

OBSERVATIONS ON CHROMOSOMES IN THE
GENUS *INDIGOFERA* L.

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This study on *Indigofera* species mainly from the West African region, will be the first of some publications dealing with the cytology of West African Leguminous plants (except trees) present in the savannah regions. These are plants which might either come into consideration as auxiliary crops or are near relatives of auxiliary plants already in use. Seeds and herbarium material were collected by the author during a two months' tour early in 1957. The tour started at Kano and proceeded via Jos, Samaru, Zinder, Fort Lamy, Bangui, Boukoko and Pobé to Adiopodoumé. From the last-mentioned place some parts of the Ivory Coast belonging to the coastal savannah area and farther inland were visited. The tour was made possible by means of a grant from the Netherlands Ministry of Agriculture on nomination by the Board of Governors of the State Agricultural University of Wageningen. Ample assistance and hospitality during my residence in West Africa were met with everywhere: I should like to tender my heartfelt thanks especially to the Director, Mr. Gisbourne, and Staff of the Government Agricultural Experiment Station at Samaru (North Nigeria), to the Director of the Service de l'Agriculture, Mr. Gontier, to the Director, Mr. Didelot, and Staff of the Station Expérimentale, Boukoko (Oubangui), to the Director, Mr. Rancoule, and Staff of the Station Expérimentale de l'IRHO at Pobé (Dahomey), to the Director, Prof. Mangenot, and Staff of the IDERT at Adiopodoumé, and to the Centre néerlandais, Adiopodoumé (Ivory Coast). But for the help of the said persons and institutions, it would not have been possible to bring together such an extensive collection of Leguminous material in such a short period.

On my request the Kew Herbarium in the person of Mr. Gillett undertook the revision of the herbarium material of my *Indigofera* specimens. Without the expert assistance from a systematic botanist it would not have been possible to find a way in the entanglement of modern nomenclature that is met with in the difficult genus *Indigofera*. The revised herbarium material has been inserted in the Herbarium Vadense, Laboratory of Systematic Botany, Wageningen.

The genus *Indigofera* L. is widespread in the tropical regions of both the Old and the New World. Not only a fair number of species have been spread by human aid, but non-cultivated taxa are known from all equatorial regions and also from regions situated at a slightly higher latitude.

