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Cytological Studies in Certain Species of Lamiaceae

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The family Lamiaceae comprises 180 genera and 3500 species, distributed in temperate and tropical regions of the world (Willis 1973). In India alone, there are about 425 species distributed under 68 genera (Mukherjee 1940). The family is of great importance from the commercial standpoint (Heywood 1978) as its members include a large number of essential oil containing plants. Most of them, though setting seeds, are cultivated mainly through vegetative means.

The members of the family have been studied by a number of taxonomists, but the views differ with regard to delimination of various taxa. Bentham and Hooker (1876) included the family in their Gamopetalae. Hutchinson (1969) placed the family at the top of Herbaceae, a group of primarily herbaceous plants. Briquet's treatment as published in Engler and Preantl's Die Natürlichen Pflanzenfamilien (1897) deals with the delimination of several genera which are subdivided into different subgenera and/or sections. Briquet divided the entire family into 8 subfamilies, namely Ajugoideae, Prostantheroideae, Prasiodeae, Ocimoideae, Scutellariodeae, Lavanduloideae, Stachydoideae and Catopheriodeae. Of these, Ajugoideae, Stachydoideae and Ocimoideae have been further split into different tribes and subtribes.

The cytological work in the family has been attempted by a number of workers. In most cases merely the chromosome number and gross morphology of the chromosome complement have been reported. The reason for such scanty cytological data is principally due to extremely small chromosomes which are difficult to analyze and the presence of essential oils, which stand against adequate clarification of chromosome details.

The genera *Ajuga* and *Leucosceptrum* selected for the present study belong to the subfamily Ajugoideae: *Prunella*, *Notochaete* and *Anisomeles* to the Stachydoideae while *Orthosiphon* belongs to the subfamily Ocimoideae. Detailed karyomorphological studies have been made for all the afore-mentioned taxa. The need for such an investigation was felt in order to provide additional data for interpreting the affinity and inter-relationship of different taxa.

Materials

Chromosome studies of 7 species belonging to 6 genera of the family Lamiaceae have been carried out. The plants were grown in the experimental garden of the Deparment of Botany, University of Calcutta. The materials were collected from various localities of the plains as well as hilly areas. Their specific names with their respective subfamily and sources of collection are given below:

	Name of the species	Subfamily	Area of collection
1	*Ajuga lobata* D. Don	Ajugoideae	Darjeeling
2	*Leucosceptrum canum* Smith.	-do-	-do-
3	*Netochaete hamosa* Benth.	Stachydoideae	-do-
4	*Prunella vulgaris* L.	-do-	-do-
5	*Anisomeles indica* Kuntz.	-do-	Calcutta
6	*Orthosiphon incurvus* Benth.	Ocimoideae	Simlipal forest, Orissa
7	*Orthosiphon scapiger* Benth.	-do-	Betla forest, Hazaribag

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Methods

a) *Study of somatic chromosomes*

The optimum pretreatment schedules followed for the respective species and their individual pretreating chemicals, temperature and periods of pretreatment are given below:

Name of the species	Pretreatment		
	Pretreating chemicals	Temp.	Duration (in hr.)
1 *Ajuga lobata*	8-hydroxyquinoline	10–14°C	2½
2 *Leucosceptrum canum*	Half-saturated PDB	12–15°C	3
3 *Notochaete hamosa*	8-hydroxyquinoline	10–14°C	3
4 *Prunella vulgaris*	Half-saturated PDB	12–14°C	2
5 *Anisomeles indica*	–do–	12–14°C	2½
6 *Orthosiphon incurvus*	–do–	12–16°C	3½
7 *Orthosiphon scapiger*	–do–	12–15°C	3

