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A SYNOPSIS OF CYTOLOGICAL STUDIES IN *GESNERIACEAE*

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Our knowledge of cytological data published on members of the family *Gesneriaceae* is summarized and critically evaluated in the light of current taxonomic treatments and phylogenetic hypotheses. There are about 1000 published chromosome counts, covering 56% of the genera but only 18% of the species. In particular the New World tribes *Beslerieae* and *Napeantheae* and the Old World tribe *Didymocarpeae* are underexplored at generic level. In *Gesneriaceae* chromosome data are a valuable source of taxonomic characters. From our current knowledge of the phylogenetic relationships in the family we know that basic chromosome numbers in the New World subfamily *Gesnerioideae* appear to be rather conserved, but that a more complex pattern of genome evolution seems to be present among the Old World tribes. Both polyploidy and dysploid changes have played a significant role in the evolution of the family. However, the number of species for which both cytological and molecular data are available is at present too low to reach firm conclusions on ancestral basic chromosome numbers, particularly for the Old World group. To facilitate wider access to cytological data on the *Gesneriaceae*, a website has been developed (http://www.rbge.org.uk/rbge/web/search/index.jsp), which is introduced in this paper.

Keywords. Basic chromosome numbers, chromosome evolution, dysploidy, 'Gesneriaceae WebCyte', phylogeny, polyploidy, taxonomy.

INTRODUCTION

Members of the *Gesneriaceae* are predominantly tropical and subtropical herbs, currently grouped into three subfamilies (Wiehler, 1983; Burtt & Wiehler, 1995): *Cyrtandroideae*, all Old World (OW) except *Rhynchoglossum azureum* (Schltdl.) B.L. Burtt; the New World (NW) *Gesnerioideae*; and the southern hemisphere *Coronantheroideae* (sometimes treated as tribe *Coronanthereae* in subfamily *Gesnerioideae*). Recent molecular analyses do not indicate subfamily status for the *Coronantheroideae* as its members are placed closer to the NW tribes *Beslerieae* and *Napeantheae* and not as sister to the entire subfamily *Gesnerioideae* (Mayer *et al*., 2003; Wang *et al*., 2004). The monotypic genus *Titanotrichum* Soler. of the monogeneric tribe *Titanotricheae* is also more closely associated with NW tribes than previously thought and may eventually have to be transferred to subfamily *Gesnerioideae* (Wang *et al*., 2004). However, the purpose of the present paper is not to introduce a new *Gesneriaceae* systematic, but to give an overview of current

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cytological knowledge and to discuss chromosome evolution in this family, where possible, in the light of phylogenetic hypotheses. Thus, we use the taxonomic system of Burtt & Wiehler (1995).

1. History of chromosome studies in *Gesneriaceae*

The first reported chromosome counts in *Gesneriaceae* were published as early as 1923 by Oehlkers. During the following 30 years, a slowly increasing number of counts was recorded. In the 1950s and 1960s, due mainly to the work of Ratter, Milne & Prentice (1963–75) on OW taxa at the Royal Botanic Garden Edinburgh (RBGE) and Lee *et al.* (1950–67) on NW taxa, the number of counts increased significantly, reaching more than 200 and 115 respectively for the two research groups. Other major contributors were Rogers (1954–62), Eberle (1956–57), Fussell (1958), Morley *et al.* (1967–72), Davidse (1970–81), Wiehler (1972–76) and, more recently, Oliver & Skog (1981–85) for NW taxa. (For original citations see Ratter, 1975; Skog, 1984.) Kiehn and co-workers in Vienna added over 120 counts for OW taxa, mainly in subfamily *Cyrtandroideae* from Malaysia (Kiehn & Weber, 1998; Kiehn *et al.*, 1998). Their papers also included discussion on the chromosome evolution of individual genera. Jong and co-workers (2000–01) continued the tradition of Ratter's work at RBGE with their contributions on *Aeschynanthus* Jack and *Streptocarpus* Lindl. Summaries of chromosome data available for *Gesneriaceae* were provided by Ratter (1975) and Skog (1984). To date about 1000 chromosome counts have been published for the family. These can be searched interactively in a database recently developed at RBGE (see section 9).

Continued cytological output in OW and NW taxa is evident from the overlapping years of publications by the major contributors (Table 1). It is

Table 1. List of selected authors of major cytological publications in *Gesneriaceae*, arranged by region of interest and date of publication. For original citations of literature before 1984 see Ratter (1975) and Skog (1984)

New World		Old World	
Main authors	Year range	Year range	Main authors
Lee *et al.*	1950–66		
Rogers	1954–62		
Eberle	1956–57		
Fussell	1958		
Lee	1962–67	1963–75	Ratter
Morley *et al.*	1967–72	1967	Ratter & Prentice
Davidse	1970–81	1970	Ratter & Milne
Wiehler *et al.*	1972–76	1975	Milne
Oliver & Skog	1981–85	1984 + 92	Sera & Karasawa
		1998	Kiehn *et al.*
		2000–01	Jong *et al.*

apparent that most cytologists work on taxa from either the OW or the NW. This compartmentalization may be due to the availability and accessibility of suitable research material, e.g. well-curated *ex situ* living collections, but also indicates centres of taxonomic research in the family.

2. Cytological coverage

Representatives of all subfamilies have been counted. At tribal level, however, cytological data for one of the 11 tribes, the monogeneric NW *Napeantheae*, are still lacking. Across the family at least one species has been counted in 56% of the genera, with 65% and 56% respectively in the NW subfamilies *Gesnerioideae* and *Coronantheroideae*, and 51% in the OW subfamily *Cyrtandroideae* (Table 2). The lower number for the latter is due mainly to the large OW tribe *Didymocarpeae*, with 70 genera the largest in the family, in which only 46% of genera have been counted. The *Gloxinieae* is the most comprehensively investigated tribe in the NW (apart from the small tribe *Gesnerieae*), with 82% of the genera and 36% of the species counted. In comparison, in the *Episcieae*, with a similar number of genera, only about two-thirds of the genera and one-fifth of the species have been analysed. The tribe *Beslerieae* has hardly been investigated and only two species have been counted, despite it containing the large genus *Besleria* L., with more than 200 species. Amongst the *Cyrtandroideae*, tribe *Epithemateae* is most comprehensively covered, with counts for 26% of its taxa, representing 86% of the genera. However, this tribe includes only seven genera and around 84 species. In the largest OW tribe, *Didymocarpeae*, comprising more than 950 species, only a quarter (24%) of the species, covering less than half of the genera (46%), have been cytologically investigated. Tribe *Cyrtandreae* is the least investigated with only 6% of the species counted. This is due mainly to the small number of species investigated in the large genus *Cyrtandra* J.R. & G. Forst. (see below).

Chromosome counts at species level across the family have been made for only about 18% (i.e. 590 out of 3347 species, see Table 2), mainly because of low coverage in some large genera (e.g. *Cyrtandra*, *Besleria*). On average, sampling of the NW and OW species has been at a similar level, 19% and 17%, respectively. Of the larger genera with more than 40 species, the NW *Sinningia* Nees (34%) and the OW *Streptocarpus* (42%) and *Henckelia* Spreng. (30%) are particularly well analysed (Table 3). On the other hand, the large genera *Alloplectus* Mart. (8%), *Besleria* (1%), *Drymonia* Mart. (7%) and *Paradrymonia* Hanst. (1%) of the NW, and *Cyrtandra* (6%) and *Agalmyla* Blume (2%) of the OW are particularly in need of investigation, as only 1–8% of their species have been counted. The low coverage in *Cyrtandra* is a result of the large number of species, around 600; of the c.60 accessions counted in 36 species the great majority reveal a diploid number of $2n = 34$; only three reports (Borgmann, 1964) give $2n = 32$.

