Published in Plant Systematics and Evolution 210, issues 3-4, 231-247, 1998
which should be used for any reference to this work

Chromosomal evolution of *Gentiana* and *Jaeschkea* (*Gentianaceae*), with further documentation of chromosome data for 35 species from western China

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Key words: *Gentianaceae, Gentiana, Jaeschkea*. – Polyploidization, dysploidization, karyotype, chromosome number, cytotype, cytotaxonomy, cytogeography.

Abstract: Chromosome numbers were recorded for 63 populations of 34 species belonging to the genus *Gentiana* from the high altitude regions of western China. Counts for 22 species were reported for the first time and new numbers were found for *G. heleonastes* ($2n = 36$), *G. prattii* ($2n = 20$) and *G. pseudoaquatica* ($2n = 40$). Incorporating previous data, a complete series of gametic chromosome numbers from $n = 6$ to 24 and 26 was established for the genus, suggesting rapid karyotypic evolution by a combination of dysploidy and polyploidy. The cytotype $2n = 20$ is proposed as the ancestral type in sect. *Chondrophyllae* s. 1. The chromosome number $2n = 16$ was found for *Jaeschkea microsperma* for the first time which, with previous reports of $2n = 18$, 20 and 22, indicates that *Jaeschkea* is a typically dysploid genus.

Gentiana L. is a very diversified genus, which is concentrated mainly in the mountainous regions of south-western China and the adjacent north-eastern Burma (Ho & Liu 1990) and is chromosomally polybasic. Following our previous karyological observations on this genus (Yuan 1993, Yuan & Küpfer 1993a, Küpfer & Yuan 1996), this paper contributes documentation of chromosome numbers and karyotype data for more species from China, especially those from the poorly-studied regions, the high-altitude Qinghai-Tibet Plateau and the adjacent Himalayas. The systematic and karyological background of the sect. *Cruciata* Gaudin, *Chondrophyllae* Bunge s. str. (excluding sect. *Dolichocarpa* T. N. Ho), *Frigidae* Kusnez., *Kudoa* (Masamune) Satake & Toyokuni ex Toyokuni (= *Monopodiae* T. N. Ho) and *Microsperma* T. N. Ho have been reviewed in our previous reports, but that of sect. *Dolichocarpa* T. N. Ho should be mentioned here. This section, established by Ho (1985), was attached to the group of species with elongated cylindrical capsules belonging to sect. *Chondrophyllae* Bunge s. 1., and 14 species were included (Ho & Pringle 1995). Except for the difference in capsule shape, almost all the other morphological aspects and the geographical distribution of the new section overlap that of sect. *Chondrophyllae* s. str.

Karyological observations have been made for three species of this group: *G. prostrata* HAENKE (2n = 32–36, JOHNSON & PACKER 1968; 2n = 36, LÖVE & LÖVE 1975a, 1986; ZHUMOVA 1982), *G. nutans* BUNGE (2n = 36, KROGULEVICH 1976, 1978) and *G. sedifolia* KUNTH (n = 20, WEAVER & RUDENBERG 1975). The other nine species are exclusively restricted to western China and the adjacent Himalayan area and none of them has been investigated cytologically.

In the course of our investigations on the genus *Gentiana*, we also examined some related genera (YUAN & KÜPFER 1993b). We have included in this paper a karyological observation on *Jaeschkea microsperma* C. B. CLARKE. *Jaeschkea* KURZ. is a small genus, endemic to the Himalayan area and comprising three species. Chromosome numbers have been reported for *J. gentianoides* KURZ. (2n = 18, KOUL & GOHIL 1973; n = 11, GOHIL & al. 1981) and *J. canaliculata* (ROYLE ex D. DON) KNOBL. (= *J. latisepala* CLARKE) (n = 10; MEHRA & VASUDEVAN 1972, VASUDEVAN 1975). Chromosome data for *Jaeschkea microsperma* are still lacking.

Materials and methods

The origins of the materials examined are listed in Table 1. The voucher specimens are deposited in the herbarium of the University of Neuchâtel (NEU), Switzerland. Seeds collected in the field were hydrated in 0.4% gibberellic acid (GA_3) for 24 hours to break dormancy, and then germinated on wet filter papers in Petri dishes. Root tips were pretreated with saturated aqueous alpha-bromonaphthalene for 105 min at room temperature and fixed with absolute ethanol and acetic acid (3:1). The squashing procedure was the same as that described before (YUAN 1993).

The terminology for centromere positions introduced by LEVAN & al. (1964), the karyotype classification of STEBBINS (1971) and the karyotype asymmetry indices defined by ROMERO ZARCO (1986) were followed. The idiograms of the karyotypes were based on the relative length of chromosomes measured from one to five cells.

Results

Chromosome numbers. The chromosome counts are given in Table 1. The metaphase chromosomes of most species studied are illustrated in Figs. 1–30. In sect. *Chondrophyllae* s. str., in addition to seven species where previous reports of chromosome numbers were confirmed, the chromosome numbers of 11 species are documented for the first time. Some numbers differing from previous reports were also found: 2n = 36 for *G. heleonastes* which had been counted previously as 2n = 12 (KÜPFER & YUAN 1996); 2n = 20 for *G. prattii* compared with 2n = 18 (KÜPFER & YUAN 1996), and both diploid (2n = 20) and tetraploid (2n = 40) cytotypes were found for *G. pseudoaquatica* for which only the diploid (n = 10) cytotype had been found before (KÜPFER & YUAN 1996). A new number both for the section and the genus, 2n = 60, was found in *G. micantiformis*. Intraspecific polyploidy was found in *G. leucomelaena* and *pseudoaquatica*. The diploid type of *G. leucomelaena* (2n = 18) was found in the higher alpine meadows of the Qinghai-Tibet Plateau, while the tetraploid type (2n = 36) was found in the lower alpine meadows east of the diploid cytotype, but in *G. pseudoaquatica* the diploid type was found in the lower eastern plateau while the tetraploid type was found in the higher western regions. Morphologically, the diploid cytotypes of both species

Table 1. The origin and chromosome numbers of materials examined. * The first documentation of chromosome number; ! new chromosome number different from previous reports

