Int. J. Plant Sci. 169(5):667–676. 2008.

1058-5893/2008/16905-0009$15.00 DOI: 10.1086/533609

ASPECTS OF GENOME EVOLUTION IN GESNERIACEAE: PATTERNS OF 45S-nrDNA SITE EVOLUTION BASED ON EVIDENCE FROM FLUORESCENT IN SITU HYBRIDIZATION (FISH)

Michael Möller,[1]* Goro Kokubugata,† and Kwiton Jong*

*Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, United Kingdom; and †Department of Botany, National Museum of Nature and Science, 4 Amakubo, Tsukuba, Ibaraki 305-0005, Japan

Fluorescent in situ hybridization is used to determine the number and position of 45S nuclear ribosomal DNA (45S-nrDNA) clusters in members of the Gesneriaceae: *Aeschynanthus*, *Agalmyla*, and *Streptocarpus* (including *Colpogyne*, *Hovanella*, *Saintpaulia*, and *Schizoboea*). All sites detected are terminal in position. Species of *Agalmyla* analyzed are uniformly diploid and possess one 45S-nrDNA locus per genome; those of *Streptocarpus* are diploid, with one or two loci per genome; and *Aeschynanthus* species are diploid or tetraploid, possessing one or two loci in each genome. In relating the nrDNA loci to the respective generic phylogenetic trees, contrasting patterns of 45S-nrDNA locus evolution become apparent. In *Streptocarpus*, gain of loci is intragenomic, i.e., within haploid genomes, whereas in *Aeschynanthus*, it involves duplications by polyploidization as well as intragenomic changes. The latter appears to have occurred early in the diversification of the genus but relatively recently in *Streptocarpus*. The relevance of this investigation to the study of evolution of nrDNA loci is discussed.

Keywords: *Aeschynanthus*, *Agalmyla*, fluorescent in situ hybridization, genome evolution, nuclear ribosomal DNA, phylogeny, *Streptocarpus*.

Introduction

Plant genomes evolve over time through structural rearrangements of chromosomes, including transposable elements, through changes in the basic number of chromosomes, and by polyploidy, resulting in quantitative and qualitative changes in DNA. In recent years, fluorescent in situ hybridization (FISH) has been widely applied to detect individual genes or chromosome regions, using sequence-specific probes (Schwarzacher and Heslop-Harrison 2000). Because of their multicopy nature and conserved sequences, nuclear ribosomal DNA (nrDNA) genes are readily detected by FISH. They are arranged in tandem clusters of several hundred to thousands of copies in the genome (Rogers and Bendich 1987; Copenhaver and Pikaard 1996). The 45S-nrDNA cluster comprises the 18S-, 5.8S-, and 26S-nrDNA genes and is located in the nucleolar organizer region (NOR) that is responsible for the formation of nucleoli in dividing cells.

The number of nrDNA copies within a single locus and the number and location of such loci in plant genomes can vary greatly (Rogers and Bendich 1987), even at the same ploidy level (Lavania et al. 2005; Marcon et al. 2005). Such numerical and positional diversity of these sites has recently been studied in a phylogenetic context at the diploid level by Datson and Murray (2006) on *Nemesia*.

A few studies have attempted to correlate variation in nrDNA loci to phylogenies as an approach to ascertaining possible patterns of their evolution. Recent examples are *Paeonia* (Zhang and Sang 1999), *Nicotiana* (Lim et al. 2000), *Thinopyrum* (Li and Zhang 2002), and *Nemesia* (Datson and Murray 2006). These studies indicate that there are no consistent patterns and no preference of location or number of nrDNA sites in particular plant groups or genera. In the case of *Nemesia*, several changes in the distribution of nrDNA loci have been demonstrated (Datson and Murray 2006). However, these studies are based on genera of widely different taxonomic affinities which are therefore not appropriate to investigation of the similarity of patterns of nrDNA evolution among closely related genera.

In order to determine whether related genera show a common pattern of nrDNA evolution, a comparative analysis of several genera within a single family for which phylogenetic and chromosomal data are available has been carried out. For this purpose, three genera in the Gesneriaceae—*Aeschynanthus* and *Agalmyla*, both from Southeast Asia, and *Streptocarpus*, from the African continent and Madagascar—were chosen for our investigation. These three genera are didymocarpoid members of the family (Weber 2004). A large number of chromosome counts are now available for *Streptocarpus* and *Aeschynanthus* but not for *Agalmyla* (table 1). The two former genera show a close similarity in their range of haploid numbers (the most frequently encountered haploid numbers being n=15, 16, 30, 32, 48) and the basic numbers of x=15 and 16 (table 2). Unlike the other two genera, *Agalmyla* appears to be uniform in chromosome number because all 11 taxa counted to date have 2n=2x=32, although some chromosome repatterning may have occurred, as reflected in the presence of at least two karyotypes (Chapman 2003).

The earlier successful application of FISH with 45S-nrDNA probes in the Gesneriaceae (Kokubugata and Peng 2002) is also an advantage. Data on phylogenetic relationships in *Aeschynanthus* (Denduangboripant et al. 2001) and *Streptocarpus* (Möller and Cronk 2001*a*, 2001*b*; Möller 2003) have been published; these analyses cover ~25% and 50%, respectively, of the

[1] Author for correspondence; e-mail: m.moeller@rbge.org.uk.

Manuscript received August 2007; revised manuscript received November 2007.

Table 1

Distribution of Haploid Chromosome Numbers in *Aeschynanthus, Agalmyla,* and *Streptocarpus*

Haploid number (n)	*Aeschynanthus* (~160 species)	*Agalmyla* (97 species)	*Streptocarpus* (~135 species)
14	2	...	1
15	4	...	16
16	22	11	42
30	2	...	1
32	6	...	2
48	1	...	1
64	...	...	1
Total	37	11	64
Coverage (%)	23	11	47

Source. WebCyte (Möller et al. 2002–).

total number of species now recognized in each genus. *Agalmyla* includes 97 species according to the most recent account (Hilliard and Burtt 2002), and an existing phylogenetic analysis of 16 species, encompassing all sections of the genus, is also available (Chapman 2003).

In our study, the number and position of 45S-nrDNA sites for a selection of species in each of the three genera have been determined, using FISH, and these, together with the chromosome numbers, are mapped onto phylogenetic trees in an attempt to ascertain whether (*a*) a correlation exists between chromosome number change and 45S-nrDNA, (*b*) related genera show a commonality of 45S-nrDNA evolution, and (*c*) evolutionary history may have an effect on the patterns observed.