Of the medium-sized NW genera with around 20 taxa, 92% of *Achimenes* Pers. species have been counted, followed by, in decreasing order: *Kohleria* Regel (77%), *Codonanthe* Hanst. (65%), *Nematanthus* Schrad. (40%), *Gesneria* s.1. (Skog, 1976)

TABLE 2. Basic chromosome numbers, numbers and percentages of genera and species analysed, and levels of ploidy recorded in tribes and subfamilies of *Gesneriaceae*. Prevalent numbers are underlined. Taxonomy and figures are based on Burtt & Wiehler (1995) and Weber & Skog (2003). Ploidy information is from Möller *et al.* (2002 ongoing)

Tribe	Genus basic numbers (*n*)	Genera	% genera counted	Species	% species counted	% polyploidy	Levels of ploidy
Subfam. Gesnerioideae							
Gloxinieae	10, 11, 12, 13	22	82	281	36	4	4*x*
Episcieae	8, 9	19	68	730	19	11	4*x*
Beslerieae	16	9	11	277	1	0	–
Napeantheae	–	1	0	23	0	0	–
Gesnerieae	14	3	100	67	22	7	4*x*
Total for subfamily		Σ54	Ø65	Σ1378	Ø19	Ø8	
Subfam. Coronantheroideae							
Coronanthereae	37, 40, 45	9	56	20	25	n.a.	–
Subfam. Cyrtandroideae							
Epithemateae	8, 9, 10, 11, 12, 16	7	86	84	26	18	4 & 6*x*
Didymocarpeae	4, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17	70	46	952	24	11	4, 6, 8, 10*x*
Trichosporeae	14, 15, 16	5	60	293	14	15	4 & 6*x*
Cyrtandreae	10, 17	3	67	619	6	0	–
Titanotricheae	20	1	100	1	100	0	–
Total for subfamily		Σ86	Ø51	Σ1949	Ø17	Ø11	
Total for family		Σ149	Ø56	Σ3347	Ø18	Ø 9	

n.a., not applicable.

TABLE 3. Percentages of species in larger genera (>40 species) with chromosome counts

New World			Old World		
Genus	Species	% counted	Genus	Species	% counted
Columnea	265+	24	*Cyrtandra*	600+	6
Besleria	200+	1	*Henckelia*	180+	30
Drymonia	140+	7	*Aeschynanthus*	160+	22
Alloplectus	75+	8	*Streptocarpus*	135+	42
Nautilocalyx	70+	19	*Chirita*	130+	16
Paradrymonia	70+	1	*Agalmyla*	95+	2
Sinningia	65+	34	*Paraboea*	90+	14
Gesneria	46+	26	*Didymocarpus*	70+	17
Mean		13	Mean		19

(26%), *Solenophora* Benth. (25%), *Diastema* Benth. (24%), *Monopyle* Benth. (7%), *Pearcea* Regel (6%) and *Rhytidophyllum* Mart. (5%). No species have been counted in the three medium-sized genera *Cremosperma* Benth., *Gasteranthus* Benth. and *Napeanthus* Gardn. Of all NW genera, 18 have no counts recorded. In subfamily *Coronantheroideae* seven out of nine genera are monotypic, and of these five (56%) have been counted. In the OW genera, Sera & Karasawa (1984) and Sera (1992) looked at 17 out of the 22 recognized species of *Saintpaulia* H. Wendl. (77%), making this the most comprehensively investigated OW genus. *Ridleyandra* A. Weber & B.L. Burtt (30%), *Monophyllaea* R. Br. (25%), *Petrocosmea* Oliv. (19%), *Lysionotus* D. Don (10%) and *Epithema* Blume (9%) have been investigated to various degrees, but in *Rhynchotechum* Blume (11%), *Briggsia* Craib (9%) and *Hemiboea* C.B. Clarke (9%) only two counts per genus are recorded, and in *Oreocharis* Benth. only one (4%).

The number of genera without chromosome counts is higher in the OW (43 genera) than the NW (18 genera). This is not surprising as there are almost twice as many genera of *Gesneriaceae* in the OW and more small, monotypic genera: OW 32 genera (37%); NW eight genera (15%). The problem with monotypic genera is that plants usually occur in small populations in few locations and are often endangered, or already extinct as is apparently the case with *Gyrogyne* W.T. Wang from China (Y.Z. Wang, pers. comm.). Cytological data are thus available for only eight of the monotypic genera of the OW and for one genus in the NW. In the absence of morphological characters or character combinations that might indicate affinities with larger genera, obtaining cytological data for monotypic genera before they become extinct is of great importance. Unlike morphological or molecular data that can be retrieved even from very old herbarium specimens (Drabkova *et al*., 2002), cytological data can be reliably obtained only from living material. This underlines the importance of *in situ* protection for such groups as well as of well-curated *ex situ* collections.

Scarcity of cytological data is a particular problem in interpreting chromosome evolution in OW genera which exhibit much variation in basic chromosome number,

e.g. *Chirita* D. Don ($n=4$, 9, 10, 14, 16, 17; 16% species counted), *Didymocarpus* s. str. ($n=10$, 11, 12, 13, 14, 16, 18; 17%), or members of tribe *Epithemateae*, such as *Monophyllaea* ($n=10$, 11, 12, 16; 28%, but see comments in Kiehn & Weber, 1998, relating to the $n=16$ count by Oehlkers, 1923), and *Rhynchoglossum* Blume ($n=10$, 18, 21, 27; 50%); see sections 5 and 7.

3. Types and significance of cytological data

Cytotaxonomy is not necessarily merely concerned with collecting chromosome numbers for the sole purpose of establishing taxonomic groups. Theories on dysploid reduction or increase in chromosome number, caused, for example, by Robertsonian events, are often put forward to explain variation in closely related groups (e.g. Hair, 1963, for *Podocarpaceae*; Wang *et al*., 1998, for *Epithemateae*; Kiehn *et al*., 1998, for *Henckelia* (*Didymocarpeae*); see section 8). In reality, however, explanations of observed differences in chromosome data are often more complex, such as comparisons of chromosome data with molecular phylogenies, e.g. the *Epithemateae* phylogeny by Mayer *et al*. (2003); see also section 8.

Gesneriaceae, in general, possess relatively small chromosomes, frequently in the <1 to 2μm range, and as a result few karyotypes have been published so far, mainly in Chinese taxa (e.g. Wang *et al*., 1998; Wang & Gu, 1999; Zhou *et al*., 2004 [this issue]). Because of their relatively small size, it is necessary to exercise great care when exceptionally large nucleolar organizer regions (NORs) are present that may tend to detach; incorrect counts can be the result of not recognizing such 'satellites'. This has apparently happened, for example, in *Lysimachia nemorum* L. (*Primulaceae*), which has a published number of $2n=18$ but a correct number of $2n=16$ (P.M. Hollingsworth, pers. comm.). In *Gesneriaceae* such large NORs are thought to exist, for example in the Chinese genus *Anna* Pellegr., where $2n=34$ was established with certainty for two species by Zhou *et al*. (2004). However, prometa- and metaphases frequently give the appearance of $2n=36$, as the stalk linking the relatively large satellite to the not very much larger chromosome is often not clearly visible (Fig. 1).

Detailed analyses of mitotic metaphase nuclei, beyond mere establishment of chromosome numbers, are essential where no difference in numbers between taxa can be established. These analyses basically involve detailed measurements of individual chromosome arms, establishment of centromere position and determination of the position and number of NORs. Interspecific comparisons of such karyotypes may well enable further species or even population distinctions, such as in *Conandron ramondioides* Sieb. & Zucc. where it was demonstrated that populations from Japan varied in NOR number in otherwise virtually indistinguishable chromosome complements (Kokubugata & Peng, 2002). Details of chromosomal rearrangements, such as inversions, duplications, deletions and translocations, can also be valuable characters for phylogenetic analyses (Borowik, 1995; Levin, 2002).

