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Cytological Studies in the Genus *Indigofera* L.

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Indigofera L. is a large genus of the family Fabaceae and belongs to the tribe Galegeae. It is comprised of 700 species of which only about 20% have been examined cytologically so far. Karyotypes have been worked out in only 20 species of the genus (Singh and Roy 1970, Bhatt and Sanjappa 1975). In view of this, a detailed study on the genus *Indigofera* was initiated in this laboratory. In this communication, results of meiosis and root tip mitosis (including karyotypes) involving 28 species of the genus are presented.

Material and methods

The seed material for different species of *Indigofera* was obtained from different agencies through correspondence and the sources are given in Table 1. The plants were raised in pots and flower beds at Meerut University Experimental Farm. Dormancy of seeds was broken by rubbing them with glass fibre paper. Treatments with concentrated sulphuric acid were also needed in some cases. Voucher specimens are deposited in the Department of Ag. Botany, Meerut University, Meerut.

For meiosis, flower buds were collected in the forenoon, fixed for atleast 24 hours in Carnoy's fluid (absolute alcohol: chloroform: acetic acid, 6:3:1) and stored in 70% ethyl alcohol. The anthers were squashed in 2.0% acetocarmine.

For mitosis, seeds were rubbed with sand glass fibre paper and then germinated. Young and healthy root tips were pretreated with saturated solution of α bromonaphthalene for $1\frac{1}{2}$ hours, fixed in acetic alcohol (1:3) for 24 hours and squashed in 2.0% acetocarmine.

Photomicrographs and camera lucida drawings were made from temporary preparations and measurements (lengths and widths of chromosomes and nuclear diameters) were made with the help of Olympus micrometer eye piece. TCl% and TF% were calculated as earlier done in *Crotalaria* (Gupta and Gupta 1978).

Results

1. *Meiosis*

Meiosis was studied in 24 collections belonging to 23 species of *Indigofera*. The data on chromosome associations and chiasmata frequencies are presented in Table 1. All collections were diploid except three species namely *I. endecephylla*, *I. parodiana* and *I. pilosa* which are tetraploid ($2n=32$). Among diploid collections, each had $x=8$ except *I. parviflora* which had $x=7$. The meiosis was regular with

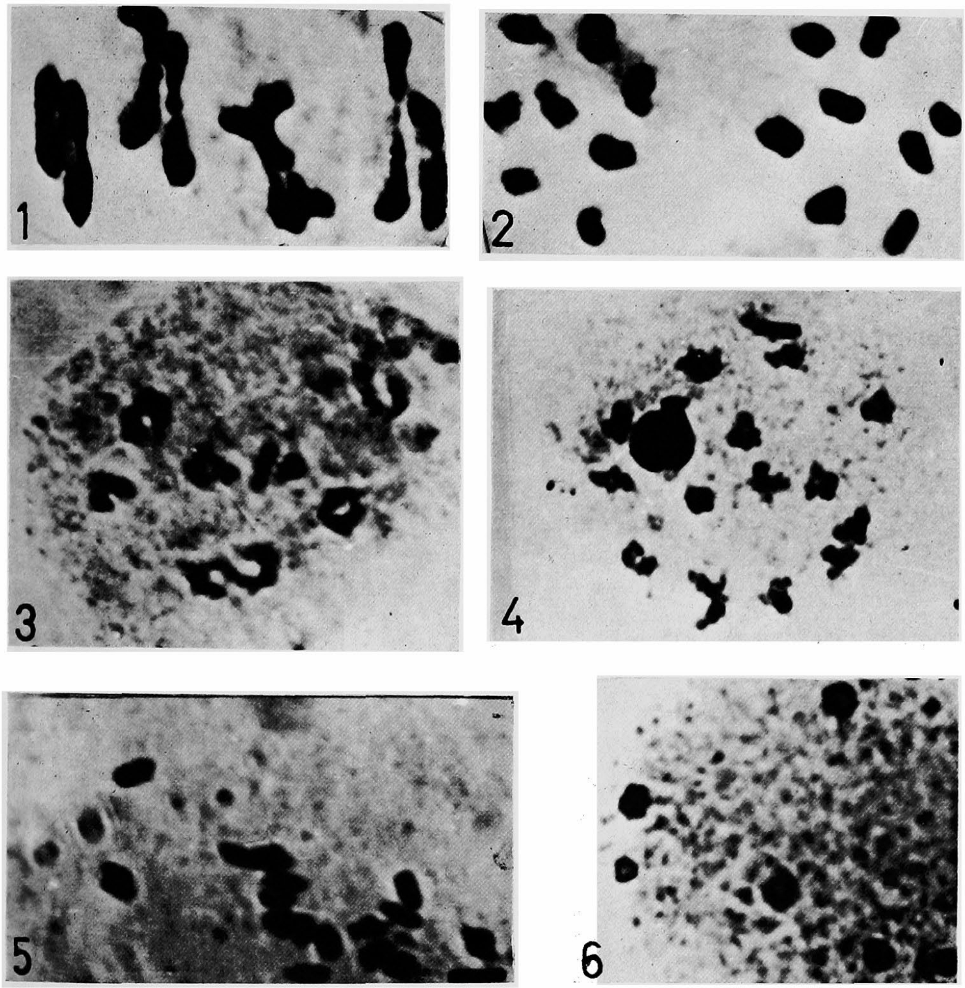
Table 1. Gametic chromosome numbers and chiasmata frequencies in 24 collections belonging to 23 species of *Indigofera* along with their sources

Species	Coll. number	Gametic number (n)	Chiasmata		Source	Accession number given by source
			Xta/PMC	Xta/bivalent		
I. alboglandulosa	1782	8	—	—	CSIRO, Canberra, Australia	CPI 20738
I. aspera	1783	8	10.85	1.35	CSIRO, Canberra, Australia	CPI 36717
I. asperifolia	1784	8	—	—	CSIRO, Canberra, Australia	CPI 34897
I. astragalina	1774	8	—	—	CSIRO, Canberra, Australia	CPI 36718
I. barberi	1785	8	13.13	1.64	CSIRO, Canberra, Australia	CPI 30098
I. brevidens	1786	8	11.20	1.40	Southern Regional Station, Georgia, U. S. A.	338608
I. colutea	1789	8	15.00	1.80	CSIRO, Canberra, Australia	CPI 30129
I. colutea	1790	8	11.60	1.45	Southern Regional Station, Georgia, U. S. A.	357751
I. cryptantha	1788	8	16.00	2.00	Plant Introduction, Pretoria, South Africa	M/71/414
I. endecephylla	1791	16	—	—	Southern Regional Station, Georgia, U. S. A.	1855325
I. enneaphylla	1778	8	13.40	1.60	Meerut University Campus, Meerut	
I. hochstetteri	1780	8	10.33	1.30	Southern Regional Station, Georgia, U. S. A.	201502
I. lespedezioides	1777	8	—	—	CSIRO, Canberra, Australia	CPI 39088
I. linifolia	1776	8	8.00	1.00	CSIRO, Canberra, Australia	CPI 32253
I. microcarpa	1793	8	8.20	1.20	Southern Regional Station, Georgia, U. S. A.	337540
I. parodiana	1795	16	31.10	1.94	Southern Regional Station, Georgia, U. S. A.	162414
I. parviflora	1775	7	10.93	1.54	Plant Introduction, Pretoria, South Africa	M/71/420
I. pilosa	1830	16	27.50	1.70	CSIRO, Canberra, Australia	CPI 21353
I. pseudotinctoria	1831	8	11.60	1.45	Southern Regional Station, Georgia, U. S. A.	225991
I. schimperi	1832	8	12.00	1.50	CSIRO, Canberra, Australia	CPI 52621
I. spicata	1803	8	15.20	1.90	Southern Regional Station, Georgia, U. S. A.	
I. subulata	1797	8	15.20	1.90	CSIRO, Canberra, Australia,	CPI 33164
I. suffructicosa	1796	8	14.20	1.80	CSIRO, Canberra, Australia	CPI 30872
I. tinctoria	1801	8	—	—	CSIRO, Canberra, Australia	

only bivalents, which disjoined regularly (Figs. 1–5). However, in *I. lespedezioides* at telophase II, sometimes 5–6 nuclei instead of 4 nuclei were observed (Figs. 6).