Material and Methods

Roots

Actively growing roots were obtained from plants raised as part of the extensive living collection of Gesneriaceae at the Royal Botanic Garden Edinburgh (RBGE). Those of *Agalmyla* and *Streptocarpus* were harvested from potted plants grown from seed, whereas those of *Aeschynanthus* were from cuttings grown in perlite for ~6 wk (table 3). Voucher specimens have been deposited at the herbarium of RBGE (E).

Chromosome Preparations and FISH

The methodology for the preparation of roots and FISH followed essentially that of Denduangboripant et al. (2007) except that the 18S-nrDNA probe was produced by PCR of genomic DNA from *Streptocarpus gardenii* Hook (Tsukuba Botanical Garden [National Museum of Nature and Science], accession no. 132947).

On average, 10 metaphases were analyzed for each species. These showed little cell-to-cell variation in the respective FISH signals.

Phylogenies

For simplicity and convenience, the reduced phylogenetic trees for *Aeschynanthus* and *Streptocarpus* are adapted from Möller and Kiehn (2004) without compromising the fundamental tree structure and section or subgeneric coverage. For *Aeschynanthus*, the phylogeny is based on the study by Denduangboripant et al. (2001) that includes 50 species. It illustrates a strongly supported two-clade structure reflecting the current taxonomic sectional divisions. For our investigation, the reduced tree consists of 15 species plus the two outgroup species. The reduced phylogenetic tree for *Streptocarpus* is based on work by Möller and Cronk (2001*a*, 2001*b*), with 77 species. The tree shows a two-clade structure as in *Aeschynanthus*. The phylogenetic positions of *Colpogyne* and *Hovanella*, both nested within *Streptocarpus*, are taken from O'Sullivan's study (1999). The reduced *Streptocarpus* phylogeny includes 22 species, 10 of which are from the African continent, with five from Madagascar and four other genera nesting inside *Streptocarpus* (*Colpogyne*, *Hovanella*, *Saintpaulia*, and *Schizoboea*), and three outgroup samples.

Mapping of Chromosome Numbers

In order to investigate the genome evolution with respect to 45S-nrDNA sites, we mapped the haploid chromosome number onto the phylogenetic trees and optimized the character state changes using MacClade, version 4.06 (Maddison and Maddison 2003). The polytomies in the *Streptocarpus* tree (the result of low divergence) were resolved randomly before optimization. This did not affect the evolution of basic chromosome numbers, and the influence on nrDNA site evolution was minimal, that is, three instead of four origins of a second locus per genome. The chromosome numbers for the species cited in the trees were retrieved from the cytology database for Gesneriaceae, WebCyte (Möller et al. 2002–; table 3).

Results

Number and Position of 45S-nrDNA Sites

All species observed, irrespective of genus, possessed terminal 45S-nrDNA sites on medium to small chromosomes (table 3). FISH signals showed variation in intensity between the loci, but there was no clear correlation with genus, ploidy level, or number of loci.

Table 2

Chromosome Numbers in *Aeschynanthus, Agalmyla,* and *Streptocarpus*

	Haploid numbers (n)	Basic numbers (x)	Geographic distribution
Aeschynanthus	14, 15, 16, 30, 32, 48	14, 15, 16	Southeast Asian
Agalmyla	16	16	Southeast Asian
Streptocarpus	15, 16, 30, 32, 48, 64	15, 16	African

Source. WebCyte (Möller et al. 2002–).

Table 3

Accession Number, Taxonomic Rank, NOR Number of *Aeschynanthus, Agalmyla,* and *Streptocarpus* Species Used for FISH and Included in the ITS Phylogenies

Taxon	RBGE accession number	Taxonomic rank[a]	2n[b]	FISH signals[c]
Aeschynanthus Jack:				
A. angustifolius (Blume) Steud.	19881452	X	30	4 (var, 1 large)
A. gracilis Parish ex C.B. Clarke	19821969	X	28	4 (var, 1 large)
A. guttatus P. Woods	19841131	M	64[d] (32)	4 (var, 1 large)
A. longicaulis Wall ex B. Br.	19621423	P	30 (28)	4 (var, 1 large)
A. nummularius (Burk. & S. Moore) K. Schum.	19682000	M	64	4 (var, 1 large)
A. radicans Jack	19672224	A	30 (32)	2 (var)
A. rhododendron Ridl.	20001550	M	32	2 (var)
A. sikkimensis Stapf	19892163	D	32	4 (var, 1 large)
Agalmyla Blume:				
A. parasitica (Lam.) Kuntze	20021921	A	32 *s*	2 (var)
A. chalmersii (F. Muell.) B.L. Burtt	19661974	D	32	2 (stalked)
A. clarkei (Elmer) B.L. Burtt	19972530	D	32 *L*	2 (inv)
A. glabra (Merrill) B.L. Burtt	19991924A	D	32	2 (inv)
A. paucipilosa Hilliard & B.L. Burtt	20000654D	E	32 *s*	2 (var)
Streptocarpus Lindl.:				
S. baudertii L.L. Britten	20030808A	SP	32	2 (inv)
S. cyaneus S. Moore	19911950A	SP	32	4 (inv)
S. dunnii Hook. f.	20020756R	SP	32	2 (var)
S. dunnii Hook. f.	20020756C	SP	32	4 (v.var, 1 minute)
S. johannis L.L. Britten	19990271K	SP	32	2 (var)
S. kentaniensis L.L. Britten & Story	19952992A	SP	32	2 (v.var)
S. polyanthus Hook.	20030945A	SP	32	2 (inv)
S. primulifolius Gand.	20030803A	SP	32	4 (var)
S. rexii Lind.	19870333E	SP	32	4 (var)
S. papangae Humbert	19972886B	SL	32	2 (inv)
S. stomandrus B.L. Burtt	20051684	SL	30	2 (var)
Colpogyne betsiliensis (Humbert) B.L. Burtt	20020562M	SP	32[d]	2 (inv)

Note. NOR = nucleolar organizer region; FISH = fluorescent in situ hybridization; ITS = internal transcribed spacer.

[a] Taxonomic ranks: *Aeschynanthus* sections: A = *Aeschynanthus*, D = *Diplotrichium*, M = *Microtrichium*, P = *Polytrichium*, X = section "X." *Agalmyla* sections: A = *Agalmyla*, D = *Dichrotrichium*, E = *Exannularia*; *s* = karyotype with small chromosomes, *L* = karyotype with large chromosomes. *Streptocarpus* subgenera: SP = *Streptocarpus*, SL = *Streptocarpella*.

[b] Numbers in parentheses indicate polymorphic chromosome numbers previously published.

[c] inv = invariable, var = variable, v.var = very variable.