Additional cytological data can be gathered from characters in interphase and prophase nuclei. Much variation in interphase nuclei was demonstrated by Tischler

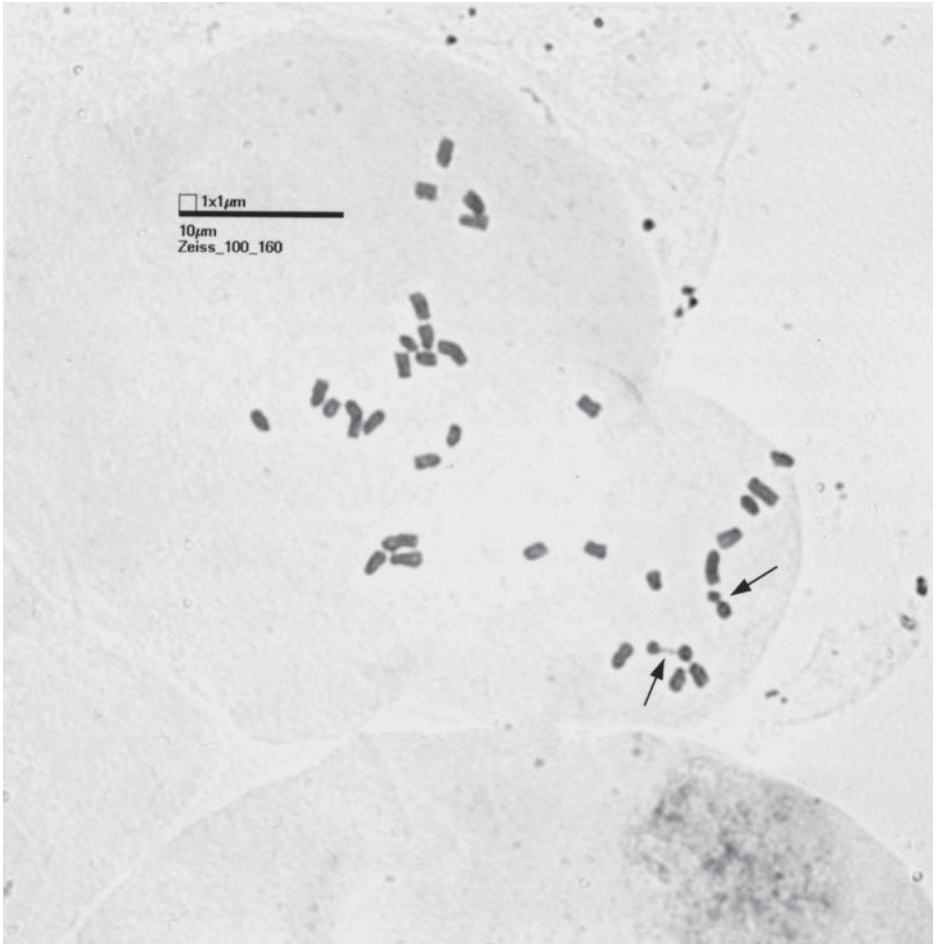

FIG. 1. Metaphase spread of *Anna submontana* Pellegr. showing $2n = 34$ and large putative NOR satellites (arrowed).

in 1934, and has been applied in a number of different plant groups, such as *Orchidaceae* (Tanaka, 1971). Zhou *et al*. (2004) have summarized their own data and those published by others on interphase and prophase characters in OW *Gesneriaceae*. Although variation was found that spanned current tribal delimitations in subfamily *Cyrtandroideae*, the authors were careful not to draw far-reaching conclusions, as too few taxa have been analysed and preliminary molecular phylogenetic data suggest that the current tribes do not form natural groups (Wang & Li, 1998; Pfosser *et al*., unpublished).

Meiotic pachytene chromosomes are still relatively long and are thus ideal for morphological analyses, particularly as at this stage fewer chromosomal bodies are apparent, because of the pairing of homologues as bivalents. Eberle (1956) analysed pachytene chromosomes from a range of taxa across the family and

noted fundamental differences between NW and OW taxa. *Rhynchoglossum* of tribe *Epithemateae* apparently possesses a chromosome type unique in the family, with all chromosome pairs being predominantly euchromatic and little heterochromatin with small chromomeres (Eberle, 1956: 310). Unfortunately Eberle did not study other members of the tribe. He also investigated one species in tribe *Trichosporeae*, *Aeschynanthus tricolor* Hook.f., and found one chromosome with a terminal NOR among the $n=16$ chromosomes. Ehrlich (1958), investigating *Saintpaulia*, used pachytene data to illustrate chromosome mutations, such as inversions, translocations and deletions. He also reported a single NOR chromosome with a small satellite.

4. Diagnostic potential of chromosome data

In *Gesneriaceae* the small size of the chromosomes frequently allows only the reporting of chromosome numbers for the taxa investigated. However, even the establishment of ploidy levels or approximate numbers can be of taxonomic importance, and this has been used to support taxonomic decisions. For instance *Didymocarpus*, in its traditional circumscription, included over 250 species, but Weber & Burtt (1998) remodelled the genus and split it into three genera, *Didymocarpus* s. str., *Hovanella* A. Weber & B.L. Burtt and *Henckelia*. In addition to morphological and biogeographical data they used differences in chromosome number to support their decision. *Henckelia* has rod-shaped chromosomes and a basic number of predominantly $n=9$; *Didymocarpus* s. str. has more globular chromosomes and a range of chromosome numbers, excluding $n=9$, while the Madagascan endemic *Hovanella* has $n=14$ (Kiehn *et al*., 1998) or $n=15$ (Möller, unpublished).

Another example is the genus *Streptocarpus*. It is divided, mainly on the basis of vegetative morphology, into two subgenera, *Streptocarpus* Fritsch and *Streptocarpella* Fritsch. The former includes mainly unifoliate and rosulate taxa, while the latter comprises mainly caulescent species (Hilliard & Burtt, 1971). All species in subgenus *Streptocarpus* have a basic number of $n=16$, while those in subgenus *Streptocarpella* have $n=15$. The exceptions, all in subgenus *Streptocarpella*, are reports of $2n=28$ for the W African *S. nobilis* C.B. Clarke, $2n=32$ for the Tanzanian *S. schliebenii* Mansfeld (Mangenot & Mangenot, 1957, 1962; see footnote in section 5(c)) and a group of woody caulescent species from Madagascar with $n=16$. However, the chromosomes of the latter have a morphology different from those in subgenus *Streptocarpus* and can be distinguished easily (Jong & Möller, 2000).

5. Distribution of haploid chromosome numbers in different taxonomic groups

(a) Gesnerioideae

Within the tribes of *Gesnerioideae* the *Gloxinieae* are relatively diverse in haploid numbers ($n=10$, 11, 12, 13), but $n=13$ prevails (Table 2). Tribe *Episcieae* is very

uniform: only two out of 21 genera have $n=8$, the rest $n=9$. The three genera in *Gesnerieae* all have $n=14$. The *Beslerieae* are seriously undersampled, only two out of more than 200 taxa having been counted, with $n=16$. Although the subfamily appears cytologically relatively uniform within tribes, it differs greatly between tribes.

(b) Coronanthereae

Plotting the frequencies of haploid numbers by subfamily reveals that the *Gesnerioideae* and *Coronanthereae* cluster in distinctly different groups (Fig. 2). While taxa in the *Gesnerioideae* have haploid numbers ranging from $n=8$ to $n=16$ (with a distinct peak at $n=9$), the *Coronanthereae* have numbers between $n=37$ and $n=45$. This might be an indication that the two groups belong to distinctly different phylogenetic lineages (but see sections 7 and 8).