Species	Collection no.	Origin	Chromosome no.		Remarks
			2n	n	
<i>Subgenus A. Acanthonotus (Benth.) Benth. & Hook. f.</i>					
1 <i>Indigofera nummulariifolia</i> (L.) Livera ex Alston	57043	Shika Grassland Farm (Nrth. Nigeria)	16		1. As " <i>Indigofera echinata</i> Willd."
<i>Subgenus C. Indigofera L.</i>					
<i>Section C2. Paniculatae (Bak.) Gillett</i>					
<i>Subsection a. Paniculatae</i>					
2 <i>Indigofera paniculata</i> Vahl ex Pers. ssp. <i>paniculata</i>	57120	Ketou (Central Dahomey)	16		
3 id.	57124	Idigny (Central Dahomey)	16		
4 id.	57130	Ketou (Central Dahomey)	16		
5 id.	57159	Dabou (Ivory Coast)	16		
6 <i>Indigofera paracapitata</i> Gillett	57092	Boukoko (Oubangui)	16		6. As " <i>Indigofera congesta</i> Welw. ex Baker"
7 <i>Indigofera congesta</i> Welw. ex Baker	57093	Boukoko (Oubangui)	16		
8 <i>Indigofera pulchra</i> Willd.	54044 (Herb. 1954/32)	Mopoyem (Ivory Coast)	16	8	
9 id.	57005	Jos (Nrth. Nigeria)	16	8	
<i>Section C3 Indigofera L.</i>					
<i>Subsection b. Brevi-erectae Gillett</i>					
10 <i>Indigofera simplicifolia</i> Lam.	57101	Bouboko (Oubangui)	16		
<i>Subsection d. Dissitiflorae (Bak.) Gillett</i>					
11 <i>Indigofera dendroides</i> Jacq.	57125	Idigny (Central Dahomey)	16		
12 <i>Indigofera heudelotii</i> Benth. ex Baker var. <i>heudelotii</i>	57006	Jos (Nrth. Nigeria)	16		
13 id.	57013	Jos (Nrth. Nigeria)	16		
<i>Subsection h. Viscosae Rydberg</i>					
14 <i>Indigofera secundiflora</i> Poir.	57121	Ketou (Central Dahomey)	16		
<i>Subsection m. Tinctoriae (Bak.) Gillett</i>					
15 <i>Indigofera macrophylla</i> Schum.	57137	Abba (Central Dahomey)	16		
16 <i>Indigofera trita</i> L.f. subsp. <i>subulata</i> Ali var. <i>subulata</i>	57153	Exp. Sta. I.R.H.O., Pobé (Central Dahomey)	16		16. Seed sample 20 years old, "Sémi-rampant"
17 <i>Indigofera arrecta</i> Hochst. ex A. Rich	Bogor 1949	General Agric. Res. Sta. Bogor, Indonesia	16		
18 id.	57071	Samaru (Nrth. Nigeria)	16		
19 <i>Indigofera suffruticosa</i> Mill.	54052 (Herb. 1954/46)	Exp. Sta. CRA, Akandjé (Ivory Coast)	16		
20 id.	57155	Exp. Sta. I.R.H.O., Pobé (Central Dahomey)	16		20. Seed sample 20 years old, " <i>Indigofera suffruticosa</i> Mill"
21 <i>Indigofera tinctoria</i> L.	Bogor 1949	General Agric. Res. Sta. Bogor, Indonesia	16		21. As " <i>Indigofera sumatrana</i> Gaertn."
22 id.	54055 (Herb. 1954/50)	Grand Bassam (Ivory Coast)	16		
23 id.	57062	Shika (Nrth. Nigeria)	16		
24 id.	57145	Exp. Sta. I.R.H.O., Pobé, (Central Dahomey)	16		
<i>Attributed to subsection m. Tinctoriae Bak. by Taubert in Engler & Prantl</i>					
<i>1st edition:</i>					
25 <i>Indigofera dosua</i> Buch.-Ham. ex D. Don.	Coimbra 1956	Bot. Garden, Coimbra, Portugal	48		
26 <i>Indigofera heterantha</i> Wall. ex Brandis	Zürich 1953	Bot. Garden, Zürich, Switzerland	48		26. As " <i>Indigofera gerardiana</i> Wall. ex Baker"
<i>Subsection n. Hirsutae Rydberg</i>					
27 <i>Indigofera hirsuta</i> L.	54058 (Herb. 1954/55)	Adiopodoumé (Ivory Coast)	16		
28 id.	57042	Shika Grassland Farm (Nrth. Nigeria)	16		28. Imported: origin unknown
29 id.	57123	Ketou (Central Dahomey)	16		
30 id.	57131	Ketou (Central Dahomey)	16		30. Dr. Wormer legit
31 id.	57152	Cové (Central Dahomey)	16		
32 id.	57177	Bingerville (Ivory Coast)	16		
33 <i>Indigofera astragalina</i> DC	57031	Shika Grassland Farm (Nrth. Nigeria)	16		33. From Plateau
<i>Subsection p. Alternifoliolae (Harvey) Gillett</i>					
34 <i>Indigofera Schimperii</i> Jaub. et Spach.	Gillett, N. Kenya 13728	Seeds received from Kew Herbarium	16		
35 <i>Indigofera spicata</i> Forsk	Bogor 1949	General Agric. Res. Sta. Bogor, Indonesia	32		
36 id.	54054 (Herb. 1954/49)	Azuretti (Ivory Coast)	16	16	35. As " <i>Indigofera endecaphylla</i> Jacq."
37 id.	57144	Exp. Sta. I.R.H.O., Pobé (Central Dahomey)	32		36. id.
38 id.	57150	Subgrowth under <i>Cocos nucifera</i> L. Semé-Podji (Dahomey)	32		37. id.
39 id.	57156	Exp. Sta. I.R.H.O., Pobé, (Central Dahomey)	32		38. id.
<i>Section unknown</i>					
40 <i>Indigofera cytisoides</i> L.	Antibes 1953	Villa Thuret, Antibes, France	48		39. id. Seed sample 20 years old, "rampant"

The use of indigo as a dyestuff is of so ancient date that it is impossible to establish the original area of several of the species which have been used for that purpose. It is a historical fact that round 2000 BC indigo dying was practised in Egypt, and it seems also to have been known in India. BURKILL (1935) supposes that the use of indigo as a dye developed independently with the Indians in America. All species signalled by Burkill for the preparation of indigo in SE Asia, come under the subgenus *Indigofera*, section *Tinctoria*. Much less data are available on the use of indigo in Africa, and no historical facts are known: the species used, however, appear to be for the greater part the same, and pertain also to the section *Tinctoria*.