[d] New chromosome count.

Agalmyla

All five species analyzed were diploids with 2n=32 chromosomes and showed two 45S-nrDNA signals in root tip cells (fig. 1). *Agalmyla chalmersii* possessed a single satellite chromosome in the pair with an nrDNA site. While *A. clarkei* and *A. glabra* had similarly sized FISH signals, those in *A. chalmersii*, *A. paucipilosa*, and *A. parasitica* were distinctly different in size between chromosome pairs.

Aeschynanthus

In *Aeschynanthus*, seven species were analyzed; in each species, either two or four 45S-nrDNA sites were observed (fig. 2), irrespective of the ploidy level and basic number. Two of the diploid species with 2n=30 or 2n=32 (*A. radicans* and *A. rhododendron*) had two sites each, but the other diploids with 2n=30 (*A. longicaulis* and *A. angustifolius*) and 2n=28 (*A. gracilis*) had four sites (fig. 2). Both tetraploids (*A. guttatus*, 2n=4x=64, and *A. nummularius*, 2n=4x=64) possessed four sites (fig. 2). All *Aeschynanthus* species showed one noticeably large signal, irrespective of ploidy level and number of nrDNA sites.

Streptocarpus

The nine species of *Streptocarpus* in subgenus *Streptocarpus* and *Colpogyne betsiliensis* of clade II all had 2n=32 chromosomes (table 3). The chromosome number for *C. betsiliensis* is reported here for the first time.

Three out of the six rosulate species sampled in the Cape Primrose clade—*S. cyaneus*, *S. primulifolius*, and *S. rexii*—had four FISH signals; the remaining species in this clade had only two signals (table 3; fig. 3). The only other species with four signals was *S. dunnii*. In this species, the two larger signals were heteromorphic, whereas the two minute signals were equal in size but were not always visible after FISH (fig. 3*D*, 3*E*). In the two caulescent species of subgenus *Streptocarpella* analyzed, *Streptocarpus papangae* and *Streptocarpus stomandrus*, both with 2n=30, two FISH signals were observed.

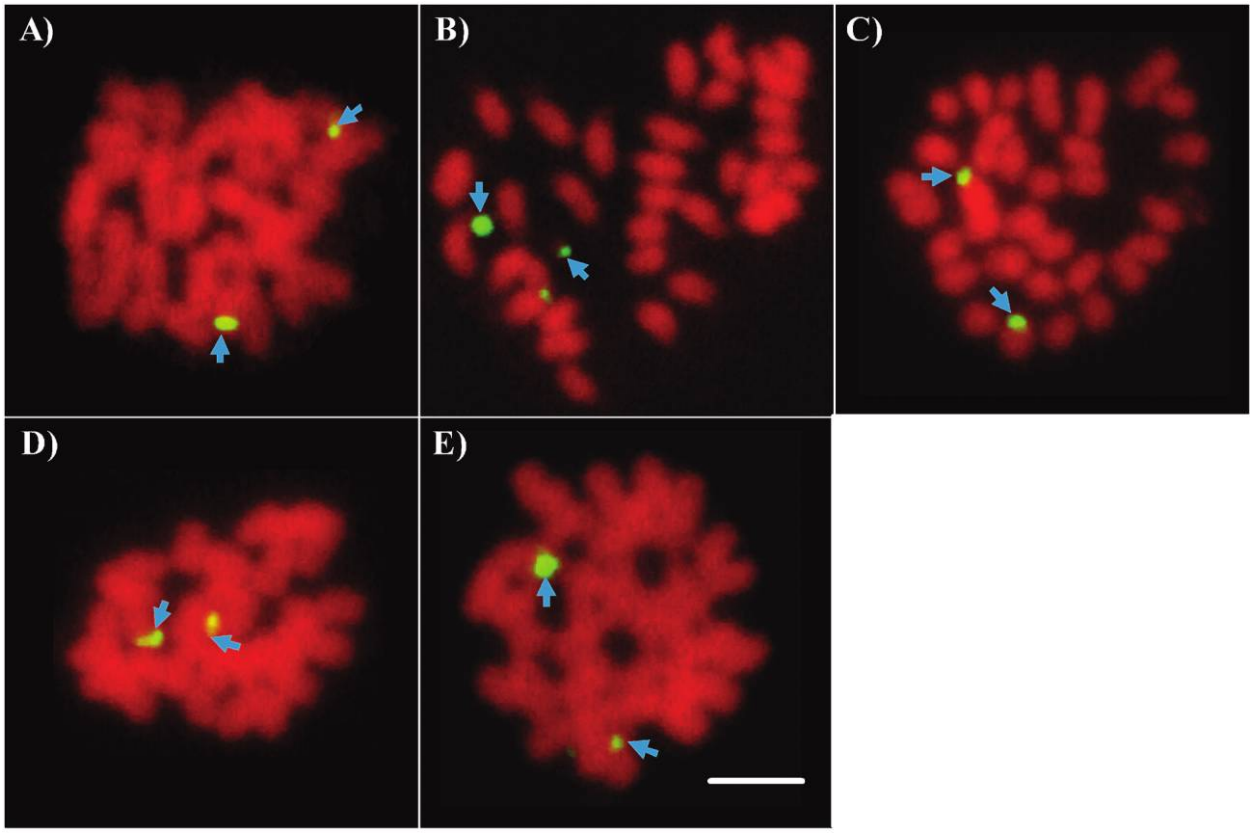

Fig. 1 Results of fluorescent in situ hybridization using an 18S-nrDNA PCR probe on *Agalmyla*. *A*, *A. parasitica* (2n=32). *B*, *A. chalmersii* (2n=32). *C*, *A. clarkei* (2n=32). *D*, *A. glabra* (2n=32). *E*, *A. paucipilosa* (2n=32). Blue arrows indicate 45S-nrDNA loci. Scale bar = 10 μm.

Discussion

Contrasting patterns of genome and nrDNA locus evolution among the three Gesneriaceae genera examined became evident in our study.

Dysploidy and Polyploidy

Agalmyla shows a constant diploid chromosome number, while *Streptocarpus* has a clade-specific basic number of x=15 for clade I and x=16 for clade II (fig. 5; Jong and Möller 2000; Möller and Cronk 2001*a*); however, the ancestral basic chromosome number for the genus cannot be ascertained from our data. *Aeschynanthus*, though, has a highly variable basic chromosome number within each of the clades (fig. 4; Denduangboripant et al. 2001; Rashid et al. 2001). In this genus, the ancestral basic number is indicated as x=16, with dysploid reductions having occurred from x=16 to x=15 and x=14 (fig. 4).