Taxon	Coll no.	Locality, altitude (a. s. l.)	Coordinate	2n	Fig.
***Gentiana* sect. *Chondrophyllae* Bunge s. str.**					
G. aristata Maxim.	92-11	Qinghai: Lajit Mt., 4000 m	36°20′N 101°26′E	14	
	92-332	Qinghai: Datung, 3000 m	36°57′N 101°46′E	14	
G. asterocalyx Diels	92-226	Yunnan: Lijiang, 2900 m	27°24′N 100°26′E	20	
**G. burkilii* H. Smith	92-82	Tibet: Langkazi, 5000 m	28°53′N 90°09′E	14	1
**G. crassula* H. Smith	92-238	Yunnan: Lijiang, 4300 m	27°25′N 100°25′E	20	
**G. crassuloides* Bureau & Franchet	92-183	Yunnan: Baimaxue Mt., 4100 m	28°24′N 98°59′E	40	
	92-265	Shaanxi: Taibai Mt., 2900 m	33°40′N 107°45′E	40	2
	92-278	Sichuan: Sungpan, 3500 m	32°56′N 103°50′E	40	
**G. flexicaulis* H. Smith ex Marq.	92-264	Shaanxi: Taibai Mt., 3400 m	33°40′N 107°45′E	14	3
**G. haynaldii* Kanitz	92-121	Tibet: Dangxiong, 4600 m	30°34′N 91°25′E	20	4
	92-158	Tibet: Dingqing, 4250 m	31°07′N 96°17′E	20	
!*G. heleonastes* H. Smith ex Marq.	92-309b	Gansu: Maqü, 3900 m	34°00′N 102°10′E	36	5
**G. leucomelaena* Maxim.	92-39	Qinghai: Kunlun Pass, 4700 m	35°42′N 94°03′E	18	
	92-45	Qinghai: Erdaogou 4700 m	34°50′N 92°45′E	18	
	92-92	Tibet: Dingri, 4500 m	28°46′N 86°11′E	18	6
	92-127	Tibet: Dangxiong, 4500 m	30°34′N 91°25′E	18	
	92-13	Qinghai: Laji Mt., 4100 m	36°20′N 101°25′E	36	
	92-34	Qinghai: Xiangpi Mt., 3850 m	36°45′N 99°36′E	36	7
	92-325	Sichuan: Ruoergai, 4200 m	33°55′N 102°50′E	36	
	92-330	Gansu: Maqü, 3900 m	34°01′N 102°10′E	36	
**G. mairei* Lévl.	92-235	Yunnan: Lijang, 3200 m	26°52′N 100°01′E	20	
**G. micantiformis* Burk.	92-50	Tibet: Dangxiong, 4700 m	30°36′N 91°31′E	60	8
**G. pantheica* Prain & Burk.	92-248	Yunnan: Dali, 3200 m	25°58′N 100°01′E	20	
G. piasezkii Maxim.	92-272	Gansu: Mingxian, 2900 m	34°30′N 104°00′E	36	
!*G. prattii* Kusnez.	92-22	Qinghai: Laji Mt., 3800 m	36°20′N 101°26′E	20	9
	92-324	Sichuan: Sungpan, 3700 m	32°56′N 103°50′E	20	
	92-303	Qinghai: Jiuzhi, 3800 m	33°42′N 101°35′E	20	10
G. pseudoaquatica Kusnez.	92-32	Qinghai: Heimahe, 3400 m	36°47′N 99°40′E	20	
	92-157	Tibet: Dingqing, 4250 m	31°07′N 96°17′E	20	11
	92-276	Gansu: Luqü, 3600 m	34°56′N 102°40′E	20	
	92-326	Gansu: Maqü, 3800 m	34°01′N 102°10′E	20	
	92-53	Tibet: Dangxiong, 4700 m	30°36′N 91°31′E	40	
	92-53	Tibet: Yanbajin, 4300 m	30°05′N 90°34′E	40	
	92-91	Tibet: Dingri, 4500 m	28°46′N 86°11′E	40	12
	92-69	Tibet: Yangbajin, 4700 m	30°08′N 90°30′E	40	13
**G. pseudosquarrosa* H. Smith	92-321	Sichuan: Sungpan, 3400 m	32°56′N 103°50′E	20	14
G. squarrosa Ledeb.	92-292	Gansu: Luqü, 3700 m	34°56′N 102°40′E	38	
G. subintricata T. N. Ho	92-198	Yunnan: Zhongdian, 3200 m	27°43′N 99°43′E	20	
**G. tricolor* Diels & Gilg	92-31	Qinghai: Qinghai Lake, 3200 m	36°38′N 100°05′E	18	15
***Gentiana* sect. *Cruciata* Gaudin**					
G. crassicaulis Duthie ex Burk.	92-188	Yannan: Zhongdian, 3300 m	27°43′N 99°43′E	26	
	92-225	Yunnan: Lijiang, 3000 m	26°52′N 100°01′E	26	
**G. lhassica* Burk.	92-162	Tibet: Dingqing, 4200 m	31°07′N 96°17′E	26	16
**G. robustra* King ex Hook. f.	92-57	Tibet: Lhasha, 3500 m	29°58′N 90°42′E	52	
	92-74	Tibet: Langkazi, 4600 m	29°14′N 90°20′E	52	

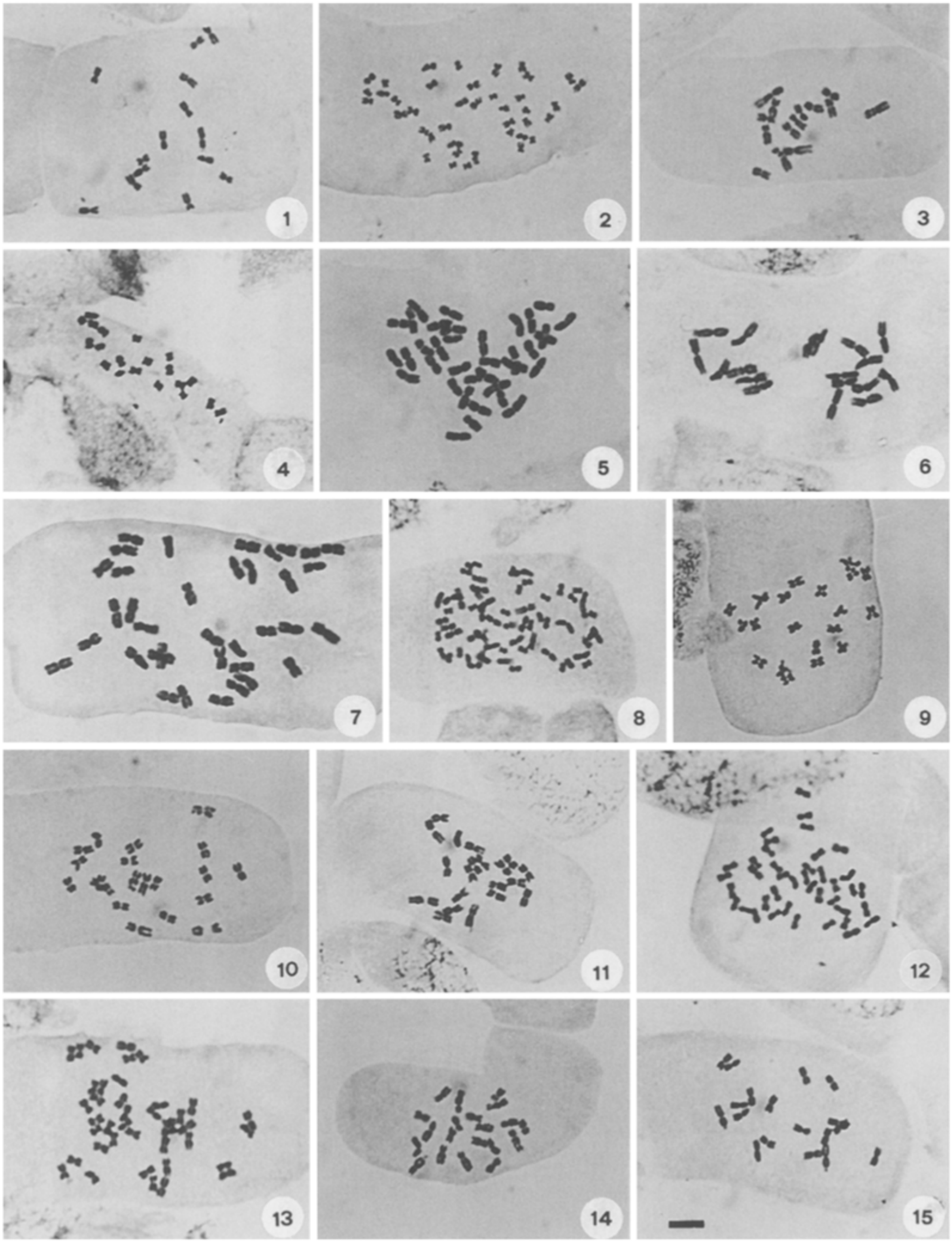
Table 1 (continued)