Only five out of the nine genera in the *Coronanthereae* have been counted so far, but all have high haploid numbers. The exact somatic number of all taxa examined has not been exactly determined, although for *Rhabdothamnus solandri* Cunn. an exact meiotic count of $n=37$ has been recorded (Hair & Beuzenberg, 1960).

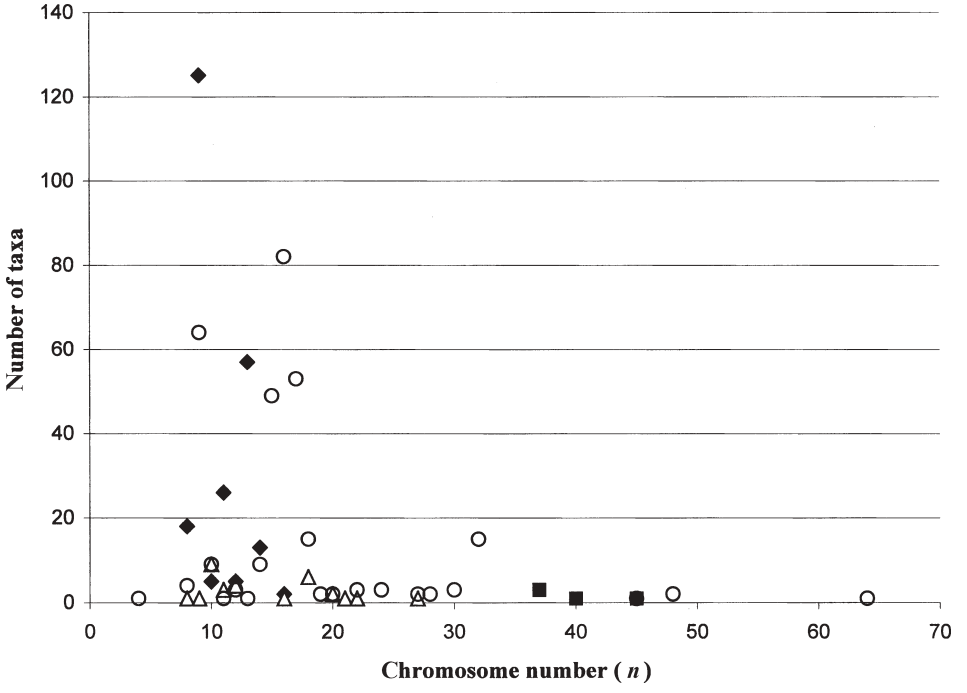

FIG. 2. Distribution of haploid numbers in tribes and subfamilies of the *Gesneriaceae*. ■ = subfamily *Coronantheroideae*, ○ = residual *Cyrtandroideae* (including tribes *Cyrtandreae* and *Trichosporeae*), △ = tribe *Epithemateae*, ◆ = subfamily *Gesnerioideae*.

(c) *Cyrtandroideae*

While the NW subfamily *Gesnerioideae* is characterized by a narrow range of low chromosome numbers, taxa in the OW subfamily *Cyrtandroideae* possess a wide range of haploid numbers from $n=4$ to $n=64$, with a peak at $n=16$ (Fig. 2). The majority have $n=9$, 15, 16 or 17. The *Didymocarpeae* are the most diverse with at least 11 different haploid numbers (Table 2). Apart from the $n=20$ for *Titanotricheae*, the *Didymocarpeae* includes all numbers recorded for the *Cyrtandroideae*. Thus, although very variable intragenerically, the tribe *Epithemateae* with $n=9-27$ fits well into the range of the rest of this subfamily.

At genus level, only subfamily *Cyrtandroideae* shows much variation in basic number, most prominently in the *Epithemateae* (e.g. $n=8$, 9, 12 in *Epithema*, $n=10$, 11, 12, (16) [see comments in Kiehn & Weber, 1998] in *Monophyllaeae*, $n=10$, 11, 18, 21, 27 in *Rhynchoglossum*). It is interesting to note that pairs of genera share haploid numbers (e.g. $n=12$ in *Epithema* and *Monophyllaea*, $n=10$ and 11 in *Monophyllaea* and *Rhynchoglossum*). However, only $x=9$ is shared by these three genera and *Stauranthera* Benth. which suggests that this is the ancestral number of the tribe (see section 8). In the *Didymocarpeae*, large genera such as *Didymocarpus* s. str. ($n=(10)$, 11, 12, (13), 14, 16, (18) [see comments in Kiehn *et al*., 1998]), *Chirita* ($n=4$, 9, 10, 14, 16, 17, 18), *Henckelia* ($n=9$, 10, 16) and *Paraboea* Ridl. ($n=16$, 17, 18), in addition to *Streptocarpus* ($n=14$[1], 15, 16), and *Aeschynanthus* ($n=14$, 15, 16) in the *Trichosporeae*, exhibit a range of basic numbers. Here again chromosome numbers overlap across tribes; e.g. $n=16$ occurs in all listed genera.

6. Polyploidy

The figures given in Table 2 and in the following discussion refer to haploid numbers and multiples thereof found within genera. Potential palaeopolyploidy, which might be assumed for species exhibiting $n=x=17$, is not taken into account, as this seems too speculative on the basis of available data.

The level of polyploidy inferred from the prevalent basic numbers shows an interesting trend congruent with the diversity of haploid numbers at subfamily level (Table 2). Polyploidy in the *Gesnerioideae* is mostly limited to tetraploidy and is greatest in tribe *Episcieae* with 11%. By contrast, the entire subfamily *Coronantheroideae* exhibits high haploid numbers, between 37 and 45. Speculation on basic numbers and ploidy levels is however hampered by two factors. First, most counts in this group are approximations, with exact numbers being unknown. Secondly, the affinities of this group with other NW groups are ambiguous (see section 8).

In the *Cyrtandroideae*, on the other hand, a high degree of polyploidy is apparent, with up to 15% of the counts for *Trichosporeae* being possibly polyploids. Levels of polyploidy may also be high, e.g. $10x$ in *Henckelia*, or $8x$ in *Streptocarpus*.

[1] Needs confirmation, as counts were performed on pollen mother cells and cleistogamy has been recorded in this species (Hilliard & Burtt, 1971).

The highest degree of inferred polyploidy is 18% of counts observed for the *Epithemateae*.

The geographical distribution of polyploids is intriguing in *Streptocarpus*, where the only polyploids observed so far come from Madagascar and adjacent islands with none on the African mainland. Furthermore, polyploids are present in both subgenera, the predominantly caulescent *Streptocarpella* and the predominantly unifoliate or rosulate *Streptocarpus*, even though they evolved before the genus split into African and Madagascan lineages (Möller & Cronk, 2001). This shows that these polyploidizations are parallel evolutionary events. The situation in *Streptocarpus* is also remarkable with regard to the general trend of chromosomal stasis observed in taxa speciating on islands (Stuessy & Crawford, 1998) and might indicate another example of rapid speciation accompanied by cytological changes in island plants such as the Hawaiian silversword alliance, *Asteraceae–Madiinae* (Carr & Kyhos, 1986) or the genus *Psychotria* L. (*Rubiaceae*) (Kiehn & Lorence, 1996).

7. Genetic versus cytological diversity

Chromosome evolution may involve structural rearrangements (see *Streptocarpus* above) and also dysploid changes in basic chromosome number. It is presumed to be a function of diversification time (Lagercrantz, 1998; Levin, 2002). It is also assumed that chloroplast DNA (cpDNA), being inherited uniparentally, evolves clock-like, steadily accumulating mutations over time, although there are exceptions (Clegg *et al.*, 1994; Muse & Gaut, 1997; Mayer *et al.*, 2003). By correlating divergence in cpDNA (here given as pairwise *trn*L-F intron/spacer sequence differences) with diversity in haploid numbers, it is possible to show whether chromosome evolution is likely to be linked to divergence time. In *Gesneriaceae* there seems to be close correlation at subfamily level (Fig. 3). At tribal level, however, the data indicate that the extant taxa in the *Didymocarpeae* represent an assemblage more diverse in chromosome numbers, while those in the *Epithemateae* are more variable in cpDNA. This supports the view that the *Epithemateae* is the oldest tribe, representing an assemblage of relict taxa.

