sister to Liliaceae) ($1C = 5.0$ pg).

Given recent advances in understanding phylogenetic relationships within Liliaceae noted above, this paper aims to summarize quantitatively the large amount of karyotype data available in the literature for all genera of Liliaceae (*sensu* APG II, 2003) and analyse them within a phylogenetic framework to assess possible modes and directions of karyotype evolution.

MATERIALS AND METHODS

Source of karyotype data

An extensive literature survey was conducted to collate available karyotype data for Liliaceae and Smilaacceae. *The index to plant chromosome numbers* (eds, P. Goldblatt and D. E. Johnson, Missouri Botanical Garden, St Louis) was consulted (hard copies up to 1979 and then electronically at http://mobot. mobot.org/W3T/Search/ipcn.html) together with other chromosome atlases (e.g. Darlington and Wylie, 1955; Fedorov, 1969) and internet resources (e.g. Kew Bibliographic Databases, ISI Web of Science, Google Scholar).

In total, karyotype data were collected for 405 accessions of Liliaceae (386) and Smilacaceae (19). Data were available for 217 species, including representatives of all genera comprising these two families. Only literature with published idiograms, and/or karyotype measurements and/or good metaphase plates with magnification or scale bar indicated were considered. When $2n$ mitotic metaphase plates were measured, the graphic method proposed by Plummer *et al.* (2003) was used to match homologous chromosomes. This involves plotting the relative length of each chromosome [= (length of individual chromosome/total length of all chromosomes)/ 100] against its arm ratio, in order to pair chromosomes.

Karyotype analysis

A data matrix of 53 karyotype features was assembled including basic chromosome number (x), ploidy, chromosome number ($2n$) and chromosome total haploid length (THL). For analysis of karyotype asymmetry, all known methods of assessing this character were used (Table 2). A review of each method and a summary of how each asymmetry index was calculated is given in Paszko (2006). For the present analyses, two coefficients of variation (CVs) were found to be particularly informative measures of asymmetry. The CV_{CI} index evaluates differences in centromere position for each chromosome in the karyotype and provides a measure of intrachromosomal asymmetry. In contrast, the CV_{CL} gives a measure of interchromosomal asymmetry as it reflects how variable the chromosome sizes are in the karyotype. In both cases, the larger the value the greater the asymmetry in the karyotype.

For each genus, a mean haploid idiogram was built as follows. Using karyoytype data for each species, chromosome pairs were arranged into decreasing lengths, then the absolute lengths of the long and short arms of each chromosome pair were taken. The data were then pooled to produce a mean length of each chromosome pair for a genus (e.g. the data for the longest chromomosome pair of all *Lilium* species were pooled to give the mean value for *Lilium* chromosome pair I). It is noted that since the study was based almost exclusively on measurements of Feulgen-stained chromosomes, there was no possibility of identifying and analysing homoeologous chromosomes between taxa. Instead the 'mean karyotype' of each genus refers only to the shape of the haploid karyotype.

TABLE 1. *Taxonomic treatment of genera currently included in Liliaceae which have appeared in the last 30 years*

| Genus | Dahlgren et al. (1985) | | | Takhtajan (1997) | | | Chase et al. (1995), Tamura (1995, 1998a, b) | | | | Patterson and Givnish (2002) | | | Fay and Chase (2000), APG II (2003) | |
	Tribe	Family	Order	Tribe	Family	Order	Tribe	Subfamily	Family	Order	Subfamily	Family	Order	Family	Order
Streptopus	Uvularieae	Uvulariaceae	Liliales	Streptopeae	Uvulariaceae	Colchicales	Tricyrtideae	–	Calochortaceae	Liliales	'Streptopoideae'	Calochortaceae	Liliales	Liliaceae	Liliales
Scoliopus	–	Trilliaceae	Dioscoreales	–	Scoliopaceae	Colchicales	Tricyrtideae	–	Calochortaceae	Liliales	'Streptopoideae'	Calochortaceae	Liliales	Liliaceae	Liliales
Prosartes	Uvularieae	Uvulariaceae (in *Disporum*)	Liliales	Streptopeae	Uvulariaceae	Colchicales	Tricyrtideae	–	Calochortaceae	Liliales	'Streptopoideae'	Calochortaceae	Liliales	Liliaceae	Liliales
Calochortus	–	Calochortaceae	Liliales	–	Calochortaceae	Colchicales	Calochorteae	–	Calochortaceae	Liliales	'Calochortoideae'	Calochortaceae	Liliales	Liliaceae	Liliales
Tricyrtis	Tricyrtideae	Uvulariaceae	Liliales	–	Tricyrtidaceae	Colchicales	Tricyrtideae	–	Calochortaceae	Liliales	'Calochortoideae'	Calochortaceae	Liliales	Liliaceae	Liliales
Medeola	–	Trilliaceae	Dioscoreales	–	Medeolaceae	Liliales	–	Medeoloideae	Liliaceae	Liliales	Medeoloideae	Liliaceae	Liliales	Liliaceae	Liliales
Clintonia	Uvularieae	Uvulariaceae	Liliales	Streptopeae	Uvulariaceae	Colchicales	–	Medeoloideae	Liliaceae	Liliales	Medeoloideae	Liliaceae	Liliales	Liliaceae	Liliales
Cardiocrinum	–	Liliaceae	Liliales	Lilieae	Liliaceae	Liliales	Lilieae	Lilioideae	Liliaceae	Liliales	Lilioideae	Liliaceae	Liliales	Liliaceae	Liliales
Notholirion	–	Liliaceae	Liliales	Lilieae	Liliaceae	Liliales	Lilieae	Lilioideae	Liliaceae	Liliales	Lilioideae	Liliaceae	Liliales	Liliaceae	Liliales
*Nomocharis**	–	Liliaceae	Liliales	Lilieae	Liliaceae	Liliales	Lilieae	Lilioideae	Liliaceae	Liliales	Lilioideae	Liliaceae	Liliales	Liliaceae	Liliales
Fritillaria	–	Liliaceae	Liliales	Lilieae	Liliaceae	Liliales	Lilieae	Lilioideae	Liliaceae	Liliales	Lilioideae	Liliaceae	Liliales	Liliaceae	Liliales
Lilium	–	Liliaceae	Liliales	Lilieae	Liliaceae	Liliales	Lilieae	Lilioideae	Liliaceae	Liliales	Lilioideae	Liliaceae	Liliales	Liliaceae	Liliales
Gagea	–	Liliaceae	Liliales	Lloydieae	Liliaceae	Liliales	Tulipeae	Lilioideae	Liliaceae	Liliales	Lilioideae	Liliaceae	Liliales	Liliaceae	Liliales
Lloydia†	–	Liliaceae	Liliales	Lloydieae	Liliaceae	Liliales	Tulipeae	Lilioideae	Liliaceae	Liliales	Lilioideae	Liliaceae	Liliales	Liliaceae	Liliales
Amana‡	–	Liliaceae (in *Tulipa*)	Liliales	Tulipeae	Liliaceae	Liliales	Tulipeae	Lilioideae	Liliaceae	Liliales	Lilioideae	Liliaceae	Liliales	Liliaceae	Liliales
Tulipa	–	Liliaceae	Liliales	Tulipeae	Liliaceae	Liliales	Tulipeae	Lilioideae	Liliaceae	Liliales	Lilioideae	Liliaceae	Liliales	Liliaceae	Liliales
Erythronium	–	Liliaceae	Liliales	Tulipeae	Liliaceae	Liliales	Tulipeae	Lilioideae	Liliaceae	Liliales	Lilioideae	Liliaceae	Liliales	Liliaceae	Liliales