Taxon	Coll no.	Locality, altitude (a. s. l.)	Coordinate	2n	Fig.
**G. siphonantha* Maxim. ex Kusnez.	92-331	Qinghai: Datung, 3000 m	36°57′N 101°46′E	26	17
!*G. straminea* Maxim.	92-29	Qinghai: Riyue Mt., 3340 m	36°27′N 101°07′E	52	
	92-153	Tibet: Baqin, 4200 m	31°50′N 94°19′E	26	18
G. tibetica King ex Hook. f.	92-165	Tibet: Changdu, 3650 m	30°42′N 97°18′E	52	
	92-174	Tibet: Mangkang, 4200 m	29°17′N 98°40′E	52	19
Gentiana* sect. *Dolichocarpa T. N. Ho					
**G. crenulato-truncata* (MARQ.) T. N. Ho	92-46	Qinghai: Erdaogou, 4700 m	34°50′N 92°55′E	18	20
**G. hyalina* T. N. Ho	92-19	Qinghai: Laji Mt., 3900 m	36°20′N 101°26′E	14	21
	92-37	Qinghai: Xiangpi Mt., 3800 m	36°45′N 99°36′E	14	
	92-89	Tibet: Dingri, 4500 m	28°44′N 86°09′E	30	22
	92-90	Tibet: Dingri, 4300 m	28°44′N 86°09′E	30	
**G. ludlowii* Marq.	92-35	Qinghai: Xiangpi Mt., 3800 m	36°45′N 99°36′E	16	23
	92-33	Qinghai: Heimahe, 3400 m	36°47′N 99°39′E	32	24
	92-88	Tibet: Dingri, 4500 m	28°44′N 86°09′E	32	
**G. ludlowii* Marq. aff.	92-99	Tibet: Nyalam, 4000 m	28°12′N 85°59′E	22	25
**G. pudica* Maxim.	G178	Gansu: Maqü, 3700 m	34°01′N 102°19′E	20	26
**G. tetrasticha* Marq.	92-128	Tibet: Dangxiong, 4500 m	30°34′N 91°24′E	24	27
Gentiana* sect. *Frigidae Kusnez.					
**G. striolata* T. N. Ho	92-148	Tibet: Dingqing, 4900 m	31°40′N 95°03′E	24	28
Gentiana* sect. *Microsperma T. N. Ho					
G. yunnanensis Franchet	92-249	Yunnan: Dali, 3700 m	25°42′N 100°09′E	24	29
Jaeschkea					
**J. microsperma* C. B. Clarke	92-95	Tibet: Nyalam, 3870 m	28°12′N 85°59′E	16	30

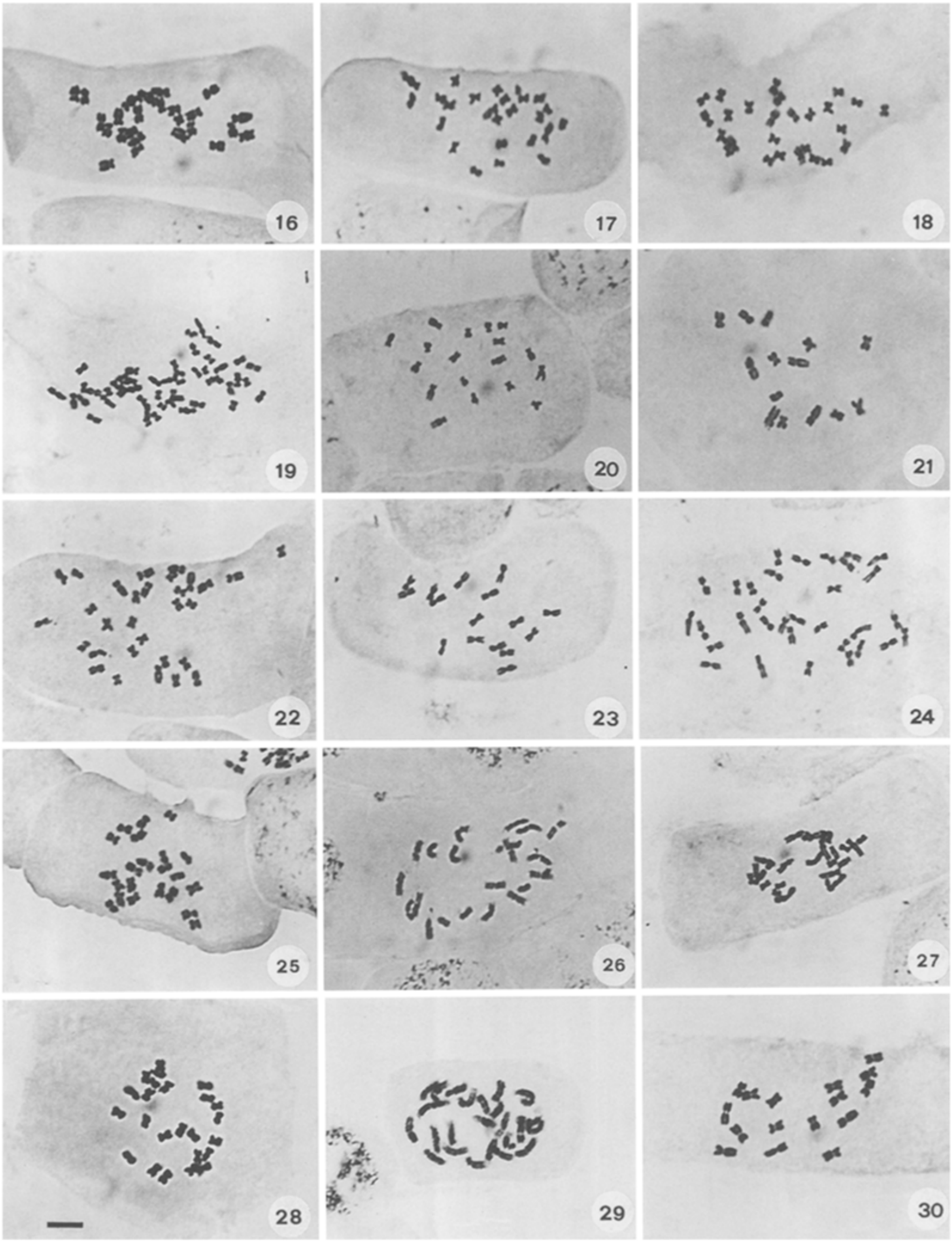
were obviously smaller than the tetraploid ones. The tetraploid number $2n = 40$ was documented for three species. This number has been reported previously in the genus *Gentiana* only as an intraspecific variation in *G. carinata* Griseb. ($n = 20$, Mehra & Gill 1968; $n = 10$, 20, Vasudevan 1975). The number $2n = 14$ was confirmed for *G. aristata* and was further recorded for two more species from very different regions (see Table 1).

In sect. *Cruciata*, the number $2n = 52$ was confirmed for *G. straminea* and *G. tibetica*, as was $2n = 26$ for *G. crassicaulis*. The number $2n = 26$ was also counted for the first time for *G. lhassica* and *G. siphonantha*, and $2n = 52$ for *G. robusta*. It was particularly interesting that a diploid cytotype of $2n = 26$ was found for *G. straminea* from northern Tibet, since only the tetraploid type has been reported before (see Yuan 1993). This was the first observation of intraspecific polyploidy in this section.