The *Coronantheroideae*, on the other hand, with their often monotypic genera and a distribution suggestive of a relict group, show, from six out of nine genera investigated, genetic divergence similar to other NW tribes, dismissing the suggestion that they are a palaeopolyploid assemblage of taxa. An explanation for their present day distribution needs further investigation.

8. Genome evolution: current knowledge

In recent years molecular data have been at the centre of numerous phylogenetic investigations. However, the continuing value of cytological data for taxonomic and evolutionary investigations cannot be emphasized enough. Knowledge of chromosome evolution (historic and recent) and inheritance in a plant group is directly relevant to a proper interpretation of any molecular analysis. After all, the nuclear

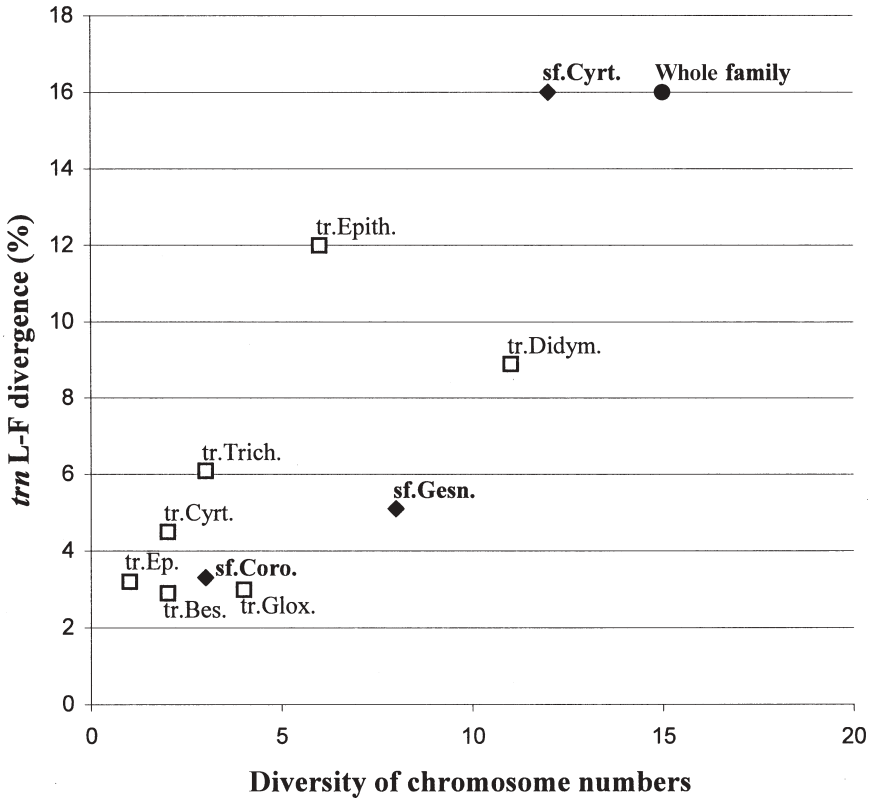

FIG. 3. Diversity of chromosome numbers plotted against maximum *trn*L-F intron/spacer diversity, across tribes and subfamilies of *Gesneriaceae*. sf.Coro. = subfamily *Coronantheroideae*, sf.Cyrt. = subfamily *Cyrtandroideae*, sf.Gesn. = subfamily *Gesnerioideae*, tr.Bes. = tribe *Beslerieae*, tr.Cyrt. = tribe *Cyrtandreae*, tr.Didym. = tribe *Didymocarpeae*, tr.Ep. = tribe *Episcieae*, tr.Epith. = tribe *Epithemateae*, tr.Glox. = tribe *Gloxinieae*, tr.Trich. = tribe *Trichosporeae*.

genes from which sequences for phylogenetic research are often obtained reside on these chromosomes and frequently represent only a very small fraction of the entire genome (e.g. one out of 25,000 genes in *Arabidopsis* L.).

Chromosome evolution may involve single base pair changes or larger chromosomal rearrangements such as translocations, insertions, deletions, duplications or inversions, but may also involve the fusion or fission of whole chromosomes (Robertsonian events, leading to dysploidy), or even of whole chromosome complements (polyploidy). The latter can be divided into autopolyploidy (unreduced gametes resulting in genome duplication – tetraploidy) and allopolyploidy (hybridization followed by chromosome duplication – amphidiploidy). Mapping available chromosome data onto molecular phylogenies provides evidence that all the mechanisms indicated above are involved in the evolution of the *Gesneriaceae* genome.

Hypotheses have been put forward suggesting that increasing or decreasing dysploid series can accompany plant evolution (for summaries see Sato, 1962; Levin, 2002). However, such assumptions are often based only on the existence of a range of basic numbers that can be arranged in, more or less complete, ascending or descending series. Examples in *Gesneriaceae* are given by Wang *et al*. (1998), Kiehn *et al*. (1998), and Kiehn & Weber (1998). If such assumed series are not backed up by other independent characters (from morphology, phytogeography, molecular phylogeny or most importantly by comparisons of karyotype morphology and pairing relationships in hybrids), they do not necessarily reflect actual events or real evolutionary patterns. Exceptions include the single discordant counts as discussed for *Henckelia* by Kiehn *et al*. (1998). Chromosome evolution is best discussed in the light of independent evolutionary hypotheses.

There is as yet no comprehensive, reliable molecular phylogeny available for the whole family *Gesneriaceae*. Recently a phylogeny of NW taxa has been published (Zimmer *et al*., 2002). Subsequent attempts to map available cytological data onto this phylogeny immediately revealed a recurring problem: many taxa that have been analysed cytologically were not included in the phylogeny and *vice versa*. Thus, the overlap between cytological and molecular data is surprisingly low: of the 57 ingroup taxa studied by Zimmer *et al*. (2002), 23 (40%) have no chromosome count. However, NW taxa of subfamily *Gesnerioideae* are relatively constant in basic chromosome number, and the phylogeny presented indicates that certain groups (e.g. *Gesneriaeae* with $n=14$) are very stable, others include a single dysploid reduction (e.g. $n=9$ to $n=8$ in *Episcieae*), while taxa with $n=13$ do not form a monophyletic clade because of a split between *Gloxinieae* and a *Sinningia* clade. It is interesting to note that $n=11$ has apparently evolved several times independently, always from $n=13$. Although the overall pattern of chromosome evolution in the NW taxa appears more complex than expected from the conserved chromosome complements within genera, there is no obvious case of an increasing or decreasing dysploid series. Zimmer *et al*. (2002) propose a basic number for subfamily *Gesnerioideae* of $n=13$ (excluding *Beslerieae* and *Napeantheae*). Although they excluded subfamily *Coronantheroideae* from their analyses, this may not have affected their results, even though it was earlier suggested (Smith *et al*., 1997) and recently proved (Wang *et al*., 2004) that subfamily *Gesnerioideae* is not monophyletic. *Beslerieae* and *Napeantheae*, both of which have been little investigated cytologically, appear basal to the rest of *Gesnerioideae* plus *Coronanthereae*. Based on the available phylogenetic and cytological evidence, the *Coronanthereae* may represent a distinct monophyletic lineage of highly polyploid species, as part of the NW subfamily *Gesnerioideae*. For constructive discussion on the basic number for all the NW taxa, it is clearly important to obtain a stable phylogeny, and to investigate comprehensively the cytology of the *Beslerieae* and *Napeantheae*.