Nomocharis has been shown to be embedded within *Lilium* (Rønsted *et al.*, 2005).
†*Lloydia* has been shown to be embedded within *Gagea* (Peterson *et al.*, 2004; Peruzzi *et al.*, 2008*a*, *b*; Peterson *et al.*, 2008; M. Zarrei *et al.*, unpubl. res.).
‡The restoration of *Amana*, which was formerly included in *Tulipa*, was based on the work by Tan *et al.* (2005).
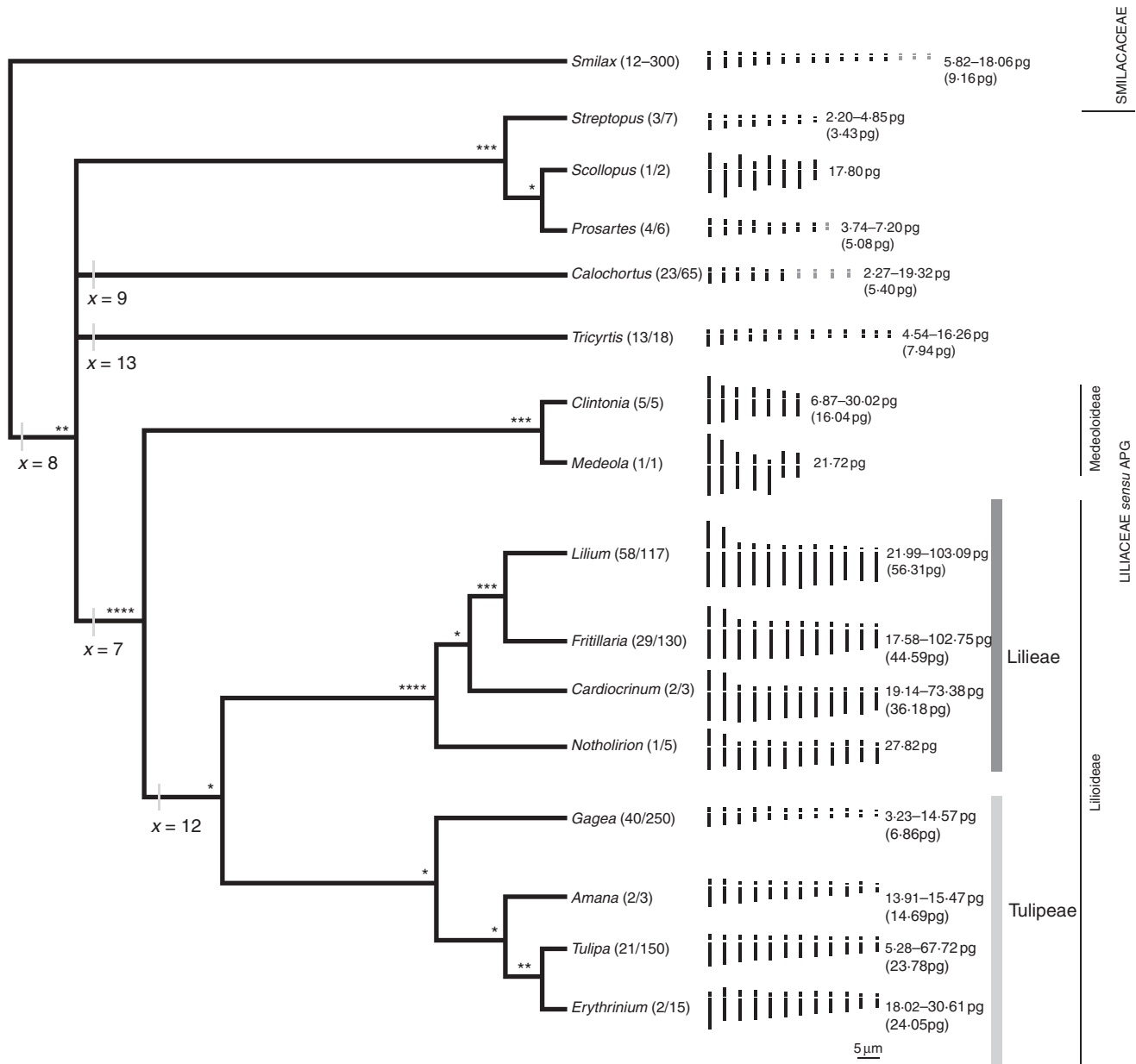
Fig. 1. Mean haploid idiograms and range of inferred 1Cx values (means in parentheses) for the genera of Liliaceae and Smilacaceae superimposed onto a phylogenetic framework. Inferred basic chromosome numbers and the strength of the clades (* = supported by one molecular marker only; ** = supported by two molecular markers; *** = supported by three molecular markers; **** = supported by four or more molecular markers) are given. Sub-divisions of Liliaceae are indicated on the right of the figure. For each genus, the number in parenthes corresponds to the number of species studied karyologically followed by the total number of species considered to comprise the genus. For genera in which the basic chromosome number is variable, chromosomes which are not found in all species are indicated in grey.

Genome size estimates inferred from the THL

According to Levin (2002, and literature cited therein), the correlation between THL and 1C values typically exceeds $r = 0.85$ within species and between species in related genera, and THL has thus been considered a good proxy for genome size. In the present study, THLs were measured for all species analysed. To transform THL into an inferred 1Cx (= monoploid genome size, which represents the DNA amount in the basic haploid chromosome complement and is calculated by dividing the 2C DNA amount by the ploidy level; Greilhuber *et al.*,

2005) estimate for each species, first a predictive regression model was built by plotting the mean THL for each genus (Table 3, based on data in Supplementary Data 1, available online) against the mean 1Cx genome size for each genus using data taken from the Plant DNA C-values database (Bennett and Leitch, 2005*b*), Leitch *et al.* (2007) and Zonneveld *et al.* (2005) (Table 3, Fig. 2A). Using these data, 11 common linear regression models were tested. All were significant at the 0·01 level. The highest r^2 value (0·92) was obtained with a Cubic curve equation, but this was rejected

TABLE 2. *Measures of karyotype asymmetry used in the present work*

Name of asymmetry index	Abbreviation (if applicable)	Reference
Stebbins asymmetry index	StebA–C	Stebbins (1971)
Stebbins asymmetry index	Steb1–4	Stebbins (1971)
Intrachromosomal asymmetry index	A_1	Romero Zarco (1986)
Interchromosomal asymmetry index	A_2	Romero Zarco (1986)
Percentage karyotype asymmetry index	AsK%	Arano (1963)
Total form %	TF%	Huziwara (1962)
Index of chromosome size resemblance	Rec	Greilhuber and Speta (1976)
Index of karyotype symmetry	Syi	Greilhuber and Speta (1976)
Karyotype dispersion index	DI	Lavania and Srivastava (1992)
Degree of karyotype asymmetry	A	Watanabe *et al.* (1999)
Coefficient of variation (CV) of the centromeric index (centromeric index is the ratio of the length of the short arm to that of the total chromosome length)	CV_{CI}	Paszko (2006)
Coefficient of variation (CV) of chromosome lengths	CV_{CL}	Paszko (2006)
Asymmetry index $(CV_{CI} \times CV_{CL}) \div 100$	AI	Paszko (2006)

TABLE 3. *Mean monoploid genome sizes (1Cx) using data taken from the literature (see Materials and Methods) and mean total haploid length (THL) used to build the predictive regression analysis shown in Fig. 2A*

Taxon	Mean 1Cx (pg)	No. of taxa analysed	Mean THL (µm)	No. of taxa analysed
Genera				
Amana	21·5	1	67·75	2
Calochortus	5·4	1	28·66	23
Cardiocrinum	38·6	1	130·62	2
Clintonia	9·5	1	71·83	5
Erythronium	31·8	4	97·67	2
Fritillaria	46·7	23	153·1	29
Gagea	6·6	2	38·52	40
Lilium	39·6	10	174·38	58
Medeola	14·2	1	90·89	1
Notholirion	35·3	1	109·4	1
Prosartes	3·4	1	30·36	4
Scoliopus	9·2	1	78·27	1
Streptopus	3·3	1	22·63	3
Tricyrtis	4·5	2	39·22	13
Tulipa	22·5	26	93·14	21
Tribe				
Lilieae	44·3	34	136·95	130
Streptopeae	5·3	3	28·92	8
Tulipeae	22·8	31	92·37	25
Subfamily				
Lilioideae	33·7	66	133·91	155
Medeoloideae	11·9	2	73·19	6
Family				
Liliaceae	32·1	70	114·2	205
Smilacaceae	5·0	1	45·11	12

because it altered the extreme values too much. Among the remaining models, a linear curve ($r^2 = 0.86$) was excluded as it gave negative 1Cx estimates for small THL values. From six other equally well fitting curves ($r^2 = 0.85–0.86$), the power regression model was selected as it gave the most reasonable outputs for both small and large THL values (Fig. 2A).

Correlations

For continuous quantitative data, Pearson's correlation coefficient was used, whereas for mixed scalar and ordinal data Spearman's rho coefficient was calculated. Correlations were considered as weak (up to 0·3), average (up to 0·5), good (up to 0·80) and high (>0·80). Only correlations significant at the 1 % level or higher are discussed.

Phylogenetic framework and inferred basic chromosome numbers

The phylogenetic framework for Liliaceae used in this study (Fig. 1) was derived mainly from the work of Patterson and Givnish (2002) and Fay *et al.* (2006), together with additional data from the phylogenetic analysis of Tan *et al.* (2005, restoration of *Amana*), Rønsted *et al.* (2005, sinking of *Nomocharis*), Peterson *et al.* (2004, 2008), Peruzzi *et al.* (2008*a*, *b*) and M. Zarrei *et al.* (unpubl. res.) concerning the sinking of *Lloydia* in *Gagea*. Smilacaceae is the sister family to Liliaceae (Patterson and Givnish, 2002; Fay *et al.*, 2006). All nodes for which bootstrap support and branch length did not fit the requirements for the 0·95 binomial confidence interval (Zander, 2004) were collapsed. The resulting phylogenetic tree is almost identical to the one used by Leitch *et al.* (2007) in their genome size study (except for the relationships between *Amana*, *Erythronium* and *Tulipa*).

The ancestral basic chromosome number for Liliaceae was inferred to be $x = 8$ based on the frequency of $2n = 32$ counts in *Smilax* (Smilacaceae) and cytological data suggesting the genus to be palaeopolyploid (Vijayavalli and Mathew, 1987). This is also considered to be the ancestral basic number for *Streptopus*, *Scoliopus* and *Prosartes* given the frequency of species in these genera based on $x = 8$. An ancestral basic chromosome number of $x = 9$ was adopted for the highly variable *Calochortus* (x ranging from 6 to 10) based on the parsimony analysis of Patterson and Givnish (2004), and $x = 13$ for the chromosomally stable genus *Tricyrtis* as suggested by Nakamura (1968). An ancestral basic number of the Medeoloideae + Lilioideae clade is more equivocal, although basic numbers of $x = 7$ for Medeloideae and $x = 12$ for Lilioideae are well supported by previous karyotype studies together with the suggestion that their karyotypes may be related by Robertsonian rearrangements (e.g. Tamura, 1998*b*).

