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CHROMOSOMAL STUDY ON *ANAX NIGROFASCIATUS* OGUMA, 1915 (ANISOPTERA:ODONATA) FROM HIMACHAL PRADESH

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SUMMARY

Chromosomes of *Anax nigrofasciatus* Oguma, 1915 belonging to family Aeshnidae of order Odonata from Shimla district of Himachal Pradesh was studied. The diploid chromosome number was found to be 2n = 27 with a pair of **m** chromosomes. Chromosomes were studied at spermatogonial metaphase and meiotic stages. For karyotype analysis, lengths of chromosomes were measured at spermatogonial metaphase from at least ten best selected plates. Total complement length as well as relative lengths of chromosomes were calculated. Idiogram was constructed based on chromosome length data and karyotype was prepared. Meiotic behaviour of chromosomes is in conformity with the earlier reports on dragonflies.

Keywords: Chromosomes, *Anax nigrofasciatus*, spermatogonial metaphase, karyotype, meiosis.

INTRODUCTION

Odonata is the insect order that includes dragonflies and damselflies. This order consists of approximately 7000 species world over (Kalkman et al. 2008) and about 470 species are reported from India (Subramanian 2009). Dragonflies belong to suborder Anisoptera. Only a few cytogenetic studies have been carried out due to lack of major morphological variation in the karyotype. Dragonflies have drawn attention to cytogenetic studies as these are biological controlling agents and indicators of habitat quality.

Aeshnidae includes large dragonflies and approximately 60 species have been cytogenetically analyzed (Mola 1992, Sandhu & Malhotra 1994). Most of the described species belong to genera *Aeshna* and *Anax* (Lefevre & Mcgill 1908, Mola 1992, 1995, Mola & Papeschi 1994, Walia & Sandhu 1999). Modal haploid chromosome number of Aeshnidae is 14 (70.7% of species), however, chromosome numbers range from 2n = 14–27 in this family (Cumming 1964, Kiauta 1967, 1971, 1975, Seshachar & Bagga 1962, Walia 2007). The decrease in chromosome number is attributed to the fusion of chromosomes.

Variation in chromosome number of *Anax nigrofasciatus* has been reported by Sandhu & Malhotra (1994) and Walia & Sandhu (1999) who reported 2n = 25 and 2n = 27 respectively in individuals from Dalhousie, Himachal Pradesh. Keeping this in view, it was considered worthwhile to investigate the chromosome number of *A. nigrofasciatus* from Shimla Hills.

MATERIAL AND METHODS

For chromosomal studies, adult male specimens of *A. nigrofasciatus* were collected from Shimla district (altitude 2200 m above sea level, 77° 17´ East Longitude and 31° 10´ North Latitude) of Himachal Pradesh during the month of July.

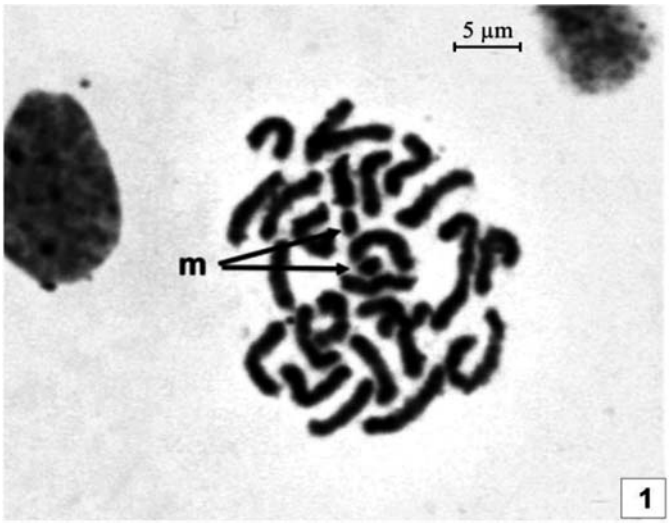
For chromosomal preparations, testes of males were dissected out. Clearing of tissue was done in 0.9% normal saline solution. The tissue was pretreated in 0.7% sodium citrate solution for 30 min. The pretreated tissue was fixed in 1:3 acetic acid-ethanol solution for 25–30 min at room temperature. After fixation, squashing of tissue was done in a drop of 45% acetic acid for 15–20 min. The tissue was then pressed by putting the slides with cover slips in between the folds of blotting paper and tapped gently. Cover slips were dislodged off from the slide with a sudden jerk. The slides and cover slips were air dried for 2 or 3 d in dust free chamber.

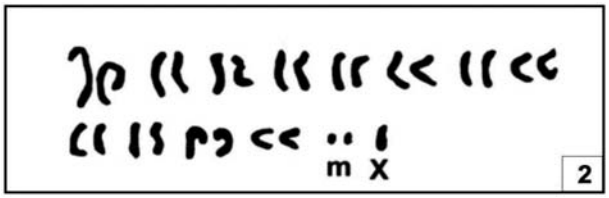
After drying, staining was done in 2% Giemsa solution and then the slides were mounted in DPX. The permanent slides were observed under binocular research microscope and photomicrographs were taken. Well spread spermatogonial metaphase complements were selected for chromosomal measurements. Lengths of chromosomes were measured using ocular micrometer and total complement length was calculated. The relative lengths (i.e., percentage of total complement length) were calculated by multiplying the actual length of chromosome pair with 100 and then dividing the product by total complement length.

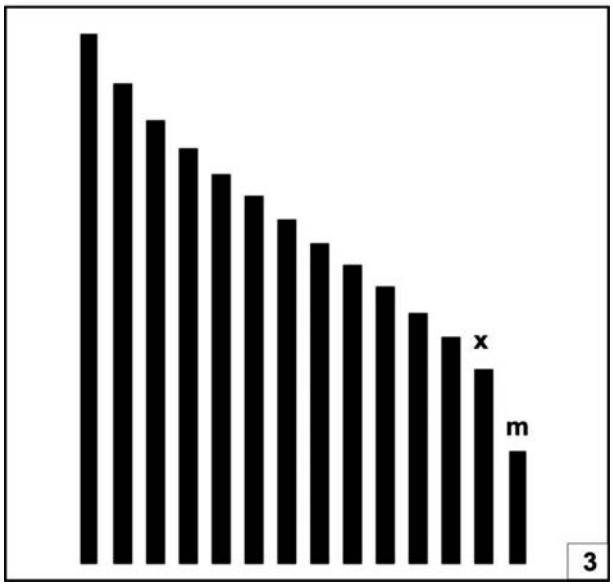
OBSERVATIONS

The present study revealed diploid chromosome number of 27 with an X chromosome and a pair of **m** chromosomes which are the smallest elements of the complement (Fig. 1). The chromosomes are holocentric. The mean lengths of chromosomes ranged from 1.08 µm ± 0.09 to 5.04 µm ± 0.33. Mean total length of the haploid complement is 44.25 µm ± 5.14. The mean relative lengths of chromosomes ranged from 2.44 ± 0.12 to 11.39 ± 0.12. The details of karyotype analysis are given in Table 1. The chromosomes in the complement show a gradual decrease in length and **m** chromosome pair is the smallest element of the complement while, X chromosome measuring 1.86 µm stands between **m** and the rest of autosomes (Figs 2, 3).

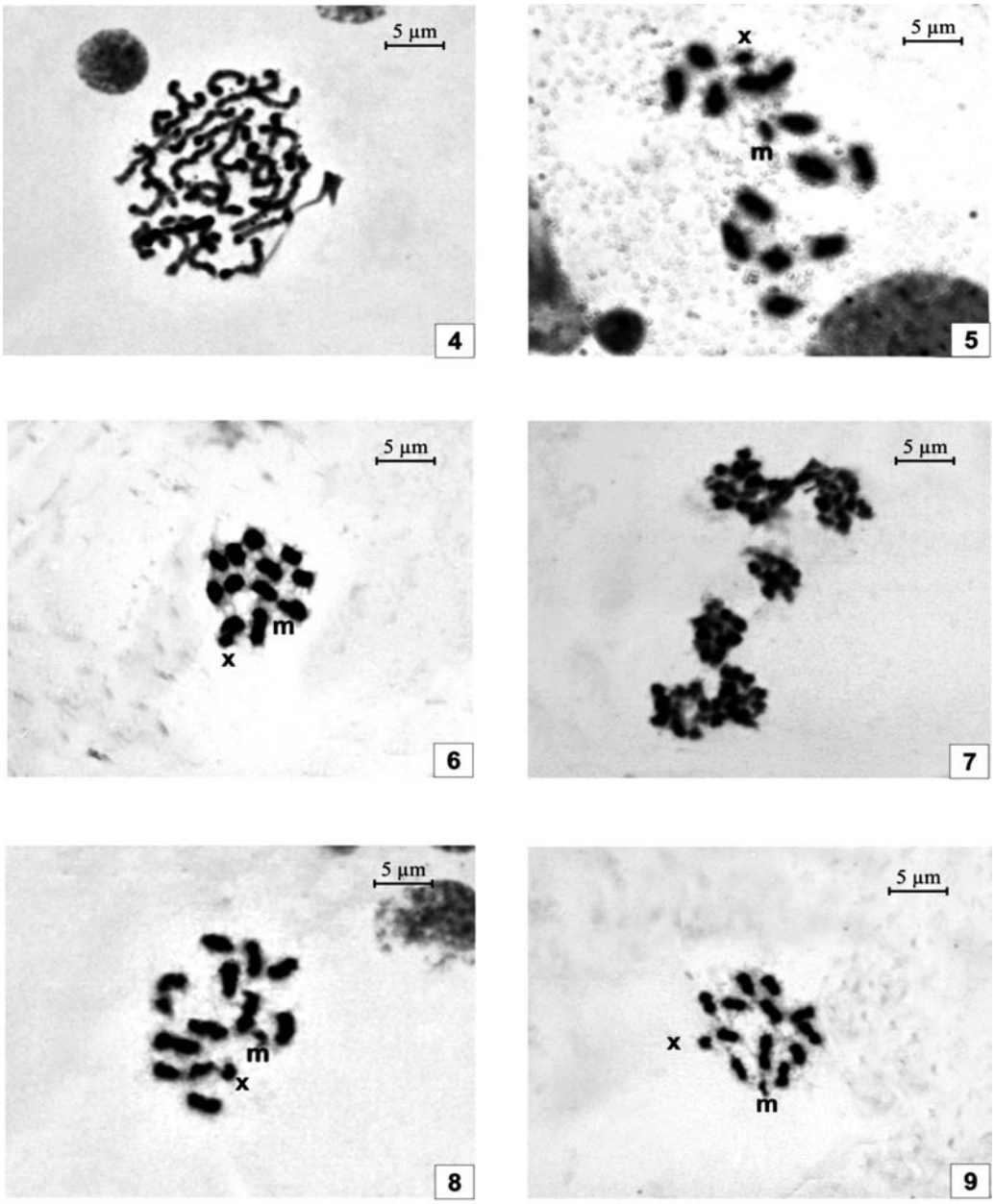
Meiotic stages observed are, pachytene, diakinesis, metaphase I, anaphase I, prophase II and metaphase II. During pachytene, the chromosomes appear as distinct entangled condensed threads (Fig. 4). At diakinesis, 14 elements are observed including the 13 bivalents and a univalent X chromosome which lies at the periphery. All bivalents have single chiasma. One large bivalent is observed, while other bivalents showed a gradual decrease in size and **m** chromosome is negatively heteropycnotic (Fig. 5). At metaphase I, 14 elements are observed which show maximum condensation and X







Figs 1–3: Cytology of *A. nigrofasciatus.* 1. Spermatogonial metaphase showing 27 chromosomes. 2. Karyotype. 3. Idiogram.. (**m**, **m** chromosomes, X, X chromosome).



Figs 4–9: *A. nigrofasciatus.* Meiosis. 4. Pachytene. 5. Diakinesis showing 13 bivalents and unpaired X chromosome. 6. Metaphase I showing 14 elements having maximum condensation. 7. Anaphase I showing complements at poles without differentiation of X chromosome. 8. Prophase II showing characteristic 'å' shaped chromosomes. 9. Metaphase II showing 14 elements with peripherally located **m** and X chromosomes. (**m**, **m** chromosome, X, X chromosome).

TABLE 1: Karyotype analysis in *A. nigrofasciatus.*

Chrom. pair	Mean length of chromosomes ± S.E. (μm)	Relative length ± S.E.
1	5.04 ± 0.33	11.39 ± 0.12
2	4.56 ± 0.23	10.31 ± 0.07
3	4.22 ± 0.22	9.54 ± 0.05
4	3.96 ± 0.22	8.95 ± 0.06
5	3.70 ± 0.21	8.36 ± 0.05
6	3.50 ± 0.20	7.91 ± 0.03
7	3.28 ± 0.22	7.41 ± 0.05
8	3.05 ± 0.21	6.89 ± 0.05
9	2.83 ± 0.20	6.40 ± 0.05
10	2.63 ± 0.19	5.94 ± 0.07
11	2.38 ± 0.16	5.38 ± 0.05
12	2.16 ± 0.16	4.88 ± 0.06
X chromosome	1.86 ± 0.14	4.20 ± 0.10
13 (m chromosomes)	1.08 ± 0.09	2.44 ± 0.12

chromosome lies at the periphery (Fig. 6). At anaphase I, chromosomes are moving toward opposite poles, while the X chromosome is not differentiated (Fig. 7). Both prophase II and metaphase II have 14 elements. At prophase II, chromosomes attained characteristic 'å' shape (Fig. 8). At metaphase II, both **m** and X chromosomes lie at peripheral position (Fig. 9).

DISCUSSION

In *A. nigrofasciatus*, diploid chromosome number was found to be 27 with a pair of **m** chromosomes. 2n = 27 is the modal diploid chromosome number for the family Aeshnidae. The present study was in agreement with the earlier findings of Kiauta (1975) and Walia & Sandhu (1999). However, Sandhu & Malhotra (1994) reported 2n = 25 in this species and has not given any explanation for this variation. Reduction in chromosome number within the family may be due to the fusion of chromosomes as suggested by various workers (Mola et al. 1999, Walia 2007). The present study was suggestive of holokinetic nature of chromosomes and inverted meiosis with XO sex determining mechanism in males. Chromosomal behaviour at meiosis was in accordance with the earlier reports of Kiauta (1975) for this species. At prophase II and metaphase II, X chromosome was present in all the cells and lies at peripheral position which suggests that during anaphase I, X chromosome divides equationally as is the case in post-reductional meiosis. Same behaviour of X chromosome was reported earlier by De Gennaro et al. (2008).

REFERENCES

CUMMING R B 1964 *Cytogenetic studies in order Odonata* Ph D Thesis Univ Texas Austin

DE GENNARO D, REBAGLIATI P J & MOLA L M 2008 Fluorescent banding and meiotic behavior in *Erythrodiplax nigricans* (Libellulidae) and *Coryphaeschna perrensi* (Aeschnidae) (Anisoptera Odonata) *Caryologia* **61** 60–67

KALKMAN V J, CLAUSNITZER V, DIJKSTRA D B, ORR A G , PAULSON D R & VAN TOL J 2008 Global diversity of dragonflies (Odonata) in freshwater *Hydrobiologia* **595** 351–363

KIAUTA B 1967 Considerations on the evolution of chromosome complement in Odonata *Genetica* **38** 430–446

KIAUTA B 1971 Studies on the germ cell chromosome cytology of some cytotaxonomically interesting or hitherto not studied Odonata from the Autonomous Region Friuli Venezia Giulia (Northern Italy) *Att Mus Civ Stor Nat Trieste* **27** 65–127

KIAUTA B 1975 *Cytotaxonomy of dragonflies with special reference to the Nepalese fauna* Nepal Research Centre Kathmandu

LEFEVRE G & MCGILL C 1908 The chromosomes of *Anasa tristis* and *Anax junius American J Anat* **7** 467–487

MOLA L M 1992 *Estudioscrmosomicos en libelulas (Orden Odonata)* Tesis de Doctorado Facultad de Ciencias Exactas y Naturales Universidad de Buenos Aires

MOLA L M 1995 Post-reductional meiosis in *Aeshna* (Aeshnidae Odonata) *Hereditas* **122** 47–55

MOLA L M & PAPESCHI A G 1994 Karyotype evolution in *Aeshna* (Aeshnidae Odonata) *Hereditas* **121** 185–189

MOLA L M, PAPESCHI A G & TABOADA CARILLO E 1999 Cytogenetics of seven species of dragonflies *Hereditas* **131** 147–153

SANDHU R & MALHOTRA I 1994 Karyological studies of four Aeschnid dragonflies from states of Jammu and Kashmir and Himachal Pradesh (India) *In* Srivastava V K (ed) *Advances in Oriented Odonatology* pp 111–115

SESHACHAR B R & BAGGA S 1962 Chromosome number and sex-determining mechanism in the dragonfly *Hemianax ephippiger* (Burmeister) *Cytologia* **27** 443–449

SUBRAMANIAN K A 2009 *A Checklist of Odonata (Insecta) of India* Zoological Survey of India Pune

WALIA G K 2007 Cytomorphological studies on *Gynacantha millardi* Fraser of the family Aeschnidae (Anisoptera Odonata) *Cytologia* **72** 57–62

WALIA G K & SANDHU R 1999 Karyotypic study of two species of family Aeschnidae (Anisoptera Odonata) *Chrom Sci* **3** 45–47

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REPEATABILITY, HERITABILITY AND ENVIRONMENTABILITY OF CERTAIN QUANTITATIVE TRAITS OF RHODODENDRONS

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SUMMARY

The quantitative morphological data of Rhododendrons from three altitudes and quadrat replicates of Wokha, Kiphiri and Kohima districts of Nagaland were analysed for phenotypic variance, genotypic variance, environmental variance, genotype × environment variance, repeatability, heritability and environmentability. The observations revealed that three altitudes 1780 m, 1802 m, and 1952 m amsl of only one district Wokha, were found favourable to the quantitative trait, girth, where repeatability is higher and correspondingly heritability of the trait is also higher indicating the faster growth of this trait in evolution. Similarly, the quantitative traits, node length, stamen length and carpel length showed higher repeatability with higher heritability and faster growth at an altitude of 1952 m amsl of the same district, Wokha.