In far more recent times the *Indigofera* species came to be useful in still another way: several members of both *Tinctoria* and other sections were applied as green manure, and in many tropical regions trials were started with either indigenous or imported species. Toxicity for cattle has been claimed for some of them, although the evidence on this matter is still contradictory in others (GILLET, 1958, p. 137). The enormous variability within certain species (*I. tinctoria*, *I. hirsuta*, *I. spicata*, and others) may have been stimulated by their world-wide dispersion; thus, according to GILLET (1958): "that the wide distribution in part by man caused obscurity as to the previous confusion of forms which is now observable". In the case of *I. spicata*, however, this remark may well be amplified by the observation that this species is a tetraploid: polyploidy in itself may be the cause of wide variation within a species.

Besides the cosmopolitical, to a certain degree cultivated, *Indigofera* species, this very extensive genus contains many representatives with a narrow area of distribution: among the latter are purely African, Asiatic, American, and Australian ones. However, quite a number have a distribution which extends over more than one continent. The monograph on the African species by GILLET (1958) and a recent article by ALI on Pakistan and Himalayan ones (1958) clearly show the immense difficulties encountered in a number of critical cases where delimitation of species in *Indigofera* becomes necessary. Our investigations on the cytology mainly contain data from a limited number of African species: the ultimate scope being to test the eventual suitability of the cytological data as a taxonomic aid in *Indigofera*. Almost all the *Indigofera* materials discussed in this study were collected in the West African region.

Table 1 contains an enumeration of the *Indigofera* specimens that were studied by us, the collection number, the origin of the sample, the chromosome number, and additional notes. From by far the greater part of the specimens seedlings were obtained, and in the root tips of the latter the diploid chromosome numbers were determined. Plates 1 and 2 show the chromosome arrangements, designs of which were made at an actual magnification of 20×100 . An attempt to ascertain the individual chromosome types in the various species met with scanty results, obviously owing to their small size. The

measuring of the entire chromosome compliments turned out to be more efficient; a list of the latter may be found in Table II.

TABLE II
Total length of mitotic chromosome complement in μ

1	Indigofera	nummulariifolia	21.4
2	"	paniculata ssp. paniculata	38.1
3	"	" " "	28.5
4	"	" " "	32.6
5	"	" " "	39.1
6	"	paracapitata	36.5
7	"	congesta	30.0
8	"	pulchra	30.1
10	"	simplicifolia	28.0
11	"	dendroides	38.9
12	"	Heudelotii var. Heudelotii	39.1
13	"	" " "	32.7
14	"	secundiflora	25.4
15	"	macrophylla	27.3
16	"	trita var. subulata	32.3
17	"	arrecta	34.3
18	"	"	32.4
19	"	suffruticosa	33.7
20	"	"	38.9
21	"	tinctoria	35.3
22	"	"	26.2
23	"	"	26.8
24	"	"	29.7
25	"	dosua	88.0
26	"	heterantha	76.0
27	"	hirsuta	18.8
28	"	"	23.8
29	"	"	21.2
30	"	"	19.4
31	"	"	23.8
32	"	"	24.7
33	"	astragalina	23.4
34	"	Schimperi	33.5
35	"	spicata	45.1
37	"	"	49.5
38	"	"	50.0
39	"	"	53.0
40	"	cytisoides	87.4

In both Tables I and II the species are arranged according to the systematic classification adopted in the monograph by GILLET (1958).

Incidental information dispersed in an extensive number of publications revealed the fact that the base number in *Indigofera* may be assumed to be 8. SENN (1938) reports a number of 6 for *I. anil*, but there seems to have been a considerable confusion in nomenclature which involves the names *I. anil*, *I. tinctoria* and *I. suffruticosa*. According

to HAGERUP (1932), *I. parviflora* Heyne has a base number of 7 and *I. endecaphylla* Jacq. was reported by KISHORE (1951) as having $n = 18$. The available publications provides the results which are listed in Table III in alphabetical order.