2. *Mitosis and karyotypes*

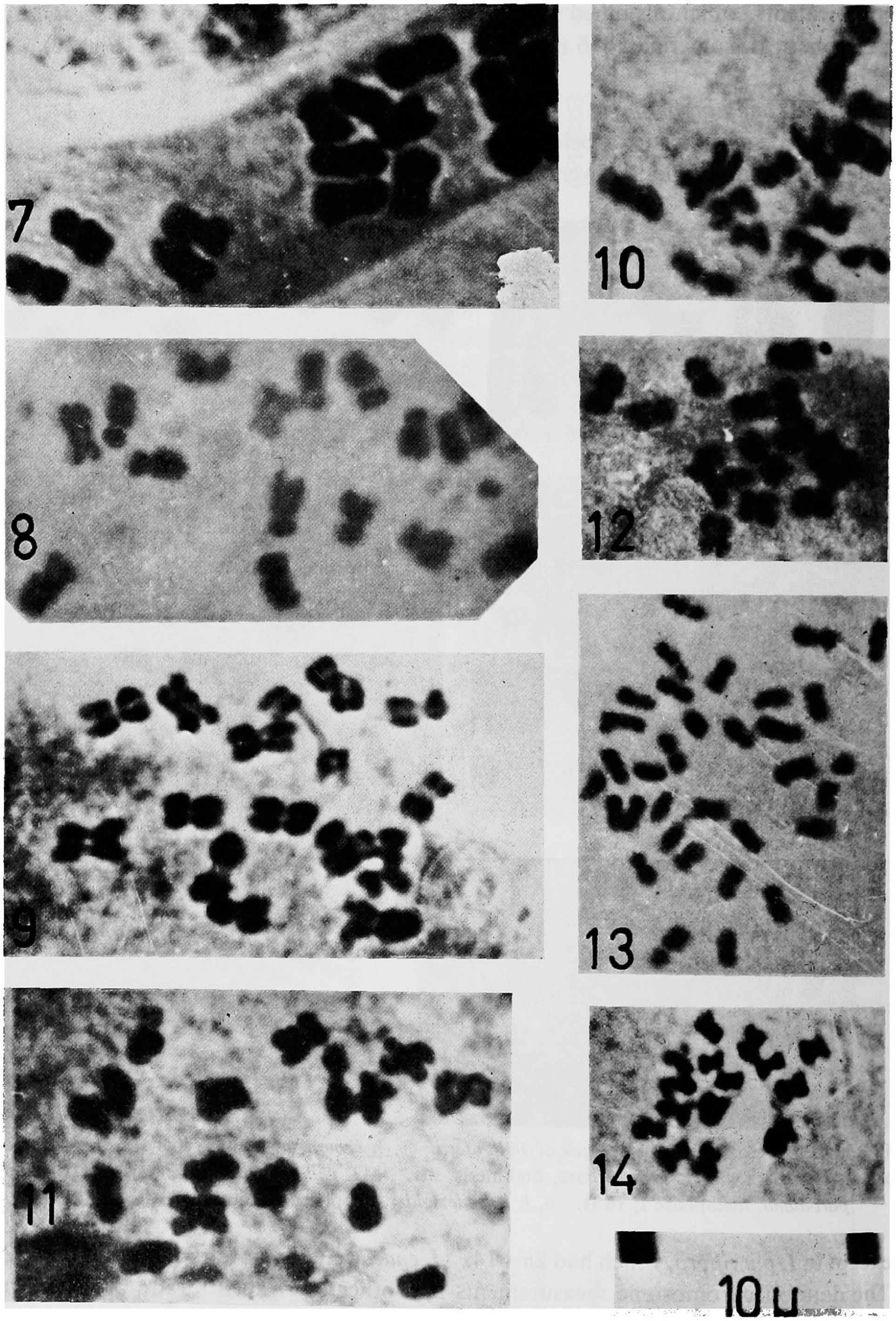
Twenty six collections belonging to twenty three species of *Indigofera* were analysed for karyotypes. Twenty five collections were diploid largely with $2n=16$,



Figs. 1–6. Meiosis in some species of *Indigofera*. 1, *I. aspera*, metaphase I, 8 II. 2, *I. aspera*, anaphase I., 8:8. 3, *I. suffructicosa*, diakinesis, 8II. 4, *I. endecephylla*, diakinesis, 16 II. 5, *I. parodiana*, metaphase I, 16 II. 6, *I. lespedezioides*, telophase II, multinucleate condition.

except in *I. parviflora*, which had $2n=14$. *I. endecephylla* was tetraploid with $2n=32$. The data on chromosome measurements and other chsracteristics are presented in Tables 2–4. Corresponding mitotic metaphase plates and idiograms are presented in Figs. 7–48.