RESULTS

Inferring genome size from THLs

In general there was a broad agreement between the mean inferred 1Cx value for each genus and values from genome size studies, although there were some discrepancies in *Lilium* and *Fritillaria*. Nevertheless this approach was considered robust enough to infer 1Cx values from THL
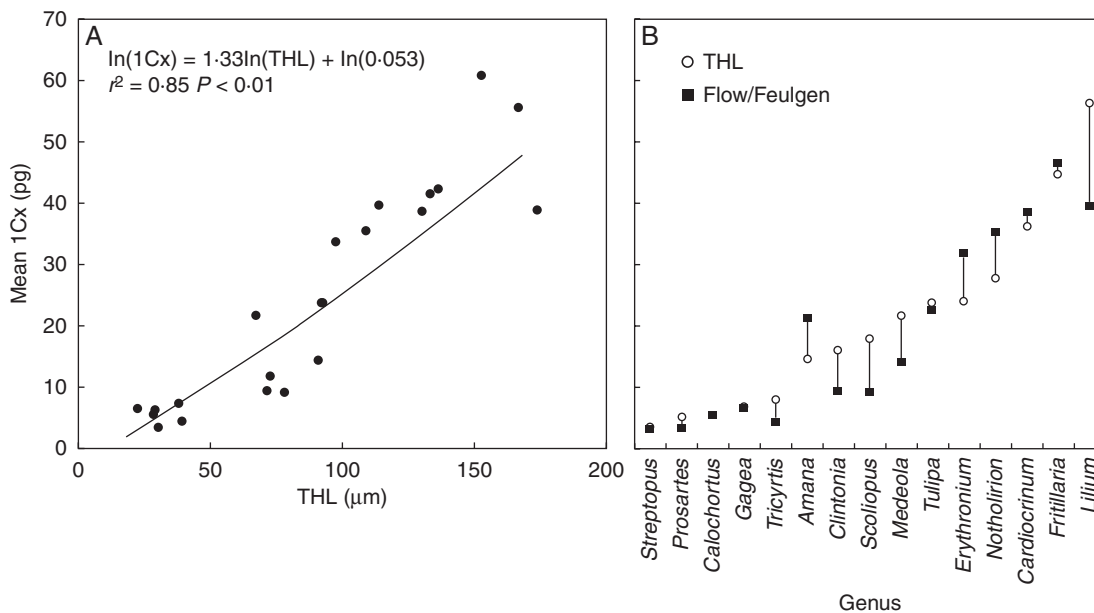
FIG. 2. (A) Scatter plot of the power regression model from chromosome total haploid length (THL; *x*-axis) to 1Cx (*y*-axis), with the related regression formula and significance parameters. Original data are taken from Table 3. (B) Comparison between mean 1Cx estimates inferred from the regression analysis of THL (circles) and 1Cx values estimated using either using flow cytometry or Feulgen microdenstometry (squares); see Materials and Methods. Data are arranged by increasing 1Cx values inferred from THLs.

measurements for all accessions studied, and these are given in Supplementary Data 1, available online.

Variabilities in inferred 1Cx values for each genus are illustrated by the boxplots shown in Fig. 3 which are superimposed onto the consensus phylogenetic framework of Liliaceae (from Fig. 1). This figure highlights the contrast between the large and wide range of genome sizes in subfamily Lilioideae, especially in tribe Lilieae, compared with the smaller and narrow range of genome sizes encountered in the remaining genera. The only exceptions to this were found in *Gagea* and *Scoliopus*. The mean genome size for *Gagea* (inferred mean 1Cx = 6·86 pg) was less than half the next smallest genome size in the tribe (for *Amana* with an inferred mean 1Cx of 14·69 pg). *Scoliopus* stood out because the genome size for this genus (inferred 1Cx = 17·80 pg) was more than three times larger than that for *Prosartes* (inferred mean 1Cx = 5·08 pg) and *Streptopus* (inferred mean 1Cx = 3·43 pg) in the same clade.

Constructing mean haploid idiograms for each genus

Mean haploid idiograms for all genera superimposed onto the consensus phylogenetic framework of Liliaceae are shown in Fig. 1 using data taken from Table 4. The different sizes of the karyotypes largely reflect the patterns already noted from the boxplots in Fig. 3, with the relatively small chromosomes of *Gagea* compared with other genera in subfamily Lilioideae and the larger chromosomes in *Scoliopus* compared with *Calochortus*, *Prosartes*, *Streptopus* and *Tricyrtis* standing out as exceptions.

Karyotype asymmetry

Results for the 13 different measures of karyotype asymmetry are given in Supplementary Data 1, available online.

Pearson's correlation coefficients between each of the different measures varied from weak to strong, negative to positive and insignificant to significant (Supplementary Data 2, available online).

Two of these measures, CV_{CL} and CV_{CI}, which represent the degree of heterogeneity within a chromosome complement in terms of chromosome length (interchromosomal variation) and centromere position (intrachromosomal variation), respectively, were analysed in greater detail. Boxplots showing the range of values obtained for these two parameters for each genus are given in Fig. 4, arranged phylogenetically. Figure 5 summarizes differences between genera in these two measures of karyotype asymmetry by plotting the CV_{CI} and CV_{CL} values for each species.

Karyotype asymmetry and genome size

In general, there was a good positive correlation between 1Cx and CV_{CI} across Liliaceae ($r = 0.65$, Fig. 6A; Supplementary Data 2, available online), indicating that increases in genome size were generally accompanied by increasing karyotype asymmetry through increasing variability in centromere position. This correlation was also found for various subgroups within Liliaceae including *Tricyrtis* ($r = 0.56$), Lilioideae ($r = 0.59$), Lilieae ($r = 0.23$) and *Lilium* ($r = 0.32$). However, the correlation was negative for *Fritillaria* (within Lilieae, $r = -0.33$) and for all Tulipeae ($r = -0.37$).

The relationship between 1Cx and CV_{CL} in Liliaceae was generally negative ($r = -0.49$, Fig. 6B). This correlation was also found for many sub-groups analysed alone, including *Tricyrtis* ($r = -0.36$), Lilioideae ($r = -0.46$) and Tulipeae ($r = -0.46$). The least negative Pearson's coefficient values were found in *Lilium* ($r = -0.22$) and Lilieae ($r = -0.17$).
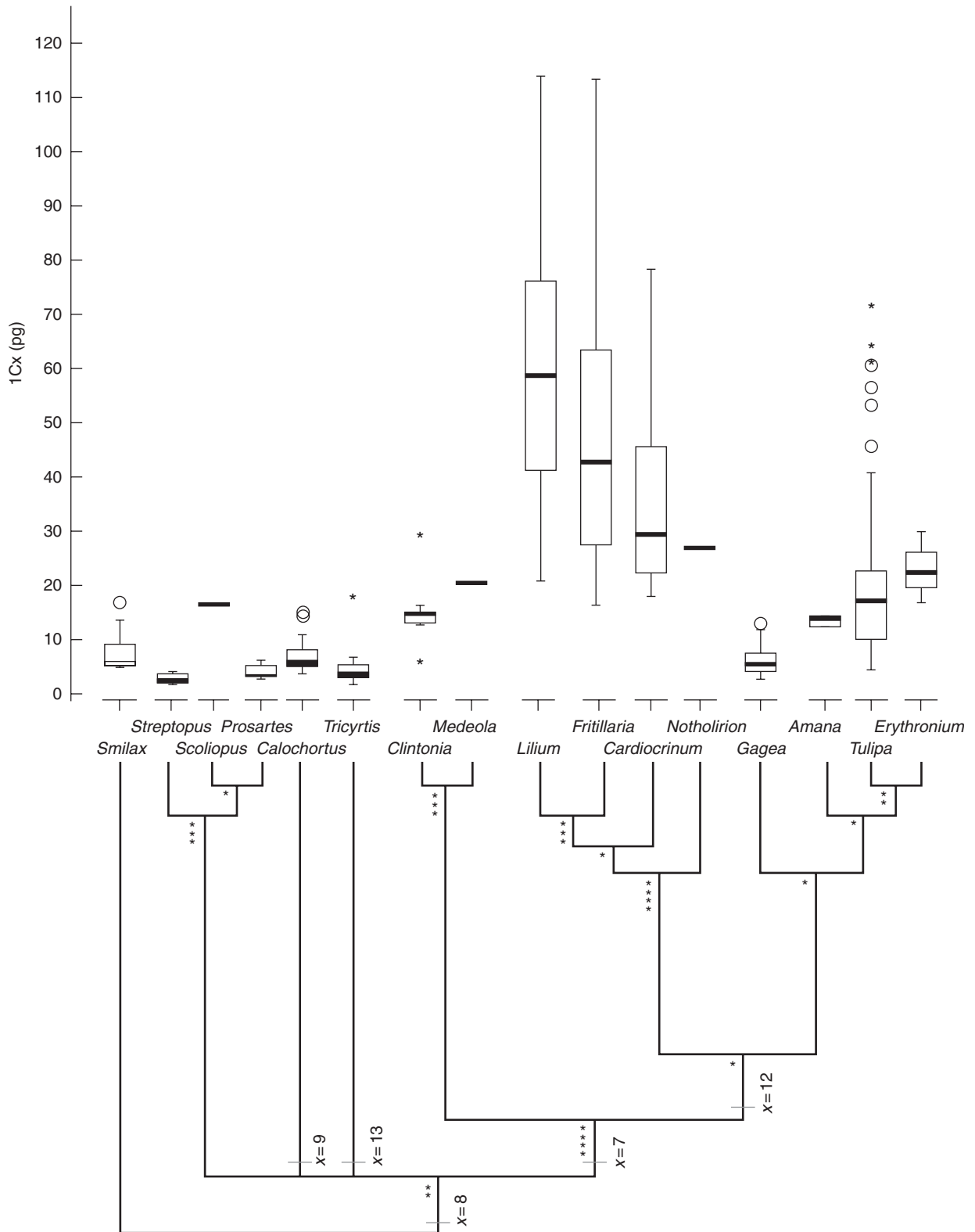
FIG. 3. Boxplots illustrating the variability of 1Cx genome size, as inferred from the regression model given in Fig. 2A. The outlined central box depicts the middle 50 % of the data extending from the upper to lower quartile; the horizontal bar is at the median. The ends of the vertical lines ('whiskers') indicate the minimum and maximum data values, unless outliers are present, in which case the whiskers extend to a maximum of 1·5 times the interquartile range. Circles indicate outliers, unless extreme outliers are present, in which case the circles extend to a maximum of three times the interquartile range and the extreme outliers are indicated as asterisks. Taxa are ordered by phylogenetic grouping (according to the phylogenetic tree on the bottom of the graph, taken from Fig. 1).