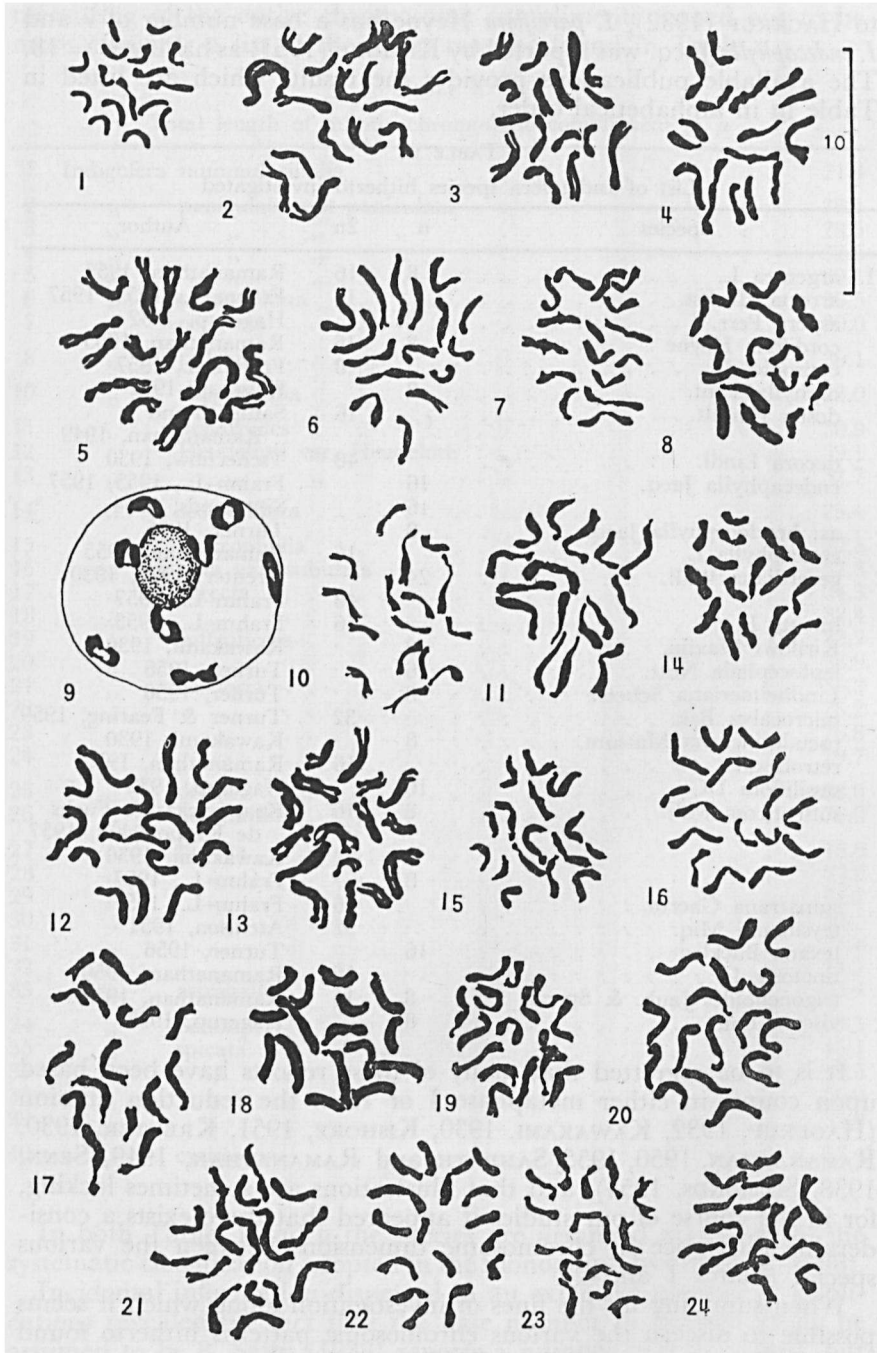
TABLE III
List of *Indigofera* species hitherto investigated