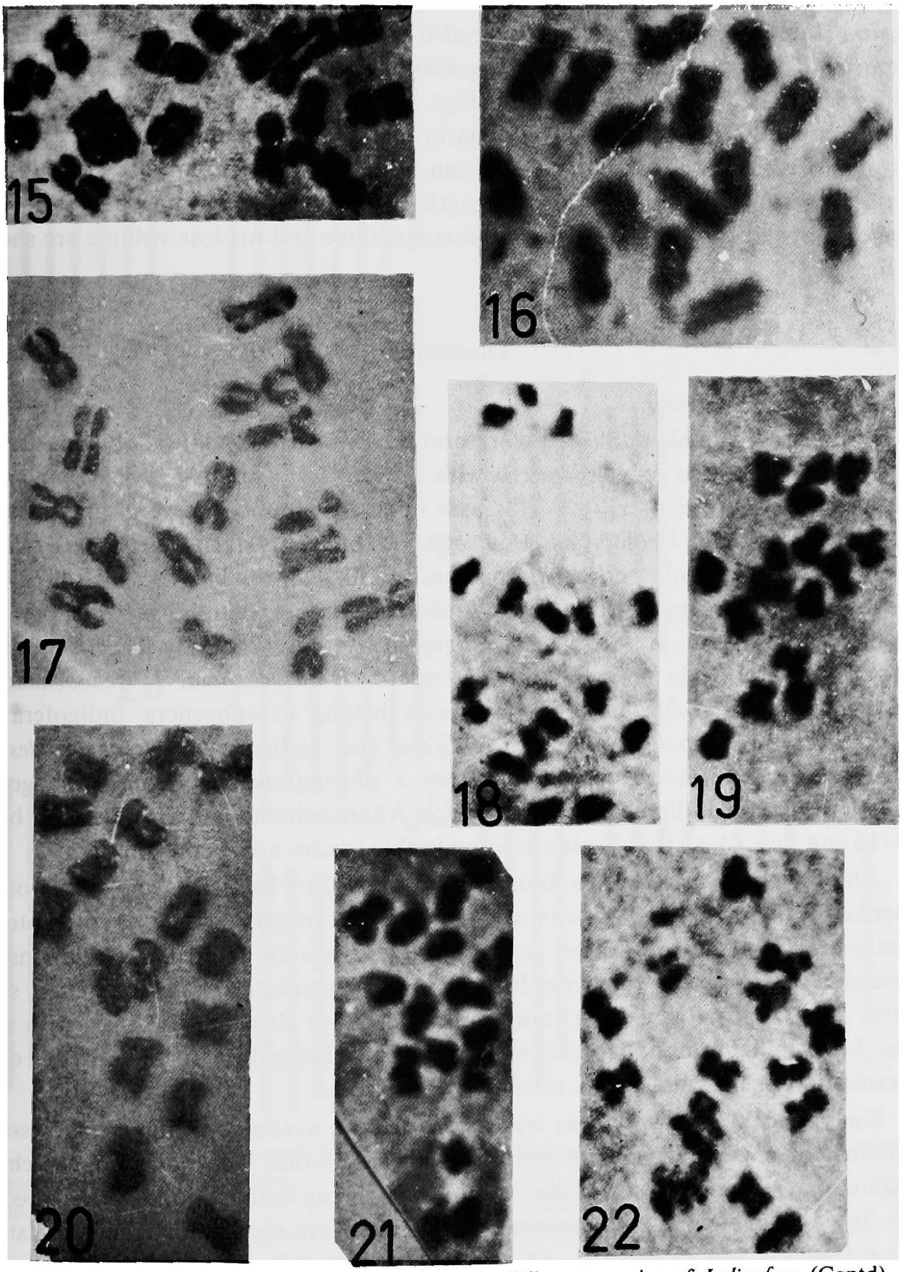
The chromosomes were designated as 1–8 in the diploids and 1–16 in the tetraploid, according to decreasing lengths. Depending upon their absolute lengths,



Figs. 7–14. Root tip mitotic metaphase plates in different species of *Indigofera*. 7, *I. alboglandulosa*, $2n=16$. 8, *I. aspera*, $2n=16$. 9, *I. barberi*, $2n=16$. 10, *I. brevidens*, $2n=16$. 11, *I. schimperi*, $2n=16$. 12, *I. colutea* (1789), $2n=16$. 13, *I. endecephylla*, $2n=32$. 14, *I. enneaphylla*, $2n=16$.

chromosomes were classified into three categories, namely A=more than 3.0 μ; B=1.5 μ to 3.0 μ and C=less than 1.5 μ.

The chromosomes were further subdivided according to the position of centromere. Superscript 'm' means median, 'sm' means submedian, 'st' means sub-



Figs. 15–22. Root tip mitotic metaphase plates in different species of *Indigofera* (Contd). 15, *I. glandulosa*, 2*n*=16. 16, *I. hochstetteri* (1780), 2n=16. 17, *I. hochstetteri* (1781), 2*n*=16. 18, *I. linifolia*, 2*n*=16. 19, *I. microcarpa*, 2*n*=16. 20, *I. suffructicosa*, 2*n*=16. 21, *I. neglecta*, 2*n*=16. 22, *I. spicata* (1821), 2*n*=16.

terminal and the subscript 'sc' preceding the alphabet means secondary constriction.

Measurements were also made on chromosome widths and nuclear diameters. These measurements were used for calculating chromatin volume and nuclear volume respectively, which are presented in Table 3. The data on chromatin length, chromatin volume and nuclear volume were also used for calculating different regression coefficients and their corresponding regression equations, which were subsequently utilized in drawing regression graphs (Figs. 49, 50). The regressions of chromatin volume and nuclear volume on chromatin length were significant suggesting that chromatin volume and nuclear volume can be utilized for evolutionary conclusions in the same manner as chromosome lengths. Frequency distributions of different species for total chromatin length, chromatin volume and nuclear volume are shown in Fig. 51.