TABLE 4. *Mean chromosome features (± s.d.) for genera studied*

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
Smilax	4·31 ± 1·55	3·75 ± 1·21	3·18 ± 0·94	2·80 ± 0·91	2·43 ± 0·89	2·28 ± 0·65	2·13 ± 0·67	2·01 ± 0·70	1·76 ± 0·57	1·60 ± 0·49	1·57 ± 0·57	1·42 ± 0·51	1·33 ± 0·38	1·20 ± 0·39	1·14 ± 0·44	0·84 ± 0·36
(N = 19)	1·11 ± 1·42	0·80 ± 0·56	0·85 ± 0·34	0·89 ± 0·34	0·89 ± 0·31	0·81 ± 0·41	0·75 ± 0·34	0·64 ± 0·29	0·71 ± 0·35	0·69 ± 0·29	0·58 ± 0·22	0·61 ± 0·23	0·60 ± 0·27	0·54 ± 0·24	0·49 ± 0·18	0·42 ± 0·26
Streptopus	3·42 ± 0·68	2·69 ± 0·70	2·07 ± 0·48	1·84 ± 0·52	1·66 ± 0·36	1·39 ± 0·44	1·27 ± 0·40	0·83 ± 0·43								
(N = 9)	1·38 ± 0·80	1·00 ± 0·28	0·94 ± 0·31	0·95 ± 0·16	0·90 ± 0·26	0·87 ± 0·21	0·87 ± 0·31	0·56 ± 0·39								
Scoliopus	7·69	9·23	5·58	6·92	4·81	5·96	6·35	3·46								
(N = 1)	5·58	2·12	4·81	2·50	4·42	3·08	2·69	3·08								
Prosartes	3·32 ± 0·80	2·87 ± 0·52	2·59 ± 0·42	1·95 ± 0·31	2·50 ± 0·86	2·15 ± 0·81	1·61 ± 0·74	1·25 ± 0·34	0·75 ± 0·51							
(N = 5)	2·32 ± 0·50	2·02 ± 0·44	1·66 ± 0·42	1·58 ± 0·36	0·99 ± 0·28	0·79 ± 0·32	0·72 ± 0·39	0·91 ± 0·18	0·38 ± 0·11							
Calochortus	3·93 ± 1·68	3·33 ± 1·47	2·98 ± 1·17	2·65 ± 1·30	2·59 ± 1·22	2·64 ± 1·35	2·00 ± 1·13	1·38 ± 1·15	1·11 ± 1·22	0·77 ± 0·88						
(N = 25)	1·21 ± 0·92	1·34 ± 0·82	1·14 ± 0·90	1·04 ± 0·56	0·84 ± 0·52	0·59 ± 0·33	0·67 ± 0·38	0·40 ± 0·40	0·37 ± 0·33	0·33 ± 0·71						
Tricyrtis	4·52 ± 1·33	3·86 ± 1·16	2·51 ± 0·92	2·17 ± 0·79	2·06 ± 0·64	1·85 ± 0·60	1·78 ± 0·51	1·64 ± 0·44	1·62 ± 0·59	1·61 ± 0·60	1·51 ± 0·57	1·42 ± 0·51	1·08 ± 0·29			
(N = 34)	1·05 ± 0·32	0·97 ± 0·31	1·24 ± 0·43	1·30 ± 0·36	1·15 ± 0·34	1·18 ± 0·32	1·11 ± 0·38	1·12 ± 0·43	1·05 ± 0·29	0·96 ± 0·26	0·92 ± 0·23	0·82 ± 0·22	0·79 ± 0·32			
Clintonia	8·03 ± 1·58	7·66 ± 1·48	6·70 ± 1·46	6·08 ± 1·50	5·63 ± 0·81	5·59 ± 1·43	5·78 ± 1·82									
(N = 13)	7·50 ± 1·66	4·28 ± 1·38	3·68 ± 1·08	3·46 ± 0·85	3·07 ± 1·06	2·53 ± 0·84	1·90 ± 0·74									
Medeola	10·07	9·64	7·88	8·76	10·07	4·38	4·38									
(N = 1)	10·07	7·99	4·38	3·29	1·75	4·38	3·83									
Lilium	13·00 ± 3·49	12·87 ± 3·63	12·94 ± 2·82	12·70 ± 2·78	13·05 ± 3·43	12·67 ± 3·61	13·36 ± 4·47	12·87 ± 4·21	12·95 ± 4·58	10·98 ± 3·45	11·10 ± 3·69	11·20 ± 4·50				
(N = 124)	9·91 ± 2·83	7·92 ± 2·29	2·59 ± 1·34	2·20 ± 1·08	1·92 ± 0·85	1·74 ± 0·78	1·72 ± 0·67	1·53 ± 0·71	1·37 ± 0·72	1·24 ± 0·69	0·96 ± 0·54	0·79 ± 0·54				
Fritillaria	11·10 ± 3·57	10·68 ± 3·57	12·19 ± 3·88	11·90 ± 3·60	11·31 ± 3·54	10·85 ± 3·81	10·34 ± 3·61	10·05 ± 3·34	9·53 ± 3·02	8·38 ± 2·92	8·10 ± 2·88	7·32 ± 2·83				
(N = 61)	7·67 ± 3·18	6·29 ± 2·74	2·79 ± 2·94	2·27 ± 2·58	2·20 ± 2·38	1·85 ± 1·04	1·89 ± 0·99	1·70 ± 0·78	1·68 ± 0·99	1·44 ± 0·84	1·25 ± 0·73	1·30 ± 1·02				
Cardiocrinum	9·67 ± 3·85	9·69 ± 3·41	10·23 ± 3·45	10·07 ± 2·94	9·55 ± 3·23	8·80 ± 2·76	8·95 ± 4·12	8·67 ± 4·14	8·11 ± 4·28	7·53 ± 2·61	7·80 ± 3·36	6·48 ± 3·42				
(N = 8)	7·15 ± 3·02	6·00 ± 3·27	1·82 ± 1·41	1·27 ± 0·77	1·33 ± 0·64	1·69 ± 0·83	1·33 ± 0·50	1·19 ± 0·53	1·13 ± 0·43	0·81 ± 0·28	0·60 ± 0·24	0·81 ± 0·39				
Notholirion	8·00	7·10	8·50	8·00	7·10	8·00	7·10	6·50	6·50	6·00	5·50	6·10				
(N = 1)	6·00	4·20	1·10	1·40	2·00	1·20	1·30	1·90	1·20	1·60	2·00	1·10				
Gagea	4·49 ± 1·31	3·98 ± 1·28	3·39 ± 1·17	2·59 ± 0·94	2·15 ± 0·69	1·99 ± 0·69	1·78 ± 0·52	1·64 ± 0·56	1·53 ± 0·48	1·37 ± 0·40	1·35 ± 0·44	1·17 ± 0·36				
(N = 56)	1·06 ± 0·47	0·97 ± 0·41	0·95 ± 0·41	1·14 ± 0·38	1·11 ± 0·42	0·94 ± 0·31	0·90 ± 0·35	0·85 ± 0·26	0·81 ± 0·30	0·82 ± 0·32	0·67 ± 0·26	0·63 ± 0·22				
Amana (N = 2)	6·89 ± 0·08	5·77 ± 1·12	5·91 ± 0·49	5·04 ± 0·53	4·40 ± 0·41	4·26 ± 1·24	4·22 ± 0·44	3·61 ± 0·86	3·33 ± 0·60	2·42 ± 0·65	1·94 ± 0·00					
	1·89 ± 0·64	2·14 ± 1·07	1·42 ± 0·67	1·25 ± 0·01	1·70 ± 0·01	1·53 ± 0·40	1·07 ± 0·04	1·44 ± 0·18	1·48 ± 0·67	0·90 ± 0·17	1·25 ± 0·12	0·78 ± 0·04				
Tulipa (N = 43)	8·71 ± 4·94	8·20 ± 4·67	7·35 ± 4·37	7·04 ± 3·92	6·66 ± 3·82	6·04 ± 3·51	5·69 ± 3·29	5·39 ± 3·07	5·16 ± 3·02	4·78 ± 2·74	4·34 ± 2·40	3·81 ± 2·29				
	2·15 ± 1·16	1·80 ± 1·08	1·92 ± 1·05	1·79 ± 1·09	1·70 ± 0·83	1·70 ± 0·94	1·54 ± 0·89	1·63 ± 0·98	1·49 ± 0·83	1·47 ± 0·79	1·45 ± 0·79	1·35 ± 0·72				
Erythronium	10·71 ± 2·17	8·00 ± 3·60	8·00 ± 2·13	7·44 ± 2·03	7·11 ± 1·34	6·61 ± 1·57	5·96 ± 1·46	5·23 ± 0·27	4·59 ± 0·53	4·45 ± 0·70	3·79 ± 1·11	3·54 ± 1·02				
(N = 3)	1·60 ± 0·41	2·91 ± 0·67	2·07 ± 0·52	2·02 ± 0·83	1·69 ± 0·71	1·66 ± 0·39	1·93 ± 0·23	1·94 ± 0·52	1·65 ± 0·43	1·50 ± 0·53	1·65 ± 0·41	1·63 ± 0·39				
Total: 405																