Compared with *Streptocarpus* (six polyploids out of 64 species analyzed; Möller et al. 2002–; Briggs 2004), the frequency of polyploids in *Aeschynanthus* is relatively high, with about a quarter of the species cytologically examined (nine out of 37 species analyzed; Rashid et al. 2001). Diploid taxa often occur together with polyploid ones in the same clade in phylogenetic analyses (Denduangboripant and Cronk 2000), indicating that the evolution of polyploidy is relatively common in this genus. There is as yet no obvious explanation for the high frequency of polyploids in *Aeschynanthus* and their absence in *Agalmyla*, despite their similar evolutionary age (table 4).

45S-nrDNA Locus Evolution

Diploid organisms possess at least one 45S-nrDNA locus in each set of chromosomes (genomes). This study examines the variation and evolution of such a locus. Either two or four 45S-nrDNA loci were observed across all diploid species analyzed by FISH, and we infer these as originating from genomes possessing either one or two loci. For example, in *Aeschynanthus*, the two polyploid species analyzed, the tetraploids *A. guttatus* and *A. nummularius*, possess four 45S-nrDNA loci, i.e., one locus per genome.

Aeschynanthus shows a high variability in chromosome number, though it has a simpler pattern of 45S-nrDNA locus variation compared with *Streptocarpus*; in the latter, several parallel within-genome gains are deduced, whereas in *Aeschynanthus*, the number of loci in each genome is constant within the two major clades (all taxa analyzed in clade I have two loci in each genome, and taxa in clade II have one locus in each genome). As noted above, polyploid *Aeschynanthus* species show one 45S-nrDNA locus in each genome, as do diploid ones within clade II. Apparently, the polyploid species have not lost a locus as has been observed in other polyploids (*Scilla*, Vaughan et al. 1993; *Thinopyrum*, Li and Zhang 2002).

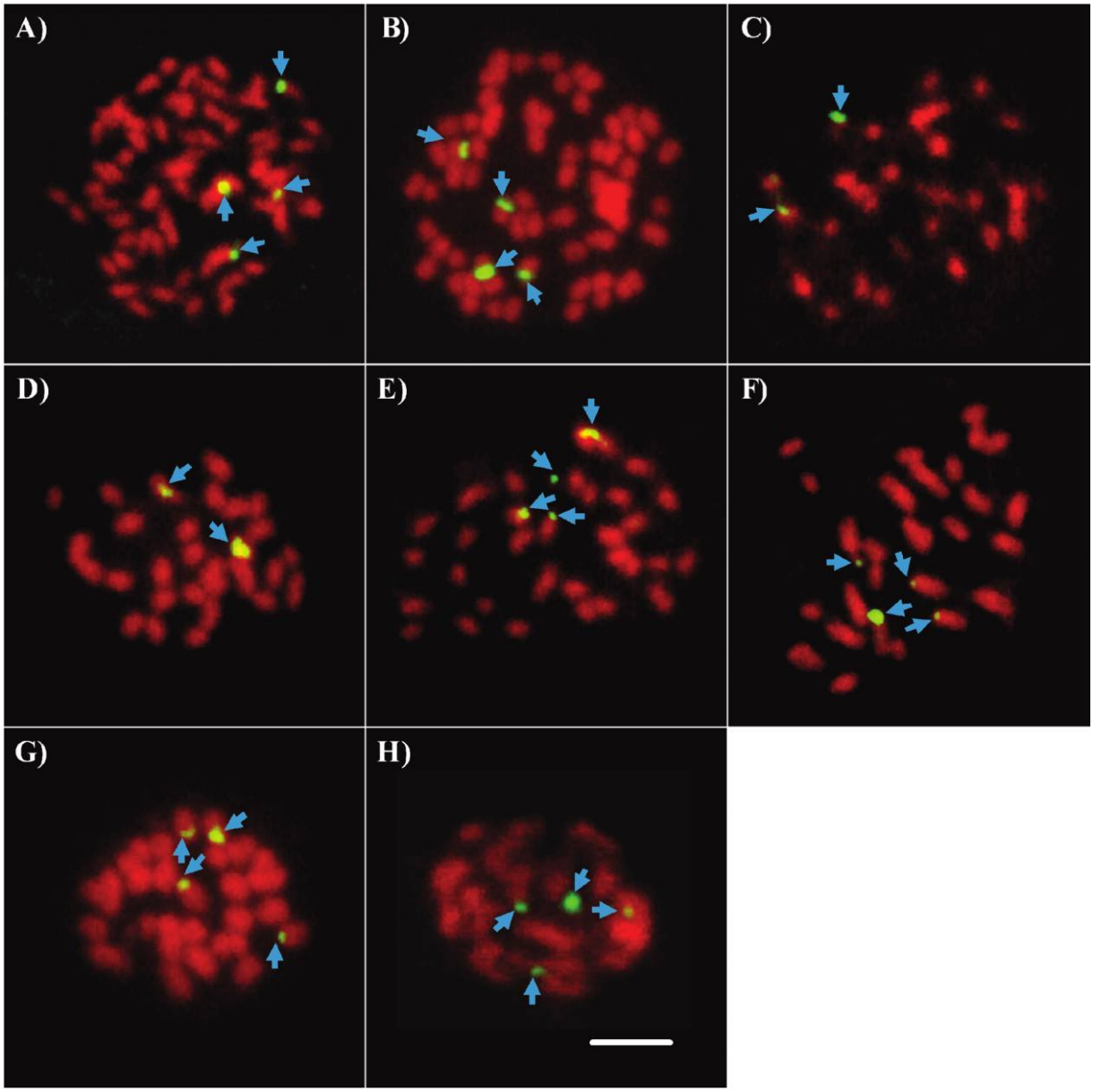

Fig. 2 Results of fluorescent in situ hybridization using an 18S-nrDNA PCR probe on *Aeschynanthus*. *A*, *A. guttatus* (2n=64). *B*, *A. nummularius* (2n=64). *C*, *A. rhododendron* (2n=32). *D*, *A. radicans* (2n=30). *E*, *A. sikkimensis* (2n=32). *F*, *A. longicaulis* (2n=30). *G*, *A. angustifolius* (2n=30). *H*, *A. gracilis* (2n=28). Blue arrows indicate 45S-nrDNA loci. Scale bar = 10 μm.

Members of the Gesneriaceae investigated here exhibit a comparatively low level of nrDNA site evolution compared, e.g., with *Nemesia* in Scrophulariaceae, a closely related family. In that genus, 12 changes to the distribution of 45S nrDNA site were estimated, involving multiple moves, gains, and losses, over an apparently short evolutionary period of 14–18 Myr (Datson and Murray 2006), an age that is younger than the estimated ages of the genera in this study (table 4).