The chromosome numbers of all six species belonging to sect. *Dolichocarpa* investigated here were documented for the first time. Very different numbers



Figs. 1–15. Mitotic metaphase chromosomes of *Gentiana* sect. *Chondrophyllae* s. str. – Fig. 1. *G. burkilii* (92-82), 2n = 14. – Fig. 2. *G. crassuloides* (92-265), 2n = 40. – Fig. 3. *G. flexicaulis* (92-264), 2n = 14. – Fig. 4. *G. haynaldii* (92-121), 2n = 20. – Fig. 5. *G. heleonastes* (92-309b), 2n = 36. – Fig. 6. *G. leucomelaena* (92-92), 2n = 18. – Fig. 7. *G. leucomelaena* (92-34), 2n = 36. – Fig. 8. *G. micantiformis* (92-50), 2n = 60. – Fig. 9. *G. prattii* (92-22), 2n = 20. – Fig. 10. *G. prattii* (92-303), 2n = 20. – Fig. 11. *G. pseudoaquatica* (92-157), 2n = 20. – Fig. 12. *G. pseudoaquatica* (92-91), 2n = 40. – Fig. 13. *G. pseudoaquatica* (92-69), 2n = 40. – Fig. 14. *G. pseudosquarrosa* (92-321), 2n = 20. – Fig. 15. *G. tricolor* (92-31), 2n = 18. – Bar: 5 µm



Figs. 16–30. Mitotic metaphase chromosomes of *Gentiana* and *Jaeschkea*. – Fig. 16. *G. lhassica* (92-162), 2n = 26. – Fig. 17. *G. siphonantha* (92-331), 2n = 26. – Fig. 18. *G. straminea* (92-153), 2n = 26. – Fig. 19. *G. tibetica* (92-174), 2n = 52. – Fig. 20. *G. crenulato-truncata* (92-46), 2n = 18. – Fig. 21. *G. hyalina* (92-19), 2n = 14. – Fig. 22. *G. hyalina* (92-89), 2n = 30. – Fig. 23. *G. ludlowii* (92-35), 2n = 16. – Fig. 24. *G. ludlowii* (92-33), 2n = 32. – Fig. 25. *G. ludlowii* aff. (92-99), 2n = 22. – Fig. 26. *G. pudica* (G178), 2n = 20. – Fig. 27. *G. tetrastica* (92-128), 2n = 24. – Fig. 28. *G. striolata* (92-148), 2n = 24. – Fig. 29. *G. yunnanensis* (92-249), 2n = 24. – Fig. 30. *J. microsperma* (92-95), 2n = 16. – Bar: 5 µm

including 2n = 14, 16, 18, 20, 22, 24, 30 and 32 were found, none of which confirmed the previous reports of 2n = 36 and n = 20 in this section (Löve & Löve 1975a, 1986; Weaver & Rudenberg 1975; Krogulevich 1976, 1978; Zhukova 1982). Among these new numbers, 2n = 16 was also new for the genus and some others such as 2n = 22, 30 and 32 were recorded only in the remotely-related European sect. *Calathianae* Froelich. Intraspecific polyploidy was found in *G. ludlowii* (2n = 16, 32), and intraspecific dysploidy in *G. hyalina*, of which the diploid cytotype had 2n = 14 chromosomes while the tetraploid cytotype had 2n = 30. A population collected from Nyalam in western Tibet (92–99) matched the description of *G. ludlowii*, but had the distinct chromosome number of 2n = 22.

Both *G. striolata* of sect. *Frigidae* and *G. yunnanensis* of sect. *Microsperma* had 2n = 24, which confirmed our previous report.

Our results revealed that *Jaeschkea microsperma* had 2n = 16, a chromosome number differing from those of the other two species of the genus.

Karyotypes. The karyotypes and the corresponding idiograms of some selected species and populations are shown in Figs. 31–54. The corresponding karyotype measurements are shown in Table 2. The karyotypes were generally very symmetrical with a fairly high proportion of metacentric and submetacentric chromosomes. The chromosome size varied from species to species. Both the diploid and the tetraploid cytotypes of *G. leucomelaena* (Figs. 6–7) had chromosomes clearly larger than those of the other species, but there was no obvious overall correlation between size and number of chromosomes in the species that we studied. However, from both the illustrations and the asymmetry indices of the karyotypes, a general trend appears: karyotypes with higher chromosome numbers tend to have more chromosomes with subterminal centromeres and thus are more asymmetrical. This could suggest the mechanism responsible for dysploidy (see Discussion). *Gentiana crenulato-truncata* is an exceptional species, since apart from the high asymmetry of its karyotype, it showed specific heteropycnosis, with very obvious chromocentres in most prophases and some interphase nuclei (Fig. 20).

Discussion

Basic chromosome numbers and their systematic implications for *Gentiana*. In the genus *Gentiana* as a whole, we are confronted with an extremely wide range of chromosome numbers: from 2n = 12 to 60 and 96. The most obvious and important characteristic of the chromosomal evolution of this genus is the heterogeneity of chromosome number variation. While some sections show very high variability, other sections are restricted to fewer numbers (Table 3).

The species of sect. *Gentiana* uniformly have 2n = 40, and all species of sect. *Ciminalis* (Adans.) Dumort have 2n = 36. They might be diploidized paleopolyploids. Some authors (e.g. Löve & Löve 1975b, 1976) suggested the basic numbers x = 5 and x = 6 respectively for these two sections, but we think their basic numbers should be x = 10 and 9, taking into account the basic numbers of other sections of the genus. These two sections are well-defined both cytologically and morphologically. No interspecific chromosome number variation was found in these two sections.