Chromosome pairs are named with roman numerals, according to their decreasing length. For each chromosome pair the absolute lengths in micrometres of the long (top) and short (bottom) arm are given. Genera are arranged in phylogenetic order according to Fig. 1. The number of accessions studied is given following the genus name.
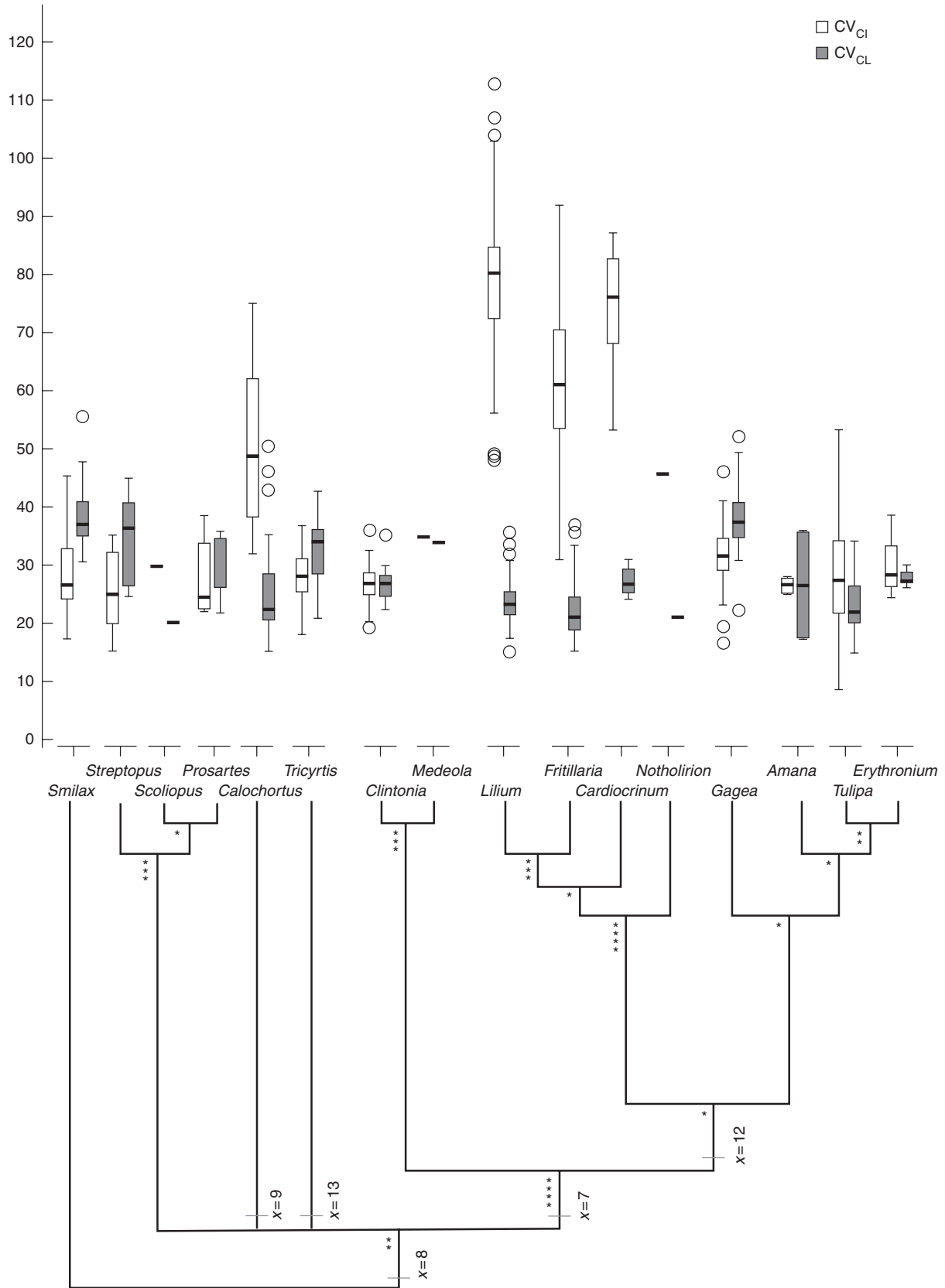
FIG. 4. Clustered boxplots illustrating the variability of both the coefficient of variation (CV) of the centromeric index (CV$_{CI}$) and chromosome length (CV$_{CL}$; see Fig. 3 for more explanations concerning the boxplots.) Taxa are ordered by phylogenetic grouping (according to the phylogenetic tree on the bottom of the graph, taken from Fig. 1).
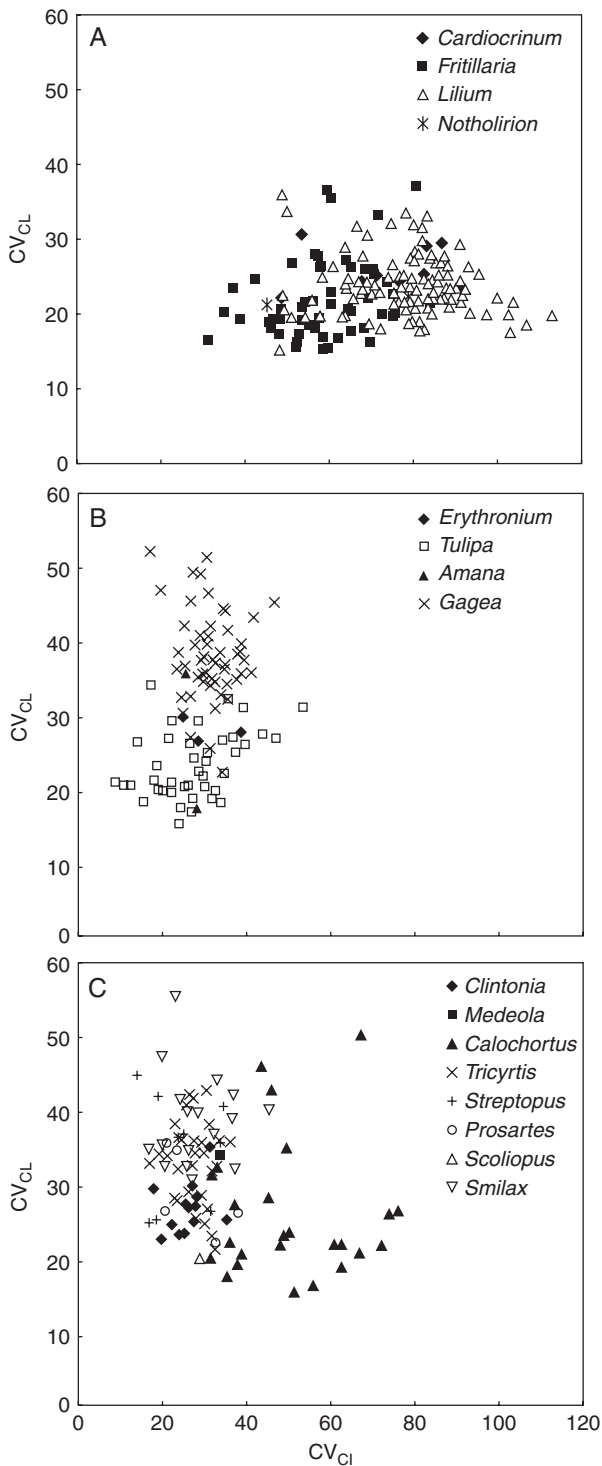
Fig. 5. Analysis of karyotype asymmetry in Liliaceae and Smilacaceae. Coefficients of variation (CV) of the centromeric index (CV_{CI}) and chromosome length (CV_{CL}) were plotted for each species studied. Increasing karyotype asymmetry is reflected in increasing values of CV_{CI} and/or CV_{CL}; (A) genera in Lilieae, (B) genera in Tulipeae, (C) the remaining genera in Liliaceae and *Smilax* in Smilacaceae.
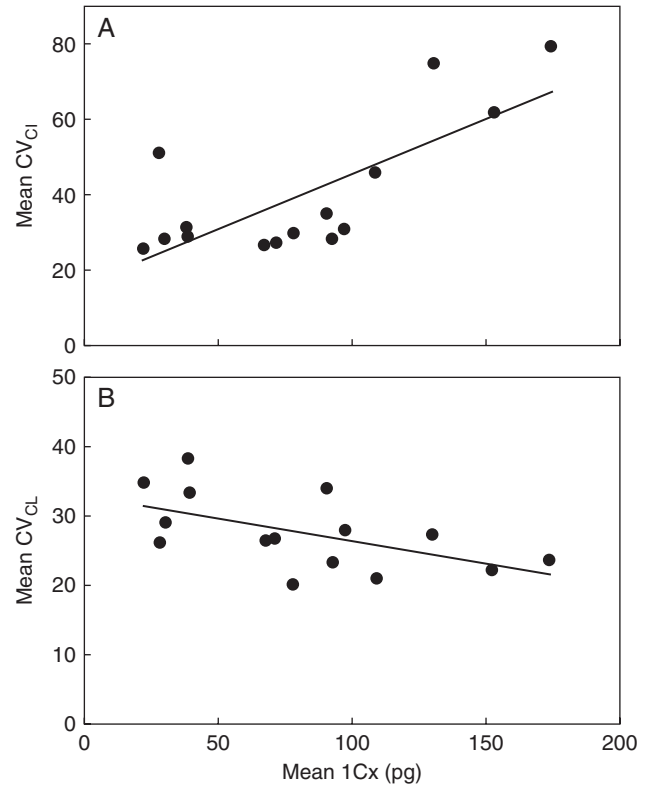
Fig. 6. Relationship between genome size (1Cx) and karyotype asymmetry. Mean 1Cx values inferred for each genus (taken from Table 3) were plotted against the mean values for the coefficient of variation (CV) of (A) the centromeric index (CV_{CI}) and (B) the chromosome length (CV_{CL}) for each genus.