Aeschynanthus appears to differ significantly in the timing of intragenomic changes in nrDNA loci compared with *Streptocarpus*. In *Aeschynanthus*, the change apparently occurred early in the diversification of the genus, probably at the time of divergence into the two major clades (fig. 4). In *Streptocarpus*, we cannot make firm inferences about the state of 45S-nrDNA sites early in its evolution because FISH data are available for only one representative of clade I, namely, *S. stomandrus*. In clade II, however, several parallel gains have occurred relatively recently in the evolution of the genus, mostly in the Cape Primrose clade. This is seen in closely related pairs of species possessing either one or two sites in each genome (*S. kentaniensis* [one site] and *S. cyaneus* [two sites], *S. baudertii* [one site] and *S. rexii* [two sites], *S. johannis* [one site] and *S. primulifolius* [two sites]), a situation comparable with that found in *Paeonia* (Zhang and Sang 1999). The changes in the number of 45S-nrDNA sites in

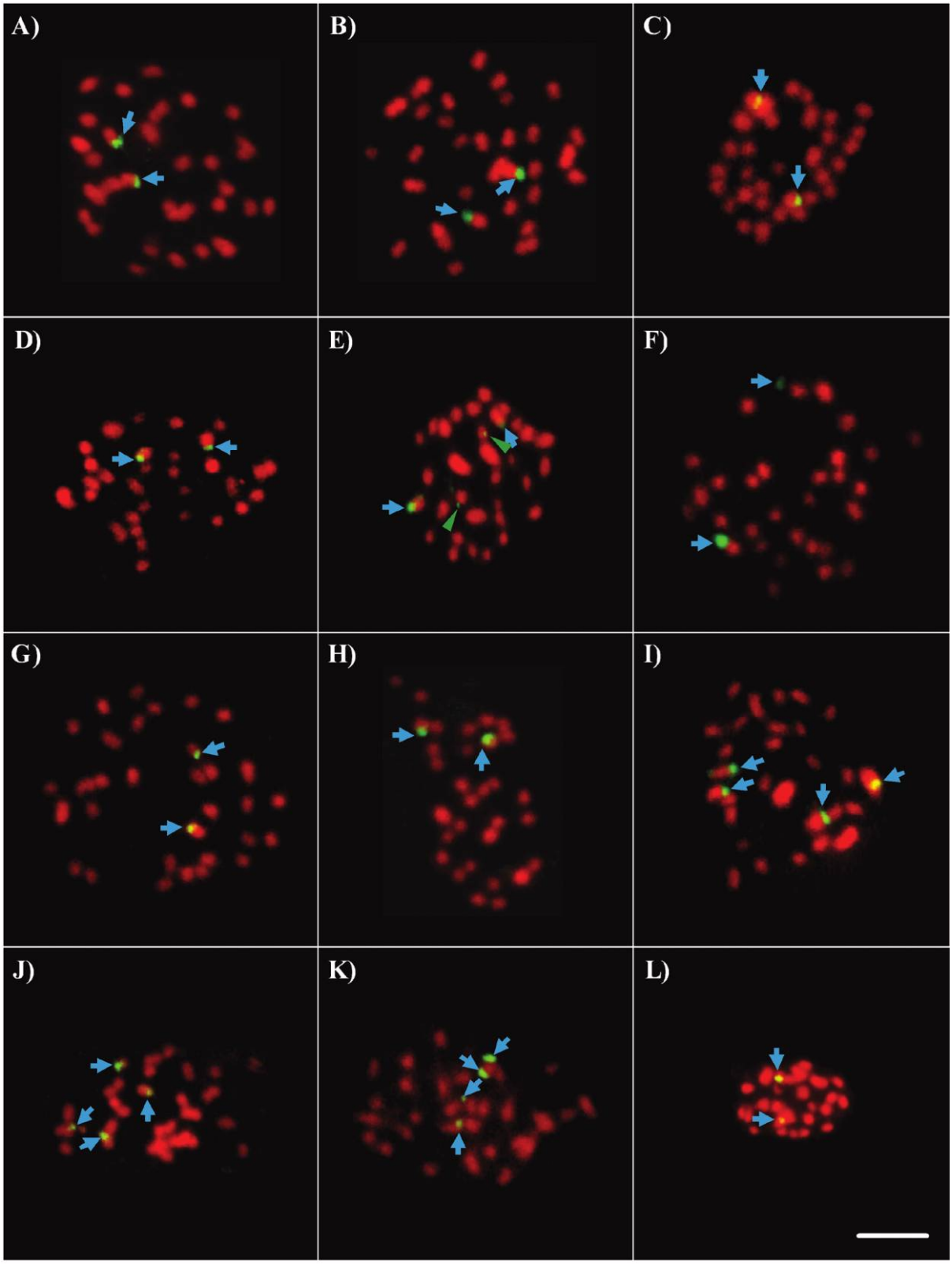

Fig. 3 Results of fluorescent in situ hybridization (FISH) using an 18S-nrDNA PCR probe on *Colpogyne betsiliensis* (*A*) and *Streptocarpus* (*B–K*). *B*, *S. papangae* (2n=32). *C*, *S. polyanthus* (2n=32). *D*, *S. dunnii* (2n=32) with two FISH signals. *E*, *S. dunnii* with four nrDNA signals. *F*, *S. kentaniensis* (2n=32). *G*, *S. johannis* (2n=32). *H*, *S. baudertii* (2n=32). *I*, *S. cyaneus* (2n=32). *J*, *S. rexii* (2n=32). *K*, *S. primulifolius* (2n=32). *L*, *S. stomandrus* (2n=30). Blue arrows indicate 45S-nrDNA loci. Dark green arrowheads in *E* indicate minute loci in *S. dunnii*. Scale bar = 10 μm.