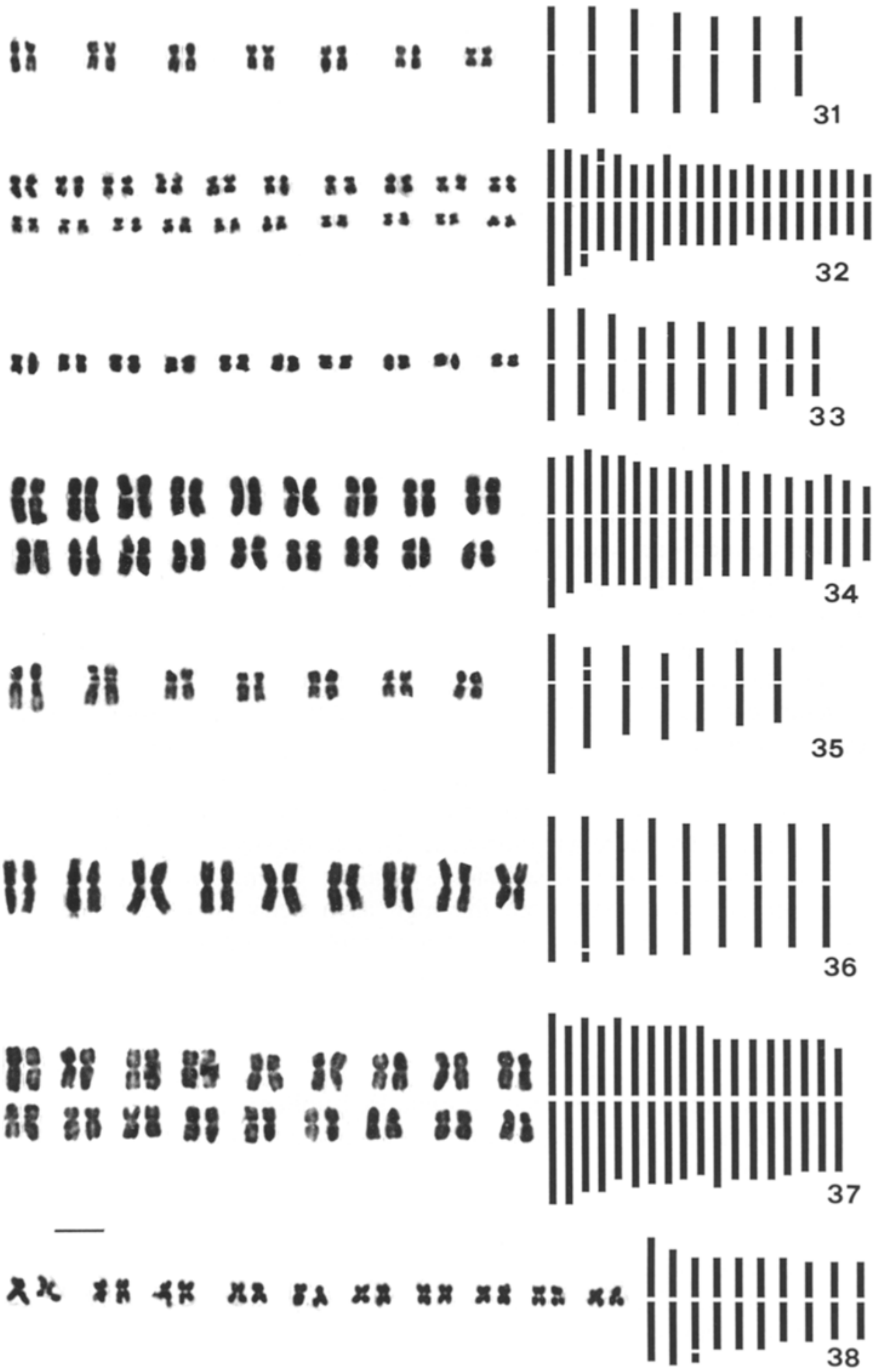
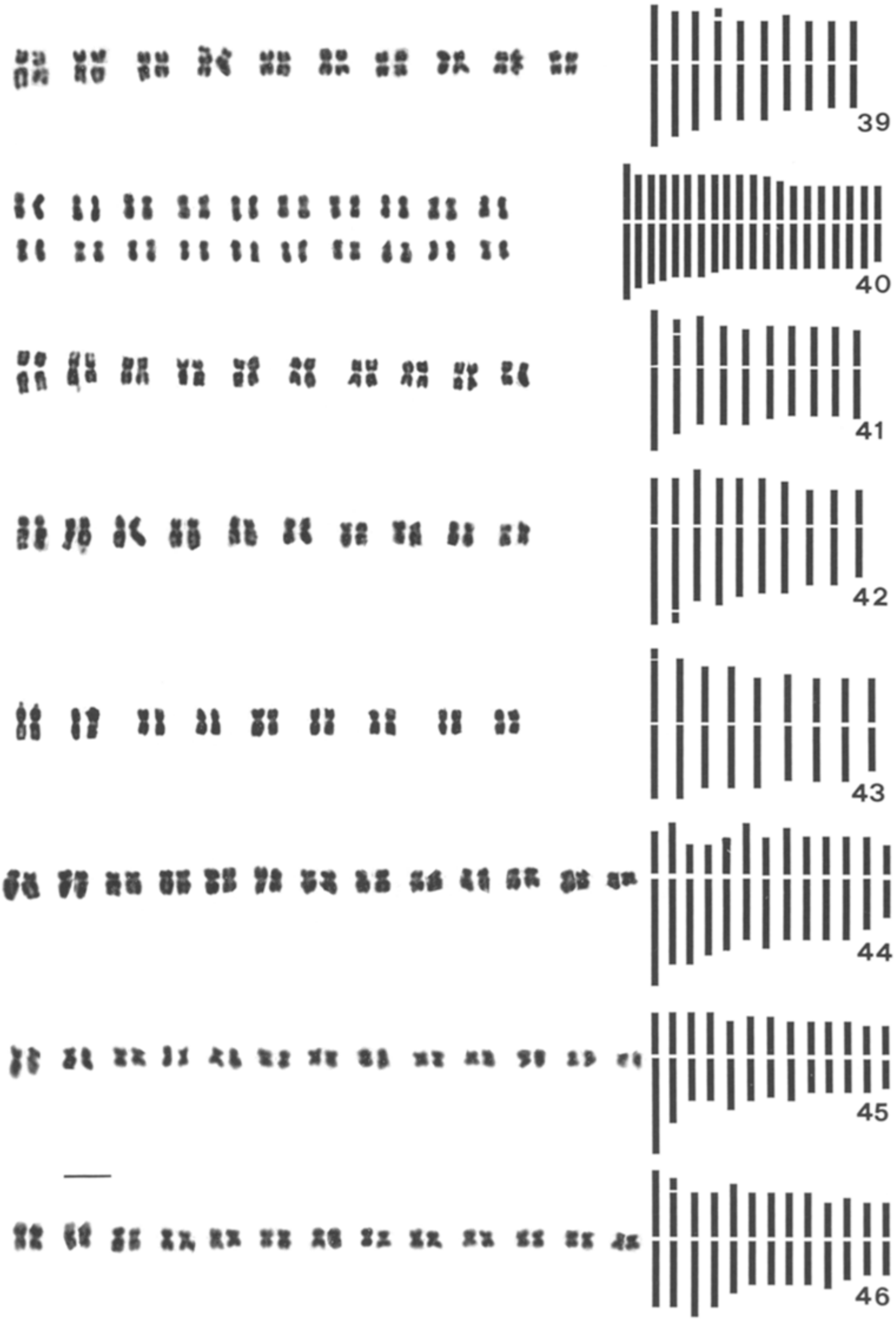
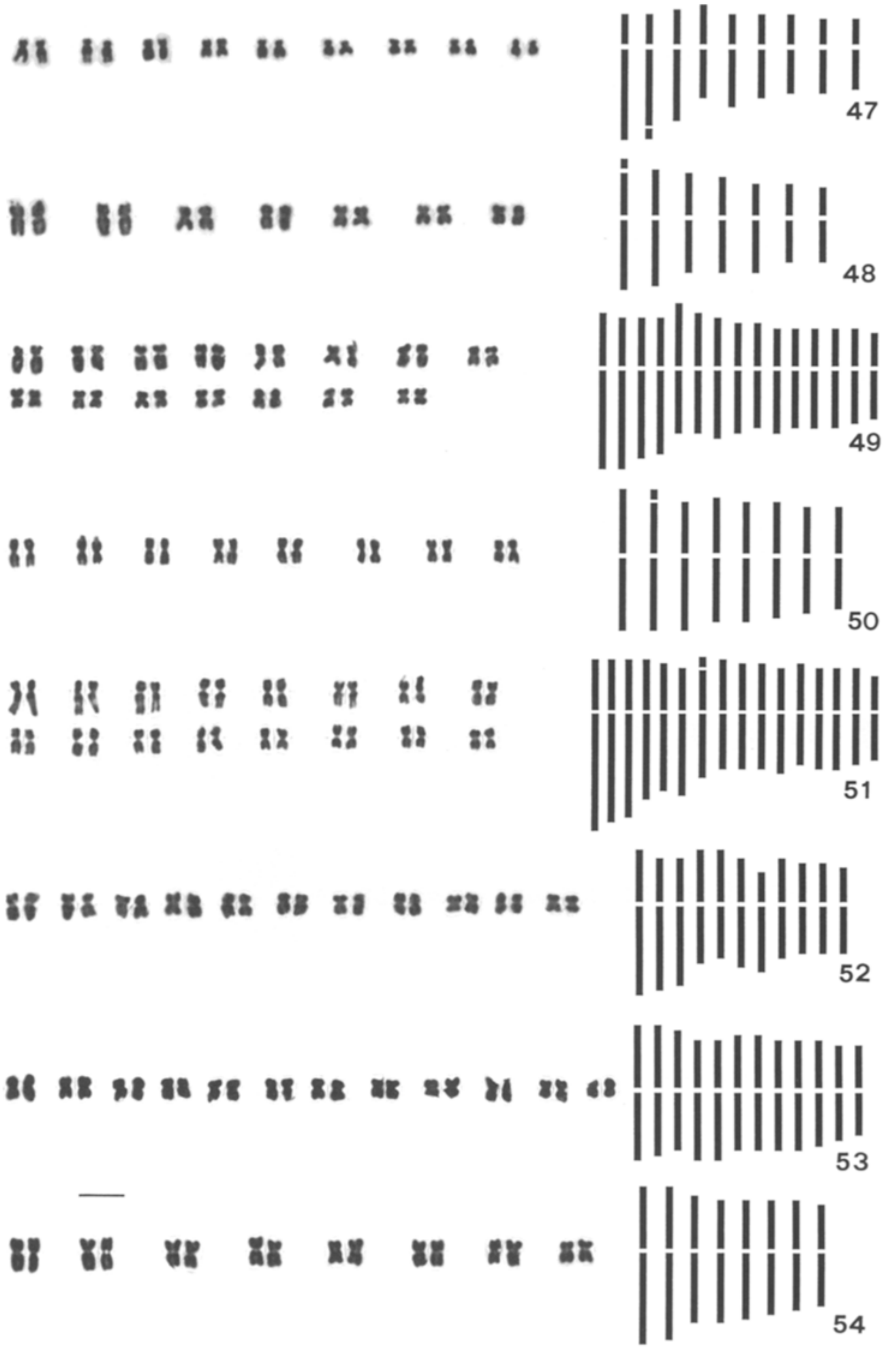