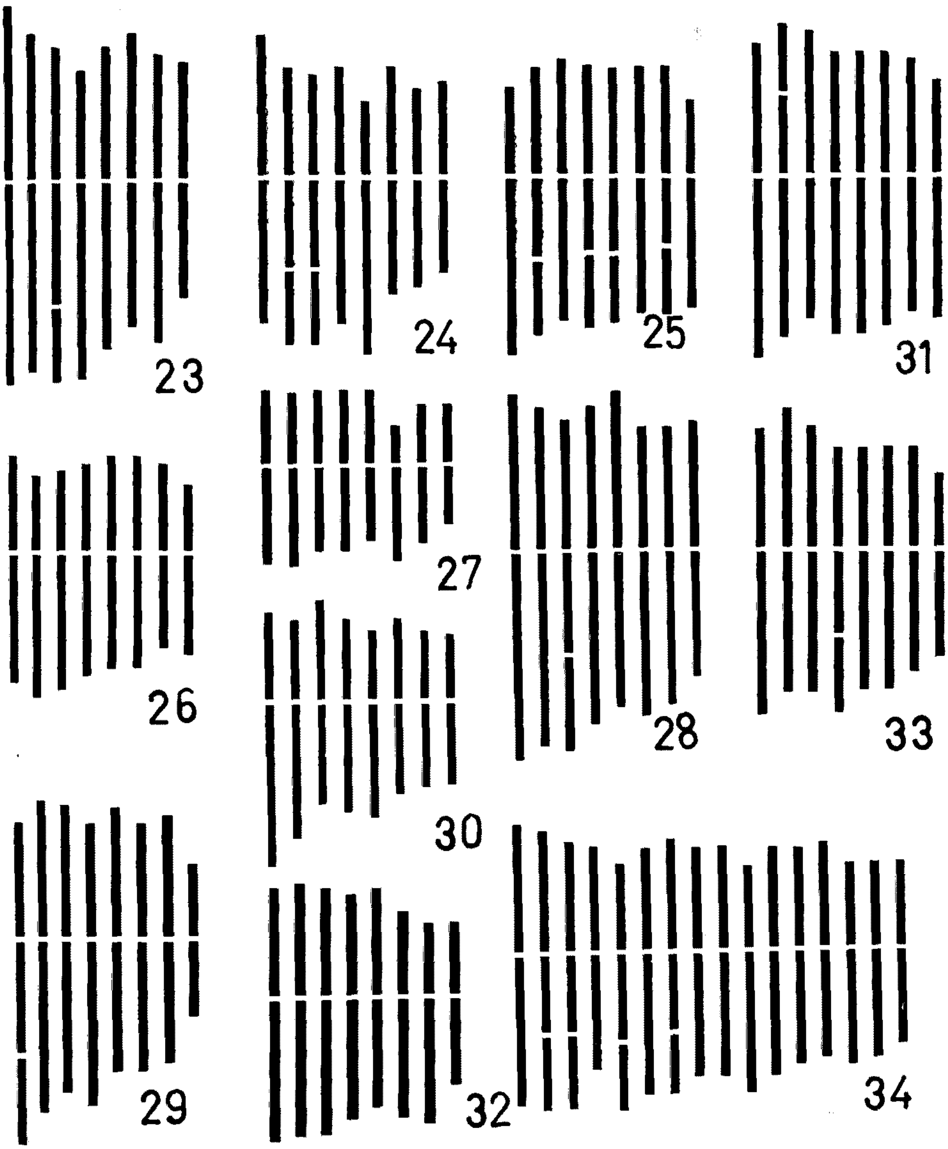
Discussion

a) *Chromosome numbers*

From the available published work and the present results, it is obvious that in the genus *Indigofera*, a polyploid series with $2n=16$, $2n=32$ and $2n=48$ is found suggesting a base number of $x=8$. This base number is found in several other genera such as *Astragalus, Crotalaria, Medicago, Melilotus, Trigonella* and *Trifolium*. There are, however, other chromosome numbers like $2n=8$, $2n=12$, $2n=14$, $2n=24$ and $2n=36$ which suggest other base numbers like $x=4$, 6 and 7 of which $x=7$ is the most important. Six species of *Indigofera* having exclusively $2n=14$ are now known, which include *I. argyroides*, *I. costata*, *I. dasycephala*, *I. ischnoclada*, *I. parviflora* and *I. strobilifera*. These species belong to subgenera Indigofera (*I. dasycephala*, *I. ischnoclada* and *I. strobilifera* and Indigastrum (*I. argyroides*, *I. costata* and *I. parviflora*). Another species *I. oblongifolia* belonging to subgenus Indigofera section Indigofera and subsection Alternifoliolae is known to have both $2n=16$ and $2n=14$, so that the latter count in this species needs confirmation.

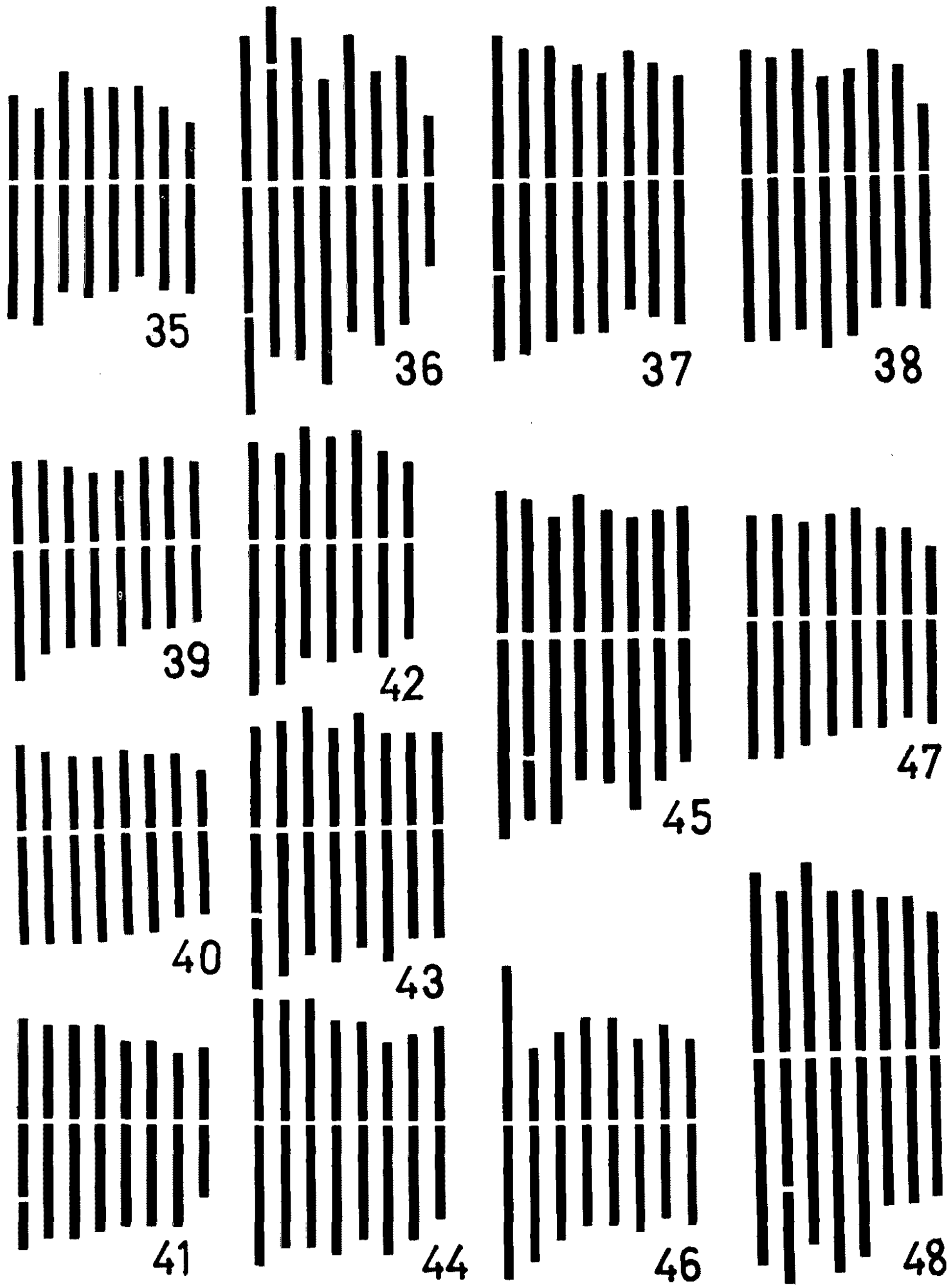
Although in the subgenus Indigastrum, only $2n=14$ is known, in the other subgenus Indigofera, both $2n=14$ and $2n=16$ are known in the sections Latestipulatae and Paniculatae. Since, genus *Cyamopsis* has $2n=14$, a close relationship between *Cyamopsis* and subgenus Indigastrum of genus *Indigofera* has been suggested. It is also obvious that Indigastrum will have closer relationship with sections Latestipulatae and Paniculatae of subgenus Indigofera than with the only other section of this subgenus i. e. section Indigofera.

Since species with $2n=14$ as well as $2n=16$ are available, it may be necessary to discuss their evolutionary relationship with each other. In doing so, the chromosome size may also be important. In *Cyamopsis* as well as in *Indigofera*, species with $2n=14$ have small chromosomes with much less chromatin content relative to that found in species with $2n=16$. In view of this, it is difficult to visualize, how $2n=14$ in this group could be derived from $2n=16$ as hypothesized in the genus *Crotalaria* (Gupta and Gupta 1978). A reduction in chromosome number particularly at the diploid level is supposd to be brought about by unequal translocations accompanied with loss of centromere and increase in chromosome size. Since loss of chromatin at the diploid level can not be tolerated, a more probable line of evolution will be the derivation of $2n=16$ from $2n=14$ by simple aneuploidy and



Figs. 23–34. Idiograms prepared from mitotic metaphase chromosomes in different species of *Indigofera*. 23, *I. alboglandulosa;* 24, *I. arrecta*. 25, *I. aspera*. 26, *I. asperifolia*. 27, *I. astragalina*. 28, *I. barberi*. 29, *I. brevidens*. 30, *I. circinella*. 31, *I. colutea* (1789). 32, *I. colutea* (1790). 33, *I. cryptantha*; 34, *I. endecephylla*.