The OW taxa, in contrast, have much more variation in basic chromosome number (Table 2). Mapping the available data onto a preliminary molecular phylogeny of OW taxa (Pfosser *et al*., unpublished) reveals interesting patterns,

although the overlap of chromosomal and molecular data is not very extensive. This cpDNA-based phylogeny reflects shared chromosome number rather than the current tribal arrangement, with groups belonging to different tribes but possessing a common chromosome number clustering together. Not surprisingly, *Chirita* with its range of basic numbers appears polyphyletic across the tree. There are however too many gaps in the cytological data for a full discussion of the chromosome evolution of this phylogenetic hypothesis.

Nevertheless, useful insights into genome evolution can be gained when focusing on phylogenies of individual genera with a higher cytological coverage. Kiehn & Weber (1998) observed that the evolutionary relationships between *Whytockia* W.W. Sm. and *Monophyllaea* and within *Monophyllaea* suggested by morphological advancements were paralleled by an increasing dysploid series with an ancestral $x=9$ (unchanged in *Whytockia*), to $x=10/11$ or 12 in *Monophyllaea*. Mapping the available chromosome numbers for this relationship onto the molecular phylogeny of Mayer *et al*. (2003) gives an impression of increasing dysploidy, though not of a continuous series. Optimizing the available chromosome numbers onto this molecular tree confirms that the basic number for *Epithemateae* is $x=9$, and that there are four undisputed increases: to $x=10$ (*Rhynchoglossum notonianum* (Wall.) B.L. Burtt), to $x=10/11$ (*Monophyllaea*), to $x=11$ (*Loxonia* Jack) and to $x=12$ (*Epithema membranacea* (King) R. Kiew), with a further increase from $x=11$ to $x=12$ (*Monophyllaea glauca* C.B. Clarke), and a possible reduction from $x=9$ to $x=8$ in *Epithema saxatile* Blume, $n=8$ and 9 (Fig. 4). To demonstrate a continuous series of dysploid increase, however, would require molecular analysis of the polymorphic races of *Monophyllaea*.

On the other hand, in the large genus *Cyrtandra* a high chromosome number stability of $2n=34$ is indicated, particularly since the available counts cover taxa representing the whole area of its distribution (Kiehn, unpublished). Differences may be present, however, at structural level even between closely related taxa (Storey, 1966; Kokubugata & Madulid, 2000). There are at present insufficient data to speculate on the age of the genus, but it may be relatively young and therefore uniform in its karyotype. The species richness of the genus may be explained by comparatively recent bird-dispersal of the fleshy fruits, in particular to islands in the eastern range of the genus where further speciation by isolation occurred.

In *Streptocarpus*, the basic chromosome number is highly conserved, having changed just once, coinciding with the origin of the subgenera (Fig. 5). Since then, speciation has occurred with no further change in basic chromosome number, although the ploidy level has increased several times independently in Madagascan taxa (see section 6). *Aeschynanthus*, by contrast, exhibits a very different chromosome evolution (Fig. 6). The resulting phylogeny has two main clades as in *Streptocarpus*. The ancestral number in *Aeschynanthus* appears to be $n=16$, and this has not been affected by the appearance of these clades. However, within the clades there is much variation in basic number, indicating that dysploid reductions in particular often coincided with, or even predated, speciation events, as seen in *A.*

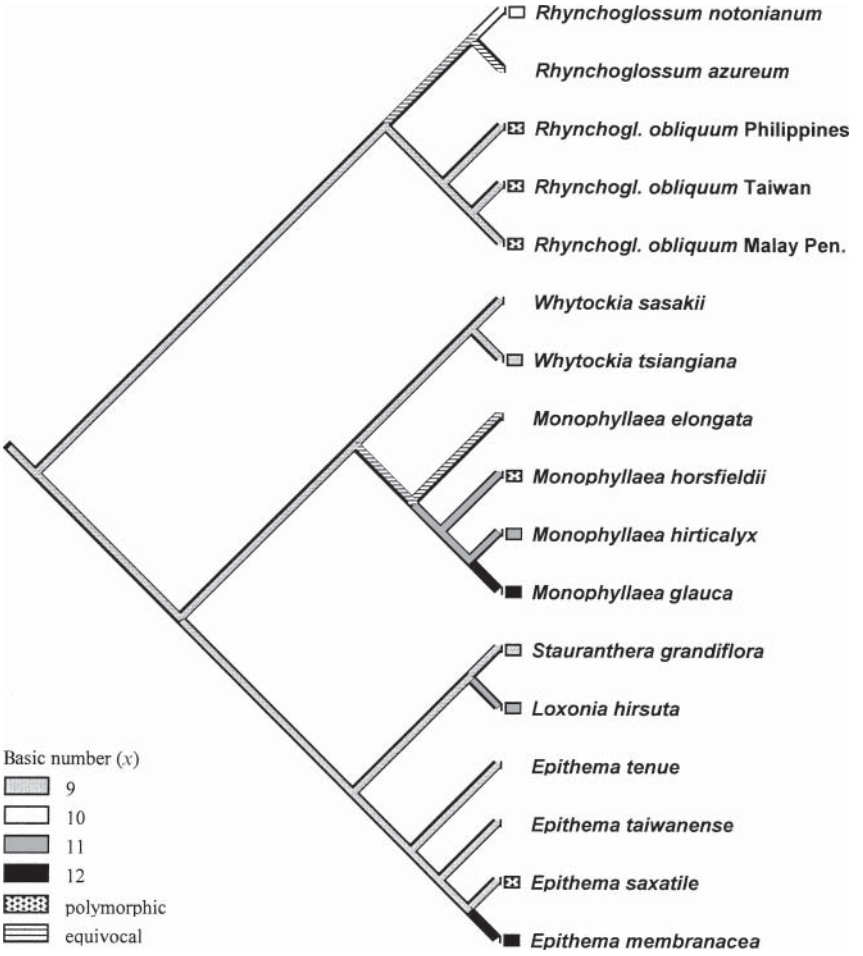

FIG. 4. cpDNA phylogeny of tribe *Epithemateae* from Mayer *et al*. (2003) with basic chromosome numbers optimized using MacClade 3.07 (Maddison & Maddison, 1997). For taxa lacking a square no cytological data are available.

radicans Jack with two cytotypes, $n = 15$ and 16. This suggests that chromosome fusion occurs relatively easily, though this needs further testing. This chromosome behaviour is fundamentally different from that of *Streptocarpus*. Although chromosome number appears to be conserved in *Streptocarpus*, there are however still significant differences in chromosome structure and karyotype of taxa with identical chromosome numbers (Fig. 7).