Fig. 31–38. Karyograms and idiograms of *Gentiana* spp. – Fig. 31. *G. burkilii* (92-82), 2n = 14. – Fig. 32. *G. crassuloides* (92-265), 2n = 40. – Fig. 33. *G. haynaldii* (92-121), 2n = 20. – Fig. 34. *G. heleonastes* (92-309b), 2n = 36. – Fig. 35. *G. flexicaulis* (92-264), 2n = 14. – Fig. 36. *G. leucomelaena* (92-92), 2n = 18. – Fig. 37. *G. leucomelaena* (92-34), 2n = 36. – Fig. 38. *G. prattii* (92-22), 2n = 20. – Bar: 5 µm



Figs. 39–46. Karyograms and idiograms of *Gentiana* spp. – Fig. 39. *G. prattii* (92-303), 2n = 20. – Fig. 40. *G. pseudoaquatica* (92-91), 2n = 40. – Fig. 41. *G. pseudoaqutica* (92-157), 2n = 20. – Fig. 42. *G. pseudosquarrosa* (92-321), 2n = 20. – Fig. 43. *G. tricolor* (92-31), 2n = 18. – Fig. 44. *G. lhassica* (92-162), 2n = 26. – Fig. 45. *G. siphonantha* (92-331), 2n = 26. – Fig. 46. *G. straminea* (92-153), 2n = 26. – Bar: 5 μm



Figs. 47–54. Karyograms and idiograms of *Gentiana* spp. and *Jaeschkea*. – Fig. 47. *G. crenulato-truncata* (92-46), 2n = 18. – Fig. 48. *G. hyalina* (92-19), 2n = 14. – Fig. 49. *G. hyalina* (92-89), 2n = 30. – Fig. 50. *G. ludlowii* (92-35), 2n = 16. – Fig. 51. *G. ludlowii* (92-33), 2n = 32. – Fig. 52. *G. ludlowii* aff. (92-99), 2n = 22. – Fig. 53. *G. striolata* (92-148), 2n = 24. – Fig. 54. *J. microsperma* (92-95), 2n = 16. – Bar: 5 µm

Table 2. Karyotype data of selected species of *Gentiana* and *Jaeschkea*. N number of cells measured;* karyological classification proposed by STEBBINS (1971); A_1 intrachromosomal asymmetry index defined by ROMERO ZARCO (1986) as $A_1 = 1 - (\Sigma(b_i/B_i))/n$, where b is the length of the short arm and B is the length of the long arm of each chromosome and n is the gametic chromosome number; A_2 interchromosomal asymmetry index defined by ROMERO ZARCO (1986) as A_2 =s/x, where s is the standard variation of the length of chromosomes in a karyotype and x is the mean of length

Taxon	Coll. no.	N	Karyotype	Total length (µm)	Size range (µm)	Type*	A_1	A_2	Fig.
Gentiana sect. *Chondrophyllae* s. str.									
G. burkilii	92-82	5	2n = 14 = 12 m +2sm	32.5	1.8–2.9	1A	0.353	0.167	31
G. crassuloides	92-265	2	2n = 40 = 4m (SAT) +26m +10sm	52.1	0.9–23	2B	0.297	0.294	32
G. haynaldii	92-121	2	2n = 20 = 18m +2sm	27.0	1.0–1.7	1A	0.170	0.260	33
G. heleonastes	92-309b	1	2n = 36 = 30m +6sm	117.3	2.0–4.4	2B	0.273	0.183	34
G. flexicaulis	92-264	5	2n = 14 = 8m +2sm (SAT) +4sm	37.4	2.0–4.3	2B	0.362	0.283	35
G. leucomelaena	92-92	2	2n = 18 = 2m (SAT) +16m	82.0	4.3–5.0	1A	0.114	0.072	36
	92-34	2	2n = 36 = 34m +2sm	116.2	2.5–4.0	1A	0.205	0.125	37
G. prattii	92-22	3	2n = 20 = 2m (SAT) +18m	35.7	1.4–3.0	1B	0.209	0.295	38
	92-303	2	2n = 20 = 2m (SAT) +18m	26.7	2.1–3.8	1A	0.216	0.200	39
G. pseudoaquatica	92-91	1	2n = 40 = 40m	83.3	1.6–3.0	1A	0.221	0.174	40
	92-157	3	2n = 20 = 2m (SAT) +16m +2sm	50.7	2.2–3.8	1A	0.277	0.198	41
G. pseudosquarrosa	92-321	2	2n = 20 = 12m +2sm (SAT) +6sm	51.2	1.9–3.3	2A	0.409	0.186	42
G. tricolor	92-31	3	2n = 18 = 2m (SAT) +16m	47.4	2.1–3.5	1A	0.148	0.182	43
Gentiana sect. *Cruciata*									
G. lhassica	92162	1	2n = 26 = 14m +12sm	56.5	1.3–3.0	2B	0.450	0.195	44
G. siphonantha	92-331	1	2n = 26 = 22m +4sm	38.9	1.1–2.8	2B	0.157	0.298	45
G. straminea	92-153	1	2n = 26 = 2m (SAT) +22m +2sm	45.6	1.3–2.5	2B	0.105	0.261	46
Gentiana sect. *Dolichocarpa*									
G. crenulato-truncata	92-46	3	2n = 18 = 4m +10sm +2st (SAT) +2st	29.3	1.1–2.3	3B	0.520	0.272	47
G. hyalina	92-19	5	2n = 14 = 2m (SAT) +12m	31.1	1.6–3.0	1A	0.324	0.241	48
	92-89	1	2n = 30 = 18m +12sm	58.5	1.3–2.7	2B	0.359	0.204	49
G. ludlowii	92-35	4	2n = 16 = 2m (SAT) +14m	37.3	1.9–2.8	1A	0.174	0.135	50
	92-33	3	2n = 32 = 2m (SAT) +20m +10sm	75.0	1.5–3.5	2B	0.331	0.252	51
G. ludlowii aff.	92-99	2	2n = 22 = 14m +8sm	44.0	1.5–2.8	2A	0.328	0.188	52
Gentiana sect. *Frigida*									
G. striolata	92-148	1	2n = 24 = 24m	45.2	1.5–2.3	1A	0.144	0.114	53
Jaeschkea									
J. microsperma	92-95	5	2n = 16 = 16m	40.9	2.0–3.3	1A	0.278	0.172	54