value against the inferred 1Cx for the accession (data not shown). [The R value is a measure of chromosomal asymmetry calculated by dividing the length of the long arm by the length of the short arm for each chromosome; see Levan *et al.* (1964). A metacentric chromosome has an R value of 1 whereas higher R values are associated with increasingly acrocentric chromosomes.] This approach revealed that the larger, often metacentric (i.e. chromosome pairs I and II) and smaller sub-metacentric to telocentric chromosomes (chromosome pairs IV and higher) undergo different patterns of karyotype evolution. While there was a strong positive correlation with smaller chromosomes from IV to XII ($r = 0.92–0.98$), indicating that the additional DNA is associated with increasing intrachromosomal asymmetry, chromosome pairs I–III had a low negative correlation with genome size (r values ranging from -0.14 to -0.18), implying that as genome size increases these chromosomes become more symmetric. This pattern was observed for all genera except *Fritillaria* and genera in Tulipeae.

When Smilacaceae were analysed alone, there were no relevant differences in the relationships between 1Cx and R values for the larger and shorter chromosomes. In all cases, the correlations were positive and high ($r = 0.70–0.98$) or not significant (depending on the chromosome).

Polyploidy

Overall the percentage of polyploids in Liliaceae was low (16 % for the whole data set). Many genera were exclusively

Trends in karyotype asymmetry and genome size were also analysed for individual chromosomes. This was done by plotting, for each chromosome in the karyotype, its R (arm ratio)

diploid (*Cardiocrinum*, *Erythronium*, *Medeola*, *Notholirion*, *Prosartes*, *Scoliopus* and *Tricyrtis*) and only three genera had 50 % or more polyploids (*Amana*, *Clintonia* and *Gagea*) (Fig. 7). Plotting the relationship between the occurrence of polyploids and mean inferred 1Cx genome size for each genus revealed that most polyploids (94 %) occurred in species with 1Cx genome sizes <25 pg (Fig. 7); above this DNA amount, the number of polyploid species was small (Fig. 8). In all genera, the species with the highest inferred 1Cx values were always diploid (Fig. 8).

DISCUSSION

The reliability of inferring genome size from THLs

Whereas previous studies have highlighted the potential for using chromosome data as a proxy for genome size (e.g. Narayan and Rees, 1976; Raina and Rees, 1983; Ceccarelli *et al.*, 1995), it has also been shown that chromosomes of a species can vary considerably in size without any change in DNA amount depending on the method of chromosome preparation as well as the phosphate concentration of the growth medium or type of root analysed (Bennett and Rees, 1967, 1969; Bennett, 1970). Given this, it was necessary to check if the karyotype data available for Liliaceae could be used to infer genome sizes. Using the approaches outlined, a new predictive regression model for inferring genome size from the THL of chromosome complements was produced specifically for Liliaceae and the closely allied Smilacaceae. In general there was broad agreement between 'real' genome size values obtained using 'best practice' genome size estimation techniques (flow cytometry and Feulgen microdensitometry) and those inferred from THL measurements (Fig. 2B). The largest differences occurred in *Lilium*. One explanation is that *Lilium* is a large genus (approx. 100 species) and, while there are karyological data for 58 species, genome size data are only available for ten species. It is possible that the

smaller mean 1Cx values from genome size data are due to the small number of species analysed. From the data given in Supplementary Data 1 (available online), *Lilium* species with the largest THLs have not had their genome sizes measured using best practice techniques. Whilst it is recognized that accurate genome size estimates will never be obtained from THLs, given the wealth of karyotype information available compared with the amount of genome size information, it was felt that the current approach was appropriate for providing some insights into patterns of genome evolution across Liliaceae.

Patterns of genome size evolution across Liliaceae

Superimposing inferred genome size data onto a phylogenetic tree of Liliaceae revealed similar patterns to those predicted from chromosome data by Tamura (1995, 1998*a*, *b*) and observed by Leitch *et al.* (2007), most notably the striking differences between the genome size profiles of (*a*) genera in subfamily Lilioideae and Medeoloideae and (*b*) the remaining genera (*Calochortus*, *Tricyrtis*, *Prosartes*, *Scoliopus* and *Streptopus*) (Figs 1 and 3). However, the considerably larger data set available in the present work (217 species compared with 78 species in Leitch *et al.*, 2007) highlighted two exceptions to this general pattern: (1) the relatively large genomes in *Scoliopus* (inferred 1Cx = 17·2 pg) compared with *Prosartes* (mean inferred 1Cx = 5·1 pg), *Streptopus* (inferred mean 1Cx = 3·4 pg), *Calochortus* (inferred mean 1Cx = 5·4 pg) and *Tricyrtis* (inferred mean 1Cx = 7·9 pg); and (2) the small *Gagea* genomes (inferred mean 1Cx = 6·9 pg) compared with the remaining genera in subfamily Lilioideae (inferred mean 1Cx range 14·7–56·3 pg).

Overall, although statistical analysis of genome size evolution across the family has revealed it to be punctuated with a significantly large shift and subsequent radiation in genome size at the base of Lilioideae (Leitch *et al.*, 2007), more localized genome size changes in the branches leading to *Gagea* and *Scoliopus* have also taken place. For *Scoliopus* the larger genomes most probably represent the result of localized genome size increases, whereas for *Gagea* genome downsizing would appear to be the more parsimonious explanation. However, the possibility that its small genome size represents the ancestral state with all other genera in the clade having experienced an increase cannot be ruled out, particularly in light of recent data suggesting that the predominant direction of genome size evolution in angiosperms is upwards (Hawkins *et al.*, 2009). The nature of the evolutionary forces driving these changes remains unknown, although analysis suggests that genome size evolution in Liliaceae is passive rather than adaptive (Leitch *et al.*, 2007).

In general, there is a positive correlation between genome size and the percentage of repetitive DNA for both tandem and dispersed repeats (Levin, 2002; Gregory, 2005). As for the effects that repetitive non-coding DNA has on the organism, there are broadly two different viewpoints. One of them interprets non-coding DNA as 'junk' DNA, whereas the other interprets repetitive DNA as adaptive in various aspects of plant structure and function, i.e. a larger genome is correlated with larger cell volumes and longer cell cycles (reviewed in Gregory, 2001; Bennett and Leitch, 2005*a*;
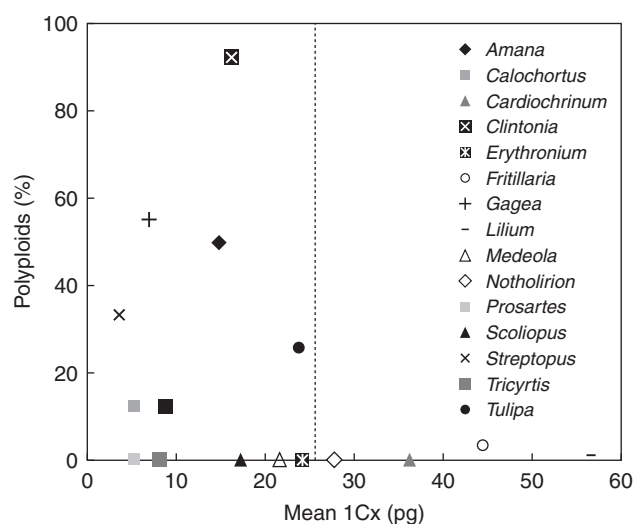
FIG. 7. The relationship between the inferred mean 1Cx genome size and percentage of polyploids (based on data in Supplementary Data 1, available online) for the 15 genera of Liliaceae. The dotted line corresponds to a mean 1Cx value of 25 pg.

Leitch and Bennett, 2007). The latter view seems to be the best supported, at least for Liliaceae, since genome size is related to morphological, ecological and/or adaptive changes (see Patterson and Givnish, 2002, for fuller discussion), although, as noted above, the analysis by Leitch *et al.* (2007) suggested that large genomes evolved passively.

Karyotype structure in Liliaceae

Figure 1, which gives a mean haploid karyotype for each genus, and data in Supplementary Data 1 (online) highlight the considerable diversity in karyotype structure in Liliaceae, already noted in previous studies (e.g. Tamura, 1995, 1998*a*, *b*). There is considerable variation in chromosome number ($x = 6–13$), ploidy ($2x–6x$; and up to $11x$ in *Gagea*; Peruzzi, 2008, and references cited therein), chromosome size ($1·00–37·73$ μm) and karyotype asymmetry. By superimposing these data onto the phylogenetic framework, patterns and trends in karyotype evolution can be seen.