Keywords: *Rhododendron*, Nagaland, repeatability, heritability, environmentability.

INTRODUCTION

The genus *Rhododendron* L. (Ericaceae) with attractive and beautiful flowers is represented by about 850 species (Mabberley 2008) to 1025 species (Chamberlain et al. 1996) world over. In India, it is represented by about 80 species with 10 subspecies and 14 varieties mostly concentrated in the temperate regions at higher elevations of 1500–5500 m amsl in the Sino-Himalayan regions with maximum concentration in western China (Pradhan 1985). Rhododendrons are distributed as trees, shrubs, terricolous and often aromatic in India and other parts the of world and it is one of the most neglected groups of plants in terms of scientific insight in India (Bhattacharya 2011).

About 98% of the Indian species is found in the Himalayan region among which 72% is found in Sikkim and the species availability decreases drastically from 4500 m amsl upwards and 2500 m amsl downwards (Singh et al. 2003).

Three species of *Rhododendron* (*R. arboretum, R. companulatum, R. anthopogon*) were recorded from nine districts and inhabiting temperate, subalpine and alpine regions of Himachal Pradesh (Kharwal & Rawat 2013). Eighteen *Rhododendron* species from Myodia district (lower

Dibang valley) and 47 species were recorded from the West Kameng and Tawang districts of Arunachal Pradesh (Mao et al. 2009). Rhododendrons were recorded from subtropical hills of Ukhrul and Senapati districts (1000–1500 m amsl), temperate hills of Siroi, Koubru peak and Mt. Esii (1600–2500 m amsl) of Manipur (Mao 2010). In Nagaland, Rhododendrons are found in subtropical hills of Zunheboto and Wokha districts, temperate forest of Mt. Saramati, Mt. Japfo, Jakhama, Khonoma, Puliebadze and Dzulakei hills (Mao 2010). The common species for both Manipur and Nagaland include *R. ellioti*, *R. formosum* var. *formosum*, *R. formosum* var. *inaequale*, *R. johnstoneanum*, *R. lepidotum*, *R. macabeanum*, *R. maddenii*, *R. triflorum*, *R. vaccinioides* and *R. wattii* (Mao 2010). Ward (1949, 1960) explored Japfo hills of Nagaland, hills of Manipur and Nagaland bordering Myanmar and contributed to the knowledge of *Rhododendron*. *Rhododendron* species from Manipur and Nagaland were first collected by Sir George Watt who surveyed Manipur and Nagaland from 1882–1885 and described four new species (*R. maccabeanum*, *R. ellioti*, *R. triflorum* var. *bauhiniflorum* and *R. wattii*) from Japfo hill range of Nagaland (Watt 1890).

The distribution of the species well documented but unfortunately, no study has been reported on its quantitative traits interaction with the environment. The quantitative traits are the characters which interact with the environment in all possible ways to cope up and survive towards fluctuating climatic conditions.

Phenotypic plasticity refers to changes in organisms' traits due to changes in internal or external environmental conditions (Pigliucci 2001). When these phenotypic changes are reversible over time, the name of phenotypic flexibility is commonly used (Piersma & Drent 2003).

Repeatability is defined as the consistency of a trait over time and allows an assessment of the probability of measuring heritability. Repeatability indicates the proportion of total variation in a trait that is due to differences between individuals (Falconer 1981). It is based on repeated measures of the same individuals followed by an analysis of variance. Repeatability is directly useful as a measure of the intra-individual consistency of displays and other aspects of behaviour. Only traits that are manifested consistently within individuals as well as differing between individuals can respond to selection.

The quantitative trait is a specific term characterized and determined by multiple genes and environmental factors. It is measurable but varies over a range among the individuals to produce a continuous distribution of phenotypes. A phenotype is a quantitative trait controlled by many genes (multiple genes) and influenced by environmental factors or in other words, a genotype is a set of genes involved in the expression of a certain phenotype. The quantitative genetics mostly applied to the crops to check their yield and other traits for future breeding prospects and new varieties.

The survey of literature suggested that a voluminous work has been done on the distribution and taxonomy of Rhododendrons but data on morphological and genetic diversity in relation to tree improvement programme are meagre. The present work was taken up to study the preliminary data

collection on certain quantitative traits of Rhododendrons from three different altitudes of three districts, Wokha, Kiphiri and Kohima of Nagaland (India) in terms of Repeatability (R), Heritability (H^2) and Environmentability (E) to observe the altitudinal effects on selected traits. It is hoped that data may be useful for designing tree breeding programmes in *Rhododendron* species.

MATERIALS AND METHODS

Morphological quantitative data

The morphological quantitative data such as plant height (ph), number of branches (nbr), girth of the tree (g), number of internodes (ni), node length (nl), leaf length (ll), leaf breadth (lb), petiole length (ptl) peduncle length (pdl), pedicel length (pcl), number of flowers per peduncle (nfpp), petal length (pl), petal breadth (pb), stamen length (sl) and carpel length (cl) were collected which served as quantitative traits for the present purpose. The data collection and measurement of the traits had been done from three different altitudes of Wokha, Kiphiri and Kohima districts of Nagaland.

Statistical analysis

The analysis of variance (ANOVA) was performed for the traits against the altitude as a factor for three districts. The ANOVA differentiated the variations between and within the traits as mean square value and within mean square value considered as environmental variance (V_E) present in the trait. Accordingly, other variations such as genotype variation (V_G), phenotype variation (V_P) and variation due to genotype and environment interaction ($V_{G \times E}$) were measured and calculated exercising Burton (1952) and Sharma (1988).

$$V_P = V_G + \frac{V_E}{r}$$

$$V_G = \frac{\text{Mean Square (Between)} - \text{Mean Square (Group)}}{\text{Number of replications (r)}}$$

V_E = Environmental variation, within mean square value (ANOVA)

$V_{G \times E}$ = Variation due to genotype and environment interaction

The other variation components such as Repeatability (R), Heritability (H^2) and Environmentability (E) were calculated using the following formulae:

$$\text{Repeatability} = \frac{V_G + V_{G \times E}}{V_P} \quad \text{(Falconer 1981)}$$

$$\text{Heritability} = \frac{V_G}{V_P} \times 100 \quad \text{(Falconer \& Mackay 1997)}$$

Environmentability = 1 – Heritability

OBSERVATIONS

The altitudinal effects on morphological traits of Rhododendrons from three districts of Nagaland have been analysed and the details are given in Table 1.

TABLE 1: Morphological parameters used for repeatability, heritability and environmentability in Rhododendrons.

Altitude (m amsl)	Estimated variation components	Morphological traits														
		ph (m)	nbr	g (m)	ni	nl (cm)	ll (cm)	lb (cm)	ptl (cm)	pdl (cm)	pcl (cm)	nfpp	pl (cm)	pb (cm)	sl (cm)	cl (cm)
							WOKHA DISTRICT									
1780	R	0.55	-2.80	0.88	-2.81	0.54	1.35	-0.02	0.57	-0.38	0.46	1.16	-0.21	-5.80	-14.84	0.10
	H^2	0.18	-0.17	0.82	-0.17	0.45	0.66	-0.02	0.45	-0.38	0.46	-0.16	-0.17	-0.51	-0.78	0.09
	E	0.82	1.17	0.18	1.17	0.55	0.35	1.02	0.45	1.38	0.54	1.16	1.17	1.51	1.78	0.92
1802	R	4.18	-218.38	0.89	-218.38	-2.41	-3.87	-0.34	-1.81	0.40	0.88	4.17	0.78	0.20	0.82	0.82
	H^2	0.64	-4.92	0.87	-4.92	-1.84	-0.55	-0.17	-1.63	0.40	0.88	0.33	0.78	0.20	0.82	0.79
	E	0.36	5.92	0.13	5.92	2.84	1.55	1.17	2.63	0.60	0.13	0.67	0.22	0.80	0.18	0.21
1952	R	1.13	1.45	0.73	1.45	0.87	0.16	0.92	0.99	0.25	0.33	11.64	0.38	0.92	0.93	0.59
	H^2	0.96	0.97	0.70	0.97	0.84	0.10	0.92	0.99	0.25	0.33	0.35	0.46	0.90	0.71	0.55
	E	0.04	0.03	0.30	0.03	0.16	0.90	0.08	0.01	0.75	0.67	0.65	0.54	0.10	0.29	0.45
							KIPHIRI DISTRICT									
3096	R	-0.25	-5.05	0.54	-5.17	-7.12	3.48	1.12	0.32	-0.00	0.00	-3.21	0.51	-0.11	-0.40	0.75
	H^2	-0.11	-1.05	0.52	-1.08	-1.65	0.82	0.60	0.24	0.00	0.00	-0.52	0.49	-0.11	-0.40	0.65
	E	1.11	2.05	0.48	2.08	2.65	0.18	0.40	0.76	0.00	0.00	1.52	0.51	1.11	1.40	0.35
3112	R	-0.57	1.34	-1.02	1.34	1.40	-4.74	0.11	0.48	-1.00	0.44	1.44	-0.02	0.15	-0.25	-0.45
	H^2	-0.09	0.31	-0.64	0.34	0.58	-0.36	0.04	0.39	-1.00	0.44	0.40	-0.02	0.15	-0.25	-0.36
	E	1.09	0.70	1.64	0.70	0.42	1.36	0.96	0.61	2.00	0.56	0.61	1.02	0.85	1.25	1.36
3430	R	8.71	2.55	0.00	2.62	0.85	17.24	5.60	1.26	0.73	-0.71	25.21	1.55	0.83	0.98	1.23
	H^2	0.86	0.55	0.00	0.57	0.33	0.75	0.78	0.86	0.73	-0.64	0.74	0.69	0.72	0.83	0.31
	E	0.14	0.45	1.00	0.43	0.67	0.25	0.22	0.14	0.27	1.64	0.27	0.31	0.29	0.17	0.69
							KOHIMA DISTRICT									
1653	R	2.12	2.17	-0.05	2.17	0.54	-1.60	-0.40	0.30	-0.50	0.29	-0.14	-0.38	-0.33	-0.46	-0.06
	H^2	0.82	0.42	-0.05	0.42	0.43	-0.34	-0.27	0.42	-0.50	0.29	0.03	-0.32	-0.33	-0.42	-0.06
	E	0.18	0.58	1.05	0.58	0.57	1.34	1.27	0.58	1.50	0.72	0.97	1.32	1.33	1.42	1.06
2050	R	-0.62	4.42	-2.33	11.70	0.21	-0.09	-0.38	-2.31	0.20	0.64	0.39	-0.74	0.40	-1.22	0.77
	H^2	-0.13	0.43	-2.17	1.13	0.18	-0.02	-0.28	-2.06	0.20	0.64	0.07	-0.45	0.40	-0.72	0.71
	E	1.13	0.58	3.17	-0.13	0.83	1.02	1.28	3.06	0.80	0.36	0.93	1.45	0.60	1.72	0.29
2284	R	0.73	3.20	-	3.20	-0.35	1.34	-10.70	-286.26	-0.86	0.45	7.29	0.98	0.68	0.81	0.69
	H^2	0.28	0.41	-2.94	0.41	-0.29	0.24	-2.08	-2.34	-0.86	0.40	0.53	0.51	0.50	0.47	0.31
	E	0.78	0.59	3.94	0.59	1.29	0.76	3.08	3.34	1.86	0.60	0.47	0.49	0.50	0.53	0.69

The value of R was over +1 or under –1 or zero 0 estimated for quantitative trait, plant height at all the altitudes for all the three districts except the values 0.55 and 0.73 at altitudes of 1780 m amsl and 2284 m amsl of Wokha and Kohima but heritability of the trait was found to be low (0.18 and 0.28) suggesting the higher effect of the environment on this trait. The environmentability of the trait is considerably higher (0.82 and 0.78).

Another quantitative trait, number of branches has shown more than 50% values (0.57, 0.57 and 0.58) for the effect of environment (environmentability) on this trait at all the altitudes (1653 m, 2050 m and 2284 m amsl) of Kohima district. Similarly, less than 50% values (0.42, 0.42 and 0.41) for heritability was recorded for the trait. The repeatability for the trait was overestimated +1 because value estimated more than unity, otherwise in normal condition it is equal to or less than one. The repeatability was over +1 or under –1 estimated for rest of the two districts (Wokha and Kiphiri) at all the altitudes. The heritability of the trait was observed to be quite high (0.96) at 1952 m amsl altitude but it may be because of the overestimation of the R value (1.45) for Wokha district.

In Wokha district, the repeatability (0.87, 0.89 and 0.72) and heritability (0.81, 0.87 and 0.70) of quantitative trait, girth was observed to be very high at all the three altitudes (1780 m, 1802 m and 1952 m amsl) respectively. The high heritability suggested the maximum changes during evolution in trait, girth from generation to generation. The high heritability also indicates the lesser effect of environment on the trait and low environmentability value. The 3 altitudes of Kiphiri (3096 m, 3112 m and 3430 m amsl) and Kohima (1653 m, 2050 m and 2284 m amsl) were not found suitable for the trait, girth with negative, zero or overestimated values of R, H^2 and E respectively.

The higher and more than 50% heritability (0.96 and 0.56) was recorded for the trait, number of internodes at 1952 m and 3430 m amsl of Wokha and Kiphiri but it may be because of the overestimation of the R. It may also be suggested that this trait has been fixed for its high heritability during space and time of evolution. The E was also over- or underestimated except the values 0.69, 0.57 and 0.58 at 1952 m, 3112 m, 1653 m and 2284 m amsl of Wokha, Kiphiri and Kohima respectively.

The R and E (0.53 and 0.55) of quantitative trait, node length was recorded more than 50% and high (0.87 and 0.84) at an altitude of 1780 m and 1952 m amsl of Wokha district respectively. It may be suggested that the altitude (1952 m amsl) is suitable for the growth of the node length. The R of the trait is high (0.85) but the low heritability (0.32) generally increases the E (0.67) which indicates that the trait is in control of environmental condition at an altitude of 3430 m amsl of Kiphiri district. The R (0.54) and E (0.57) estimated more than 50% and low R (0.20) and H^2 (0.17) but high E (0.82) at an altitude of the 1653 m and 2050 m amsl of Kohima district respectively. It was observed that the growth of the trait is slow and environment dependent.

The trait, leaf length is under the control of environment (E = 0.89) and also controls the growth (R = 0.16) and heritability (H^2 = 0.10) of the trait at an altitude of the 1952 m amsl of Wokha district.

On the other hand, the trait leaf length is heritable (H^2 = 0.818) in Kiphiri at an altitude of 3096 m amsl but R and E values are not supportive. The heritability of the trait may be the result of overestimation of the R. The trait, leaf breadth showed equal R (0.91) and H^2 (0.91) but low E (0.08) and high E (0.96) and low R (0.10) and H^2 (0.03) at an altitude of 1952 m and 3112 m amsl of Wokha and Kiphiri districts respectively. The same trait shows two different results, one is environment favourable and other is unfavourable. Both the traits, leaf length and leaf breadth are influenced by the environment at both the altitudes of both the districts.

For the trait, petiole length, both heritability (0.45) and environment (0.45) are equally contributing or having equal effect towards the repeatability (R = 0.56) and growth of the trait in the environmental conditions at an altitude of 1780 m amsl. On the other hand, high heritability (H^2 = 0.98) contributes towards the high repeatability (R = 0.98) and vice versa of the trait at an altitude of 1952 m amsl in Wokha district respectively. More than 60% environmental effect (E = 0.75, 0.61) was observed on this trait at an altitude of 3096 m and 3112 m amsl of Kiphiri and also more than 50% environmental effect (E = 0.582) was observed on this trait at an altitude of 1653 m amsl of Kohima district. Since this trait is under the control of environmental conditions of the altitudes, low heritability and repeatability was observed.

The trait, peduncle length was also observed under the influences of environmental condition of the altitudes. Approximately, 60–70% environmental influences (E = 0.60, 0.75) were recorded for this trait at an altitude of 1802 m and 1952 m amsl of Wokha and almost 80% environmental influences (E = 0.80) recorded for the trait at an altitude of 2050 m amsl of Kohima district. The good heritability (H^2 = 0.727) of the trait was observed in Kiphiri district at 3430 m amsl.

The length of pedicel is under the control of environmental condition or environment dependent at all the altitudes of all districts. Whereas higher heritability of the trait (0.87) was observed at 1802 m amsl, it is found moderate (0.63) at 2050 m amsl in Wokha and Kohima districts.