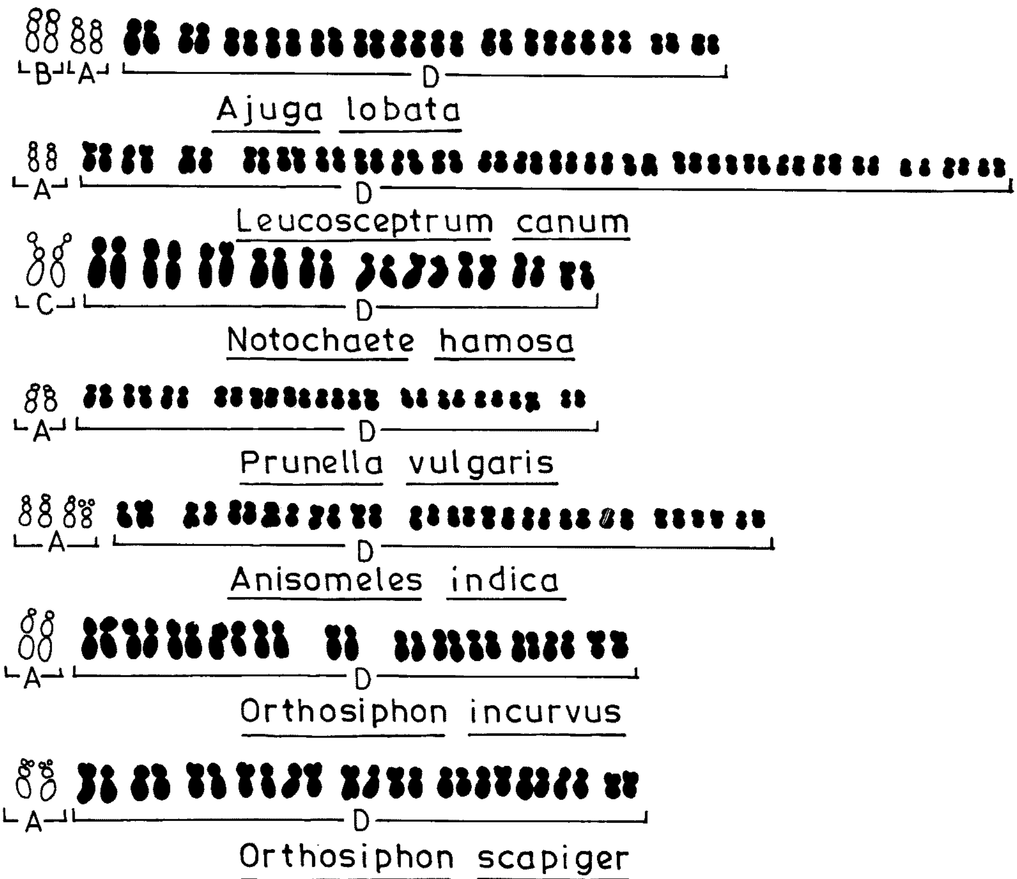


Fig. 1. Karyogram tables of the seven different species of the family Lamiaceae. ×2800 approx.

After the pretreatment the root tips were fixed in acetic ethanol (1: 3) for 1 to 3 hours. Usual acetic orcein/N HCl method was followed for staining.

b) *Study of meiotic chromosomes*

Flower buds of suitable size were fixed overnight in acetic ethanol (1: 2). The anthers were then transferred to 45% acetic acid for five minutes and smeared in 2% acetic carmine.