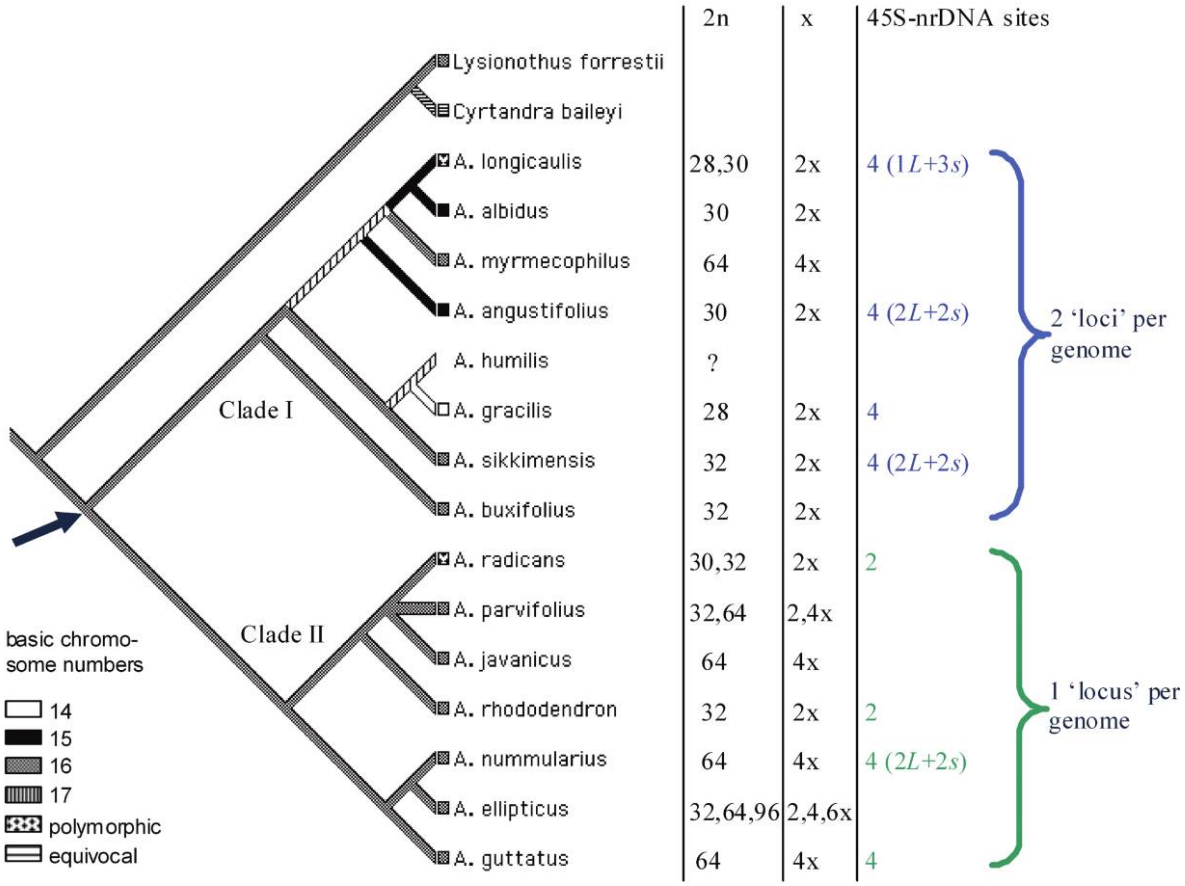

Fig. 4 Simplified internal transcribed spacer phylogeny of *Aeschynanthus* based on Denduangboripant et al. (2001), onto which the basic chromosome numbers are optimized using MacClade (Maddison and Maddison 2003; shaded branches). Somatic chromosome numbers (2n), ploidy levels (x), and 45S-nrDNA locus number are indicated on the right. Arrow indicates hypothesized site of nrDNA locus number change early in the evolution of the genus. L = large fluorescent in situ hybridization (FISH) signal; s = small FISH signal.

clade II are clearly not linked to the dysploid alteration in the basic chromosome numbers that happened early on in the evolution of *Streptocarpus* (fig. 5).

Support for a difference in the timing of changes of 45S-nrDNA sites in the Gesneriaceae genera investigated comes from sequencing: PCR amplification using universal primers and direct sequencing of nrDNA internal transcribed spacer (ITS) sequences in members of *Streptocarpus* in the Cape Primrose clade, whether the species possess one or two loci in each genome, is straightforward, revealing only two single-site polymorphisms in ITS sequences (0.4% divergence; Hughes et al. 2005), which may be explained by incomplete intralocus homogenization.

Table 4

Summary of Genome Characteristics, Chromosome Numbers, nrDNA Sites, ITS Divergence, and Estimates of Maximum Divergence Times of the Genera of Gesneriaceae Analyzed

Genus	Chromosome numbers	nrDNA sites per genome in clades	Maximum molecular divergence	
			ITS (%)	Myr[a]
Aeschynanthus	Variable	Constant	16.9[b]	24.0 ± 2.8
Agalmyla	Constant	Constant	14.1[c]	20.0 ± 2.4
Streptocarpus	Clade constant	Variable	23.5[d]	33.5 ± 3.9

Note. ITS = internal transcribed spacer.
[a] Based on average rates of ITS evolution (Richardson et al. 2001).
[b] Denduangboripant et al. (2001).
[c] Chapman (2003).
[d] Möller and Cronk (2001*b*).