The trait, number of flowers per pedicel was supported by the environmental condition (E = 0.92) at an altitude of 2050 m amsl of Kohima district only.

The high repeatability (R = 0.78 and 0.98) of the trait, petal length was observed at 1802 m amsl and 2284 m amsl in Wokha as well as Kohima districts. The repeatability (R = 0.91, 0.82 and 0.68) of petal breadth was observed at altitudes 1952 m, 3430 m and 2284 m amsl of all the three districts.

The H^2 and R of the stamen length were recorded at the altitudes 1802 m and 1952 m amsl of Wokha district and 3430 m amsl of Kiphiri district. The R of the trait was high (0.80) at 2284 m amsl of Kohima district. The R and H^2 of the carpel length were supported at the altitudes 1802 m and 1952 m amsl of Wokha, 3096 m amsl of Kiphiri and 2050 m amsl of Kohima districts.

DISCUSSION

Repeatability permits the estimation of sample size which could be properly used to measure the heritability of the sample size. Heritability may help to design the breeding programmes by reducing the Standard Error of work involved in designing the programme (Falconer 1981, Shaw 1987). Therefore, the preliminary measurement of repeatability of a trait could be satisfactorily used to identify a particular trait for further genetical analysis. Also, repeatability can be used to indicate whether efforts to measure heritability are likely to be worthwhile.

The statistical explanation of repeatability and heritability suggests that low repeatability cannot accompany high heritability, unless small sample sizes have resulted in erroneous estimates. The low repeatability puts a ceiling on heritability and results in slow evolution (little change in a phenotype from generation to generation), even if a trait is subject to strong selection. The change in phenotype between generations can be predicted by multiplying the heritability of a trait by the selection differential on that trait.

Higher repeatability indicates that repeated measures of the same individual or trait have substantially less variation than measures of different individuals or traits. Higher repeatability may accompany higher heritability, in which case environmental variation is low and most of the genetic variation is additive in nature.

The possible reason of repeatability being considerably or significantly higher than heritability could be that environmental variation is high, or that nonadditive variance (such as dominance effects) makes a major contribution to genetic variance.

A combination of high repeatability and low heritability could indicate that a trait has been under strong selection in the past and is still closely associated with fitness. The strong past selection would reduce additive genetic variance and increase the role of dominance variance (Mather & Jinks 1971).

Repeatability could be low for two reasons because it is computed as a ratio: 1) the numerator can be relatively small, which will occur if all individuals or traits are very similar. The similarity might be attributable to either genetic or environmental effects, but further experimentation would be necessary to understand the relative influence of each effect. 2) A second cause of low repeatability is a relatively large denominator, which is a consequence of environmental influences.

All the altitudes of all the three districts favour one or more trait for its repeatability, heritability and environmentability. The high value of repeatability suggests the high heritability of the character, but it has not been observed in most of the traits except the traits such as girth at all altitudes of Wokha district, node length, stamen length and carpel length at 1952 m amsl of Wokha district. The

altitude (1952 m amsl) may favour the growth of the girth, node length, stamen length and carpel length. It might be concluded that the traits have potential towards high evolutionary trends and could be utilized further in plant breeding programmes.

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REFERENCES

BHATTACHARYA D 2011 Rhododendron species and their uses with special reference to Himalayas: a review *Ass Univ J Sci Tech Biol Env Sci* **7** 161–167

BURTON G W 1952 Qualitative inheritance in grasses *Proceedings of the 6th International Grassland Congress* 17–23 August Vol **1** pp 277–283 Pennsylvania State College Pennsylvania

CHAMBERLAIN D F, HYAM R, ARGENT G, FAIRWEATHER G & WALTER K S 1996 *The genus Rhododendron: its classification and synonymy* Royal Botanic Garden Edinburgh

FALCONER D S 1981 *Introduction to Quantitative Genetics* 2nd ed Longman New York

FALCONER D S & MACKAY T F C 1997 *Introduction to quantitative genetics* 4th ed Longman Scientific and Technical Edinburgh

KHARWAL A D & RAWAT D S 2013 Ethanobotanical studies and distribution of different *Rhododendron* species in Himachal Pradesh India *Plant Sciences Feed* **3** 46–49

MABBERLEY D J 2008 *Mabberley's Plant Book A Portable Dictionary of Plants their classifications and uses* 2nd ed Cambridge University Press Cambridge

MAO A A 2010 The genus Rhododendron in north-east India *Botanica Orientalis-J Plant Sci* **7** 26–34

MAO A A, GOGOI R & APANG O 2009 Studies on *Rhododendron* species of Mayodia Lower Dibang Valley Arunachal Pradesh India *Bull Arunachal For Res* **25** 93–99

MATHER K & JINKS J L 1971 *Biometrical genetics* Chapman and Hall London

PIERSMA T & DRENT J 2003 Phenotypic flexibility and the evolution of organismal design *Trends Ecol Evol* **18** 228–233

PIGLIUCCI M 2001 *Phenotypic Plasticity: Beyond Nature and Nurture* 1st ed John Hopkins University Press Baltimore

PRADHAN U C 1985 A preliminary enumeration of Rhododendrons of Indian region Part 1 *Himalayan Plant Journal* **3** 110–123

SHARMA J R 1988 *Statistical and biometrical techniques in plant breeding* New Age International Limited Publishers New Delhi

SHAW R G 1987 Maximum-likelihood approaches applied to quantitative genetics of natural populations *Evolution* **41** 812–826

SINGH K K, KUMAR S, RAI L K & KRISHNA A P 2003 Rhododendrons conservation in the Sikkim Himalaya *Curr Sci* **85** 602–606

WARD K F 1949 Plant hunting in Manipur *J Roy Hort Soc* **74** 288–295

WARD K F 1960 *Pilgrimage for Plants* George G Harrap and Co Ltd London

WATT G 1890 Forests of Manipur *Indian Forestry* **14** 291–387

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KARYOTYPIC STUDIES ON THREE SPECIES OF *LABEO* FROM HIMACHAL PRADESH

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SUMMARY

Karyotypic investigations on three species belonging to the genus *Labeo* namely, *L. calbasu*, *L. dero* and *L. rohita* were carried out. Diploid chromosome numbers of 2n = 50, 54 and 50 with karyotypic formulae 8m + 4sm + 38t, 10m + 6sm + 8st + 30t and 8m + 4sm + 6st + 32t were observed in *L. calbasu*, *L. dero* and *L. rohita* respectively. Such studies may prove useful in solving taxonomic problems in the closely related species as well as in determining the evolutionary pathways in this important group of organisms.

Keywords: Chromosomes, idiogram, karyotype, fish, *Labeo.*

INTRODUCTION

Chromosomal studies on fishes have received considerable attention in recent years as they constitute the most important group of organisms for the evolutionary and cytotaxonomic studies. They are found in many kinds of environments and show wide genetic variability both at chromosomal and molecular levels (Kosswig 1973). Different techniques are used to study the fish karyotypes like culturing leucocytes and fibroblasts, C-banding, Q-banding, NOR-banding and DAPI fluorescent staining etc. But the air-drying technique, originally developed for mammals is the most common procedure for chromosome preparation in fishes. Fish karyotypes are generally characterized by a large number of small chromosomes.

About 28400 species of fishes have been reported all over the world (Nelson 2006). Out of these, 2000 species of inland and marine fishes have been analyzed for karyological aspects and over 200 species have been investigated karyologically from India (Das & Barat 1995). In Himachal Pradesh, there are many natural as well as man-made waterbodies which support a variety of fish life. At present, 97 species of fresh water fishes, belonging to 51 genera,18 families and 6 orders have been reported from Himachal Pradesh (Sharma 2010). Fish species of Himachal Pradesh belong to families Cyprinidae, Notopteridae, Cobitidae, Siluridae, Bagridae, Amblycipitidae, Sisoridae, Schiebeidae, Belonidae, Muglidae, Channidae, Anabantidae and Mastocemlidae, with the first one i.e., Cyprinidae dominating the region.

Genus *Labeo* is one of the three Indian major carps and belongs to family Cyprinidae. So far, no karyological work has been done on this genus from Himachal Pradesh. Keeping in view the increasing importance of karyotypic studies on fishes in general and the lack of data on fish karyotypes in Himachal Pradesh in particular, it is desirable to investigate the chromosomes of some fresh water fishes from Himachal Pradesh.The present study was undertaken to study the chromosomes of three species of *Labeo*, *L. calbasu*, *L. dero* and *L. rohita* from Himachal Pradesh.

MATERIALS AND METHODS

For chromosomal studies, live specimens of *L. calbasu*, *L. dero* and *L. rohita* were collected from different waterbodies. The first two species were collected from Seer Khad, Ghumarwin (Bilaspur district) and the third from Pong Dam (Kangra district). An air dried technique of Thorgard & Disney (1990) with some modifications was used. Anterior kidney tissue was used to make the chromosomal preparations. Colchicine was used as pretreatment agent and an intra-muscular injection of 0.05% colchicine was given to each fish at the rate of 1ml/100 g body weight. After 2 to 3 h of pretreatment, the fishes were dissected from the ventral sides and anterior kidneys were removed. Tissue was minced into smaller pieces and placed in hypotonic solution of 0.56% KCl for 30 to 40 min. Then it was fixed in freshly made Carnoy's fixative (3:1 methanol:glacial acetic acid). Then tissue was taken from the fixative and centrifuged at 1000–1500 rpm for 10 min. After that, supernatant was discarded and tissue was fixed in small amount of fresh fixative. This suspension was dropped on clean slides by using splash method from 30–60 cm height and slides were air dried in dust free chamber. After drying, staining was done in 2% Giemsa solution. Then the slides were washed in running tap water and then air dried for 2 or 3 d. These air dried slides were made permanent by mounting in DPX. The permanent slides were scanned under binocular research microscope and photomicrographs were taken. Well spread, nonoverlapping and optimally stained metaphase plates were used for chromosomal measurements. Karyotypes were prepared by arranging the chromosomes in decreasing order of their length and the chromosomes were categorized according to Levan et al. (1964). Chromosomal measurements were taken by using ocular micrometer and idiograms were constructed by using haploid complements.

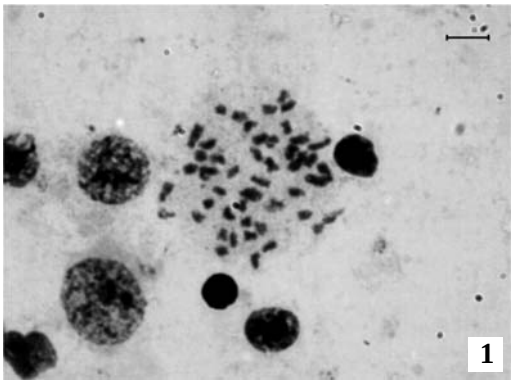
OBSERVATIONS

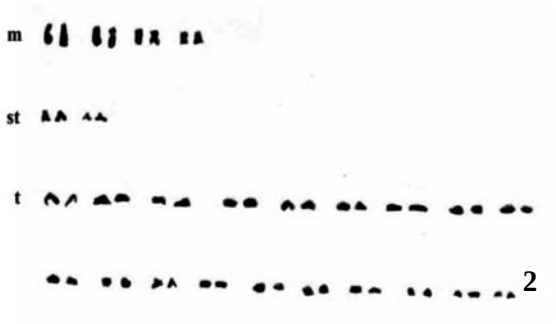
L. calbasu

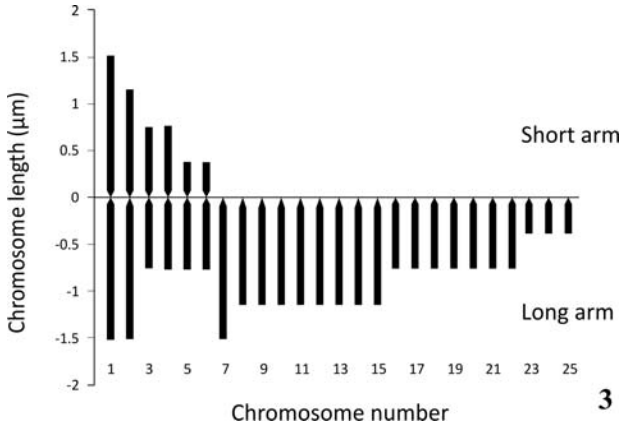
2n = 50 has been observed in this species with karyotypic composition as 8 metacentric, 4 submetacentric and 38 telocentric chromosomes (Figs 1, 2). The lengths of chromosomes ranged from 0.38 µm to 3.06 µm. The total haploid complement length is 28.38 µm. Arm ratio of the complement ranged between 1–∞ and the centromeric index ranged between 0–50. Idiogram of this species showed gradual decrease in the size of chromosome pairs (Fig. 3) with karyotype formula 8m + 4sm + 38t.

L. dero

This species has a diploid chromosome number of 54 with karyotypic composition of 10 metacentric, 6 submetacentric, 8 subtelocentric and 30 telocentric chromosomes (Figs 4, 5). The lengths of chromosomes ranged from 0.38 µm to 3.06 µm. The total length of haploid complement is 35.64 µm. Arm ratio of the complement ranged between 1–∞ and the





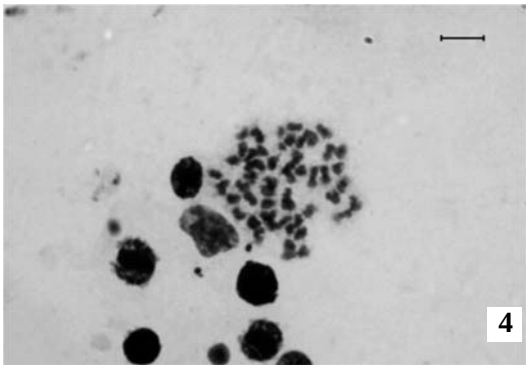


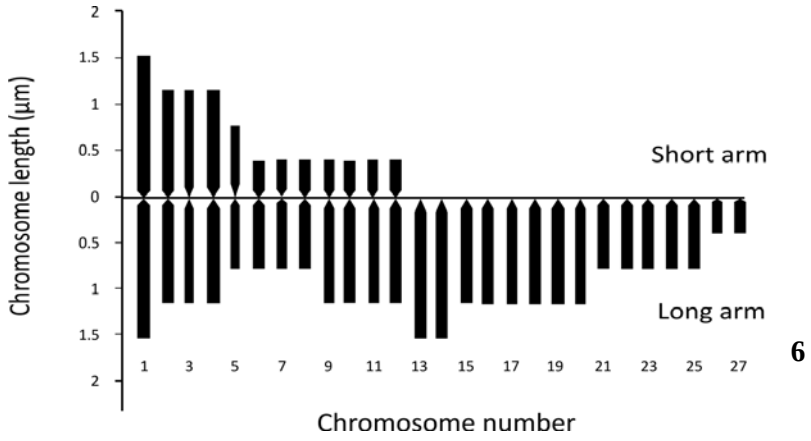
Figs 1–3: *L. calbasu*.1. Metaphasic plate showing 50 chromosomes. 2. Karyotype. 3. Idiogram. (m, metacentric, st, subtelocentric, t, telocentric). (Bar = 5µm)

centromeric index ranged between 0–50. Idiogram of this species showed gradual decrease in the size of chromosome pairs (Fig. 6) with karyotype formula 10m + 6sm + 8st + 30t.

L. rohita

2n = 50 has been observed in this species with karyotypic composition of 8 metacentric, 4 submetacentric, 6 subtelocentric and 32 telocentric chromosomes (Figs 7, 8). The lengths of chromosomes ranged from 0.43 µm to 2.56 µm. The total length of haploid complement was 35.87 µm. Arm ratio of the complement ranged between 1–∞ and the centromeric index ranged between 0–50. Idiogram of this species showed gradual decrease in the size of chromosome pairs (Fig. 9) with karyotype formula 8m + 4sm + 6st + 32t.



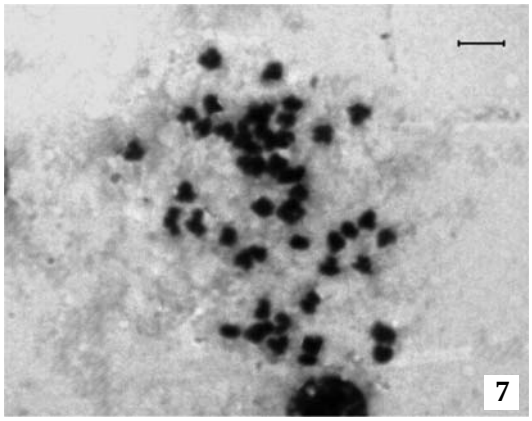


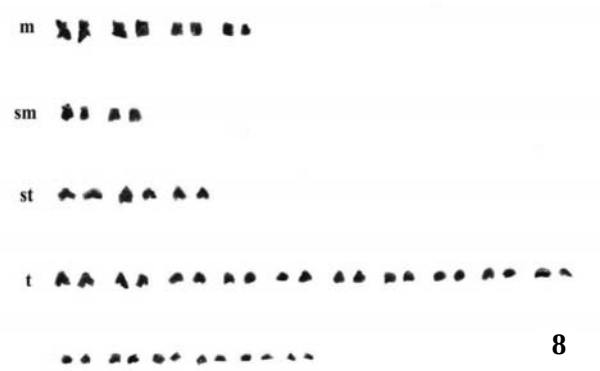
Figs 4–6: *L. dero*. 4. Metaphasic plate showing 54 chromosomes. 5. Karyotype. 6. Idiogram. (m, metacentric, sm, submetacentric, st, subtelocentric, t, telocentric). (Bar = 5µm)

DISCUSSION

The diploid chromosome number in *L. calbasu* was found to be 50 with 8 metacentric, 4 submetacentric and 38 telocentric chromosomes. 2n = 50 is probably the modal chromosome number for this genus. Earlier, same diploid chromosome number (2n = 50) with different karyotype formulae had been reported by various workers such as 6m + 8sm + 22st + 8t + 6T (Manna & Khuda-Bukhsh 1977), 10m + 10sm+ 14st + 16t (John et al.1993) and 10m + 14sm + 4st + 22a (Jana & Nagesh 2007). Translocation, duplication, deletion, inversion and Robertsonian fusion/fission might be the reasons for the structural changes in the chromosomes (Gold 1979, Dhar & Chatterjee 1986, Padhi & Mandal 2000).