structural changes. Such an increase in chromosome number from $2n=14$ to $2n=16$ was artificially brought about in barley by Tsuchiya (1969). A study of meiosis in an interspecific hybrid with $2n=15$ may give an answer to this question, but the



Figs. 35–48. Idiograms prepared from mitotic metaphase chromosomes in different species of *Indigofera* (Contd.). 35, I. *enneaphylla*. 36, *I. glandulosa*. 37, *I. hochstetteri* (1780). 38, *I. hochstetteri* (1731). 39, *I. linifolia*. 40, *I. microcarpa*. 41, *I. neglecta*. 42, *I. parviflora*. 43, *I. schimperi*. 44, *I. specata* (1821). 45, *I. subulata* (1797). 46, *I. subulata* (1798). 47, *I. suffructicosa*. 48, *I. trifoliata*.

possibility of a much more complex relationship between species with $2n=14$ and $2n=16$ can not be ruled out. That $2n=14$ may be primitive and not advanced as in *Crotalaria*, gets support from the presence of symmetric karyotype in species with $2n=14$ as recorded in the present study. A similar trend is speculated in the genera *Medicago* and *Trifolium*, where again the chromatin content is less in species with $2n=14$ than in species with $2n=16$.

It may also be necessary to consider the implications of the report of $2n=8$ in the genus *Indigofera* giving a base number of $x=4$. This chromosome number is reported in only one species, namely *I. richardsieae* belonging to subgenus Microcharis. Reports of $2n=16$ in other spcies of Microcharis are also known. Frahm-Leliveld (1966) argued that in view of the availability of $2n=8$ in the genus *Indigofera*, species with $2n=16$ should be regarded as tetraploids with $x=4$ and not diploids with $x=8$. Wanscher (1934) had also suggested $x=4$ as basic number for the family. It may, however, be necessary in this connection to examine the chromosome size in species with $2n=8$ and those with $2n=16$. In *I. richardsieae*, four giant chromosomes are available, which are much bigger in size than those in the species having $2n=16$. Increase in chromosome size accompanied with reduction in chromosome number can be easily explained on the basis of several steps involving unequal interchanges. Therefore, it is only logical to assume that species with $2n=16$ are only diploids and that $x=4$ is a secondarily derived base number. Singh and Roy (1970) also supported the view that $x=8$ should be regarded as the primary base number and the other base numbers like $x=4$, 6, 7 should be considered as derived. Further, it was observed that there is relationship between increase in chromosome number and reduction in chromosome size (Dowrick 1952, Darlington 1955, Sharma and Sharma 1959, Singh and Roy 1970).

Frahm-Leliveld (1966) described in the genus *Indigofera*, 10 species which are tetraploid with $2n=32$. These 10 species represented seven subsections of section Indigofera of subgenus Indigofera. He, however, considered some of these reports to be chance duplications, so that frequency of tetraploidy with $2n=32$ was actually an overestimate. In any case, tetraploidy with $2n=32$ was known to be confined to subgenus Indigofera. Subsequently polyploidy was described in additional species, namely *I. asplathoides* (Singh and Roy 1970) and *I. hochstetteri* (Bhatt and Sanjappa 1975). While *I. asplathoides* belongs to subgenus Indigofera, *I. hochstetteri* falls outside the subgenus Indigofera and belongs to subgenus Amecarpus. *I. hochstetteri* is predominantly a diploid species as obvious from several reports of $2n=16$ in this species (Frahm-Leliveld 1962, Singh and Roy 1970). In the present study also, $2n=16$ was recorded in *I. hochstetteri*. The report of $2n=32$ in this species as given by Bhatt and Sanjappa (1975) is based on a material collected from Gujarat, India. No significance to this count ($2n=32$ in *I. hochstetteri*) can be attached, unless the identity of material is ascertained and the count confirmed. However, if this report of polyploidy is confirmed, polyploidy with $2n=32$ will be no longer confined to the subgenus Indigofera.

During the present study, polyploidy with $2n=32$ was observed in three species, namely *I. endecephylla*, *I. pilosa* and *I. parodiana*, each belonging to the subgenus Indigofera. Since *I. parodiana* was worked out for the first time, the present study

Table 2. Analysis of mitotic chromosomes in different diploid species of *Indigofera*. (In each species, first row represents absolute length of chromosome in μ; the second row gives arm ratios; the third row gives relative chromosome lengths and fourth row gives TCl%)

Species and collection	Chromosome pairs							
	1	2	3	4	5	6	7	8
I. alboglandulosa (Engl.) Gillett.	3.59	3.17	3.11	2.90	2.83	2.76	2.69	2.21
	1.17	1.30	1.50	1.80	1.28	1.00	1.29	1.00
	100.00	88.46	86.54	80.77	78.85	76.92	75.00	61.54
	15.40	13.60	13.30	12.40	12.10	11.80	11.50	9.80
I. arrecta Hochst et Rich.	2.69	2.55	2.48	2.42	2.35	2.14	1.86	1.79
	1.05	1.47	1.57	1.33	2.40	1.07	1.25	1.00
	100.00	94.87	92.31	89.74	87.18	79.49	69.23	66.67
	14.72	13.96	13.58	13.21	12.83	11.70	10.19	9.81
I. aspera Perr.	2.55	2.48	2.48	2.42	2.35	2.35	2.28	2.21
	1.96	1.40	1.25	1.33	1.27	1.27	1.20	1.29
	100.00	97.33	97.33	94.63	91.93	91.93	89.22	86.52
	13.30	12.90	12.90	12.60	12.20	12.20	11.90	11.50
I. asperifolia Bong ex Benth.	2.14	2.07	2.07	2.00	2.00	2.00	1.73	1.59
	1.38	2.00	1.73	1.42	1.23	1.23	1.08	1.56
	100.00	96.77	96.77	93.55	93.55	93.55	80.65	74.19
	13.70	13.20	13.20	12.80	12.80	12.80	11.00	10.10
I. astragalina DC.	1.66	1.62	1.52	1.52	1.38	1.28	1.28	1.10
	1.29	1.47	1.20	1.20	1.00	2.36	1.32	1.00
	100.00	97.91	91.67	91.67	83.33	77.08	77.08	66.67
	14.59	14.29	13.37	13.37	12.16	11.25	11.25	9.73
I. barberi Gamble	3.52	3.24	3.11	3.04	3.04	2.76	2.69	2.48
	1.32	1.35	1.50	1.20	1.00	1.35	1.29	1.00
	100.00	92.16	88.23	86.27	86.27	78.43	76.47	70.59
	14.70	13.50	13.00	12.70	12.70	11.50	11.20	10.40
I. brevidens Benth.	3.04	2.97	2.69	2.66	2.48	2.35	2.35	1.38
	1.75	1.26	1.67	1.48	1.00	1.13	1.00	1.00
	100.00	97.73	88.64	87.50	81.82	77.27	77.27	45.45
	15.25	14.90	13.52	13.34	12.48	11.79	11.79	6.93
I. circinella Baker	2.42	2.07	1.93	1.79	1.72	1.66	1.45	1.38
	1.92	1.73	1.00	1.36	1.78	1.18	1.33	1.50
	100.00	85.71	80.00	74.29	71.43	68.57	60.00	57.14
	16.70	14.30	13.30	12.40	11.90	11.40	10.00	9.50
I. colutea (Burm. f.) Merr. Coll. No. 1789	3.00	2.97	2.76	2.69	2.69	2.62	2.42	2.27
	1.42	1.10	1.00	1.29	1.29	1.24	1.19	1.54
	100.00	98.85	91.95	89.65	89.65	87.36	80.46	75.86
	14.01	13.85	12.88	12.56	12.56	12.24	11.27	10.63
I. colutea (Burm. f.) Merr. Coll. No. 1790	2.42	2.42	2.35	2.14	2.07	1.97	1.93	1.52
	1.33	1.19	1.27	1.21	1.00	1.37	1.80	1.20
	100.00	100.00	97.14	88.57	85.71	81.40	90.00	62.86
	14.37	14.37	13.96	12.73	12.32	11.71	11.50	9.04

Table 2. Continued.