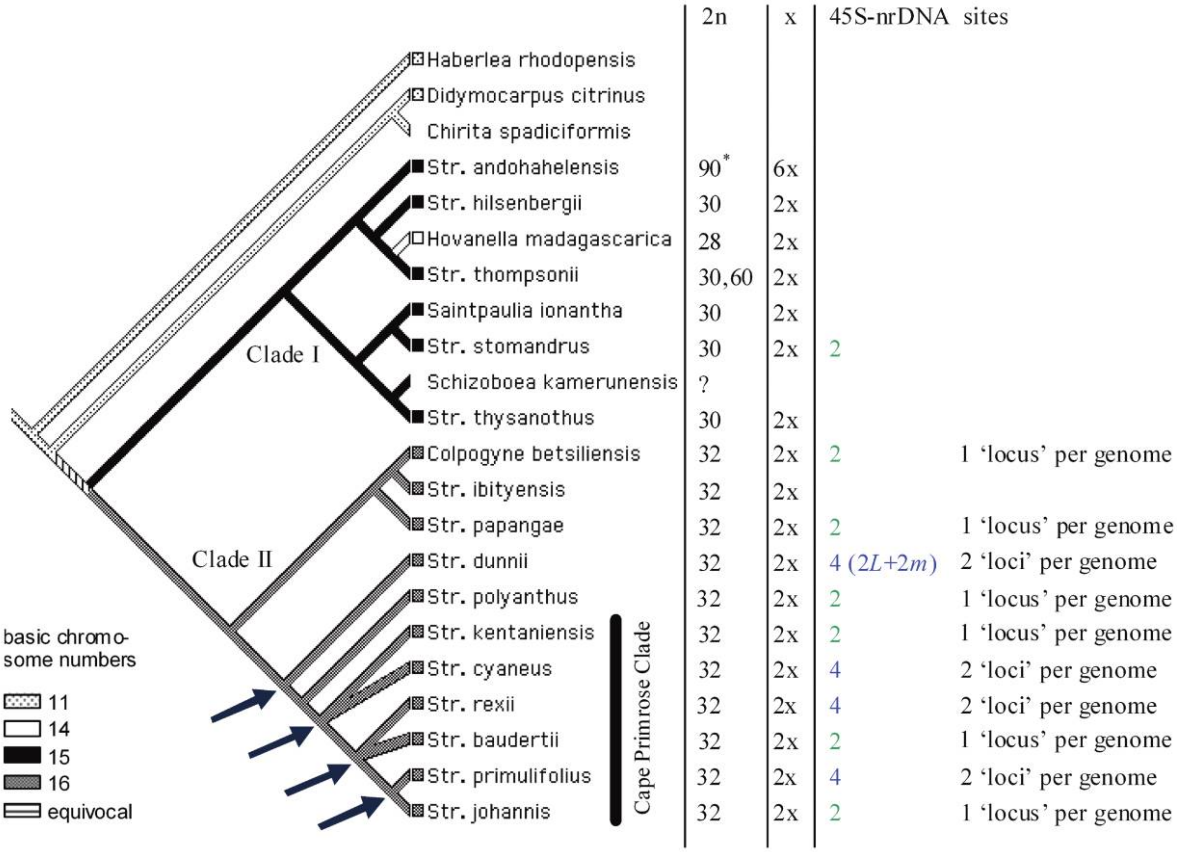

Fig. 5 Simplified internal transcribed spacer phylogeny of *Streptocarpus* based on Möller and Cronk (2001*a*, 2001*b*) and Möller and Kiehn (2004), onto which the chromosome numbers are optimized using MacClade (Maddison and Maddison 2003; shaded branches). Somatic chromosome numbers (2n), ploidy levels (x), and 45S-nrDNA loci number are indicated on the right. Arrows indicate sites of hypothesized nrDNA locus gains late in the evolution of the genus. L = large fluorescent in situ hybridization (FISH) signal; m = minute FISH signal. * = from Briggs (2004).

This indicates that all intraspecific 45S-nrDNA loci are very similar in these species and that the gain has indeed occurred relatively recently.

In *Aeschynanthus*, however, direct sequencing is not possible because of the presence of high intraindividual ITS length variation among cloned ITS fragments that show up to 5.01% sequence divergence (*A. guttatus*; Denduangboripant and Cronk 2000). Denduangboripant and Cronk (2000) propose a low molecular drive to explain this high divergence and argue for an origin of clone divergence that does not significantly predate the divergence of the species. Support was given by the observation that most intraindividual ITS clones formed sister groups in phylogenetic trees. Our data for *Aeschynanthus*, however, indicate a long persistence since the split into the two main clades ~24 Myr ago of two 45S-nrDNA loci on nonhomologous chromosomes within a genome. A low molecular drive between these loci should have allowed high levels of divergence to develop, with paralogue copies not forming sister pairs. To reconcile this apparent paradox, we propose a high molecular drive (Arnheim 1983; Dover 1994; Elder and Turner 1995) with frequent interlocus exchanges (Arnheim et al. 1980; Wendel et al. 1995; Rauscher et al. 2004) that resulted in sequence similarities of 45S-nrDNA copies of the two loci at levels that would maintain the sister relationships of individual copies.

Not much is known about the mechanisms by which the number of nrDNA loci varies or the nature of these variations. Differential increases or reductions at different locations in the number of nrDNA units above or below levels detectable by FISH may account for some variation in loci number (Datson and Murray 2006). Variation in FISH signal size has been observed within most species studied here, irrespective of whether two or four FISH signals are present (figs. 1–3). These size differences are unlikely to be artifacts because they are seen in most of the cells observed. These differences may indeed reflect variation in nrDNA copy number (Zurita et al. 1997). This has also recently been shown to be the case for *Streptocarpus* (Denduangboripant et al. 2007). Whether this can be linked to function or quality of the 45S-nrDNA alleles is at present unclear and would require additional parallel analyses such as silver staining (Neves et al. 1995) to reveal whether or not these nrDNA sites are transcribed.

Our data on genome and nrDNA evolution presented here for *Aeschynanthus* indicate an interplay of an ancient intragenomic nrDNA locus change, repeated polyploidizations, and frequent

interchanges and homogenization between nonhomologous chromosomes, coupled with several unrelated dysploid reductions in basic chromosome numbers. In *Streptocarpus*, the basic numbers are found to be more stable, but the genus underwent parallel duplications in 45S-nrDNA sites, while *Agalmyla*, closely related to *Aeschynanthus* (M. Möller et al., unpublished manuscript), is uniform in both chromosome number and 45S-nrDNA sites. Interestingly, the genus *Conandron*, another Asian genus, shows the highest variation in nrDNA among the Gesneriaceae genera analyzed to date, with different populations varying in 45S-nrDNA locus number (Kokubugata and Peng 2002).

This analysis of three genera of the Gesneriaceae indicates that very different patterns of nrDNA and genome evolution exist within a single plant family and that these are not necessarily correlated with chromosome or genome change. The variation appears to differ notably from genus to genus, irrespective of their taxonomic affinities. Furthermore, the evolutionary history of the genera seems to have no effect on the changes in nrDNA patterns observed because lineages of comparable age show great variation.

Acknowledgments

We thank the Royal Botanic Garden Edinburgh (RBGE) horticulture staff, especially Steve Scott and Andy Ansell, for their role in the maintenance of the living research collection and the provision of study plants. We also gratefully acknowledge the RBGE Sibbald Trust and the project Integration of Systematics and Molecular Phylogenetics in All Groups of Organisms of the National Museum of Nature and Science for funding study visits of G. Kokubugata to the RBGE. The RBGE is supported by the Scottish Government Rural and Environment Research and Analysis Directorate.

Literature Cited

Arnheim N 1983 Concerted evolution of multigene families. Pages 38–61 *in* M Nei, RK Koehn, eds. Evolution of genes and proteins. Sinauer, Sunderland, MA.

Arnheim N, M Krystal, R Schmickel, G Wilson, O Ryder, E Zimmer 1980 Molecular evidence for genetic exchanges among ribosomal genes on non-homologous chromosomes in man and apes. Proc Natl Acad Sci USA 77:7323–7327.

Briggs M 2004 The taxonomy of Madagascan *Saintpaulia*-like *Streptocarpus*. MSc thesis. University of Edinburgh.

Chapman A 2003 Relationships in the genus *Agalmyla* (Gesneriaceae), inferred from molecular, morphological and cytological data. MSc thesis. University of Edinburgh.