In *L. dero*, 2n = 54 with karyotypic composition of 10 metacentric, 6 submetacentric, 8 subtelocentric and 30 telocentric chromosomes have been observed. 2n = 54 with all rod-shaped chromosomes has also been reported by Nayyar (1962). 27 rounded chromosomes of same size





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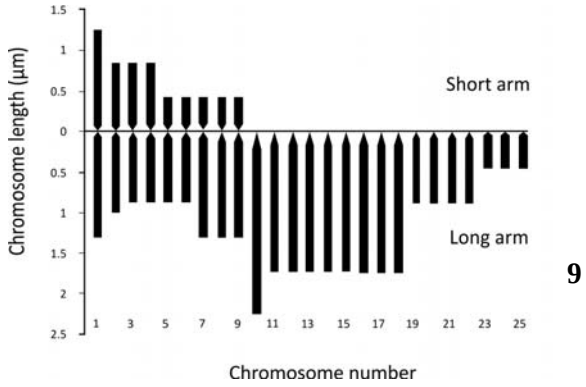

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Figs 7–9: *L. rohita*. 7. Metaphasic plate showing 50 chromosomes. 8. Karyotype. 9. Idiogram. (m, metacentric, sm, submetacentrix, st, subtelocentric, t, telocentric). (Bar = 5µm)

have been reported in primary and secondary spermatocytes. According to him, in addition to rod-shaped chromosomes, a large number of V-shaped chromosomes are present in many cyprinids and each V-shaped chromosome is formed due to centric fusion of two rod-shaped chromosomes. He also suggested that originally all cyprinids had 2n = 54 as reported in his studies. However, Khuda-Bukhsh & Chanda (1983) reported 26 metacentric, 12 submetacentric, 2 subtelocentric and 10 telocentric chromosomes in this species.

L. rohita has 2n = 50 with karyotypic composition of 8 metacentric, 4 submetacentric, 6 subtelocentric and 32 telocentric chromosomes. The karyotypic studies on *L. rohita* had been made by many workers in India as well as in neighbouring countries such as China, Thailand, Vietnam and Bangladesh. In all these studies, the diploid chromosome number in this fish was reported to be 50 with different karyotype formulae such as 14m + 6sm + 4st/26a, FN = 70 (Donsakul & Magtoon 1993), 14m + 16sm + 8st/12a, FN = 80 (Magtoon & Arai 1993), 16m + 10sm + 14st + 10a, FN = 76 (Dung 1990, 1992, Trong & Dung 1990), 6m + 16sm + 8st + 20t (Nagpure

1997), 10m + 14sm + 8st + 18t and FN = 74 (Nagpure et al. 2001), 8m + 12sm + 22st + 8t/a (Tripathy et al. 2010) and 18m + 6sm + 1st (Sarower-E-Mahfuj et al. 2014). Thus, the diploid chromosome number of 2n = 50 found in the present study is in conformity with the reports of earlier workers.

REFERENCES

DAS P & BARAT A 1995 Application of genetics in fisheries can help blue revolution in India *In* G K Manna & S C Roy (eds) *Perspectives in Cytology & Genetics* **8** pp 25–33

DHAR N J & CHATTERJEE K1986 A distinctive chromosomal race of channid fish *Channa punctata Microbios Lett* **33** 15–18

DONSAKUL T & MAGTOON W 1993 A chromosome study of three cyprinid fishes *Labeo erythrurus L bicolor* and *L rohita Proceedings of the 31st Kasetsart University Annual Conference* Bangkok Thailand pp 221–226

DUNG N D 1990 *Study on karyotype of some popular cyprinid species in Vietnam* Ph D Thesis University of Hanoi Vietnam (in Vietnamese)

DUNG N D 1992 Study on karyotype of some cyprinid species *National Workshop on Aquaculture* (in Vietnamese) pp 10

GOLD J R1979 Cytogenetics *In* W S Hoar, D J Randall & J R Brett (eds) *Fish Physiology* vol **8** Academic Press New York pp 353–405

JANA D & NAGESH T S 2007 Karyological analysis of three threatened freshwater fishes of West Bengal *J Inland Fish Soc India* **39** 60–63

JOHN G, BARAT A & LAKRA W S 1993 Localization of nucleolar organizer regions in *Labeo* (Cyprinidae) *La Kromosoma* **2** 2381–2384

KHUDA-BUKHSH A R & CHANDA T 1983 Somatic chromosomes of three species of hill stream fishes of Assam *In* P Das & A G Jhingran (eds) *Fish Genetics in India* pp 69–73

KOSSWIG C 1973 The role of fish in research on genetics and evolution *In* J H Schroder (ed) *Genetics and Mutagenesis* Springer Verlag Berlin pp 3–16

LEVAN A, FREDGA K & SANDBERG A A 1964 Nomenclature for centromeric position on chromosomes *Hereditas* **52** 201–220

MAGTOON W & ARAI R 1993 Karyotypes and distribution of nucleolus organizer regions in cyprinid fishes from Thailand *Jpn J Ichthyol* **40** 7785

MANNA G K & KHUDA-BUKHSH A R 1977 Karyomorphology of cyprinid fishes and cytological evaluation of the family *Nucleus* **20** 119–127

NAGPURE N S 1997 Distribution of C-band heterochromatin in *Labeo rohita* Ham (Cyprinidae) *Chrom Sci* **1** 45–46

NAGPURE N S, KUSHWAHA B, SRIVASTAVA S K & PONNIAH A G 2001 Comparative cytogenetic studies in Indian major carps *Labeo rohita Catla catla* and *Cirrhinus mrigala* (Cyprinidae Pisces) *Chrom Sci* **5** 153–158

NAYYAR R P 1962 Karyotype studies in two cyprinids *Cytologia* **27** 229–231

NELSON J S 2006 *Fishes of the World* 4th ed John Wiley and Sons New York

PADHI B K & MANDAL R K 2000 *Applied Fish Genetics* Fishing Chimes Chimes House Vishakhapatnam

SAROWER-E-MAHFUJ M, HOSSAIN M B, RAHMAN M M & HOSHAN M I 2014 Chromosomal studies and quantitative karyotypic analysis of Rohu *Labeo rohita Pak J Biol Sci* **17** 490–496

SHARMA I 2010 Diversity and status of fish fauna of the river drainage systems of Himachal Pradesh in western Himalaya India *Biosystemica* **4** 5–23

THORGARD G H & DISNEY J E 1990 Chromosome preparation and analysis *In* C B Schreck & P B Moyle (eds) *Methods for Fish Biology* **IV** American Fisheries Society Maryland Bethesda pp 171–190

TRIPATHY S K, GAUR K K & SARANGI N 2010 Cytogenetic characterization of B1R and C1F2 backcross progenies of *Catla catla* (Ham) and *Labeo rohita* (Ham) *J Occup Health* **10** 34–37

TRONG T D & DUNG N D 1990 Karyotype of silver carp (*H molitrix Rastarinus lineatus* and *Toxabramis swinbonis*) *J Biol* **12** 1–3

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AUTOPOD TYPE LIMB ABNORMALITIES IN MAN AND THEIR GENETIC BEARING

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SUMMARY

A general discussion is provided highlighting the features and genetic bearing of human limb abnormalities with special reference to a few autopod type of abnormalities such as polydactyly, syndactyly, synpolydactyly and ectrodactyly, primarily based on the findings in certain Kerala Kindreds. Polydactly is characterized by the presence of extra digits in the palms and feet which may be preaxial/postaxial, bilateral/unilateral with varying degrees of expression. Syndactly refers to digital anomaly, in which adjacent fingers and/or toes webbed. This may be bilateral/unilateral with inter- and intrafamilial phenotypic variation. In most cases, both polydactyly and syndactyly coexist (synpolydactyly) and both held as expressions of the same genetic factor – autosomal dominant with incomplete penetrance and variable expressivity. Ectrodactyly, also known as split hand-foot malformation (SHFM) or lobster-claw abnormality is a congenital defect in man characterized by a deep median cleft of the hand and/or foot due to absence of central rays, often occurring in combination with other congenital defects. There is disagreement concerning the mode of inheritance such as autosomal dominant with incomplete penetration and variable expressivity, autosonal recessive and X-chromosomal. Cytogenetic studies have suggested the critical chromosomal locus as 7q 21.1 – q 22.1. Gene targeting studies have suggested Dactylin as a probable candidate gene for ectrodactyly.

Keywords: Autopod, human, limb, abnormalities, genetics.

Congenital limb abnormalities in man occur in about one in 1000 neonates which include both reduction defects and more subtle alterations in the number, length and anatomy of the digits (Wilkie 2003). The major causes of limb abnormalities are abnormal genetic programming and intra-uterine disruption of development the former arising due to alterations of the limb development gene function, and the latter caused by disruption of the hedgehog (Hh) signalling pathway which regulates the digital pattern in the limbs (Wilkie 2003, Anderson et al. 2012). After the rediscovery of Mendel's work in 1900, the first human disorder recognized following the principle of Mendelian inheritance was the limb malformation, now termed bracthycephaly type A1

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(BDA1) characterized by absent or short middle phalanges in the hands and feet. Several decades later, a Chinese group demonstrated that heterozygous mutations in Indian hedgehog (IHH), a member of a key family of developmental regulators, was the cause of BDA_1 (Gao et al. 2001). The observations spanning the history of genetics from its inception to its current apotheosis encapsulate the ability of studying human limb abnormalities. In spite of the use of the limb over several decades as a classical developmental model, the genetic analysis of human limb abnormalities has been relatively a neglected subsect, probably for two reasons (1) their clinical management tends to be the province of surgeons who are traditionally not very interested in genetics and (2) the disorders in limb development are not life threatening (Wilkie 2003). The study, however, has much to contribute both to human health and to developmental biology.

The genes implicated to limb bud development are numerous, and they work together to trigger limb bud growth, its polarization and patterning which are largely pleiotropic. Increasing number of genes are being identified, which when functionally perturbed, result in limb abnormalities. The abnormalities are often part of complex syndromes that affect other systems in the body, and hence clear genotypic-phenotypic correlations are difficult to establish owing to the interlocking network of genetic signals underlying limb development (Hanu et al. 1999).

There have been a number of classifications of limb abnormalities, of which the current and the more preferred is the one which divides the limb abnormalities into seven types (Moore & Persaud 2008) such as:

1. Amelia (complete absence of limb)
2. Meromelia (Partial absence of limb)
3. Hemimelia (absence of half a limb)
4. Phocomelia (a flipper-like appendage)
5. Acheiria (missing hand/foot)
6. Adoctyly (absence of metacarpals/metatarsals)
7. Aphalangia (digits absent)

The human hands and feet comprise bones of a particular size and shape. The developing limb can be described along the proximodistal axis having three main regions with abnormalities along the axis characterized by changes in their components (Moore & Persaud 2008) such as (1) **Stylopod** which concerns the humerus of the forelimb and femer of the hind limb, (2) **Zeugopod** concerning the radius/ulna of the fore limb and tibia/fibula of the hind limb, and (3) **Autopod** concerning the musculoskeletal hands of the forelimb and feet of the hind limb. This paper is aimed at highlighting the features and genetic bearing of a few autopod type of limb abnormalities in man such as polydactyly, syndactyly, synpolydactyly and ectrodactyly based primarily on the finding in certain Kerala Kindreds (Mathew & Jyothilekshmi 2017).

Polydactyly

This is characterized by the presence of extra digits in the palm and feet. Hexadactyly is the main type in man, in which the extra digit can be preaxial (ulnar) or postaxial (radial). Based on the nature of the extra digit, there are three types (1) the extra digit is a small attachment not adherent to the skeleton, and often without bone or even cartilage, (2) the extra digit more or less like an ordinary finger or toe and (3) the extra digit deficient in the composition of metacarpals/metatarsals. In a Muslim Kindred studied from Kerala (Mathew 1988), which comprised 65 subjects spread over in four generations, there were 11 polydactylous subjects who showed bilaterial hexadactyly in the palm in five, of which the extra digit was a small attachment without bones, and in all, the extra finger was postaxial. Polydactyly in the feet was less frequent in the group, only six, of which four bilateral and two unilateral, and all were preaxial. Three expression types were evident such as 6.6:6.6, 6:6:5.6, 6.6:5.5). A deviant type also is known (Brachydactyly) in which the middle phalanges very short, often associated with endocrine abnormality leading to short stature, round face, short fourth and fifth metacarpals (Moore & Persaud 2008).

Syndactyly

It is a digital malformation in which adjacent fingers and/or toes are webbed as they fail to separate during limb development. This is one of the common hereditary malformations with prevalence of 3–10 in 10000 birth. Clinically this is one of the most heterogeneous developmental deformities known in the medical literature. A number of combinations are known in which the adjacent fingers and toes webbed in diverse degree; can be bilateral or unilateral, symmetrical/ asymmetrical. Moreover, inter- and intrafamilial phenotypic variability is very common. The condition can be so variable that the same individual may display asymmetrical phenotypes in the fore and hind limbs in both right and left. Syndactyly could be partial or complete, cutaneous or bony and involving only the phalanges or further extending up to the metacarpal, metatarsal or capal/tarsal levels. In the Kerala Kindreds, syndactyly (webbing and/or fusion) was present in 10 subjects, among whom seven grades could be recognized, the least severe type being the one involving two digits and the severest with webbing in both palms and feet involving several digits (Mathew 1988). A deviant type known as Grieg cephalopoly syndactyly (GPCS) characterized by postaxial polydactyly of the hands, preaxial polydactyly of the feet and syndactyly. Additional features include a large head with broad forehead and hypotelorism (wide spaced eyes) The identification of translocations involving the chromosome band 7 p13 enabled Vortkamp et al. (1991) to pinpoint GL13 which encodes the zinc-finger protein as the gene mutated in GPCS. Several nonsyndromatic syndactylies with additional subtypes have been characterized (Malik 2012). Despite considerable progress in the understanding of syndactyly at clinical and molecular levels, the fundamental question concerning the disturbed developmental mechanisms leading to fused digits remains unknown.

Synpolydactyly

It is characterized by association of both polydactyly and syndactyly in the same limb and is a less frequent phenomenon. This was present in eight of the affected subjects in the Kerala Kindred. In great bulk of the reported cases, polydactyly and syndactyly are suggested as dominant traits, while a few cases of recessive inheritance also reported. According to Stern (1960), a dominant gene (*D*) controls the number of rays formed in the embryonic buds of palms and feet. In the *dd* genotype, five rays of metacarpals or metatarsals and phalangeal bones are formed leading to normal digital condition. In the Kerala Muslim pedigree, all the affected sibs invariably had one of their parents affected and the other unaffected. This, together with the incidence of both affected and unaffected subjects (male/ female) in the same sibship was held suggestive of autosonal dominant inheritance of the trait (Mathew 1988). Since both polydactyly and syndactyly occur in the same limb, although in different degrees, the two conditions may be considered as expressions of the same genetic factor. The extent of variation in the degree of expression of the traits could be due to incomplete penetrance and variable expressivity of the dominant gene in the heterozygous state. In the Kerala Kindred, the abnormality skipped in one generation, in which two subjects, despite being sons of the same affected father, were themselves unaffected, and these could be nonmanifesting heterozygotes. Such 'skipped generation' subjects may show up minor expressions which if detected, could serve as a basis for genetic counselling (Cross et al. 1968)

Ectrodactyly

Ectrodactyly, otherwise known as lobster-claw abnormaliy or splithand foot malformation (SHFM) is a congenital defect in man. It is characterized by aberrant development of the central digital rays with absence of one or more of the digits, coupled with a deep median cleft and fusion of the remaining phalanges (Ozen et al. 1999, Duijf et al. 2003). This is clinically a heterogeneous condition manifested in an isolated form or in combination with a variety of congenital defects involving different organs or organ systems. Detailed analysis of a Latin Catholic Kindred in the capital city, Thiruvananthapuram of the state of Kerala comprising 47 subjects spread over in four generations, showed 22 affected members (Balachandran et al. 2001, Mathew & Jyothilekshmi 2017) exhibiting great variation in the degree of expression of the anomaly. The anomaly was bilaterial/unilateral and symmetrical/asymmetrical and the expression varying from bilateral monodactyly in both limbs with acute impairment of form and function of the digits to unilaterial tetradactyly with near normal function of the digits. The lobster claw appearance was striking in the hands, and in a few subjects a deep cleft resulting in a V-shaped malformation was evident. Subjects with severe defects showed marked reduction in digital rays with rudimentary digits, symphalangism, double nails, epistasis/ hypostasis of metacarpals and phalanges, synostasis and bent bones. The cleft in the feet were less impaired with deformation including less severe hypoplasia of the middle toe with deep cleft formation, metracarpal hypoplasia and even monodactyly.