Karyotype asymmetry. Significant differences in karyotype asymmetry are apparent within Liliaceae based on the various measures of karyotype asymmetry analysed. Most of the correlations found among different karyotype asymmetry measures (Supplementary Data 2, available online) agreed with data reported by Paszko (2006) for *Calamagrostis* (Poaceae), and overall the present study supports her conclusions concerning the general validity of the different measures of karyotype asymmetry tested. However, from the present data, the asymmetry index (AI) which Paszko proposed as a new measure of asymmetry, aiming to capture the degree of asymmetry in a single value, is strongly and positively correlated with CV_{CI} ($r = 0·85$, a measure of asymmetry based on variation in centromere position between chromosomes in the karyotype) but not significantly correlated with CV_{CL} ($r = 0·08$, a measure of asymmetry based on variation in chromosome lengths between chromosomes in the karyotype). For this reason, it was felt that the AI value proposed by Paszko does not adequately reflect all aspects of karyotype asymmetry in Liliaceae. Instead it seems more meaningful to retain these two separate parameters which reflect two different aspects of karyotype asymmetry, variation in chromosome length (CV_{CL}) and centromere position (CV_{CI}).

Boxplots summarizing the values of these two parameters for each genus (Fig. 4) highlight the wide range and large values of CV_{CI} in *Calochortus* and tribe Lilieae compared with other genera. This indicates that their karyotypes are considerably more asymmetric in terms of variation in the position of the centromeres than those of other genera of Liliaceae. In contrast, there was less variation in the range of values obtained for CV_{CL} between and within different genera.

Plotting CV_{CL} and CV_{CI} values for each genus highlights further differences in karyotype asymmetry between different genera (Fig. 5). For example, the asymmetric karyotypes that characterize species in Lilieae are due more to variation in the position of the centromere (large CV_{CI} values) than to differences between the chromosome sizes in the karyotype (i.e. lower values of CV_{CL}; Fig. 5A). Karyotypes in Tulipeae are generally less asymmetric than in Lilieae as most have a narrower range of values for both CV_{CL} and CV_{CI} (Figs 4

and 5B), as also found in Medeoloideae (Figs 4 and 5C). An exception to this pattern is the relatively high CV_{CL} values in *Gagea* within Tulipeae. For the remaining genera in Liliaceae, increasing karyotype asymmetry arises through increasing variation in chromosome lengths (wide range of CV_{CL} values) rather than shifts in centromere position (narrow range of CV_{CI}; Fig. 5C), a pattern also observed in *Smilax* in Smilacaceae. There were no species with large CV_{CL} and CV_{CI} values, indicating that there are no karyotypes that are asymmetric in terms of both large differences in chromosome length and centromere position in Liliaceae. The only comparable study using CV_{CL} and CV_{CI} to describe karyotype asymmetry was that of Paszko (2006). However, this study was restricted to just eight species of *Calamagrostis* (Poaceae) and the data were not viewed within a phylogenetic framework. To what extent the patterns observed here are specific to Liliaceae or represent more general patterns of karyotype asymmetry evolution is currently unknown.

Karyotype asymmetry and genome size. Overall, an analysis of the relationship between 1Cx and CV_{CI} showed these two parameters were positively correlated (Fig. 6A), indicating that genome size increases are generally accompanied by increasing intrachromosomal asymmetry through increased variability in centromere position within the karyotype ($r = 0·65$). Such a pattern suggests that the additional DNA is not being added uniformly across the karyotype. This correlation was also found for various subgroups within Liliaceae including *Tricyrtis* ($r = 0·56$), Lilioideae ($r = 0·59$), Lilieae ($r = 0·23$) and *Lilium* ($r = 0·32$). However, for *Fritillaria* (in Lilieae) and the whole of Tulipeae, the correlation was negative ($r = -0·33$ for *Fritillaria* and $-0·37$ for Tulipeae), suggesting that the distribution of the additional DNA followed a different pattern from that noted for the rest of the family.

The relationship between 1Cx and CV_{CL} was generally negative (Fig. 6B), indicating that an increase in genome size is accompanied by decreasing size differences between chromosomes of the karyotype (i.e. a more symmetrical karyotype). This correlation was also found for some sub-groups analysed alone: *Tricyrtis*, Lilioideae and Tulipeae.

The analysis of changes in individual chromosome asymmetry with genome size provided some insights into how the additional DNA is distributed across the karyotype. Whereas the large metacentric chromosomes (e.g. chromosome pairs I and II) became more symmetrical with increasing genome size, the smaller sub-metacentric and telocentric chromosomes (i.e. chromosome pairs IV and above) became more asymmetrical. Such a pattern implies that the additional DNA is added mainly to the long arms of the smaller chromosomes rather than being distributed evenly between all chromosome arms of the karyotype complement. As far as we are aware, this type of chromosome evolution pattern has not been noted before.

To date, two main patterns for the addition of DNA in a chromosome complement have been described (reviewed by Levin, 2002). For 'proportional increase', the amount of DNA added to each chromosome arm is proportional to its length. This pattern does not result in a change in karyotype asymmetry with increasing DNA amount and thus the values
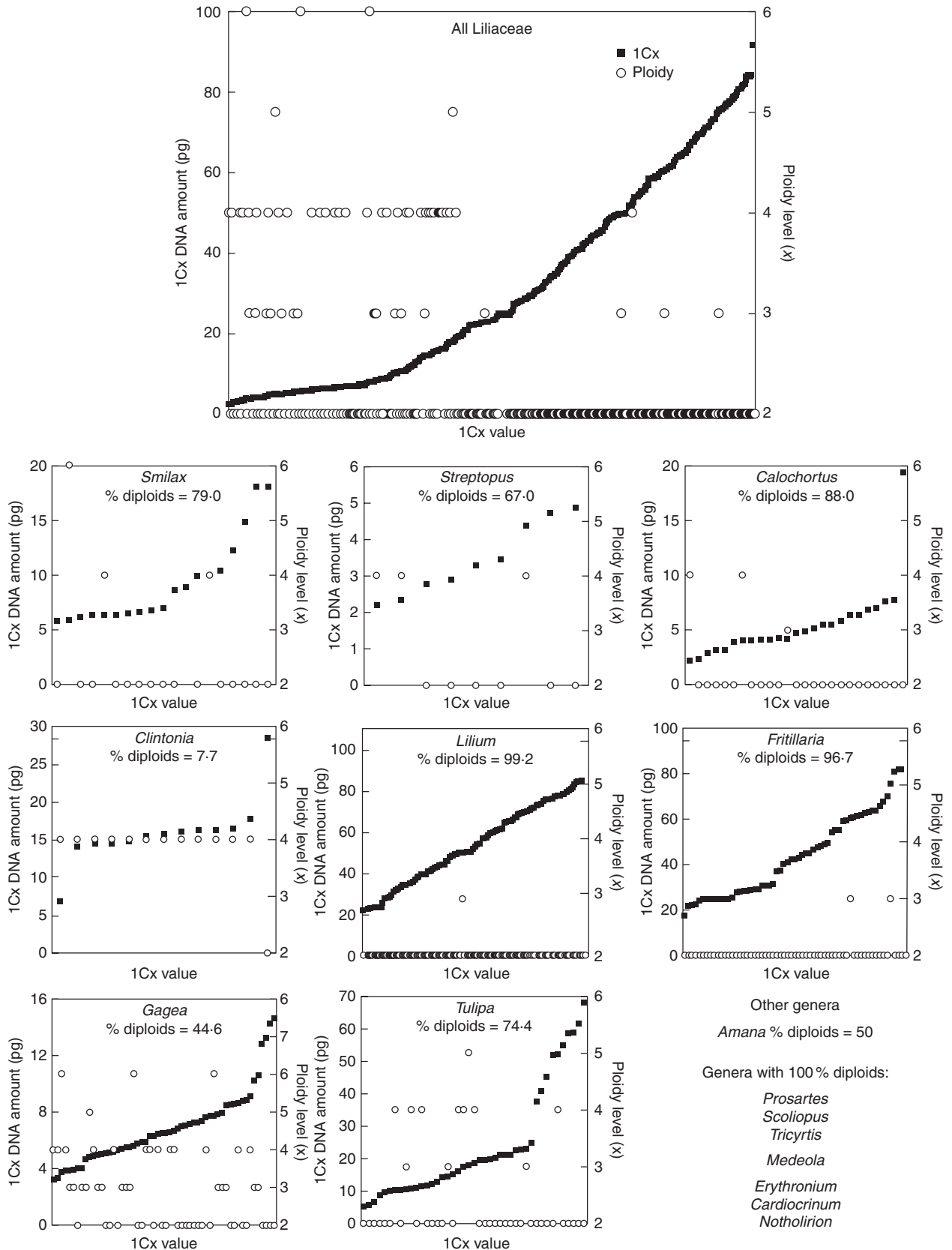
FIG. 8. Scatter plots of the 386 accessions of Liliaceae and of eight selected genera (including *Smilax* from Smilacaceae), arranged by increasing 1Cx values (*x*-axis). The left-hand *y*-axis represents inferred 1Cx values (squares), and the right-hand *y*-axis represents ploidy levels (circles). The percentage of diploids is reported for all genera.

for CV_{CI} and CV_{CL} remain unchanged as the genome size changes. This pattern has been observed in several genera including *Aloe* and *Gasteria* (Xanthorrhoeaceae) (Brandham and Doherty, 1998) and in some species of *Oxalis* (Oxalidaceae) (De Azkue and Martinez, 1988). For 'equal increase', the same amount of DNA is added to each chromosome arm regardless of its size. This will result in an increase in karyotype symmetry, and hence a decrease in CV_{CI} and CV_{CL} with increasing genome size. Examples of genera showing this pattern include *Vigna* (Fabaceae) (Parida *et al.*, 1990), *Vicia* (Fabaceae) (Raina and Rees, 1983), *Papaver* (Papaveraceae) (Srivastava and Lavania, 1991) and *Hypochaeris* (Asteraceae) (Cerbah *et al.*, 1998*a*, *b*).