Observations

a) *Mitotic studies*

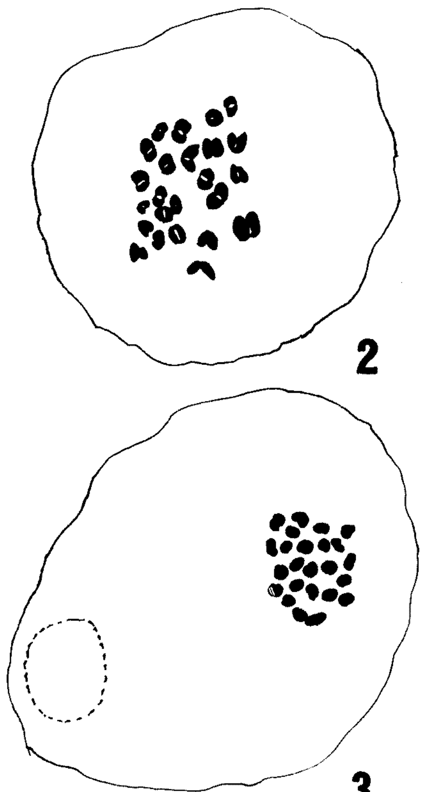
In the chromosomes of the seven different species belonging to six genera of the family Lamiaceae, both the numerical and structural differences have been noted. The normal somatic chromosome number has been found to vary from 2n=22 to 2n=48 in the different taxa. The morphology of the chromosomes shows little variation in having median to nearly submedian primary constriction in all the investigated taxa. In general, the chromosomes are very small. The chromosome size ranges from 0.89 to 3.39 μm. The TF% varies from 34.36 to 39.94%. The number of nucleolar chromosomes varies from two to four. Chromosome analysis of different species has revealed the presence of following four types of chromosomes:

Type A: Chromosomes are with two constrictions. One constriction is median to nearly median and the other is nearly submedian to nearly subterminal in position. The chromosome length ranges from 1.78 to 3.39 μm.

Type B: Chromosomes are with two constrictions. One constriction is nearly median and the other is nearly submedian in position. Both the distal segments are same in length. The chromosome length is 2.5 μm.

Type C: Chromosomes bear two constrictions of which one is satellite. One constriction is nearly median and the other is nearly submedian in position. The chromosome length is 3.21 μm.

Type D: Chromosomes are with nearly median to nearly submedian primary constrictions. The chromosome length ranges from 0.89 to 3.21 μm.



Figs, 2 and 3. *Leucosceptrum canum*—Meiotic metaphases I and II (n=24). ×2000 approx.

b) *Meiotic studies*

In *Leucosceptrum canum*, the mitotic chromosome number (2n=48) has been confirmed by the occurrence of 24 bivalents at first meiotic metaphase (Fig. 2) and 24 chromosomes at second meiotic metaphase (Fig. 3). Meiotic study of other species could not be done due to unavailability of flower buds.

Discussion

The chromosome numbers of seven species has been determined. These represent first counts for *Orthosiphon scapiger* (2n=26), *Orthosiphon incurvus* (2n=26) and *Notochaete hamosa*

(2n=22), new reports for *Ajuga lobata*, (2n=32, previously reported as n=8 and 2n=16; Arora 1961) and *Leucosceptrum canum* (n=24 and 2n=48, previously reported as n=12; Malla *et al.* 1977), confirmation for *Prunella vulagris* (2n=28) and *Anisomeles indica* (2n=34). The somatic chromosome numbers in the present materials are multiples of 11, 12, 13, 14, 16 and 17 chromosomes. A scrutiny of the chromosome numbers recorded so far in the family Lamiaceae including the present record, clearly indicates that each different subfamily, as established by Briquet (1897), is characterized by a series of different chromosome number in its species. The presence of identical numbers in unrelated genera is a very noteworthy feature in this family (Fedorov 1969). The presence of such widely different series of chromosome numbers in the species of even the same genus and in genera placed under different subfamilies, indicates that the different chromosome numbers can be derived one from the other. The previous observation taken in conjunction with the present one suggests that aneuploidy has been one of the main features in the evolution of different species of this family. The difference in chromosome numbers alone, between different genera, therefore, does not provide any conclusive evidence with regard to the systematic status and phylogenetic position of the different members of this family.