9. 'GESNERIACEAE WEBCYTE', A CYTOLOGICAL DATABASE

Chromosome data on *Gesneriaceae* are scattered in the literature, and they have been compiled and discussed only twice in the past (Ratter, 1975; Skog, 1984). The

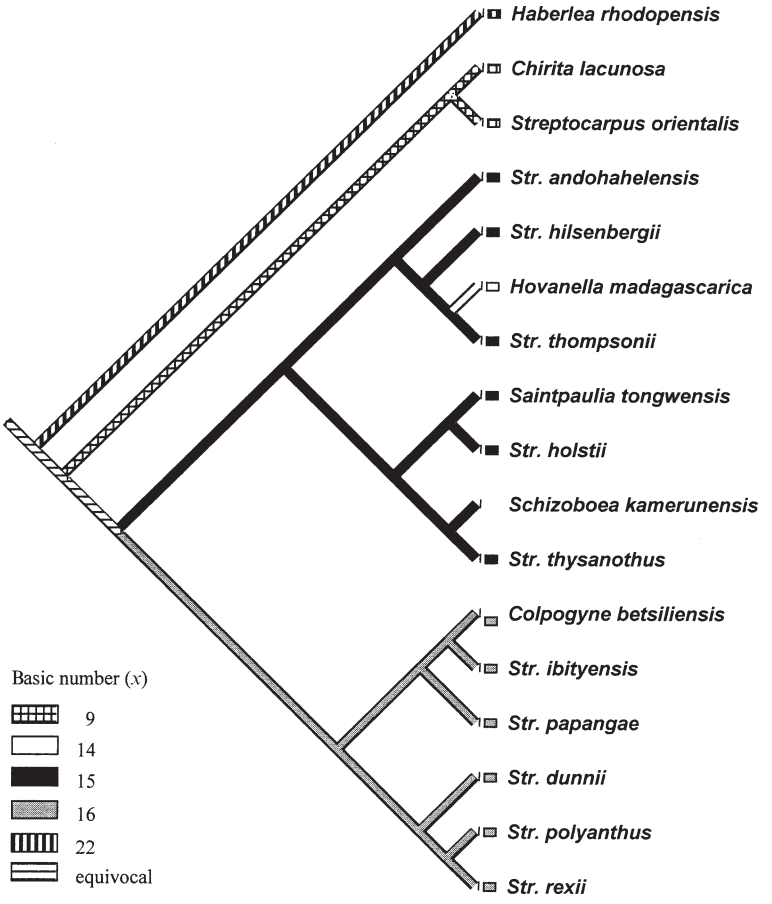

FIG. 5. Simplified *Streptocarpus* Lindl. ITS phylogeny based on Möller & Cronk (2001) and O'Sullivan (1999). For *Schizoboea* (Fritsch) B.L. Burtt no cytological data are available.

indexes to plant chromosome numbers (last issue: Goldblatt & Johnson, 2000) merely summarize chromosome number reports, and normally refer only to literature covering the two years before their publication. Currently, there are 55 species of *Gesneriaceae* listed. No doubt there are also many unpublished cytological data on *Gesneriaceae*. The plan to create a platform holding all chromosome data on *Gesneriaceae*, with the possibility of contributing previously unpublished data, was supported by all participants in the *Gesneriaceae* workshop held at RBGE in 2002 (Möller *et al*., 2002). The ability to access up-to-date knowledge on cytology quickly would increase effective use of existing data and encourage the closing of gaps in our knowledge. In order to fill this need, the cytological database 'Gesneriaceae WebCyte' (http://www.rbge.org.uk/rbge/web/search/index.jsp) has been developed at RBGE in collaboration with M.K. (second author of this paper) and Larry Skog of the Smithsonian Institution, USA (Möller *et al*., 2002 ongoing). It is regularly updated, includes all cytological data available for the *Gesneriaceae* and allows

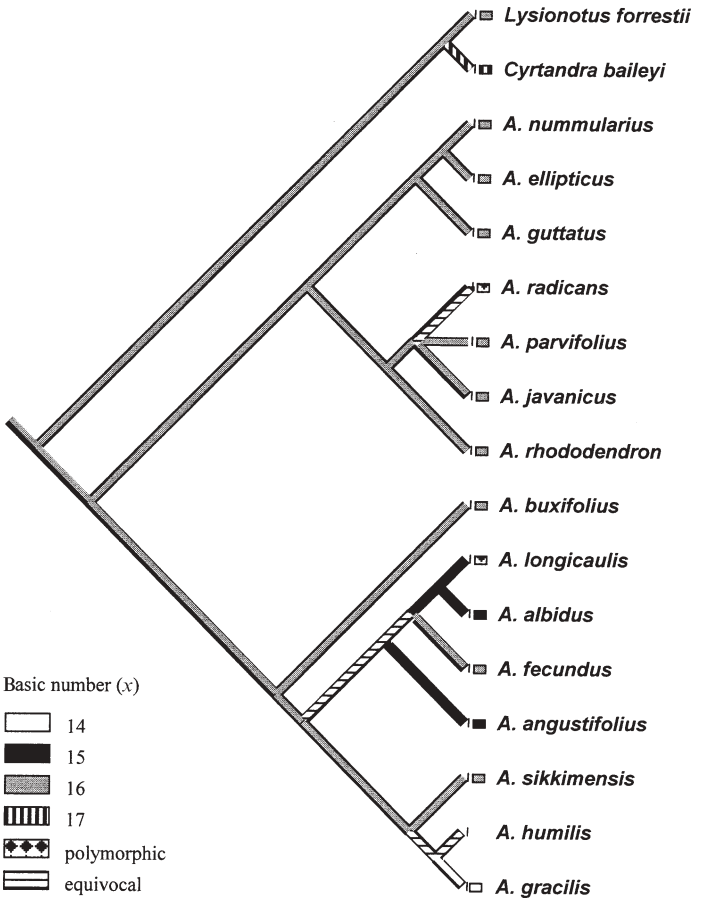

FIG. 6. Simplified *Aeschynanthus* Jack ITS phylogeny based on Denduangboripant *et al.* (2001). For *A. humilis* Hemsl. no cytological data are available.

their instant display, including statistics and images of chromosome preparations if available. Thus all these data are now immediately accessible to those in the research community who may be interested in *Gesneriaceae* cytology (Fig. 8).

10. CONCLUSIONS AND OUTLOOK

Although tremendous efforts have been made to accumulate chromosomal data in *Gesneriaceae*, with about 1000 published counts, there are still significant gaps in the coverage of the family, particularly in certain NW and OW tribes and small monotypic genera. The NW tribes *Beslerieae* and *Napeantheae* and the large, heterogeneous OW tribe *Didymocarpeae* are in particular need of investigation. There is also a frustrating lack of correspondence between the availability of molecular and of cytological data, a lack which should be rectified. Of particular interest would be a more detailed investigation of the genera in tribe *Epithemateae* with their variable

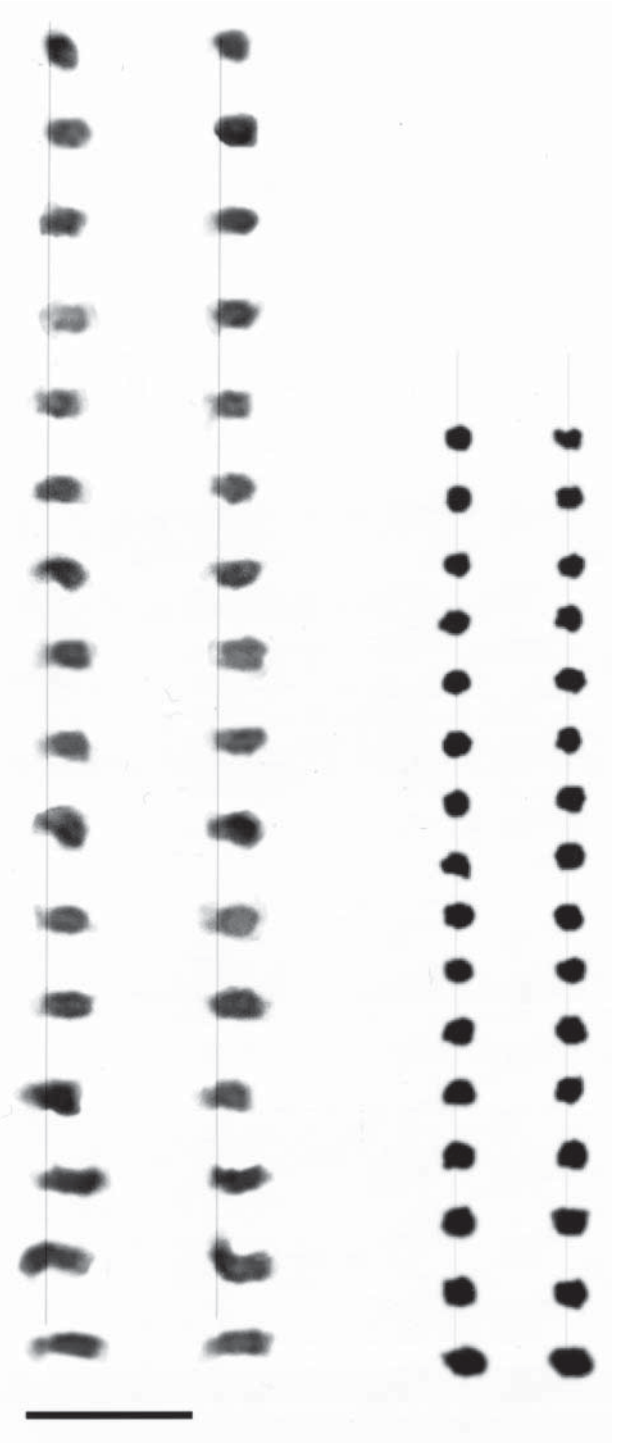

FIG. 7. Karyogram of *Streptocarpus ibityensis* Humbert (top) and *S. suffruticosus* Humbert (bottom), both $2n = 32$. Scale bar for both = 10µm.