Species	n	2n	Author
<i>I. argentea</i> L.	8	16	Ramanathan, 1955
<i>arrecta</i> Hochst.		16	Frahm-L., 1953, 1957
<i>aspera</i> Perr.	8		Hagerup, 1932
<i>cordifolia</i> Heyne	8	16	Ramanathan, 1955
<i>cytisoides</i> L.		48	Frahm-L., 1957
<i>diphylla</i> Vent.	8		Hagerup, 1932
<i>dosua</i> Hamilt.		16	Sampath and Ramanathan, 1949
<i>decora</i> Lindl.		48	Tschechow, 1930
<i>endecaphylla</i> Jacq.	16		Frahm-L., 1953, 1957
	16		Simmonds, 1954
as: <i>hendocephylla</i> Jacq.	8		Turner, 1956
<i>enneaphylla</i> L.		16	Ramanathan, 1955
<i>gerardiana</i> Wall.	24		Kreuter, 1929, 1930
		48	Frahm-L., 1957
<i>hirsuta</i> L.		16	Frahm-L., 1953
<i>Kirilowi</i> Maxim.	8		Kawakami, 1930
<i>leptocephala</i> Nutt.	16		Turner, 1956
<i>Lindheimeriana</i> Scheele	8		Turner, 1956
<i>microcalyx</i> Bak.		32	Turner & Fearing, 1959
<i>pseudotinctoria</i> Matsum.	8		Kawakami, 1930
<i>retroflexa</i> ?		16	Ramanathan, 1955
<i>sessiliflora</i> DC	16		Hagerup, 1932
<i>suffruticosa</i> Mill.	8	16	Krapovickas & Fuchs de Krapovickas, 1957
		32	Kawakami, 1930
	8		Frahm-L., 1957
<i>sumatrana</i> Gaertn.		16	Frahm-L., 1957
<i>teysmanni</i> Miq.		32	Atchison, 1951
<i>texana</i> Buckley	16		Turner, 1956
<i>tinctoria</i> L.		16	Ramanathan, 1950
<i>trigonelloides</i> Jaub. & Spach.	8	16	Ramanathan, 1955
<i>viscosa</i> Lam.	8		Hagerup, 1932

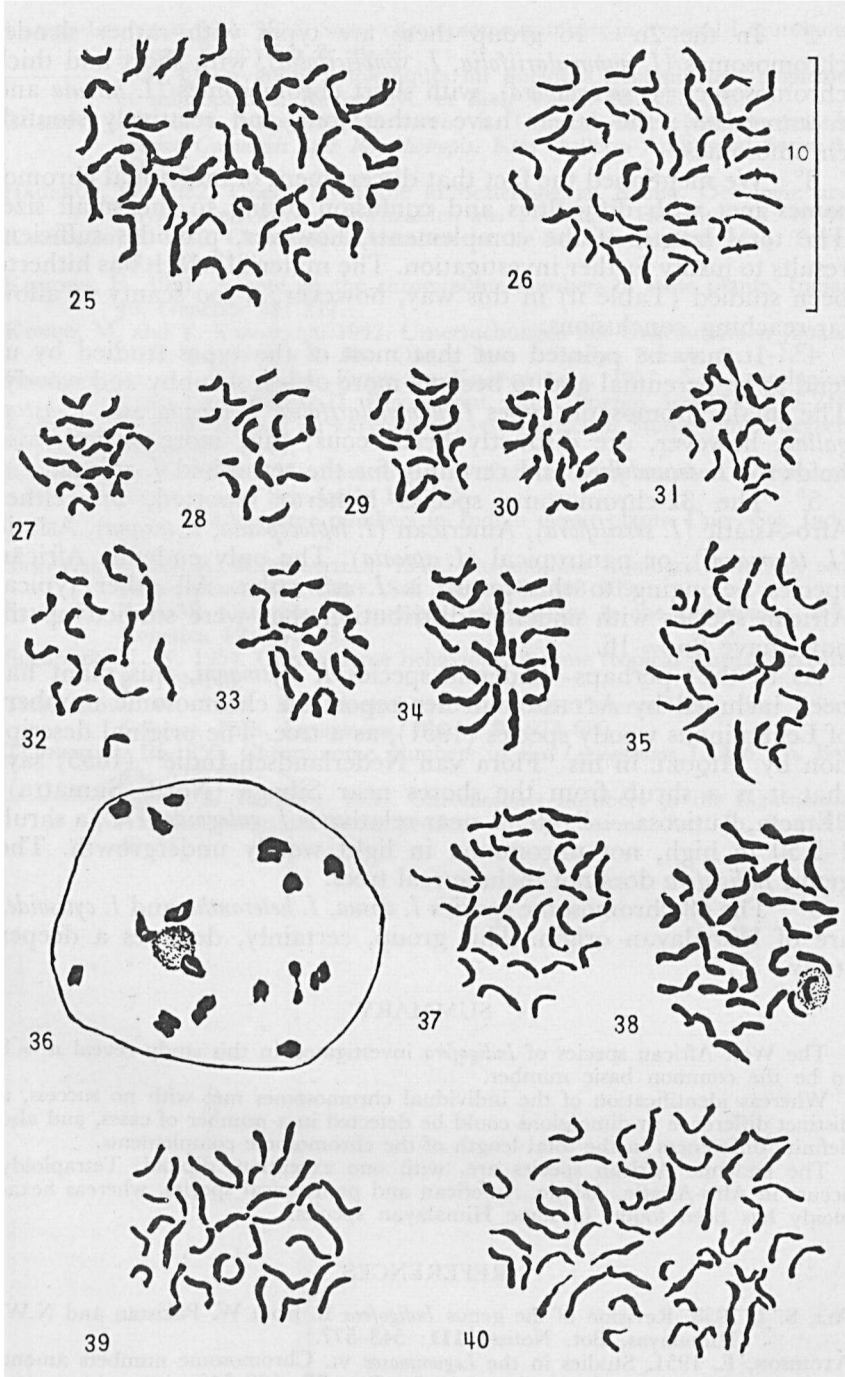
It is to be regretted that many of these reports have been based upon counts in either metaphase I or II of the reduction division (HAGERUP, 1932, KAWAKAMI, 1930, KISHORE, 1951, KREUTER, 1930, RAMANATHAN, 1950, 1955, SAMPATH and RAMANATHAN, 1949, SENN, 1938, SIMMONDS, 1954), and that illustrations are sometimes lacking, for in the course of our studies it appeared that there exists a considerable difference in chromosome dimensions between the various species (Plates 1 and 2).