In many genera in Liliaceae, however, a third pattern is apparent. Here there is an 'unequal increase', i.e. the amount of DNA added varies between longer and shorter chromosome arms unequally, leading to an overall increase in karyotype asymmetry with genome size. This pattern gives rise to the observed increase in CV_{CI} but a slight decrease in CV_{CL} with increasing genome size.

Such a pattern may, in part, be influenced by an upper limit on chromosome size for a particular karyotype, as reported for both monocots and eudicots (Schubert and Oud, 1997; Hudakova *et al.*, 2002). Nevertheless, other factors such as the involvement of Robertsonian translocations will also play a role as exceptions to the unequal increase pattern are found in *Fritillaria* (with some of the largest chromosomes of Liliaceae) and Tulipeae. In these groups, the increase in genome size is accompanied by a general decrease in asymmetry (decreasing CV_{CI}) and is typical of the equal increase pattern noted above.

Of course there are other types of chromosome change which can contribute to asymmetry variation between species and genera (reviewed by Schubert, 2007) including pericentric inversions and/or differential translocations of DNA between smaller and larger chromosomes (if there is no change in chromosome number) and Robertsonian fissions and fusions (if accompanied by changes in chromosome number); such karyotype changes have also been reported in some species of Liliaceae. Indeed the low, but significant, negative correlation between CV_{CI} and CV_{CL} probably in part reflects the occurrence of Robertsonian translocations which are found in several genera including *Cardiocrinum*, *Fritillaria*, *Streptopus* and *Tricyrtis* (Darlington, 1973; Zhongyan *et al.*, 1989; Tamura, 1995; Zhang and Gu, 2005).

Ploidy, genome size and karyotype asymmetry

The average negative correlation between the percentage of polyploid species and genome size found in almost all investigated taxa (Fig. 8) agrees with many other published studies for other groups of angiosperms (e.g. D'Ovidio and Marchi, 1990; Levin, 2002) and across angiosperms as a whole (Leitch and Bennett, 2004). The occurrence of polyploidy was also found to be related to genome size (Fig. 7). Thus genera with mean $1Cx$ values up to 25 pg showed a wide range of polyploidy from 0 to 80 % depending on the genus, whereas the occurrence of polyploidy in genera with mean $1Cx$ values above 25 pg was low (0·8 % in *Lilium* and 3 % in *Fritillaria*) or non-existent (in *Notholirion* and

Cardiocrinum). These results agree broadly with those previously reported by Grif (2000) based on an analysis at family level, namely that the percentage of polyploids in a family decreased with increasing mean genome size for the family. It is noted that out of the 11 monocot families analysed by Grif, Liliaceae had the largest mean genome size ($1C$ = approx. 20 pg) and the lowest percentage of polyploids (20 %).

Trends and patterns of karyotype evolution in Liliaceae

According to the present study (and also Leitch *et al.*, 2007, concerning genome size evolution), ancestral karyotypes in Liliaceae are likely to have had small genomes ($1Cx$ = approx. 6 pg), low CV_{CI} values, relatively high CV_{CL} values (similar to *Smilax*) and $x = 8$. From this evolutionary starting point, different trends in karyotype evolution at the generic level are apparent.

Streptopus, Scoliopus and Prosartes*.* In the small clade comprising *Streptopus*, *Scoliopus* and *Prosartes* (15 species in total), although there was some variation in chromosome number (in *Prosartes*; $2n = 12$, 16 and 18) and ploidy (*Streptopus*; up to $6x$ based on $x = 8$), the karyotypes are largely similar to those in the outgroup Smilacaceae in terms of structure (i.e. range of CV_{CL} and CV_{CI}) and genome size, with the notable exception of the >2-fold increase in genome size in *Scoliopus*. Given that only one species of *Scoliopus* (out of two recognized) was analysed, the mode of DNA addition is difficult to determine.

Tricyrtis. In *Tricyrtis* the karyotype has maintained similar karyotypic features to those in Smilacaceae, *Streptopus* and *Prosartes* (small and narrow range of genome sizes and a karyotype that is not strongly asymmetric). The main difference in *Tricyrtis* was the increase in the basic chromosome number from $x = 8$ to $x = 13$ which may have arisen via an ancient palaeopolyploid event followed by loss of DNA. The predominant chromosome number for *Tricyrtis* is $2n = 26$ although counts of $2n = 24$ have been reported in *T. hirta*, possibly linked to Robertsonian translocations (Takahashi, 1991).

Calochortus. Karyotypically, *Calochortus* (comprising approx. 65 species) is more variable than the genera mentioned above, with considerable variation in basic chromosome number ($x = 6$, 7, 8, 9 and 10), genome size (inferred $1Cx$ values range 8·5-fold) and asymmetry (especially CV_{CI}, Fig. 5C) compared with the presumed ancestral state in, for example, *Streptopus* and *Smilax*. The mechanisms involved in generating this karyotype diversity are currently unknown, although a role for translocations, fusions and fissions has been proposed (Beal, 1939). (For a more detailed discussion of chromosome diversity and evolution in the genus see Patterson and Givnish, 2004.)

Lilioideae + Medeoloideae.. The Lilioideae + Medeoloideae clade is the most diverse of Liliaceae. This clade has experienced large increases in genome size (2- to 25-fold), and the two types of karyotype asymmetry (i.e. intra- and interchromosomal, reflected in the values for CV_{CI} and CV_{CL}, respectively) have contrasting influences on karyotype evolution, depending on the taxonomic group concerned.

Subfamily Medeoloideae. The most plesiomorphic karyotype in the Lilioideae + Medeoloideae clade is probably found in Medeoloideae which, apart from an increase in genome size ($2-$ to 3-fold) and a reduction in the basic chromosome number from $x = 8$ to $x = 7$, has maintained low karyotype asymmetry features with a karyotype consisting of seven large, more or less metacentric chromosome pairs. Of the five species of *Clintonia*, only one is diploid (*C. udenesis*, $2n = 2x = 14$). The remaining four are tetraploid, making this the genus with the highest percentage of polyploids in Liliaceae. *Medeola* is monotypic (*M. virginiana* is the sole species) and has $2n = 14$ chromosomes.

Subfamily Lilioideae. In this subfamily the basic chromosome number increased to $x = 12$. It has been suggested that this arose through multiple reciprocal centric fissions (Tamura, 1995) probably involving the five smaller chromosome pairs of a Medeoleae-like ancestor or, less probably, through polyploidy. The majority of species in this subfamily have retained a base number of $x = 12$, with just a few exceptions in some *Fritillaria* (e.g. *F. montana* and *F. ruthenica* with $2n = 18$) and *Gagea* species. However, whereas polyploidy is fairly common in Tulipeae (40·7 %, according to the present data set), it is rare in Lilieae (1·54 %).

Tribe Lilieae. In this tribe the karyotype evolved towards a further increase of intrachromosomal asymmetry (up to 3-fold increase in CV_{CI}, Fig. 5A) and genome size (up to 7-fold in some *Fritillaria* and *Lilium* species), mostly through unequal addition of DNA to the long arms of the ten smaller telocentric pairs (III–XII; with *Fritillaria* being an exception). As a consequence of this unequal addition of DNA, this mode of karyotype evolution was accompanied by a small reduction in interchromosomal asymmetry ($0·5-$ to 0·7-fold, Fig. 5A compared with 5C).

Tribe Tulipeae. In contrast to the sister tribe Lilieae, the main mechanism of chromosome evolution in Tulipeae was shown to be an equal change in the amount of DNA for each chromosome arm, regardless of its length, although there was some evidence of preferential addition or subtraction of DNA from the smaller chromosomes. Consequently, the two largest chromosome pairs are telocentric/sub-telocentric rather than typically metacentric (as in Lilieae), and these changes are accompanied by variations in interchromosomal asymmetry (i.e. CV_{CL}, Fig. 5B). Within this framework, two distinct pathways of karyotype evolution are apparent: (1) a massive reduction in genome size (up to 0·3- to 0·5-fold) in *Gagea* accompanied by an increase in interchromosomal asymmetry (CV_{CL} up to 2- to 3-fold), leaving the intrachromosomal asymmetry (CV_{CI}) almost unchanged; and (2) an increase in genome size in *Amana*, *Erythronium* and *Tulipa* through the addition of equal amounts of DNA to each chromosome regardless of length, leading to a reduction in both their inter- and intrachromosomal asymmetries (i.e. CV_{CL} and CV_{CI}) with respect to *Gagea* (Fig. 5B).

CONCLUSIONS

In summary, through this combined approach of collating all available information (karyomorphometric data, genome size, phylogeny) for species of Liliaceae it has been possible to reconstruct the most likely patterns of chromosome evolution in this family. Such an approach has highlighted the diverse modes of karyotype evolution to be found even within this comparatively small angiosperm family.

SUPPLEMENTARY DATA

Supplementary Data are available at *Annals of Botany* online and consist of (1) karyotype features in the studied literature accessions of Liliaceae and Smilacaceae; and (2) Pearson's correlation coefficients among all published modalities of karyotype asymmetry measurements and genome size.

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