Copenhaver GP, CS Pikaard 1996 Two-dimensional RFLP analyses reveal megabase-sized clusters of rRNA gene variants in *Arabidopsis thaliana*, suggesting local spreading of variants as the mode for gene homogenization during concerted evolution. Plant J 9:273–282.

Datson PM, BG Murray 2006 Ribosomal DNA locus evolution in *Nemesia*: transposition rather than structural rearrangement as the key mechanism? Chromosome Res 14:845–857.

Denduangboripant J, QCB Cronk 2000 High intraindividual variation in internal transcribed spacer sequences in *Aeschynanthus* (Gesneriaceae): implications for phylogenetics. Proc R Soc B 267: 1407–1415.

Denduangboripant J, QCB Cronk, G Kokubugata, M Möller 2007 Variation and inheritance of nuclear ribosomal DNA clusters in *Streptocarpus* (Gesneriaceae) and their biological and phylogenetic implications. Int J Plant Sci 168:455–467.

Denduangboripant J, M Mendum, QCB Cronk 2001 Evolution in *Aeschynanthus* (Gesneriaceae) inferred from ITS sequences. Plant Syst Evol 228:181–197.

Dover G 1994 Concerted evolution, molecular drive and natural selection. Curr Biol 4:1165–1166.

Elder JF Jr, BJ Turner 1995 Concerted evolution of repetitive DNA sequences in eukaryotes. Q Rev Biol 70:297–320.

Hilliard OM, BL Burtt 2002 The genus *Agalmyla* (Gesneriaceae-Cyrtandroideae). Edinb J Bot 59:1–210.

Hughes M, M Möller, DU Bellstedt, TJ Edwards 2005 Refugia, dispersal and divergence in a forest archipelago: a study of *Streptocarpus* in eastern South Africa. Mol Ecol 14:4415–4426.

Jong K, M Möller 2000 New chromosome counts in *Streptocarpus* (Gesneriaceae) from Madagascar and the Comoro Islands and their taxonomic significance. Plant Syst Evol 224:173–182.

Kokubugata G, CI Peng 2002 Taxonomic study of *Conandron ramondioides* from Japan and Taiwan. Page 29 *in* M Möller, M Mendum, QCB Cronk, eds. Gesneriaceae workshop (abstracts). Royal Botanic Garden Edinburgh, Edinburgh.

Lavania UC, S Basu, S Srivastava, Y Mukai, S Lavania 2005 In situ chromosomal localization of rDNA sites in "Safed Musli" *Chlorophytum* Ker-Gawl and their physical measurement by fiber FISH. J Hered 96:155–160.

Li DY, XY Zhang 2002 Physical localization of the 18S-5.8S-26S rDNA and sequence analysis of ITS regions in *Thinopyrum ponticum* (Poaceae: Triticeae): implications for concerted evolution. Ann Bot 90:445–452.

Lim KY, R Matyásek, CP Lichtenstein, AR Leitch 2000 Molecular cytogenetic analysis and phylogenetic studies in the *Nicotiana* section *Tomentosae*. Chromosoma 109:245–258.

Maddison DR, WP Maddison 2003 MacClade, version 4.06. Sinauer, Sunderland, MA.

Marcon AB, ICL Barros, M Guerra 2005 Variation in chromosome numbers, CMA bands and 45S rDNA sites in species of *Selaginella* (Pteridophyta). Ann Bot 95:271–276.

Möller M 2003 Gesneriaceae. Pages 421-425 *in* SM Goodman, JP Benstead, eds. The natural history of Madagascar. University of Chicago Press, Chicago.

Möller M, QCB Cronk 2001*a* Evolution of morphological novelty: a phylogenetic analysis of growth patterns in *Streptocarpus* (Gesneriaceae). Evolution 55:918–929.

——— 2001*b* Phylogenetic studies in *Streptocarpus* (Gesneriaceae): reconstruction of biogeographic history and distribution patterns. Syst Geogr Plants 71:545–555.

Möller M, M Kiehn 2004 A synopsis of cytological studies in Gesneriaceae. Edinb J Bot 60:425–447.

Möller M, M Pullan, M Kiehn, LE Skog 2002– RBGE "WebCyte"—Gesneriaceae cytology database. http://elmer.rbge.org.uk/webcyte/webcyteintro.php.

Neves N, JS Heslop-Harrison, W Viegas 1995 rRNA gene activity and control of expression mediated by methylation and imprinting during embryo development in wheat × rye hybrids. Theor Appl Genet 91:529–533.

O'Sullivan S 1999 Molecular phylogenetics of Madagascan *Streptocarpus* (Gesneriaceae) using chloroplast *trn*L-F data. MSc thesis. University of Edinburgh.

Rashid MH, K Jong, M Mendum 2001 Cytotaxonomic observations in the genus *Aeschynanthus* (Gesneriaceae). Edinb J Bot 58:31–43.

Rauscher JT, JJ Doyle, AHD Brown 2004 Multiple origins and nrDNA internal transcribed spacer homologue evolution in the *Glycine tomentella* (Leguminosae) allopolyploid complex. Genetics 166:987–998.

Richardson JE, RT Pennington, TD Pennington, PM Hollingsworth 2001 Rapid diversification of a species-rich genus of Neotropical rain forest trees. Science 293:2242–2245.

Rogers SO, AJ Bendich 1987 Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. Plant Mol Biol 9:509–520.

Schwarzacher T, P Heslop-Harrison 2000 Practical *in situ* hybridization. BIOS Scientific, Oxford.

Vaughan HE, M Jamilena, C Ruiz Rejón, JS Parker, MA Garrido-Ramos 1993 Loss of nucleolus organizer regions during polyploid evolution in *Scilla autumnalis*. Heredity 71:574–580.

Weber A 2004 Gesneriaceae. Pages 63–158 *in* JW Kadereit, ed. Flowering plants: dicotyledons: Lamiales (except Acanthaceae including Avicenniaceae). Vol 7 of K Kubitzki, ed. The families and genera of vascular plants. Springer, New York.

Wendel JF, A Schnabel, T Seelanan 1995 Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). Proc Natl Acad Sci USA 92:280–284.

Zhang D, T Sang 1999 Physical mapping of ribosomal RNA genes in peonies (*Paeonia*, Paeoniaceae) by fluorescent in situ hybridization: implications for phylogeny and concerted evolution. Am J Bot 86: 735–740.

Zurita F, A Sánchez, M Burgos, R Jiménez, R Díaz de la Guardia 1997 Interchromosomal, intercellular and interindividual variability of NORs studied with silver staining and *in situ* hybridization. Heredity 78:229–234.