The lobster-claw defects in man are of two types (1) a deep palmer cleft separating the two central metacarpals, one or more rays absent, and the remaining digits confluent and of unequal length and (2) the central ray absent, only the short radial and ulnar rays remaining and showing webbing between the thumb and little finger. The phalanges may be short or absent. The subjects of the Thiruvananthapuram Kindred all conformed to the first type. Generally, the abnormality co-exists with a constellation of congenital defects of which the most frequent is the EEC (ectrodactyly-ectodermal cleftlip/palate) syndrome (Obel et al. 1993, Leung et al. 1995). Other less frequent associations include genito-urinary and cardio-vascular defects (Ozen et al. 1999), pituitary dysfunction (Van Maldergen et al. 1992), congenital heart defects and mental retardation (Ahmed et al. 1987). In the Thiruvananthapuram Kindred, no major associated defects, except minor cases of mental retardation were evident, and hence the condition here should be largely of the isolated type.

There exists some confusion concerning the mode of inheritance of the ectrodactyly deformity, and this may be due to conclusions drawn based on inadequate number of subjects in the pedigree. The existing suggestions are autosomal dominant with full or incomplete penetrance and variable expressivity (Leung et al. 1995), autosomal recessive (Freire-maia 1971) and X-chromosomal (Ahmed et al. 1987). There are also reports suggesting chromosomal structural aberrations associated with ectrodactyly which include interstitial deletion in the long arm of chromosome 7 (Cobben et al. 1995), complex chromosome rearrangements resulting in breaks in chromosomes 5, 7 and 9 (Sharland et al. 1991), balanced translocation involving chromosome 6 (Genuardi et al. 1993), reciprocal translocations between chromosomes 7 and 9 (Hasegawa et al. 1991). Analysis of the karyotypes of a few affected male as well as female subjects along with unaffected male and female controls by the conventional karyomophological study as well as G-banding study in the Thiruvananthapuram Kindred did not show up any type of chromosome structural abnormalities.

During the past few decades, especially in the 1990s, quite a few in depth studies have been known on the cytogenetic and molecular aspects of the ectrodactyly disorder aimed at locating the genetic locus, and for targeting the genes which control the condition. Sharland et al. (1991) have proposed that the break at chromosome 7q, 21 had disrupted a gene for complete hand ray development. Most of the studies have implicated chromosome 7, and it has been suggested that the critical region of the chromosome for ectrodactyly could be 7q21.1–q22.1 (Ignatius et al. 1996). According to Palmer et al. (1994) the association between SHFM and cytologically visible rearrangements of chromosome 7 at bands q21–22 provides compelling evidence for the location of the causative gene at this locus, and they designated the locus as SHFM-1. Ianakiew et al. (2000) have suggested that the lobster-claw abnormality in man is analogous to the naturally occurring murine dactylaplasia mutant (Dac) embryo in which the central segment of the apical ectodermal ridge (AER) degenerates leaving the anterior and posterior segments intact. The results of gene targeting studies have demonstrated that P63, a homologue of the cell cycle regulater TP 53 plays a crucial role in the regulation and formation and differentiation of the AER. By positional cloning, EST database researching and RT- PCR, Ianakiew et al. (1999) have generated a cDNA encoding human Dactalyn, and

they assigned Dactylin to the critical region of SHFM-3 on chromosome 10q 24, and have also suggested that Dactylin is likely to be involved in signalling pathways crucial for limb development, and as a probable candidate gene for ectrodoctyly.

Johnson et al. (1995) suggested that the anomalous inheritance pattern of ectrodactyly often skipping generations and displaying disturbed segregation, might be the consequence of polymorphic epistatic genes. All the affected sibs in the Thiruvananthapuram Kindred over generations had one of their parents (male/female) affected and the other unaffected. The disorder has not skipped any generation, and there were several cases of male to male and also female to female transmission. This, coupled with the incidence of both affected and unaffected male/female in the same sibstrip appears to be strongly suggestive that the lobster claw anomaly in the Kindred is an autosonal trait and that the affected subjects are heterozygotes with *Dd* genotype. Since none of the parents or close relatives of the oldest subject in the Kindred (I.1) was known to have had any symptoms of the disorder, it may be presumed that the ectrodactyly malformation in the Kindred must have originated *de novo* in this subject as an antosomal mutation, and from him the trait transmitted to his descendants in successive generations in tune with the simple autosomal dominant pattern of inheritance.

The study of inherited human limb abnormalities has continued to throw up new genes not previously suspected to play a role in limb development. The identification of the causative genes involved in limb abnormalities is important for genetic counselling, in addition to providing insights into the mechanisms controlling limb development. The study, however, is beset with a vista of complicate factors which include complex interactions of morphogens, involvement of modifier genes, multiple genes and a long range of regulatory elements. Much work needs to be undertaken for understanding and delineating the precise role of genes and their functions and pathogenesis of different human limb abnormalities.

REFERENCES

AHMED M, ABBAS H & FLATZ G 1987 X-chromosomally inherited split hand foot/abnormally in a Pakistan Kindred *Hum Genet* **75** 169–173

ANDERSON E, PELUSO S, LETTIES L A & HILL E R 2012 Human limb abnormalities caused by disruption of hedgehog signalling *J Trend Genet* **28** 364–378

BALACHANDRAN G, MATHEW P M & PILLAI P G 2001 Ectrodactly (lobster-claw) anomaly in a fishermen kindred in Kerala *J Cytol Genet* **2 (NS)** 17–23

COBBEN J M, VERHEIJ J B M, SISMA W H, ROBINSON P H, ZWIERSTER R P, LEEGTE B & CASTEDO S 1995 Bilateral splithand foot malformation and INV (7) P 22q21-3 *J Med Genet* **32** 375–378

CROSS H E, LERBERG D B & KUSICK V A 1968 Type II syndactyly *Amer J Hum Genet* **20** 368–380

DUIJF P H G, BROKHOVEW H V, & BRUNNER H G 2003 Pathogenesis of split hand/split-foot malformation *Hum Mol Genet* **12** 51–60

FREIRE-MAIA A 1971 A recessive form of ectrodactyly and its implication for genetic counselling *J Hered* **62** 53–86

GAO B, GUO J, SHE C, SHU A, YANG M & TAN Z 2001 Mutations in JHH encoding Indian hedgehog caused brachydactyly by – type A1 *Nat Genet* **28** 386–388

GENUARDI M, POMPON M G, SAMMITO V, BELLUSSI V, ZOLLING M & NERI G 1993 Splithand-foot anomaly in a family segregating a balanced translocation with break point on 7q22-1 *Am J Med Genet* **47** 823–831

HASEGAWA T, HASEGAWA Y, ASAWURA S, NAGI S T, TSUCHIYA Y, NINOMIYA M & FUKUSHIMA Y, 1991 EEC syndrone (ectrodactyly ectodermal dysplasia and cleft lip/palate) with a balanced reciprocal translocation between 7q11.21 and 9p12 in three generations *Clin Genet* **40** 202–206

HANU S M, ESPINASSE M N & LYONNET S 1999 Genetics of limb abnormalities in humans *J Trend Genet* **15** 409-417

IANAKIEW P, KILPATRICK M W, DEALY C, KOSHER R, KORENBERG J R, CHEN X B & TSIPOURAS P 1999 A novel human gene encoding an F-box/WD40 containing protein maps in the SHFM critical region on 10q24 *Biochem Biophys Res* **261** 64–70

IANAKIEW P, KILPATRICK M W, TOUDJARSKA T, BASEL D, BELGHTON P & TSIPOURAS P 2000 Splithand-foot malformation is caused by mutations in the p63 gene on 3q27 *Am J Hum* Genet **67** 59–66

IGNATIUS J, KNUTILA S, SCHERER W, TRASK B & KERE J 1996 Splithand-foot malformation involving 7q21.3 *Am J Med Genet* **33** 507–510

JOHNSON K R, LANE P W, WARD-BAILEY P & DAVISSON M T 1995 Mapping the mouse dactylaplasia mutation Dac and a gene that controls its expression *Genomics* **29** 457–464

LEUNG K Y, MCLACHIAN N A & SEPULVEDA W 1995 Prenatal diagnosis of ectrodactyly the lobster-claw anomaly *Ultrasound Obstet Gynocol* **6** 433–446

MALIK S 2012 Syndactyly phenotypes genetics and current classification *Eur J Hum Genet* **20** 817–824

MATHEW P M 1988 Polydactyly and syndactyly in a predegree of Muslims in Kerala *J Cytol Genet* **23** 89–94

MATHEW P M & JYOTHILEKSHMI P 2017 *Fundamentals of population Genetics with emphasis on human inbreeding* Southern Book Star Thiruvananthapuram

MOORE K L & PERSAUD T V N 2008 *Musculoskelital System-Limb abnormalities –Embryology* Cell Press Publishers Cambridge

OBEL N, HANSEN B & BLACK F T 1993 Normal immulogical status in four patients with ectrodoctyly-endodermal dysplasia clefting syndrome (EEC syndrome) *Clin Genet* **43** 146–149

OZEN R S, BASAL B E, DEVLIN B, FARR J E, GORRY M, ERLICH G D & RICHARD C W 1999 Fine mapping of the split hand/foot locus at 10q24 evidence for anticipation and segregation distortion *Am J Hum Genet* **64** 1646–1654

PALMER S E, SCHERER S W, KUKOLICH M, WIJSMAN E M, TSUI L C, STEPHENS K & EVANS J P 1994 Evidence for locus heterogeneity in human autosomal dominant split hand/foot malformation *Am J Hum Genet* **55** 21–26

SHARLAND M, PATTON M A & HILL L 1991 Ectrodactyly of hands and feet in a child with a complex translocation including 7q21.2 *Am J Med Genet* **39** 413–414

STERN C 1960 *Principles of Human Genetics* Freeman San Francisco

VAN MALDERGEM I, LILLEROT Y, VAMOS E, TOPPETT M, WATTILLON P & VAN VIET G 1992 Vassopressim and Gonadotropin in a boy with ectrodactyly ectrodermal dysplasia-clefting syndrome *Acta Paediatr* **81** 365–367

VORTKAMP A, GESSLER M & GRZESC-HIK K H 1991 Zinc finger gene translocation in Greig syndrome *J Anat* **190** 539–590

WILKIE A O M 2003 Why study human limb malformation *J Anat* **202** 27–35

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SOCIOECONOMIC CORRELATES AND GENETIC DETERMINANTS OF CONSANGUINITY

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SUMMARY

A general discussion is provided highlighting the impact of socioeconomic factors and genetic determinants on the phenomenon of consanguinity. The major socioeconomic factors are, education, occupation and family income, all inducing strong influence on the consanguinity rate. In almost all known cases, the literacy factor is negatively correlated with the rate of inbreeding. Increased educational, occupational and financial status play a prominent role in younger generations inhibiting the propensity for related marriages. In most population groups, a descending trend of consanguinity is evident from the older generations to the younger. The genetic determinants include, the degree of spousal relationship, the nature, number and frequency of deleterious recessive genes, coupled with duration of inbreeding. The increase of recessive homozygosity is maximum for the highest degree of spousal relationship and vice versa. The risk of inbreeding very much depends on the degree of lethality of the recessive gene such that if the gene is fully lethal and severely detrimental, serious harmful effect may be expected. The harmful effects also depend largely on the number of deleterious recessive genes and of their frequency. The risk effects increase exponentially with an increasing number of lethal genes, and a very low frequency of such genes matters exceedingly much implying that inbreeding is highly harmful in populations with rare hereditary diseases and defects.

Keywords: Consanguinity, socioeconomic, genetic factors.

INTRODUCTION

Consanguinity or human inbreeding is referred to as the phenomenon of marital union between spouses who are related to each other by common ancestry. As the likelihood of two related spouses to possess the same genes inherited from the common ancestor, concealed in heterozygous state is pretty high, inbreeding would bring them together at fertilisation resulting in higher frequencies of homozygous offspring in consanguineous families. Many harmful traits in man are known to be recessives which find expression as affected offspring in higher frequencies in such families, and they are likely to meet with developmental abnormalities *in utero* or after birth, and become nonviable leading to mortality at various pre-reproductive stages and of morbid conditions of genetic

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predispositions and succumb to diverse genetic diseases and congenital defective conditions. (Mathew 2017). The level of inbreeding and the associated risk effects on mortality and morbidity are dependent on a variety of socioeconomic covariables, customs and traditions and on certain demographic and genetic determinants. The prominent factors which influence the incidence of related marriages are parental consanguinity and the socioeconomic covariables like education, occupation and family income, coupled with demographic factors like year of birth and marriage and spousal age at marriage *vis-a-vis* geographic correlates, urban-rural effect and marital distance (Mathew & Jyothilekshmi 2017). The genetic determinants include, the degree of spousal relationship, the nature, number and frequency of recessive genes and duration of inbreeding. This paper aims at highlighting the role of these factors, particularly the socio- economic covariables and genetic determinants.

Parental consanguinity

An advantage of parental inbreeding is that their children would have better chances of childhood and juvenile friendship and familiarity with the children of relatives of both the parents. (Bener et al. 1996). This would minimise the fear of the 'unknown'. It has been held that the children of blood-related parents would cultivate better knowledge of relatives of their age group which can ultimately lead to marital union between them by their own personal decision, often coupled with added parental recommendations and consent (Imaizumi 1987). It was pointed out that the prospective related mates know each other well consequent on the consanguineous relationship of their parents which prompted them contract marital union themselves (Ichiba 1953). Consanguinity studies carried out in several communities of the Kerala region have yielded convincing data of influence of parental consanguinity (Mathew et al. 2006).

Socioeconomic correlates

Social classes are generally considered and identified based on three major socioeconomic parameters such as (1) education, (2) occupation and (3) family income. This stratification allows (a) definition of the appropriate control variables to be devised on the effects of consanguinity on public health parameters, (b) description of the social profile of the target population to be addressed by public health programme and (c) providing valuable information regarding the cotroversial question of the social functions of endogamous marriages (Khlat 1988). Among populations in which consanguinity is preferential, high rates of close kin alliances are consistently reported in rural areas and in the least educated sections of the society. In most communities studied, the measured frequencies of consanguinity are more among such groups (Bittles 1994).

Education

Consanguinity studies carried out in diverse population groups the world over have indicated the social status defined in terms of the educational level of the members of the community as having strong influence on the consanguinity rates. In almost all known cases, the literacy factor was negatively correlated with the rate of inbreeding such that lower the literacy level, higher the

frequency of consanguinity and vice versa (Hussain & Bittles 1998, Mathew et al. 2006). The role of literacy on consanguinity rate on the Kerala communities were studied by considering four literacy classes such as illiterate, low literacy, medium and high (Mathew et al. 2006). A consistently declining trend was noticed with increasing literacy status. However, in certain Brahmin groups in Kerala and among many Muslim groups, there is a clear propensity to pursue close kin alliance despite high literacy level. The strongly positive association of literacy with consanguinity rate in the immigrant Tamil Brahmin group of the region has been suggested to be due to their strong adherence to their original Tamil custom and tradition in matters of mate selection and kinship ties (Jyothilekshmi 2015). Strong negative association between consanguinity rate and literacy was evident in most tribal groups in Kerala. This trend was particularly evident among the tribal groups of Attapadi in Palakkad district and in a few tribal groups of the Idukki district in which the consanguinity rates were very high, over 70% in the former and over 90% in the latter (Mathew & Jyothilekshmi 2017).

Occupation

Most studies carried out for determining the role of occupation on consanguinity rates have suggested that the practice of close-kin marriage goes beyond social and occupational boundaries (Shami et al. 1991). A multivariate analysis performed in certain middleeast Muslim groups demonstrated that occupational status accounts heavily on the association between educational level and status of marriage, and the finding has revealed occupational status as a relevant indicator of social class for the purpose of controlling potential confounding variables (Khlat 1985). Almost a similar pattern of association was noticed in the Kerala study in several social classes. High occupation increases the style and status of life which might change the attitude to marriage relation, often favouring an alliance with a related spouse with equal or better occupational status. However, in the case of the tribals of the state at large, no such association was evident, and in many of them the association was negative.

Family income

The family income is considered to be a major indicator of social level. Consanguineous alliance has been felt as a feasible choice for many communities to avoid financial liabilities and uncertainities, and this is often associated with dowry and bridal payments (Bittles 2001). The association between consanguinity and family income was assessed in the Kerala communities by stratifying the families under four groups based on their family income and in great many cases, a clear positive association was evident (Mathew & Jyothilekshmi 2017). The association was highly significant in many backward communities like Ezhava, Muslim etc. However, a bivariate analysis has revealed negative association in some Scheduled castes and most tribal communities of the state. A glaring exception was evident in the Christian Malai Arayas of the Kottayam district in which the upper middle and high income groups constitute the bulk (Mathew et al. 2006).