Species and collection	Chromosome pairs							
	1	2	3	4	5	6	7	8
I. cryptantha	2.73	2.73	2.55	2.48	2.31	2.31	2.14	1.76
Benth. ex.	1.40	1.03	1.18	1.57	1.39	1.39	1.21	1.43
Harv. and Stand.	100.00	100.00	93.69	91.16	84.81	84.81	78.50	64.55
	14.30	14.30	13.40	13.05	12.20	12.20	11.30	9.25
I. enneaphylla L.	2.14	2.07	2.07	2.00	1.93	1.79	1.73	1.66
	1.58	2.00	1.00	1.23	1.15	1.00	1.50	2.00
	100.00	96.77	96.77	93.55	90.32	83.87	80.64	77.42
	13.90	13.40	13.40	13.00	12.50	11.60	11.20	10.70
I. glandulosa	3.59	3.28	3.07	2.90	2.83	2.59	2.55	1.35
Willd.	1.54	1.02	1.22	2.00	1.00	1.50	1.18	1.44
	100.00	91.35	85.58	80.77	78.85	72.11	71.15	37.50
	16.20	14.80	13.86	13.08	12.77	11.68	11.53	6.07
I. hochstetteri	3.04	2.90	2.83	2.55	2.48	2.48	2.41	2.35
Baker f.	1.20	1.33	1.16	1.31	1.40	1.00	1.19	1.43
Coll. No. 1780	100.00	95.45	93.18	84.09	81.82	81.82	79.54	77.27
	14.43	13.77	13.44	12.13	11.80	11.80	11.48	11.15
I. hochstetteri	2.76	2.69	2.66	2.55	2.52	2.45	2.28	1.93
Baker f.	1.14	1.44	1.26	1.85	1.61	1.09	1.28	2.11
Coll. No. 1781	100.00	97.50	96.25	92.50	91.25	88.75	82.50	70.00
	13.91	13.57	13.39	12.87	12.70	12.35	11.48	9.74
I. linifolia	2.14	1.86	1.73	1.66	1.66	1.66	1.66	1.52
Retz.	1.21	1.25	1.27	1.40	1.40	1.00	1.00	1.00
	100.00	87.10	80.64	77.42	77.42	77.42	77.42	70.97
	15.40	13.40	12.40	11.90	11.90	11.90	11.90	10.90
I. microcarpa	1.86	1.79	1.73	1.73	1.73	1.66	1.52	1.31
Desv.	1.25	1.36	1.50	1.50	1.27	1.40	1.20	1.53
	100.00	96.30	92.59	92.59	92.59	88.89	81.48	70.37
	13.99	13.47	12.95	12.95	12.95	12.44	11.39	9.84
I. neglecta	2.14	2.00	2.00	1.93	1.73	1.73	1.59	1.38
Brown	1.21	1.23	1.23	1.15	1.27	1.27	1.56	1.00
	100.00	93.55	93.55	90.32	80.64	80.64	74.19	64.51
	14.70	13.80	13.80	13.30	11.90	11.90	10.90	9.50
I. parviflora	2.38	2.21	2.17	2.10	2.07	1.93	1.66	
Heyne ex	1.55	1.56	1.03	1.18	1.00	1.33	1.29	
Wight et arm	100.00	92.75	91.34	88.41	86.96	81.16	69.56	
	16.39	15.20	14.98	14.49	14.25	13.33	11.40	
I. schimperi	2.42	2.42	2.35	2.21	2.21	2.14	1.93	1.93
	1.50	1.38	1.00	1.28	1.00	1.38	1.15	1.15
	100.00	100.00	97.14	91.43	91.43	88.57	80.00	80.00
	33.30	33.30	13.30	12.50	12.50	12.10	10.90	10.90
I. spicata	2.48	2.35	2.35	2.21	2.07	2.00	1.93	1.79
Fosk.	1.12	1.00	1.00	1.28	1.42	1.64	1.33	1.00
	100.00	94.44	94.44	88.89	83.33	80.55	77.78	72.22
	14.40	13.60	13.60	12.80	12.00	11.60	11.20	10.40

Table 2. Continued.

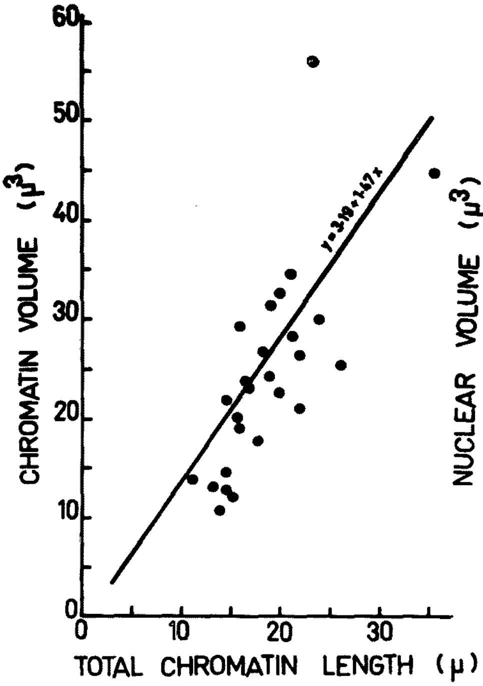
Species and collection	Chromosome pairs							
	1	2	3	4	5	6	7	8
I. subulata	3.31	2.97	2.90	2.62	2.59	2.55	2.55	2.42
Fosk.	1.40	1.32	1.62	1.00	1.21	1.85	1.18	1.00
Coll. No. 1797	100.00	89.58	87.50	79.17	78.12	77.08	77.08	72.92
	15.12	13.54	13.23	11.97	11.81	11.65	11.65	11.02
I. subulata	2.90	2.00	1.93	1.93	1.93	1.79	1.79	1.73
Vahl.	1.00	1.90	1.21	1.00	1.00	1.36	1.00	1.27
Coll. No. 1798	100.00	69.05	66.67	66.67	66.67	61.90	61.90	59.52
	18.10	12.50	12.00	12.00	12.00	11.20	11.20	10.70
I. suffructicosa	2.28	2.28	2.07	2.07	2.07	1.87	1.76	1.66
Mill.	1.36	1.36	1.42	1.31	1.00	1.25	1.13	1.53
	100.00	100.00	90.91	90.91	90.91	81.99	77.27	72.73
	14.19	14.19	12.90	12.90	12.90	11.64	10.97	10.32
I. trifoliata	3.73	3.66	3.60	3.59	3.45	2.90	2.90	2.69
Mill.	1.16	1.41	1.00	1.36	1.27	1.00	1.00	1.05
	100.00	98.15	96.56	96.30	92.59	77.78	77.78	72.22
	14.00	13.80	13.58	13.54	13.02	10.94	10.94	10.16

extends the list of polyploid species known in the subgenus Indigofera.