When summing up the lines of investigation along which it seems possible to discern the various chromosome patterns hitherto found in *Indigofera* we come to the following results.

1° Apart from a few devious reports, there are three main chromosome groups, viz. $2n = 16$, $2n = 32$ and $2n = 48$.



Plates 1 and 2. The numbers of the chromosome complements correspond to the numbers of the species listed in Table 1. All the designs were made from metaphase plates in root-tip cells, except for the PMC diakinesis figures 9 and 36. The unit



of magnification is 10μ . The drawings were made from sectioned material previously fixed in Navashin fluid and subsequently stained with crystal violet.

2° In the $2n = 16$ group there are types with rather slender chromosomes (*I. nummularifolia*, *I. simplicifolia*), with short and thick chromosomes (*I. secundiflora*), with short chromosomes (*I. hirsuta* and *I. astragalina*); the others have rather long and relatively stoutish chromosomes.

3° We mentioned the fact that discernment of individual chromosomes met with difficulties and confusion owing to the small size. The total length of the complements, however, provides sufficient results to justify further investigation. The material which has hitherto been studied (Table II) in this way, however, is too scanty to allow far-reaching conclusions.

4° It may be pointed out that most of the types studied by us tend to be perennial and to become more or less shrubby and woody. The small-chromosome types *I. nummularifolia*, *I. hirsuta* and *I. astragalina*, however, are distinctly herbaceous; this, more or less, also holds for *I. secundiflora* and certainly for the tetraploid *I. spicata*.

5° The 32-chromosome species hitherto reported, are either Afro-Asiatic (*I. sessiliflora*), American (*I. leptcephala*, *I. texana*), Asiatic (*I. teysmanni*), or pantropical (*I. spicata*). The only endemic African species belonging to this group is *I. microcalyx*. All other typical African species with endemic distribution that were studied up till now, have $2n = 16$.

As to the—perhaps—endemic species *I. teysmanni*, this plant has been included by ATCHISON in her report on chromosome numbers of Leguminous woody species (1951), as a tree. The original description by MIQUEL in his "Flora van Nederlandsch-Indië" (1855) says that it is a shrub from the shores near Siboga (North Sumatra): "Erecta, fruticosa—", etc. A near relative is *I. galegoides* DC, a shrub 1–2.50 m high, not uncommon in light woody undergrowth. The genus *Indigofera* does not include real trees.

6° The 48-chromosome species *I. dosua*, *I. heterantha* and *I. cytisoides* are of Himalayan origin. This group, certainly, deserves a deeper study.

SUMMARY

The West African species of *Indigofera* investigated in this study reveal $n = 8$ to be the common basic number.

Whereas identification of the individual chromosomes met with no success, a distinct difference in dimensions could be detected in a number of cases, and also definite differences in the total length of the chromosome complements.

The endemic African species are, with one exception, diploid. Tetraploidy occurs in Afro-Asiatic, Asiatic, American and pantropical species, whereas hexaploidy has been found in some Himalayan species.

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