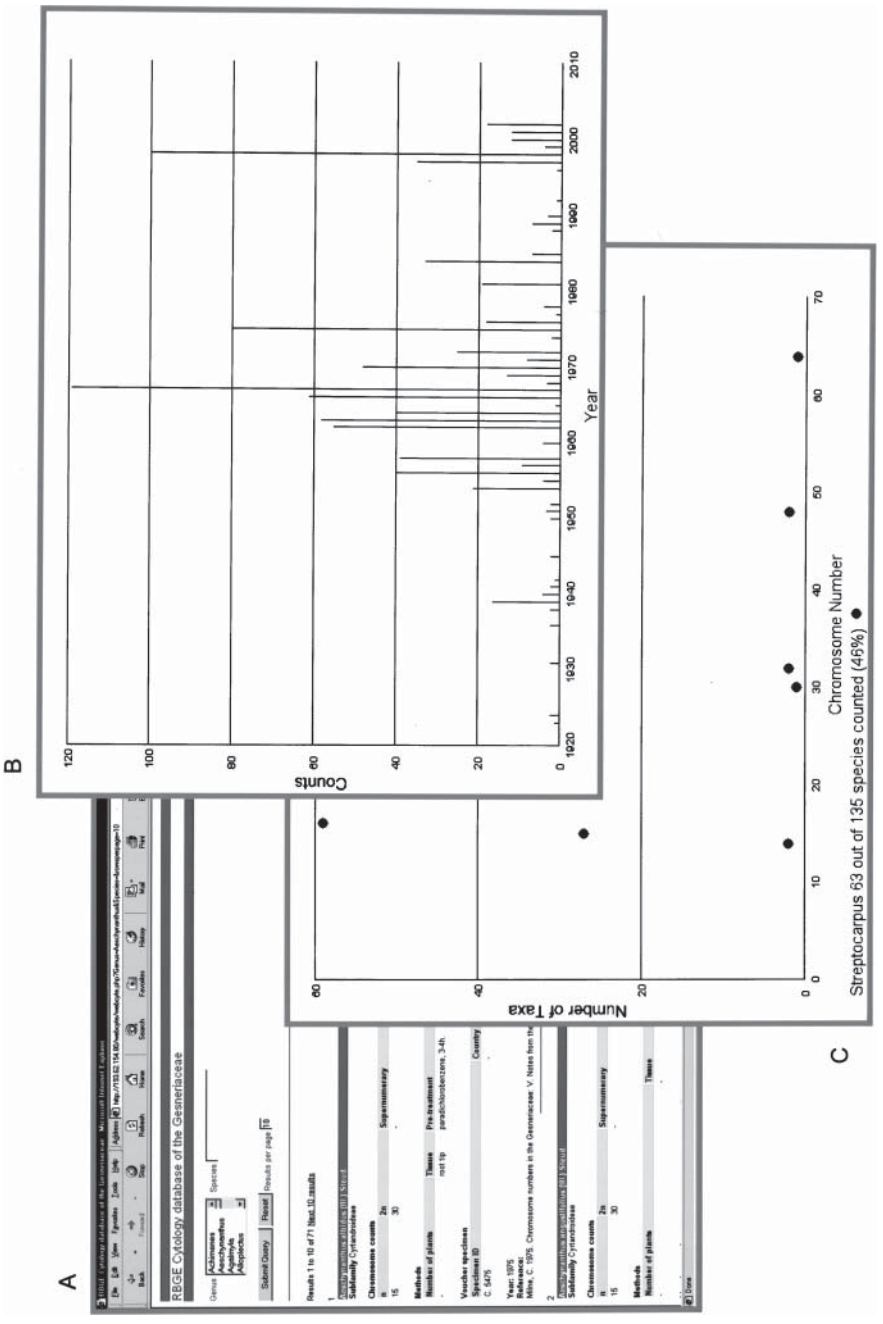

FIG. 8. Sample datasheets from 'Gesneriaceae WebCyte' (Möller *et al.*, 2002 ongoing). A. Output format for cytological queries. B. Statistical output of summary of counts. C. Statistical output of counts by selection, here for *Streptocarpus* Lindl.

basic numbers. Although investigated previously, species in subfamily *Coronantheroideae* need re-evaluation to establish their exact chromosome numbers. This should allow establishment of their basic number and, together with improved molecular data, provide better information on their phylogenetic position. At present, this group of species appears to be a highly polyploid lineage within subfamily *Gesnerioideae*.

Knowledge of chromosome morphology becomes more important for closely related taxa in cases where they possess the same basic chromosome number, especially in the light of molecular phylogenies. Therefore, besides the need to count chromosome numbers for additional species, more studies of chromosome structure and gene locations are desirable. Although some recent publications include images of sufficient quality to show morphological features, older publications relied on drawings which may or may not reflect reality. For comparative studies it seems prudent to produce new preparations. This is of particular importance in cases where different pretreatment or staining techniques can affect chromosome morphology (Zhou *et al.*, 2004). Karyotyping was hampered in the past by the generally small size of *Gesneriaceae* chromosomes. This may now be easier with the introduction of new, highly sensitive 'chromosome painting' techniques such as:

- Fluorescent *in situ* hybridization (FISH) for the localization of conserved multicopy loci.
- Genomic *in situ* hybridization (GISH) for detection of hybrids and quantification of introgression.
- Primed *in situ* hybridization (PRINS), for localization of single genes or determination of loci number of low-copy genes.

These techniques may even allow the acquisition of new data at nucleotide level.

Living research collections are an important source of cytological material, but are often not fully exploited at present. Resources should be channelled into this area to recover and secure as much information as possible. *Gesneriaceae* are often short-lived and may not persist for more than a few months or years in cultivation. Also they should be analysed while physiologically in peak condition to maximize cytological success. This involves a battle for resources and time.

How can we tackle the task of closing existing gaps in our data and knowledge? Extended, targeted field work, preferably including the collection of living plants or seeds, and collaborative work will be the main tools to achieve maximum impact with limited institutional resources. This includes the establishment and maintenance of well-curated, decentralized *ex situ* living collections, crucial for cytological investigations, and the organization of regular workshops bringing together experts on the family and providing a platform for discussion, such as the workshop at RBGE in 2002 (Möller *et al.*, 2002). The new cytological database at RBGE, 'Gesneriaceae WebCyte', is intended to provide an optimized and continually updated overview of chromosome data in *Gesneriaceae*. It should serve as a depository for cytological

data submitted by all researchers. On the basis of the data gathered there, collaborative project proposals to close existing gaps in our knowledge should be easier to draw up, thus stimulating a greater interest in the cytology of this exciting plant family.

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