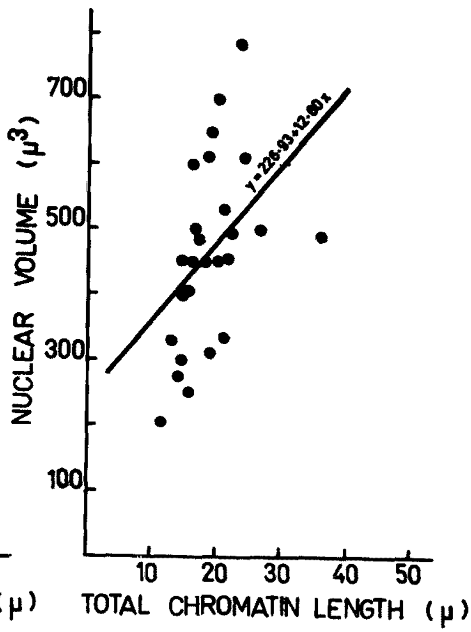
The polyploids with somatic number other than $2n=32$ are also known e. g. $2n=24$ in one species (*I. emarginella*, Frahm-Leliveld 1966), $2n=28$ in one species (*I. cordifolia*, Bir and Sidhu 1967), $2n=36$ in one species (*I. endecephylla*, Kishore 1951) and $2n=48$ in six species (*I. cystisoides*, *I. dosua*, *I. gerardiana*, *I. heterantha*, *I. decora* and *I. divaricata*—Darlington and Wylie 1955, Fedorov 1969). In view of several reports of $2n=16$ in *I. cordifolia* and that of $2n=32$ in *I. endecephylla*, the reports of $2n=28$ in *I. cordifolia* and of $2n=36$ in *I. endecephylla* can not be attached any significance unless identity of material is verified and chromosome count confirmed. However, there is no doubt about the correctness of $2n=24$ and $2n=48$ in the genus. While the count $2n=24$ (*I. emarginella*) should be a tetraploid with $x=6$ in view of the availability of $2n=12$ in two species (*I. macrocalyx*, *I. anil*), $2n=48$ (available in six species as enumerated above) can either be a hexaploid with $x=8$ or it may be octoploid with $x=6$. Frahm-Leliveld (1966) treated these polyploids with $2n=48$ as octoploid as against the view of Gillett (1958), who believed that these were hexaploids. Frahm-Leliveld (1966) gave no reason for his interpretation except the availability of reports of $2n=12$ and $2n=24$ with which $2n=48$ can make a polyploid series. Since $2n=16$ and $2n=32$ are more common in the genus *Indigofera*, and since hexaploidy is an optimal ploidy level in many cases, it may not be necessary to regard $2n=48$ as octoploids.

b) *Karyotypes*

The karyotypic studies in the genus *Indigofera* were undertaken in the past by Singh and Roy (1970) in 17 species and by Bhatt and Sanjappa (1975) in seven species. Four of the species examined by Bhatt and Sanjappa (1975) were also included in the list of species examined by Singh and Roy (1970), so that karyotype analysis of only 20 species of *Indigofera* was hitherto available. Besides these karyotypes, measurements without giving details of karyotypes were also reported by Frahm-Leliveld (1960, 1962) in 38 species, which though give an idea of the size of chro-



49



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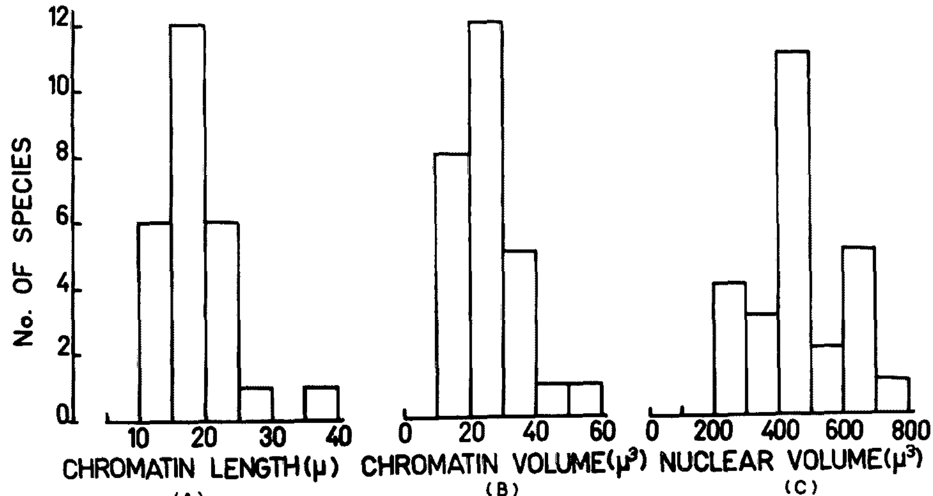

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Figs. 49–51. 49, graph showing regression of chromatin volume on total chromatin length. 50, graph showing regression of nuclear volume on total chromatin length. 51, frequency distribution of different species for (A) total chromatin length, (B) chromatin volume and (C) nuclear volume.

mosome and gradient index (shortest chromosome/longest chromosome ×100), but not quite about the symmetry of karyotypes which is determined not only on the basis of variation in chromosome size, bot also on the basis of position of centromeres in different chromosomes. In the present study karyotypes were prepared for 26 collections belonging to 23 species of which 13 species were examined for karyotypes for the first time, and of these 13, five species (*I. asperifolia*, *I. brevidens*, *I. circinella*, *I. cryptantha* and *I. neglecta*) were never examined before for any kind of cytological study.

While determining karyotype asymmetry, Singh and Roy (1970) considered only TF% (total sum of short arms/total sum of chromosome length ×100).

Table 3. Chromosome data in 26 collections of *Indigofera*

Species and collection no.	Total chromatin (μ)	Mean chromosome length (μ)	Longest/shortest ratio	TF%	Chromatin volume (μ^3)	Nuclear volume (μ^3)
I. alboglandulosa	23.26	2.91	1.62	43.90	14.15	788.32
I. arrecta	18.28	2.28	1.50	42.64	6.64	610.23
I. aspera	19.12	2.39	1.15	42.40	7.79	658.32
I. asperifolia	15.60	1.95	1.35	41.10	5.02	408.28
I. astragalina	11.35	1.42	1.51	43.46	3.43	215.81
I. barberi	23.88	2.98	1.42	44.50	7.44	622.31
I. brevidens	19.92	2.49	2.20	44.89	8.11	690.45
I. circinella	14.42	1.80	1.75	40.60	3.55	409.50
I. colutea (1789)	21.42	2.68	1.32	44.61	7.11	461.58
I. colutea (1790)	16.82	2.10	1.59	43.94	5.58	503.45
I. cryptantha	19.01	2.38	1.55	43.37	6.12	317.79
I. endecephylla	35.81	2.24	1.55	43.83	11.16	480.65
I. enneaphylla	15.39	1.92	1.29	42.10	3.02	259.80
I. glandulosa	22.15	2.77	2.66	43.15	5.26	489.08
I. hochstetteri (1780)	21.04	2.63	1.29	44.59	8.57	377.46
I. hochstetteri (1781)	19.84	2.48	1.43	40.87	5.61	447.85
I. linifolia	13.89	1.73	1.41	44.70	2.73	274.64
I. microcarpa	13.33	1.67	1.42	42.23	3.28	332.66
I. neglecta	14.50	1.81	1.55	44.70	5.42	291.71
I. parviflora	14.52	2.07	1.43	44.18	3.20	450.18
I. schimperi	17.61	2.20	1.25	45.00	4.34	446.87
I. spicata (1821)	17.18	2.15	1.39	46.10	5.88	483.07
I. subulata (1797)	21.91	2.74	1.37	43.46	6.61	530.28
I. subulata (1798)	16.00	2.00	1.68	45.60	4.68	469.42
I. suffructicosa	16.04	2.01	1.37	44.52	7.28	600.49
I. trifoliata	26.52	3.31	1.39	46.35	6.30	492.04