Table 3. Recorded chromosome numbers (2n), and suggested basic chromosomal number and (x), or ancestral basic number where multiple basic numbers exist, for the sections of *Gentiana*. Question marks refer to unknown or less certain numbers

Section	2n	x	Comments
Calathianae	14, 20, 22, 24, 28, 30, 32, 42	15?	incomplete dysploidy complex
Chondrophyllae	12, 14, 18, 20, 24, 26, 36, 38, 40, 44, 48, 60, 96	10	dysploidy complex (see text)
Ciminalis	36	9	x = 18 may be a secondary basic number
Cruciata	26, 42?, 52	13	
Dolichocarpa	12, 14, 16, 18, 20, 22, 24	10	dysploid complex (see text)
	30, 32, 36, 40	10	
Fimbricorona	20, 40	12	
Frigidae	24, 26, 42, 48	12	the species with 2n = 42 is probably misplaced in this section, and the number 2n = 26 requires confirmation
Gentiana	40	10	x = 20 may be a secondary basic number
Isomeria	24	12	
Microsperma	24, 26	12?	
Monopodiae	24, 26, 48	12	
Otophora	no count available	?	
Phyllocalyx	26	13	
Pneumonanthe	26, 44	13	the species with 2n = 44 is probably misplaced in this section
Stenogyne	34, 42, 46	23?	

Several morphologically defined sections showed only polyploid variation without alteration of their basic numbers or, occasionally, a few exceptional basic numbers in addition to a dominant one. Section *Phyllocalyx* T. N. Ho consists of two species, one with 2n = 26 and the another of unknown number. Section *Isomeria* has 2n = 24, but chromosome numbers for most species of the section remain unknown. Section *Cruciata* has 2n = 26 and 52, with a basic number of x = 13. Section *Kudoa* has 2n = 24, 26 and 48 and sect. *Microsperma* 2n = 24 and 26. Section *Pneumonanthe* (GLED.) GAUDIN has 2n = 26 with only one exceptional species with 2n = 44 (*G. asclepiadea* L.) and sect. *Frigidae* has 2n = 24 with one exceptional species with 2n = 42 (*G. froelichii* JAN). Among these sections, sect. *Cruciata* and sect. *Pneumonanthe* are rather isolated from the others. Morphologically, the remaining sections form a closely related complex, with basic numbers of x = 12 and x = 13, which has been defined as sect. *Frigidae* s. l. by SMITH (NILSSON 1967, 1970). The chromosome numbers of *G. asclepiadea* and *G. froelichii*, which differ from those of the other species of their sections, indicate they have been wrongly attributed. Molecular phylogenetic studies, based on chloroplast DNA sequences (Gielly & Taberlet 1996) and nuclear ribosomal DNA sequences (Yuan & al. 1996), have revealed their closer relationships to sect. *Gentiana* than to sect. *Pneumonanthe* and *Frigidae*.

In sect. *Cruciata*, diploid and tetraploid cytotypes (2n = 26, 52) do not usually occur in the same species. *Gentiana straminea* was thought to be tetraploid (Yuan 1993), but we found a diploid population (92–153) in northern Tibet. Most species of the section in the highland of western China are diploid, but those in other regions such as western Asia and Europe are predominantly tetraploid. Since the evolution from diploid to tetraploid is generally an irreversible process, the highland region of western China may represent the centre of origin of *G. straminea*, and might be the primary diversification centre of the whole sect. *Cruciata*.

Section *Stenogyne* Franchet is a fairly specific group in the genus, since it has higher and obviously secondary basic numbers of x = 17 (2n = 34), x = 21 (2n = 42), and x = 23 (2n = 46) (Yuan & Küpfer 1993a). These high basic numbers, especially x = 23, suggest a relationship to the genera *Crawfurdia* Wall. and *Tripterospermum* Blume that is closer than to the genus *Gentiana*. Molecular phylogenetic studies based on nuclear ribosomal DNA sequences have confirmed this close affinity (Yuan & al. 1996).

The greatest variation of chromosome numbers is found in sect. *Calathianae, Chondrophyllae* s. str. and *Dolichocarpa*. *Calathianae* is a morphologically well-defined group. Müller (1982) investigated its chromosome numbers extensively and found 2n = 14, 20, 22, 28, 30, 32 and 38. These numbers may represent an incomplete series of dysploidy (and polyploidy). The incompleteness may be caused by the extinction of some intermediate cytotypes. It is difficult to determine the original ancestral number and the polarity of the dysploidy, although Müller (1982) proposed a scheme showing polyploidization and dysploidization from a supposed ancestral basic number of x = 5 (Müller 1982: scheme 3). A rapid radiation and starlike phylogeny have been suggested for this section, based on the phylogenetic study of chloroplast DNA sequences (Gielly & Taberlet 1996). Section *Dolichocarpa* used to be included within sect. *Chondrophyllae* s. l. The splitting by Ho (1985) was based mainly on the elongated capsule of the latter. While Ho (1985) and Ho & Liu (1990) treated them as different sections, Laìnz (1976) and Löve & Löve (1975a, b, 1976, 1978, 1986) established three independent genera, based only on the three European species of these sections. The segregated 'new genera' are *Chondrophylla* A. Nelson, *Holubogentia* Löve & Löve and *Kuepferella* Laìnz, based on *G. prostrata* (2n = 36), *G. pyrenaica* (2n = 26) and *G. boryi* (2n = 20) respectively. The discriminating characters of the segregates were the different basic chromosome numbers of the three species. The available karyological data, however, do not support the splitting of sect. *Chondrophyllae* s. l., either into different sections or different genera (see below for further discussion). Molecular phylogenetic studies of nuclear ribosomal DNA sequences also strongly suggest that this group forms a single monophyletic complex (Yuan & Küpfer 1997).