Demographic factors

Many studies have established correlations of consanguinity with demographic factors which was effectively used to interpret the ascending and descending temporal trends of consanguinity (Bittles & Black 2010). Association of various demographic attributes with consanguinity was examined in many population groups in Kerala, particularly in respect of elements like year of birth, year of marriage, age at marriage of spouses and spousal age difference (Mathew & Jyothilekshmi 2017). Highest rate of consanguinity was evident in most of the backward class communities associated with older generations, and lowest in the younger generation. Increased education and occupational status and higher health awareness might have played a prominent role in the younger generations creating reluctance to related alliances. In great many of the Scheduled castes and tribals, a descending trend of consanguinity was evident from the older generation to the younger.

Assessment of consanguinity with the year of marriage is helpful for understanding the direction of temporal trends of consanguinity. The study in the Kerala group of communities showed great many of them, surpassing social class difference, registering higher consanguinity rates in marriages contracted in the earlier period (before 1960). The declining trend with period of marriage was highly significant in many of them. Age at marriage is demographically important as it determines the length of marital life and of the fertility span of the couples. Many studies the world over and also those among the Kerala group of communities have shown higher rates of close-kin alliances associated with low age of spouses. The low age at marriage, especially of women, provides longer cohabitation period as well as of reproductive span. In the absence of effective family planning programmes, this becomes an issue and cause of concern of population growth.

Genetic determinants

The magnitude of the risk effects of inbreeding depends on a variety of genetic and nongenetic factors. The genetic factors are primarily (a) the degree of spousal relationship, (b) the nature, number and frequency of deleterious genes and (c) the duration of inbreeding. The probability of the offspring of the related spouses to be homozygous dominant and recessive would be greater than p^2 and q^2 respectively by an amount each by ‘e’, and to be heterozygous correspondingly less than ‘2pq – 2e’. The inbreeding coefficient is F = 1 when the population is completely inbreeding, and F = 0 when absolutely panmictic, and the value of F may vary from ‘0–1’ in a population. The increase factor ‘e’ by inbreeding is a function of the inbreeding coefficient ‘F’ and the two gene frequencies ‘p’ and ‘q’ such that ‘e = Fpq’.

Spousal relationship

Assuming a hypothetical panmictic population with frequencies of the dominant (*A*) and recessive (*a*) genes at a given locus as p = 0.4 and q = 0.6, its genotypic constitution according to Hardy-Weinberg Law would be *AA* = p^2 = 0.16, *Aa* = 2pq = 0.48 and *aa* = q^2 = 0.36. Considering the population as resorting to different mating patterns such as Uncle–Niece (UN), First cousin (1C),

Second cousin (2C) and panmixis (RM) in which the corresponding F values are for UN = 1/8, 1C = 1/16, 2C = 1/64 and for RM = 0, the respective numerical equivalents of the 'e' values would be e_1 for UN = 0.03, e_2 for 1C = 0.15; e_3 for 2C = 0.0037 and e_0 for RM = 0.00. The resulting genotype frequencies in respect of the four mating patterns are furnished in Table 1. They are positively associated with the degree of spousal relationship. The increase of the recessive homozygosity is maximum for the highest degree relationship (UN) and lowest for the least, which implies that the effect of inbreeding on homozygosity is dependent on the degree of spousal relationship.

TABLE 1: The 'e' values and genotype frequencies in different mating patterns.

Mating types	e (Fpq)	*AA*	*Aa*	*aa*
Uncle–Niece (UN)	e_1 = 0.03	0.190	0.420	0.390
First cousin (1C)	e_2 = 0.015	0.175	0.450	0.375
Second cousin (2C)	e_3 = 0.0037	0.163	0.472	0.365
Panmixis (RM)	e_0 = 0.00	0.160	0.480	0.360

Nature of genes

The risk of inbreeding depends very much on the degree of lethality of the deleterious recessive gene. If the gene is fully lethal and severely detrimental, serious risk effect may be expected, and conversely very little or no risk if the gene is less lethal or not lethal. In the case of highly deleterious recessive genes, the recessive genotype would be prone to severe selection pressure (s = 1) resulting in its complete elimination every generation so much so the recessives do not pass on the recessive gene to the next generation. In such cases, a sharp decline of the recessive gene frequency is expected in the next few generations. If the recessive gene is less lethal/sublethal, the rate of decline of the recessive gene frequency is only marginal.

Number of genes

The harmful effect of inbreeding also depends largely on the number of detritimental recessive genes present in a population. Since the coefficient of inbreeding (F) for first cousins is 1/8, the probability of any one of the children of this type of marriage being affected is 1/4 × 1/8 = 1/32 = 3.1%, and being unaffected is 1–3.1 = 96.9%. If the first cousins carry a second deleterious recessive gene, the probability of a child being affected by any one of the two genes is 2 × 0.969 × 0.031, and affected by both is 0.031^2. If the related first cousins carry 'n' deleterious recessive genes, the probability of the child being affected is $1 - 0.969^n$, and accordingly the risk with

1 gene = 1 – 0.969 = 3.1%

2 genes =1 – 0.969^2 = 6%

3 genes = 1 – 0.969^3 = 9%

8 genes = 1 – 0.969^8 = 22.4%

This would imply that the harmful effect of inbreeding increases exponentially with increasing number of lethal recessive genes in a population.

Frequency of recessive genes

The expected harmful effect of inbreeding, e.g. First cousin in terms of deleterious recessive gene ‘q’ is denoted by the expression

$$\frac{R_1}{R_0} = \frac{q2 + \frac{1}{16}pq}{q2} = \frac{q(1 + 15q)}{16 + q2}$$

where, p and q are the dominant and recessive gene frequencies, 1/16 the F for the first cousin pattern, R_0 recessives without inbreeding and R_1 with inbreeding (Mathew & Jyothilekshmi 2017). The values of different ‘q’ values, Fpq and R_1/R_0 are furnished in Table 2. It may be noted that the values of harmful effect (R_1/R_0) are very low for higher values of ‘q’ (0.4, 0.2, 0.1), while this for very low ‘q’ values (eg. q = 0.001) the harmful effect is exceptionally high in which the Fpq is the major component. This implies that the frequency of the lethal recessive gene matters very much indicating that inbreeding is highly harmful in populations with very rare hereditary diseases and defects.

TABLE 2: Recessive gene frequencies and harmful effect of inbreeding.

q_0	R_0	Fpq	R_1	R_1/R_0
0.4	0.16	0.015	0.175	1.09
0.2	0.04	0.010	0.050	1.25
0.1	0.01	0.0056	0.0156	1.56
0.01	0.0001	0.00062	0.00072	7.19
0.001	0.000001	0.0000624	0.0000634	63.4

q_0 , Initial recessive gene frequency; R_0, R_1, recessive genotype frequency without and with inbreeding.

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REFERENCES

BENER A, AL-GAZALI Y M, MICALLEF R, AL-KHAYAT A L & GABER T 1996 Consanguinity and associated socio-demographic factors in the United Arab Emirates *Hum Hered* **46** 256–264

BITTLES A H 1994 The rate and significance of consanguinity as a demographic variable *Popul Dev Rew* **20** 561–584

BITTLES A H 2001 Consanguinity and its relevance in clinical genetics *Clin Genet* **60** 89–98

BITTLES A H & BLACK M L 2010 The impact of consanguinity on neonatal and infant health *Early Hum Dev* **86** 737–741

HUSSAIN R & BITTLES A H 1998 The prevalence and demographic characteristics of consanguineous marriages in Pakistan *J Biosoc Sci* **30** 261–275

ICHIBA M 1953 Studies in the children of consanguineous marriage I Frequency and causes of consanguineous marriage *Niham Ika Daigaku Zasshi* **20** 314–321

IMAIZUMI Y 1987 Reasons for consanguineous marriages in Japan *J Biosoc Sci* **19** 97–106

JYOTHILEKSHMI P 2015 Consangunity study in the immigrant tamil Brahmins of Palakkad district Kerala *J Cytol Genet* **16** (NS) 31–37

KHLAT M 1985 Consanguineous marriages in Beirut: time trends special distribution *Soc Biol* **35** 324–330

KHLAT M 1998 Consanguineous marriage and reproduction in Beirut Lebanon *Am J Hum Genet* **43** 188–196

MATHEW P M 2017 Hidden hazards of human inbreeding – impact and remedy *J Cytol Genet* **18** (NS) 31–38

MATHEW P M & JYOTHILEKSHMI P 2017 *Fundamentals of Population Genetics with emphasis on human inbreeding* Southern Book Star Thiruvananthapuram

MATHEW P M, VIJAYAVALLI B & DEVIPRIYA V 2006 Consanguinity and its effect with special reference to inbreeding communities of Kerala *J Cytol Genet* **7** (NS) 185–195

SHAMI S A, QUAISAR R & BITTLES A H 1991 Consanguinity and adult morbidity in Pakistan *Lancet* **338** 954–955

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KARYOTYPIC STUDY ON *NETELIA DILATATA* (THOMSON) (HYMENOPTERA : ICHNEUMONIDAE)

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SUMMARY

The karyotype of *Netelia dilatata* was investigated for the first time from Himachal Pradesh. This wasp species exhibits haplo-diploid sex determination mechanism wherein the males have been found to be haploid and the females diploid. The chromosome numbers were found to be 6 in males and 12 in females. The chromosome length ranged from 0.37 to 2.98 ìm both in males and females with total lengths of complements being 12.66 ìm in males and 26.08 ìm in females. Haploid complement of this species consists of 4 metacentric chromosomes and 2 telocentric chromosomes.

Keywords: *Netelia dilatata*, parasitic Hymenoptera, karyotype.

INTRODUCTION

Parasitic Hymenoptera is one of the most abundant and taxonomically complicated groups of insects (Rasnitsyn 1980, Quicke 1997). Although, they are economically important as biological control agents of agricultural pests (Godfray 1994, Viktorov 1976), but are poorly studied cytogenetically (Gokhman 2004). Ichneumonidae is one of the large families in Hymenoptera with 24281 described species worldwide (Ghahari & Jussila 2016, Yu et al. 2012), but some earlier workers consider that its species richness may well exceed 100000 (Wahl & Sharkey 1993, Yu & Horstmann 1997).

Netelia Gray is the largest genus of the subfamily Tryphoninae with worldwide species distribution with all members being koinobiont ectoparasitoids of lepidopteran larvae. So far, more than 330 species under 12 subgenera have been described (Bennett 2015). *N. dilatata* has been reported as a Palaearctic in distribution (Kolarov 2007). Main characteristics of these individuals are the manner of oviposition of eggs and their structure (Yu et al. 2005). Strongly twisted mandibles, fully pectinate claws and fore wing vein 2*m-cu* distal to 2*rs-m* are some others characters of these individuals. In addition, the male and female individuals are cytologically different, the former being haploid and the latter diploid.

Parasitic Hymenoptera are widely distributed all over India. The fauna of North India especially of Kashmir and Himachal Pradesh are very much related to Palaearctic fauna (Narendran 2002). Chromosomes of more than 400 species of parasitic wasps have been studied (Gokhman 2009) and in these, haploid chromosome number varies from n = 2 to 23. Chromosomes of these insects are monocentric.

Considering the economic importance of this species as a biological control agent, it was considered worth analyzing its chromosomes. Karyotyping may be effectively used as a rapid and inexpensive screening method for detecting sibling species and population polymorphism.

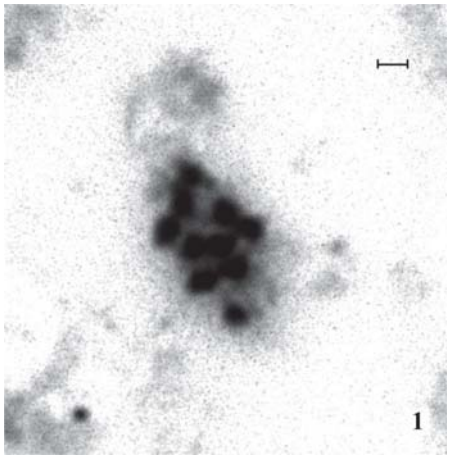
MATERIAL AND METHODS

Samples were collected from Shimla district of Himachal Pradesh (31.10°N latitude and 77.17°E longitude). Chromosomal preparations were made from cells/tissues obtained from testes of male individuals and ovaries of female individuals. The material was pretreated in colchicine hypotonic solution (0.005% colchicine) for 45 to 60 min and fixed in fixative I (3:3:4 glacial acetic acid:absolute ethanol:distilled water) for 20–25 min at room temperature. Fixative I spreads outwards at first and then retracts. Just before retraction, two drops of fixative II (1:1 glacial acetic acid:absolute ethanol) were added. After a few minutes, fixative III (glacial acetic acid) was added. After squashing, the tissue was stained in 2% Giemsa for about 25 to 30 min followed by mounting in DPX. The slides were observed under the binocular research microscope. For photomicrography, well spread chromosome plates were selected. Photomicrographs were taken with the help of camera LEICA DFC 320. Standard procedure has been employed for karyotype analysis. Karyotypes were prepared by arranging the chromosomes in decreasing order of their length. Chromosomal measurements were taken by using ocular micrometer and idiograms were constructed by using haploid complements. The classification of chromosome types was made according to Levan et al. (1964). Total length of the chromosome is measured as sum total length of short and long arm. Arm ratio is calculated as the ratio of long arm to short arm while centromeric index is calculated using the formula short arm/total length of chromosome × 100.

OBSERVATIONS

The chromosome number in male (haploid) individuals of *N. dilatata* is 6 (Fig. 1). The chromosomes are relatively small ranging in length from 0.37 to 2.98 ìm with the total length of the complement being 12.66 ì m. The karyotype formula is 4m + 2t. The karyotype includes 4 metacentric and 2 telocentric chromosomes (Figs 2, 3). The details of chromosome measurements are given in Table 1.

In female (diploid) individuals, 12 chromosomes have been observed at metaphase (Fig. 4). There is a remarkable similarity between male and female individuals in regard to the karyological details. The female karyotype consists of 4 pairs of chromosomes with median centromeres and 2 pairs of chromosomes with terminal centromeres. One of the telocentric pairs is double the size of the other telocentric pair which is the smallest chromosome pair of the complement (Figs 5, 6). The chromosome measurements are detailed in Table 2.



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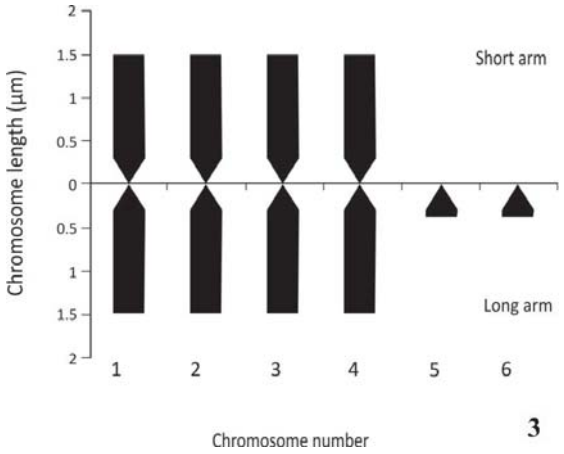

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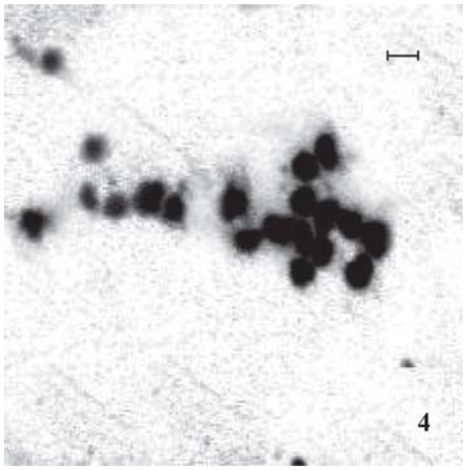

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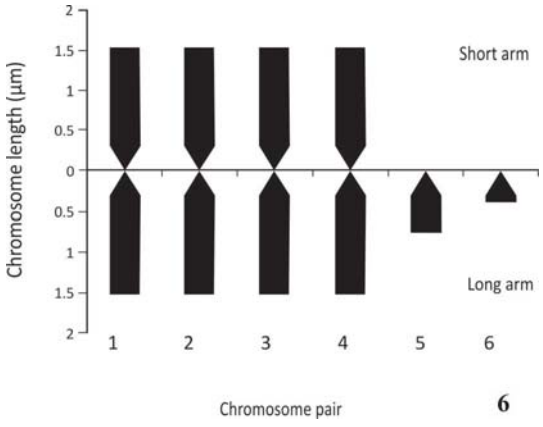

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Figs 1-6: *N. dilatata*. 1–3. Male (n = 6). 1. Metaphase chromosomes. 2. Karyotype. 3. Idiogram. 4–6. Female (2n = 12). 4. Metaphase chromosomes. 5. Karyotype. 6. Idiogram. (Bar = 5µm)

TABLE 1: Chromosome morphometry of male *N. dilatata*.