Bhatt and Sanjappa (1975) on the other hand, considered TF% along with other features like number of metacentric chromosomes and ratio of longest to shortest chromosome. However, no attempt in thses studies was made to classify the examined species into 12 categories given by two way classification by Stebbins (1971).

During the present study, detailed karyotype analysis was done and degree of asymmetry was worked out on the basis of chromosome size and centromere position. Only four classes of the classification of Stebbins (1971) were represented.

I. brevidens belonged to 1B, *I. glandulosa* belonged to 2B, five species namely *I. arrecta, I. asperifolia. I. astragalina, I. enneaphylla* and *I. hochstetteri* (Coll. 1781) belonged to 2A and the remaining 17 collections belonged to 1A. This indicates that the level of asymmetry was moderate as is also evident from similar values of TF% for different species. Variation was, however, observed in the total chromatin length which ranged from 11.35 μ to 26.52 μ among diploids. Although this variation can be partly attributed to different levels of contraction, but atleast part of the

Talbe 4. Karyotypic formulae of 26 collections belonging to 23 different species of *Indigofera* (A, B and C represent long, medium and short chromosomes respectively; 'sc' used as superscript and subscript represent secondary constriction in short and long arms respectively; superscripts 'm', 'sm' and 'st' represent respectively the median, submedian and subterminal positions of centromeres)

S. no.	Species	Karyotypic formulae
1.	*I. alboglandulosa*	$1A^{m}+1scA^{sm}+1A^{sm}+2B^{m}+3B^{sm}$
2.	*I. arrecta*	$3B^{m}+2scB^{sm}+2B^{sm}+1B^{st}$
3.	*I. aspera*	$1scB^{m}+3scB^{sm}+4B^{sm}$
4.	*I. asperifolia*	$1B^{m}+6B^{sm}+1C^{sm}$
5.	*I. astragalina*	$2B^{sm}+4C^{m}+1C^{sm}+1C^{st}$
6.	*I. barberi*	$2A^{m}+1scA^{sm}+2A^{sm}+1B^{m}+2B^{sm}$
7.	*I. brevidens*	$1scA^{sm}+3B^{m}+3B^{sm}+1C^{m}$
8.	*I. circinella*	$2B^{m}+4B^{sm}+2C^{sm}$
9.	*I. colutea* (Coll. 1789)	$1A^{sm}+1^{sc}B^{m}+2B^{m}+4B^{sm}$
10.	*I. colutea* (Coll. 1790)	$2B^{m}+5B^{sm}+1C^{m}$
11.	*I. cryptantha*	$2B^{m}+1scB^{sm}+5B^{sm}$
12.	*I. endecephylla*	$4B^{m}+4scB^{sm}+8B^{sm}$
13.	*I. enneaphylla*	$3B^{m}+5B^{sm}$
14.	*I. glandulosa*	$1scA^{m}+1^{sc}A^{sm}+1A^{sm}+2B^{m}+2B^{sm}+1C^{sm}$
15.	*I. hochstetteri* (coll. 1780)	$1scA^{m}+3B^{m}+4B^{sm}$
16.	*I. hochstetteri* (coll. 1781)	$2B^{m}+5B^{sm}+1B^{st}$
17.	*I. linifolia*	$2B^{m}+5B^{sm}+1C^{m}$
18.	*I. microcarpa*	$6B^{sm}+2C^{sm}$
19.	*I. neglecta*	$1B^{m}+1scB^{sm}+4B^{sm}+1C^{m}+1C^{sm}$
20.	*I. parviflora*	$3B^{m}+4B^{sm}$
21.	*I. schimperi*	$4B^{m}+1scB^{sm}+3B^{sm}$
22.	*I. specata* (Coll. 1821)	$4B^{m}+4B^{sm}$
23.	*I. subulata* Coll. 1797)	$1A^{sm}+1scB^{m}+1B^{m}+1scB^{sm}+4B^{sm}$
24.	*I. subulata* (Coll. 1798)	$4B^{m}+4B^{sm}$
25.	*I. suffructicosa*	$2B^{m}+6B^{sm}$
26.	*I. trifoliata*	$2A^{m}+1scA^{sm}+2A^{sm}+3B^{m}$

differences in chromatin length must be real as also evident from differences in the estimates of chromatin volumes and nuclear volumes (Table 3). It has earlier been demonstrated in wheat by Pegington and Rees (1970) that chromosome volume and chromosome weights are better estimates of chromatin content than the chromosome length. Moreover, Sparrow (1965) demonstrated that a direct correlation exists between nuclear DNA content and nuclear volume, so that nuclear volume can give a fairly good estimate of nuclear DNA content.

As shown above only seven of the 23 species have relatively asymmetric karyotypes and were placed in classes 1B, 2B and 2A. The only species placed in class 2B was *I. glandulosa*, which thus had the most asymmetric karyotype in the collections used in the present study. It should therefore, be regarded as most advanced. This observation gets support from Bhatt and Sanjappa (1975), who also regarded *I. glandulosa* as one of the most advanced species. *I. hochstetteri* has also been regarded as an advanced species by Singh and Roy (1970) and by Bhatt and Sanjappa (1975) on the basis of chromosome lengths and TF%. During the present study of karyotypes, this was placed in class 2A suggesting that this species had a relatively asymmetric karyotype and was an advanced species in relation to other species placed in class 1A.

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Summary

In the genus *Indigofera* L., meiosis was studied in 24 collections belonging to 23 species and karyotypes with the help of root tip mitosis were examined in 26 collections belonging to 23 species. Three species, namely *I. endecephylla*, *I. parodiana*, and *I. pilosa* were tetraploid. Among diploid taxa, *I. parviflora* had $2n=14$ and other remaining collections had $2n=16$. Karyotype asymmetry was assessed following the two way classification of Stebbins (1971). *I. glandulosa* belonging to class 2B had the most asymmetric karyotype and was therefore considered most advanced. The length of individual chromosome ranged from 3.59 μ to 1.10 μ. The total chromosome length varied from 26.52 μ to 11.35 μ in diploid collections and was 35.81 μ in the solitary tetraploid collection of *I. endecephylla*. The chromatin volumes and nuclear volumes were also worked out and had significant linear regression on total chromatin lengths. Five diploid species (*I. asperifolia*, *I. brevidens*, *I. circinella*, *I. cryptantha*, and *I. neglecta*) and one tetraploid species (*I. parodiana*) were cytologically examined for the first time and karyotypes were worked out in 13 species for the first time.

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