Karyotypic evolution through the combination of dysploidization and polyploidization in *Gentiana* sect. *Chondrophyllae* s. l. We use the name sect. *Chondrophyllae* s. l. to include both sect. *Chondrophyllae* and sect. *Dolichocarpa* of Ho & Liu (1990). In our present and previous reports, the following chromosome numbers have been recorded for *Chondrophyllae* s. str.: 2n = 12 (1 sp.), 14 (3 spp.), 18 (6), 20 (26), 24 (1), 26 (4), 36 (7), 38 (1), 40 (4), 44 (1), 48 (2) and 60 (1). For *Dolichocarpa*, they are 2n = 14 (1), 16 (1), 18 (1), 20 (1) 22 (1), 24 (1), 30 (1), 32 (1), 36 (2) and 40 (1). These numbers confirm the typically polybasic and dysploid nature of the two sections. The chromosome numbers *per se* do not support the splitting of sect. *Chondrophyllae* s. 1. into two sections. The cytotypes of intraspecific polyploids and dysploids of this group, such as *G. heleonastes* (2n = 12, 36), *G. pseudoaquatica* (2n = 20, 40), *G. leucomelaena* (2n = 18, 36), *G. ludlowii* (2n = 16, 32), *G. prattii* (2n = 18, 20), and *G. hyalina* (2n = 14, 30), may indicate cryptic speciation, where either no recognizable morphological differentiation has occurred, or the differentiation is too slight to merit any taxonomic attention. Thus, extreme care should be taken when making any further taxonomic considerations regarding this group and more thorough morphological studies are needed. The high karyotypic diversity suggests that rapid karyotypic evolution has occurred in sect. *Chondrophyllae* s. 1., involving chromosomal rearrangements. Considering the existing chromosome data, we suggest that the combination of dysploidization and polyploidization may have played a major role in the karyotypic evolution of the group. The number 2n = 20 (x = 10) could be the original ancestral number of the dysploidy series: (1) it is the most common number in the group, occurring in nearly half of the chromosomally-known species; (2) it has quite a wide polytopic distribution, including New Guinea, Japan, south-western China, the Himalayas, as well as western Europe; and (3) it is represented by species such as *G. anisostemon* Marq., *G. asterocalyx, G. crassula, G. intricata* Marq., *G. praticola* Franchet and *G. mairei* from Yunnan, south-western China, which differ quite considerably from one another morphologically,

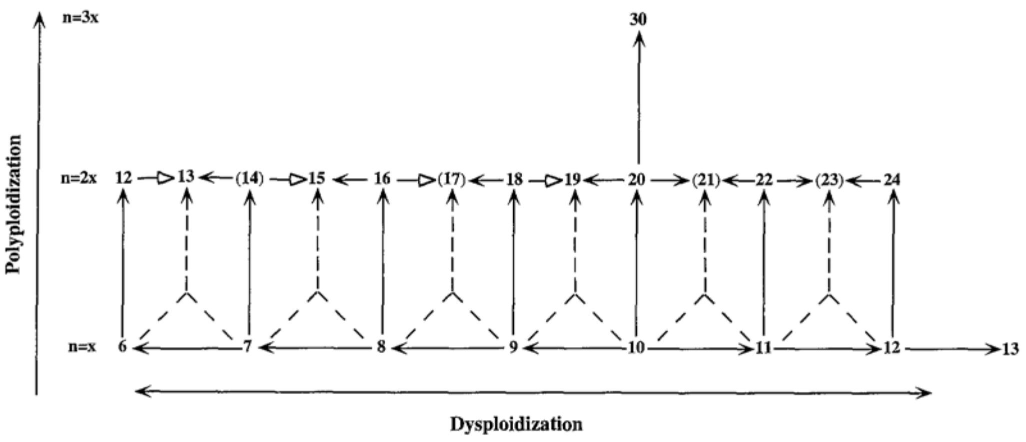


Fig. 55. A suggested scheme of karyotypic evolution by a combination of dysploidization and polyploidization within *Gentiana* sect. *Chondrophyllae* s. 1. Numbers refer to the gametic chromosome numbers of the different cytotypes. Numbers in brackets are suggested, but without direct documentation for the group. "x" refers to the basic chromosome number. Broken line and empty arrows refer to a less certain possibility

but share the same number of 2n = 20 (Table 1; KÜPFER & YUAN 1996). We therefore suppose that both ascending and descending dysploidy are involved in the karyotypic evolution of the group, and that dysploidization could have happened at both diploid and higher ploidy levels. 2n = 18, 16, 14 and 12 might be descending dysploids at the diploid level and 2n = 22 an ascending one; 2n = 24 could be an ascending dysploid or the tetraploid of a descending dysploid; 2n = 26, 30, 32, 36, 38, 40, 44, and 48 could be tetraploids of the above dysploids or dysploids at tetraploid level. We suggest a scheme showing the evolutionary relationships among the different cytotypes of *Gentiana* sect. *Chondrophyllae* s. 1. (Fig. 55). However, suggestions merely based on chromosome numbers cannot bring deeper insight to the understanding of the relationships among those dysploids and polyploids, since conventional karyological analysis cannot tell whether a specific number, say 2n = 24, is diploid or polyploid, unless clear morphological chromosome markers can be seen, which are not present in *Gentiana*. Thus the scheme should be considered only as an arbitrary depiction of the relationships among the cytotypes. It is hoped that cytogenetic analyses and DNA molecular phylogenetic studies will shed new light on the relationships among those cytotypes, and the lineages of their karyotypic evolution.

Karyotype variation and the possible mechanism of dysploidization in *Gentiana*. On the whole, the genus *Gentiana* has rather symmetrical karyotypes. This can be seen both from the illustrations of karyotypes (Figs. 31–54) and from the parameters shown in Table 2. The dysploid changes of chromosome numbers did not significantly influence the asymmetry of karyotypes. However, the species with higher chromosome numbers do tend to have more submetacentric or subtelocentric chromosomes and thus more asymmetrical karyotypes. Correspondingly, the karyotypes of those species with lower chromosome numbers tend to be more symmetrical. This trend does not follow the usual pattern of dysploid variation via centric fission and fusion, which is very common in karyotypic evolution of most animals and some groups of plants (e.g. *Tradescantia* sect. *Cymbispatha*, see JONES & al. 1981), neither does it correspond to STEBBINS' (1971) model of dysploidy. Among the four models of dysploid variation summarized by GREILHUBER & EHRENDORFER (1988), only JACKSON's (1985) partially covers the case of *Gentiana*. In this model, two different arms, each from one homologue of a metacentric pair, are translocated onto one homologue of another metacentric pair, resulting in balanced n + 1 and n – 1 gametes, which can then combine into homologous dysploid zygotes (see GREILHUBER & EHRENDORFER 1988: fig. 6). This model could explain the mechanism on dysploid variation in the genus *Gentiana*. However, selection at the karyotype level in this genus, if there is any, is presumably in favour of descending, rather than ascending dysploidy, since descending dysploids are more common than ascending ones, assuming the ancestral basic number of x = 10 can be confirmed.

Karyological observation of *Jaeschkea*. The small genus *Jaeschkea* is another typical example of dysploidy, since besides the previously reported chromosome numbers of 2n = 28, 20 and 22, we found a lower number, 2n = 16, for *J. microsperma*. Dysploidization may also play an important role in the karyotypic evolution of this genus. However, the direction of the dysploidy and the ancestral basic number of the genus are not yet clear.

We thank Prof. J.-R. Chen and Prof. S.-W. Liu for organising the field excursions and Dr B. de Montmollin, Mr J.-Q. Liu for taking part in them. Profs. T.-N. Ho and S.-W. Liu very kindly checked our determinations of the voucher specimens. Our appreciation is also due to Mr E. Fortis for printing the micrographs, and to Prof. C. Favarger and two anonymous reviewers for their critical comments. Miss C. Fischer has improved the English of the manuscript. This study forms part of the Ph.D. Thesis of Y.-M. Yuan, presented at the University of Neuchâtel and financially supported by grant No. 3100-039'741.93/1 of the Swiss National Scientific Research Foundation (FNRS). The field excursions were supported by the Jean-Marcel Aubert Foundation.

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