Table 1. A comparative representation of different chromosome parameters in seven different species

Taxa	Chromosome number	Karyotype formula	No. of chromo somes with secondary constriction	Total chromatin length in haploid complement (μm)	TF%	Range of chromosome length (μm)
1 *Ajuga lobata* D. Don	2n=32	2A+2B+28D	4	27.60	38.85	1.07–2.50
2 *Leucosceptrum canum* Smith.	2n=48	2A+46D	2	30.80	37.62	0.89–1.78
3 *Notochaete hamosa* Benth.	2n=22	2C+20D	2	27.28	34.36	1.78–3.21
4 *Prunella vulgaris* L.	2n=28	2A+26D	2	17.46	38.86	0.89–1.78
5 *Anisomeles indica* Kuntz.	2n=34	4A+30D	4	23.87	39.94	1.07–2.14
6 *Orthosiphon incurvus* Benth.	2n=26	2A+24D	2	29.25	39.68	1.60–3.39
7 *Orthosiphon scapiger* Benth.	2n=26	2A+24D	2	25.94	38.13	1.42–2.50

The karyotype analyses of seven different species of this family reveal that chromosomes possess median, nearly median or nearly submedian types of centromeres only. No chromosome with terminal or subterminal centromere has been observed. The chromosome size, represented in a graded karyotype ranges from 0.89 to 3.39 μm in length (Table 1). The number of nucleolar chromosomes varies from two to four and these are evidences of both numerical and structural changes in their evolution. Of the seven different genera and species studied here, it has been noted that inspite of the general similarity of the karyotype, *Notochaete hamosa* and two species of *Orthosiphon* have larger chromosome than the rest (Table 1, Fig. 1). This may be taken as an index of their primitiveness. The TF% vary from 34.36 to 39.94%, indicating the symmetrical nature of karyotypes of all the investigated taxa.

On the basis of anatomical characters of the flower, subfamily Ajugoideae has been regarded as the most primitive and Stachydoideae as most highly evolved (Hillson 1959). The karyotypes of the investigated taxa belonging to two different subfamilies, however, exemplify a close resemblance among the different members placed under different subfamilies. That the division of the entire family into 8 different subfamilies as suggested by Briquet is quite unnatural, has already been indicated by Heywood (1978). This also gets full confirmation from the cytological data.

The family Lamiaceae shows a range of chromosome numbers, mostly multiples of 8 to 17 chromosomes. One may feel inclined to suggest a polyphyletic origin for the different taxa of this family. But the similarities in habit, highly specialized vegetative and floral morphology, inflorescence, anatomy and essential oil bearing glands, undoubtedly indicate that it is a natural family of homogenous assemblage.

From a cytological standpoint, it seems possible that the different species with different chromosome numbers, may be derived from a common genome, as specially evidenced in the idiogram (Fig. 1), The general similarity in chromosome morphology, consisting primarily of small chromosomes with median to nearly submedian constrictions, signify that the different species are closely allied to each other. In such a homogenous family, a polyphyletic origin of the different members, therefore, seems unlikely. Thus, it may be assumed that the diverse chromosome numbers owe their origin from a deep seated basic number through numerical changes in the chromosomes. It is, however, difficult to state as to which number is basic for this family. All but two species in this family are in fact herbs or undershrubs. The karyotype of the only arborescent member found in India (*Leucosceptrum canum*) also resembles the karyotype of the remaining herbaceous forms. The tree habit may, therefore, be regarded as a "sport" since it has not contributed to other such forms in the course of evolution.

Abstract

Chromosome study has been carried out on seven species belonging to six genera of the family Lamiaceae, with the aid of improved techniques. The general feature noted in this family is the wide range of chromosome numbers with graded symmetrical karyotypes. All the chromosomes are very small and are submetacentric to nearly submetacentric in nature. The species differ from one another in minute karyotypic details and in number of chromosomes with secondary constrictions.

Both numerical and structural alterations in chromosomes have been operative in the evolution of the different members of this family.

A monophyletic origin of the different members from a common deep seated base number has been indicated. The tree habit observed in this family has been regarded as a "sport" not contributing to any further evolutionary progress.

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