Chrom. No.	Long arm (µm)	Short arm (µm)	Total length (µm)	Arm ratio	Centromeric index	Category
1	1.49	1.49	2.98	1	50	m
2	1.49	1.49	2.98	1	50	m
3	1.49	1.49	2.98	1	50	m
4	1.49	1.49	2.98	1	50	m
5	0.37	0	0.37	∞	0	t
6	0.37	0	0.37	∞	0	t

TABLE 2: Chromosome morphometry of female *N. dilatata.*

Chrom. Pair	Long arm (µm)	Short arm (µm)	Total length (µm)	Arm ratio	Centromeric index	Category
1	1.49	1.49	2.98	1	50	m
2	1.49	1.49	2.98	1	50	m
3	1.49	1.49	2.98	1	50	m
4	1.49	1.49	2.98	1	50	m
5	0.75	0	0.75	∞	0	t
6	0.37	0	0.37	∞	0	t

DISCUSSION

In *N. dilatata,* the chromosome number was 6 in males and 12 in females. Haplo-diploid sex determination mode operates in this wasp species. The karyotype formula for females was observed to be 8m + 4t. Chromosome length ranges from 0.37 to 2.98 ìm in both males and females with total mean of 12.66 ìm in males and total haploid complement length of 13.04 ìm in females. Karyotypes are symmetrical in both male and females. Male and female karyotypes though differ in terms of ploidy levels, exhibit remarkable similarity in regard to chromosome size and morphology. Minor differences in lengths may be due to different stages of condensation as well as different orientation of chromosomes in the plates. Same chromosome numbers were reported earlier in *N. latungula* by Gokhman (2001). An analysis of previous chromosome number reports showed that in most parasitic wasps, chromosome numbers fall within a range of n = 9–11 (Gokhman & Quicke 1995) with exceptions of many Chalcidoidea (n = 5–6) and Gasteruptiidae (n = 14–16). The modal chromosome number for the family Ichneumonidae, n = 11 is also modal number for Ichneumoniformes (Adelognathinae, Cryptinae and Ichneumoninae) and Ophioniformes (Tryphoninae, Anomaloninae,

Ctenopelmatinae, Metopiinae, Mesochorinae, Banchinae, Campopleginae and Cremastinae) with n = 14–21 for Pimpliformes and n = 15 for Orthopelmatiformes. From karyotypic data, differences in variation ranges of chromosome number were observed between two tribes of subfamily Tryphoninae i.e., Phytodietini and Tryphonini with different chromosome numbers, n = 6 and 10 respectively. Two main trends prevailed in karyotype evolution of parasitic wasps. These are chromosome number reduction mainly, due to tandem fusions and less frequently due to centric ones and karyotypic dissymmetrization through an increase in size differentiation of chromosomes and in the share of acrocentrics in chromosome set (Gokhman 2006).

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REFERENCES

BENNETT A M R 2015 Revision of the world genera of Tryphoninae (Hymenoptera: Ichneumonidae) *Mem Am Entomol Inst* **86** 1–387

GHAHARI H & JUSSILA R 2016 The Ichneumonidae (Hymenoptera) of northern Iran a faunistic study *Acta Mus Mor Sci Biol (Brno)* **101** 55–62

GODFRAY H C J 1994 *Parasitoids behavioral and evolutionary ecology* Princeton University Press Princeton

GOKHMAN V E 2001 Chromosomes of parasitic wasps of the Family Ichneumonidae (Hymenoptera) *Zoologicheskii Zhurnal* **80** 968–975

GOKHMAN V E 2004 Karyotype evolution in parasitic Hymenoptera *Entomol Rev* **84** 161–169

GOKHMAN V E 2006 Karyotypes of parasitic Hymenoptera diversity evolution and taxonomic significance *Insect Sci* **13** 237–241

GOKHMAN V E 2009 *Karyotypes of parasitic Hymenoptera published* Dordrecht

GOKHMAN V E & QUICKE D L J 1995 The last twenty years of parasitic Hymenoptera karyology: an update and phylogenetic implications *J Hymenopt Res* **4** 41–63

KOLAROV J 2007 A catalogue of the Ichneumonidae from Greece (Hymenoptera) *Entomofauna* **30** 405–452

LEVAN A, FREDGA K & SANDBERG A A 1964 Nomenclature for centromeric position on chromosomes *Hereditas* **52** 201–220

NARENDRAN T C 2002 Systematics studies of parasitic Hymenoptera in India *In* Melika G & Thuróczy C (eds) *Parasitic wasps evolution systematics biodiversity and biological control* Systematic parasitioid laboratory pp 40–43

QUICKE D 1997 *Parasitic wasps* Chapman and Hall London

RASNITSYN A 1980 The origin and evolution of Hymenoptera *Tr Paleontol Inst Akad Nauk SSSR* **174** 1–192

VIKTOROV G 1976 *The Ecology of Entomophagous Parasites* Nauka Moscow

WAHL D B & SHARKEY M J 1993 Superfamily Ichneumonoidea *In* Goulet H & Huber J T (eds) *Hymenoptera of the world An identification guide to families* Agriculture Ottawa pp 358–509

YU D S & HORSTMANN K 1997 A catalogue of Ichneumonidae (Hymenoptera) *Mem Am Entomol Inst* **58** 15–58

YU D S, VAN ACHTERBERG C & HORSTMANN K 2005 *World Ichneumonoidea taxonomy biology morphology and distribution* Taxapad Vancouver

YU D S, VAN ACHTERBERG C & HORSTMANN K 2012 *Taxapad 2012 Ichneumonoidea 2011 Database on flash-drive* Retrieved from www.taxapad.com Ottawa

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CYTOLOGY IN THE SYSTEMATICS, PHYLOGENY AND EVOLUTION IN PLANTS I. PTERIDOPHYTES

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SUMMARY

Extensive cytological study on the South Indian ferns and fern allies was carried out in the Department of Botany, University of Kerala since 1950s. The study on the eusporangiate ferns covered species of *Psilotum, Lycopodium, Botrychium, Isoetes, Selaginella, Ophioglossum* etc. and on the leptosporangiate ferns comprising over 100 species belonging to a number of families *sensu* Copeland. A highlight of the implications of cytology in the systematics, phylogeny and evolution of the group is provided. Fairly high chromosome numbers abound in many ferns, particularly in the eusporangiate group, soaring up to n = 630 as in *Ophioglossum*. In the leptosporangiate group, the numbers are medium/low. The very high chromosome numbers in the ferns should be the product of polyploidy/secondary hybrid polyploidy and aneuploidy at different ploidy levels. Great many of the polyploids in ferns are allopolyploids (Palaeopolyploids), and a few auto-/auto-allopolyploids. Existence of a very high ploidy levels in the South Indian region may be suggestive that cytological evolution in the group could have been very dynamic in this tropical belt. Of the various classifications of the Pteridophytes, the Smith et al's, based on morphological as well as molecular evidences is the most tenable and in conformity with the cytological data. Their splitting of the large Pteridaceae into different coherent groups and division of the Aspidiaceae appears appropriate, but creation of many splinter families, superfluous.

Keywords: Pteridophytes, cytology, systematics, phylogeny, evolution.

INTRODUCTION

Cytology has been well recognized as a potential supplementary tool helpful for tackling taxonomic problems and for elucidation of systematic relationships, phylogeny and evolution of related plant groups. There are unequivocal examples of chromosome data having played decisive role in dealing with such issues in different plant groups. Extensive study on the cytology of the ferns and fern allies, carried out since 1950s in the Department of Botany, University of Kerala, has yielded a rich store of chromosome data on the group indigenous to South India. The study was initially

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on the eusporangiate group by C. A. Ninan, the major findings of which published in a series of papers (Abraham & Ninan 1954, 1958, Ninan 1955, 1956 a–e, 1958 a–c). This was followed by study in a very large number of leptosporangiate ferns by C. A. Ninan and P. M. Mathew, and the results on 100 species published as a monograph (Abraham et al. 1962). The study of the eusporangiate ferns covered species of *Psilotum, Lycopodium, Selaginella, Isoetes, Ophioglossum* etc. and those of leptosporangiate ferns comprised species belonging to a number of families *sensu* Copeland (1947) such as Schizaeaceae (*Schizaea, Lygodium, Anemia),* Gleicheniaceae *(Gleichinia, Dicranopteris*), Pteridaceae (*Microlepis, Sphenomeris, Schizoloma, Pteridium, Pteris, Acrostichum, Cheilanthes, Doryopteris, Hemionitis, Pitygramma, Adiantum*), Cyatheaceae (*Cyathea*), Aspidiaceae (*Polystichum, Cyrtomium, Rumohra, Bolbitis, Egenolphia, Doryopteris, Dryopteris, Tectaria, Quercifilix, Thelypteris, Cyclosorus, Ampelopteris, Athyrium, Anisocampium, Diplazium*), Blechnaceae (*Lomaria, Blechnum, Stenoclaena*), Aspleniaceae (*Asplenium*), Polypodiaceae (*Platycerium, Pyrrosia, Microsorum, Drynaria, Crypsinus*) and Marsileaceae (*Regnellidium, Marsilea*). This paper provides a highlight of the major findings emerged from the above chromosome study in the pteridophytes, both eusporangiate and leptosporangiate discussed in relation to the systematics, phylogeny and evolution of the group.

Cytology and evolution

For meaningful discussion of cytological evolution, it is essential to make use of valid data of relevant cytological parameters such as (1) chromosome behaviour at meiosis, (2) the number and morphology of somatic chromosomes, (3) the basic chromosome constitution and (4) the modes and magnitude of factors which bring about numerical and structural changes in chromosomes at different taxonomic levels. The factor of basic chromosome number at various taxonomic levels has played a vital role in shaping the prevailing concept of evolution in plants, and this has formed one of the major criteria considered for formulating phylogenetic speculations. It also serves as a reliable and stable marker of the direction of evolution. Diminution of basic chromosome numbers, rather than their increase, often occurs playing significant role in chromosome evolution (Stebbins 1971). Findings emerged from the cytological study accomplished here over the years in different plant groups have yielded convincing evidence favouring the concept of diminishing trend of the basic chromosome constitution. The phenomenon of polyploidy, aneuploidy and secondary hybrid polyploidy have been known to have played a major role in the derivation and evolution of high basic chromosome numbers in many fern families. The high chromosome numbers in some eusporangiate genera, particularly *Ophioglossum* (Ninan 1956a) is an example of the basic chromosome numbers soaring up to profound heights. Such highly evolved levels of chromosome constitutions are referred to as ‘palaeopolyploids’ (Ehrendorfer 1970). A perusal of the chromosome data of the ferns show that very high numbers occur in many ferns, particularly in the eusporangiate group, which is exceptionally so in the Ophioglossaceae (Ninan 1958c), in which the species studied constitute a polyploid series on x = 120, (n = 240, 480, 630). Other relatively high haploid numbers in the family are, n = 94 in *Helminthostachys* and n = 45, 90 in *Botrychium.* In another eusporangiate genus, *Lycopodium*, the

observed haploid numbers show that the genus is cytologically very dynamic and heterogeneous (Ninan 1958b). The distribution of haploid numbers in the entire eusporangiate group shows a wide spectrum ranging from a very low of n = 8, 9, 10 as in *Selanginella*, n = 11 in *Osmunda* and *Azola* and to a very high of n = 630 in *Ophioglossum*. Many ferns in the leptosporangiate group show high-medium, medium and low haploid numbers ranging from n = 19/20 as in *Regnellidium/Marselia*, to n = 78/80 as in *Marattia/Angiopteris*. The high medium haploid numbers are frequent in families like Gleicheniaceae (n = 33–77), Polypodiaceae (n = 33–37), Thelypteridaceae (n = 30–36), Aspidiaceae (n = 40–42) etc. and medium haploid numbers common in the rest of the fern families which are around n = 28–30.

Stebbins (1971) and Grant (1982) have held that relatively high basic numbers so dominant among certain angiosperm families and pteridophytes, particularly those above x = 9 or 10, are derived ones from lower ancestral ones by various processes like polyploidy, aneuploidy and secondary hybrid polyploidy. On such a consideration, the very high basic numbers in most of the pteridophytes must be the product of progressive polyploidy and aneuploidy at different ploidy levels. Abraham et al. (1962) have contended that the present-day high-chromosome numbered taxa of the group could have originated and evolved from two groups of ancestral basic numbers such as (1) x = 9, 10, 11 and (2) x = 13, 15. The ancestral x = 10 is postulated to have originated from a still lower primary ancestral n = 5 state, and that the x = 9 and 11 derivatives from x = 10 by descending and ascending aneuploidy respectively, while the x = 13 and 15 derived from an ancestral x = 14 by aneuploidy which in turn derived from a primary ancestral n = 7. The very high chromosome-numbered x = 120 species of *Ophioglossum* and those of *Botrychium* (n = 45, 90) and *Helminthostachys* (n = 94) could be derivations from an ancestral x = 15 by progressive polyploidy. The lower haploid numbers as in Gleicheniaceae, Blechnaceae, Polypodiaceae etc. could be derivations from ancestral x = 9 by polyploidy and aneuploidy at different levels, while those in Marsileaceae, Aspidiaceae, Adiantaceae etc. from an ancestral x = 10, the Osmundaceae from x = 11 and Dicksoniaceae descended from x = 13.

Polyploidy

The phenomenon of polyploidy is the most widespread and distinctive cytogenetic process which has greatly influenced evolution (Stebbins 1971). This is owing to their ability to breakdown the reproductive barriers and also to their higher tolerance to adverse environmental conditions. They are endowed with better genetic makeup which enhances their adaptive and invasive potential. The available chromosome data of the pteridophytes, and their distribution in a wide range of habitats, show that great many of them are polyploids. There is some discord concerning the causative mechanism of the phenomenon of polyploidy. There is a strong view that polyploid plants are more tolerent to extreme climatic conditions than their diploid conspecifics (Love & Love 1949). But many others are opposed to this view. Stebbins (1950), whose contention is that polyploidy is associated with habit of the plant such that increased frequency of polyploids occur in

perennial herbs, while the annuals and woody plants are predominantly diploids. But, the renowned pteridologist, Manton (1950), based on her comparative survey and study of fern floras of different world regions, has held that polyploidy in ferns is not associated with environmental effect. Her view is that evolution proceeds faster in tropics than in temperate latitudes and that the well-watered equatorial regions have always been the most powerful centres of evolutionary versatility. Mehra (1961) who made extensive study of the fern floras of Eastern and Western Himalayas, and also Abraham et al. (1962) who made prolific study of the South Indian counterparts, are also of the view held by Manton. The results of the South Indian ferns appear suggestive that there is a tendency to replace the low chromosome-numbered taxa by high-numbered descendants. The findings of the highest ever ploidy levels in species of *Ophioglossum* from South India may be suggestive that cytological evolution of ferns could have been dynamic as well as rampant in the tropical belt. The view held by Abraham et al. (1962) is relevant in this connection that in regions like South India, which are geologically very old and which have longest escaped great geological uphaevals have produced opportunities for the existence of a continued vegetation cover for a pretty long span of evolutionary time, high levels of ploidy may well be expected. In great many of the polyploid taxa of both eusporangiate and leptosporangiate groups studied from South India, meiosis was fairly normal characterized by regular bivalent formation and normal anaphase separation resulting in high spore fertility. All such polyploid taxa could be reckoned as allopolyploids. However, certain polyploid species of *Lycopodium* and also a few leptosporangiate taxa displayed irregular meiosis with abnormal anaphase separation due to formation of varying frequencies of multivalents and univalents. These could be autopolyploids/autoallopolyploids.

Systematic consideration

The pteridophytes, generally grouped as ferns and fern allies, are the pioneer vascular plants originated in the Cambrian period (Madhusoodhanan 2015). They are primitive vascular plants devoid of cambium barring a few exceptions (*Selaginella, Marsilea* etc.), and they are generally grouped under two discrete categories, the ferns and fern allies. The fern allies are considered under the classes, Psilopsida, Lycopsida and Sphenopsida. The Psilopsida has only two extant genera, *Psilotum* and *Tmesipteris*, and the other fern allies are club mosses (*Lycopodium*) and little club mosses (*Selaginella*). The genus *Lycopodium* has been subdivided into three (*Hupersia, Lycopodiella and Lycopodium*). The recent classification of Smith et al. (2006) keeps all names at family and ordinal ranks as in the previous classifications, which were generated based on morphological data. The new classification of Smith et al. (2006) is based on a variety of morphological as well as molecular evidences. They have recognized four classes, 11 orders and 37 families. Bower (1928) was the first to attempt a phylogenetic classification of the ferns, and this was followed by a few others as that of Copeland (1947), Holtum (1949), Pichi-Sermolli (1959), Pryer et al. (2004) and Smith et al. (2006) of which the Smith et al.'s is the most comprehensive and recent. Abraham et al. (1962) who examined the distribution of different monoploid chromosome numbers in genera of various families *sensu* Copeland (1947) have found that the arrangement of genera and families in this treatment to be broadly

in conformity with cytological results excepting two prominent discontinuities concerning the Pteridaceae. The chromosome data on the South Indian group of ferns show that *Pteris* and subsequent genera of Copeland's Pteridaceae form a coherent group with monoploid numbers around n = 29/30, while the other genera preceding *Pteris* reveals disharmonious relationship characterized by uneven numbers like n = 34, 41, 44, 47, 50 and 52. Manton & Sledge (1954), viewing this discontinuity, have suggested separation of *Pteris* and subsequent genera of Copeland's Pteridaceae as a separate family. However, Smith et al. (2006) have consolidated the entire group in their Pteridaceae. Mehra (1961) also contributed favourably to this treatment. Abraham et al. (1962) were of the view that, while it is feasible to separate *Pteris* and subsequent genera into distinct family, creation of many splinter families is unwarranted and superfluous, and the present authors are also inclined for a treatment of the entire assemblage of *Pteris* and allies under the broad family Pteridaceae as done by Smith et al. (2006).

The Schizaeaceae contains three distinct cytological elements such as *Lygodium* (n = 30/29), *Anemia* (n = 28) and *Schizaea* (n = 77) representing paths of three separate evolutionary lines such as (1) the genera with n = 29/30, (2) Marsileaceae (n = 19/20), and (3) Parkireaceae (n = 38, 40, 76, 77, 78). Abraham et al. (1962) have held the view that this ancient family could be ancestral to the three lines of evolution referred to above. However, they have opted for a view of preferring Copeland's treatment of the Parkireaceae as a separate family. A prominent cytological discontinuity may be noticed in Copeland's Aspidiaceae, in which great bulk of the genera possess n = 41 along with n = 40, while many others possessing clearly deviant numbers such as n = 36 (*Cyclosorus*, *Leptogramma*, *Ampelopteris* etc.) and several aneuploid derivations of n = 36 such as n = 35, 34, 32, 31, 30) as in *Thelypteris*. This appears to warrant splitting of Copeland's Aspideaceae into at least two discrete groups. Smith et al. (2006) treatment appears to assume importance here. They have dispensed with the family epithet of Aspideaceae, and instead added two new families, the Woodsiaceae and Thelypteridaceae, the former holding mostly Copeland's genera possessing n = 41/40 and the latter with n = 36 and its derivations. In the new family of Smith et al. (2006), the Thelypteridaceae, having a heterogeneous cytological framework, appears to have enjoyed great deal of step-wise descending aneuploid evolution from n = 36. The phylogenetic significance of the chromosome constitution in the genus *Thelypteris* was emphasized earlier by Abraham et al. (1962). Although Copeland (1947) has retained this heterogeneous genus in his Aspidaceae, Holtum (1949) and Pichi-Sermmolli (1958) had both separated this from Aspidiaceae, keeping it in their monotypic Thelypteridaceae, and this treatment has been justified in the Smith et al.'s (2006) modern classification. Abraham et al. (1962) have suggested a common origin of Cyatheaceae and Thelypteridaceae, together with Blechnaceae, Polypodiaceae and Aspleniaceae and with certain other ancestral types. In their scheme of cytological evolution of ferns, they have posed a possibility of n = 36 being a derivation from an ancestral x = 9, a view the present authors also favour. There is however, need for exercising caution in applying cytological data for deducing phylogenetic relationships blindly, since the same number or numbers which appear to be closely related may occur in completely unrelated taxa as well.

REFERENCES

ABRAHAM A & NINAN C A 1954 Chromosomes of *Ophioglossum reticulatum* L *Curr Sci* **23** 213–214

ABRAHAM A & NINAN C A 1958 Cytology of *Isoetes Curr Sci* **27** 60–61

ABRAHAM A, NINAN C A & MATHEW P M 1962 Studies on the cytology and phylogeny of the Pteridophytes VII Observations on 100 species of South Indian ferns *J Indian Bot Soc* **41** 339–421

BOWER F O 1928 *The Ferns* Vol III Cambridge University Press London

COPELAND E B 1947 *Genera Filicum* Chronica Botanica Co Waltham Mass

EHRENDORFER F 1970 Evolution: pathways and patterns Evolutionary patterns and strategies in seed plants *Taxon* **19** 185–195

GRANT V 1982 Periodities in the chromosome number of the angiosperms *Bot Gaz* **143** 379–389

HOLTUM R E 1949 The classification of ferns *Biol Rev* **24** 267–296

LOVE A & LOVE D 1949 The geobotanical significance of polyploidy I Polyploidy and latitude *Portug Aca Biol* R B Goldschmidt Vol 272–352

MADHUSOODHANAN P V 2015 *Handbook of Ferns and Fern allies of Kerala* Malabar Botanical Garden Kozhikode

MANTON I 1950 *Problems of Cytology and Evolution in the Pteridophytes* Cambridge University Press London

MANTON I & SLEDGE W A 1954 Observations on the cytology and taxonomy of the Pteridophyte Flora of Ceylon *Phil Trans* **238 B** 127–158

MEHRA P N 1961 Cytological evolution of ferns with particular reference to Himalayan ferns 48th Indian Science Congress Abstract

NINAN C A 1955 Cytology of *Equisetum debile J Indian Bot Soc* **34** 112–114

NINAN C A 1956a Cytology of Ophioglossaceae *Curr Sci* **25** 161–162

NINAN C A 1956b Studies on the cytology and phylogeny of the Pteridophytes I Observations on the Marattiaceae *J Indian Bot Soc* **35** 233–239

NINAN C A 1956c Cytology of *Psilotum nudum La Cellule* **57** 307–318

NINAN C A 1956d Studies on the cytology and phylogeny of the Pteridophytes III Observations on *Osmunda regalis* L *J Indian Bot Soc* **35** 248–251

NINAN C A 1956e Studies on the cytology and phylogeny of the Pteridophytes IV Systematic position of *Ceratopteris thalictroides* L *J Indian Bot Soc* **35** 93–103

NINAN C A 1958a Studies on the cytology and phylogeny of the Pteridophytes *J Indian Bot Soc* **37** 93–103

NINAN C A 1958b Studies on the cytology and phylogeny of the Pteridophytes II Observations on the genus *Lycopodium* *Proc Nat Ins Sci India* **24 B** 54–66

NINAN C A 1958c Studies on the cytology and phylogeny of the Pteridophytes VI Observations on the Ophiglossaceae *Cytologia* **23** 291–316

PICHI-SERMMOLLI R E G 1958 The higher taxa of the Pteridophytes and their classification *In* Hedberg O (ed) *Systematic of today Upsala Univ Arsskr* pp 70–90

PICHI-SERMMOLLI R E G 1959 *Pteridophyta In* Turril W B (ed) *Vistas in Botany* Pergaman Press Ltd London pp 421–493

PRYER K M, SCHUETTPELS E, WOLF P G, SCHNEIDER H, SMITH A R & CRANFILL R 2004 Phylogeny and evolution of ferns with focus on the early leprosporangiate divergence *Amer J Bot* **91** 1582–1598

SMITH A R, KATHLEEN M, PRYER K M, SCHUETTPELZ E, KORRAL P, SCHNEIDER H & WOLF P G 2006 A classification of extant ferns *Taxon* **55** 705–731

STEBBINS G L 1950 *Variation and Evolution in Plants* Columbia Univ Press New York

STEBBINS G L 1971 *Chromosomal Evolution in Higher Plants* Edward Arnold London

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GENETIC DIVERGENCE ANALYSIS IN *BACOPA MONNIERI* OCCURRING IN KERALA

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SUMMARY

Bacopa monnieri (L.) Wettst. (Plantaginaceae) is a pantropical, perennial, herbaceous species. The profound medicinal utility and consequent great demand in the pharmaceutical industry has resulted in its indiscriminate collection from the wild in Kerala and consequent gene erosion. Multivariate analysis has been carried out in this species based on eight agrobotanic characters in 60 accessions collected from all over Kerala. On the basis of the results obtained during analysis, the accessions were clustered under 12 groups. The clusters IV and X displayed the highest intercluster distance (3378.6) and the lowest (180) was between clusters IV and IX. The results of the study is useful for assessing the extent of genetic variability existing in the gene pool and also for selecting appropriate genotypes as parents for hybridization programmes aimed at exploiting hybrid vigour.

Keywords: *Bacopa monnieri*, Mahalanobis D^2 statistics, genetic divergence, brahmi.

INTRODUCTION

Bacopa monnieri (L.) Wettst. (Plantaginaceae) is a perennial herbaceous species distributed pantropically, and known as 'Brahmi' or 'Neerbrahmi' in Malayalam. This species is widely used in traditional systems of medicine such as Ayurveda, Siddha and Unani, and also in Homoeopathy (Singh & Singh 1980), for the treatment and management of a line of mental defects and as an energizer for the nervous system and heart (Mukherjee & Dey 1966). It also possesses an array of therapeutic properties such as antioxidant (Tripathi et al. 1996), anti-cancer (Elangovan et al. 1995), immuno-modulatory (Dahanukar & Thatte 1997), anti-stress (Chowdhuri et al. 2002) and adaptogenic activities (Rai et al. 2003). The principal active factors in *Bacopa* are two steroidal saponins, bacosides A and B known as 'memory chemicals', that help repair damaged neurons by enhancing proteins involved in the regeneration of neural-cell synapses (Rastogi et al. 1994). The profound medicinal value and consequent high demand in the pharmaceutical industry has resulted in its over exploitation from the wild leading to extinction of its genotypes. D^2 analysis is employed mainly for grouping the genotypes of a species based on their genetic similarity. Hence, the study is useful for assessing genetic diversity

in a gene pool of a crop and to understand their genetic interrelationships. In this context, it has been pointed out that for developing better varieties, it is desirable to classify the germplasm on the basis of genetic similarity and to make crosses between genotypes belonging to divergent groups which share maximum diversity. Maximum amount of heterosis of recombination can be expected from crosses involving the parents belonging to the most divergent clusters (Narasimhayya & Rao 1974). In this context, D^2 analysis has been undertaken in 60 accessions of the species based on eight agrobotanic characters for assessing genetic diversity in the gene pool and to classify the accessions based on their genetic similarity. The study is particularly useful for selecting appropriate parent genotypes for hybridization programmes for exploiting hybrid vigour and also for understanding genetic interrelationships between the accessions.

MATERIALS AND METHODS

Field surveys were conducted throughout Kerala and 60 accessions of *B. monnieri* were located and collected from different parts of the State (Table 1). Planting materials of the accessions collected during the field surveys were

TABLE 1: Details of collection localities of the 60 accessions of *B. monnieri*.

Acc. No.	Place of collection	District	Date of collection	Latitude	Longitude	Altitude (mamsl)
Bm 1	Viraly	Thiruvananthapuram	25-12-2011	8°18'N	77°05'E	14
Bm 2	Kadakkavoor	Thiruvananthapuram	27-12-2011	8°40'N	76°45'E	14
Bm 3	Karikkakom	Thiruvananthapuram	30-12-2011	8°30'N	76°54'E	15
Bm 4	Aakkulam	Thiruvananthapuram	30-12-2011	8°31'N	76°54'E	16
Bm 5	Vettamukku	Kollam	28-12-2011	9°01'N	76°33'E	17
Bm 6	Panayam	Kollam	07-04-2012	8°57'N	76°37'E	18
Bm 7	Kochuplamoodu	Kollam	07-04-2012	9°00'N	76°39'E	9
Bm 8	Dalavapuram	Kollam	28-12-2011	8°56'N	76°33'E	12
Bm 9	Padukkottukal	Pathanamthitta	02-06-2012	9°11'N	76°43'E	34
Bm 10	Thakazhi	Alappuzha	28-12-2011	9°22'N	76°24'E	7
Bm 11	Pattaniyidukku	Alappuzha	28-12-2011	9°30'N	76°19'E	10
Bm 12	Pathirapally	Alappuzha	28-12-2011	9°32'N	76°19'E	9
Bm 13	Vattayil	Alappuzha	28-12-2011	9°28'N	76°20'E	10
Bm 14	Nerekadavu	Kottayam	30-05-2012	9°38'N	76°30'E	48
Bm 15	Koduppadom	Kottayam	30-05-2012	9°47'N	76°23'E	8
Bm 16	Kumarakom	Kottayam	30-05-2012	9°36'N	76°25'E	9
Bm 17	Vazhikkadavu	Kottayam	30-05-2012	9°40'N	76°53'E	972
Bm 18	Kumbalam	Ernakulam	14-01-2012	9°54'N	76°18'E	10
Bm 19	Thripunithura	Ernakulam	13-01-2012	9°57'N	76°20'E	11
Bm 20	Charthedam	Ernakulam	13-01-2012	10°11'N	76°13'E	12

(Continued)

TABLE 1 : (Concluded)

Bm 21	Near Aarch dam	Idukki	31-05-2012	9°50'N	76°58'E	562
Bm 22	Vellayamkudi	Idukki	31-05-2012	9°46'N	77°05'E	907
Bm 23	Munnar	Idukki	27-10-2012	10°05'N	77°03'E	1462
Bm 24	Chellarkovil	Idukki	31-05-2012	9°40'N	77°10'E	1095
Bm 25	Kumily	Idukki	31-05-2012	9°37'N	77°09'E	892
Bm 26	Chelakkara	Thrissur	20-05-2011	10°40'N	76°21'E	54
Bm 27	Karuppadannapalam	Thrissur	29-12-2011	10°15'N	76°12'E	12
Bm 28	SNpuram beach	Thrissur	29-12-2011	10°16'N	76°10'E	9
Bm 29	Undaikadavu	Thrissur	14-01-2012	10°13'N	76°10'E	13
Bm 30	Kodakara	Thrissur	29-12-2011	10°22'N	76°18'E	17
Bm 31	Pattambi	Palakkad	27-04-2012	10°48'N	76°11'E	35
Bm 32	Kambram	Palakkad	28-04-2012	10°53'N	76°11'E	97
Bm 33	Pulakad	Palakkad	28-04-2012	10°47'N	76°39'E	78
Bm 34	Varakulam	Malappuram	28-04-2012	10°20'N	76°17'E	36
Bm 35	Paravanna	Malappuram	28-04-2012	10°54'N	75°53'E	11
Bm 36	Kannoorkettu	Kozhikode	12-04-2012	11°27'N	75°46'E	15
Bm 37	Musankandi	Kozhikode	12-04-2012	11°21'N	75°43'E	10
Bm 38	Kakkoor	Kozhikode	12-04-2012	11°22'N	75°49'E	20
Bm 39	Pathinonnammile	Kozhikode	12-04-2012	11°22'N	75°50'E	17
Bm 40	Chelapram	Kozhikode	12-04-2012	11°20'N	75°47'E	10
Bm 41	Manicherry hills	Kozhikode	12-04-2012	11°30'N	75°51'E	478
Bm 42	Payyannur	Kannur	13-05-2012	12°05'N	75°11'E	10
Bm 43	Muttukatti	Kannur	13-05-2012	12°00'N	75°15'E	12
Bm 44	Kakkad	Kannur	13-05-2012	11°53'N	75°23'E	20
Bm 45	Andalloorkavu	Kannur	13-05-2012	11°47'N	75°28'E	11
Bm 46	Sasimala	Wayanad	13-04-2012	11°48'N	76°11'E	761
Bm 47	Channothukolli	Wayanad	13-04-2012	11°49'N	76°12'E	728
Bm 48	Seethamount	Wayanad	13-04-2012	11°50'N	76°12'E	752
Bm 49	Nadavayal	Wayanad	13-04-2012	11°44'N	76°07'E	797
Bm 50	Meppadi	Wayanad	13-04-2012	11°55'N	76°13'E	870
Bm 51	Padannakkadu	Kasaragode	12-05-2012	12°15'N	75°06'E	13
Bm 52	Kumbla	Kasaragode	12-05-2012	12°35'N	74°56'E	9
Bm 53	Edayilekkadu	Kasaragode	12-05-2012	12°08'N	75°09'E	10
Bm 54	Madakkara	Kasaragode	12-05-2012	12°13'N	75°07'E	14
Bm 55	Manakkakadavu	Kottayam	30-05-2012	9°48'N	76°24'E	8
Bm 56	Channikkadavu	Alappuzha	28-12-2011	9°51'N	76°18'E	6
Bm 57	Pedikkattuthuruthu	Ernakulam	13-01-2012	10°58'N	76°20'E	8
Bm 58	Cheriyathuruthu	Ernakulam	13-01-2012	10°03'N	76°14'E	10
Bm 59	Chungam	Alappuzha	28-12-2011	9°29'N	76°20'E	8
Bm 60	Kinanoor	Kasaragode	12-05-2012	12°16'N	75°10'E	15

planted in a Nursery at Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram and on establishment, the accessions were transplanted to the Field Gene Bank (FGB) at the institute. In the FGB, the accessions were maintained and propagated vegetatively for four successive generations. Five noded stem cuttings of the fourth generation vegetatively propagated plants of the accessions collected from the FGB were planted in an Experimental Plot (EP) in three replications and in the EP, all the 60 accessions were grown under uniform environmental conditions.

Data on the eight agrobotanic characters such as (1) internode length (2) leaf area (3) leaf thickness (4) stem thickness (5) biomass yield (6) percentage content of bacoside A (7) bacopaside I and (8) total bacosides of each accession were used for the analysis. Five observations were scored for each character from each replication. Simple random sampling was followed for collecting the data on morphological traits. Biomass yield was calculated as percentage dry weight. Percentage contents of bacoside A, bacopaside I and total bacosides were estimated by HPTLC densitometric method. The data collected on the 60 accessions relating to eight traits were subjected to multivariate analysis using Mahalanobis (1936) D^2 Statistics.

OBSERVATIONS

In the present study, 60 accessions of *B. monnieri* were clustered under 12 groups, based on the relative magnitude of D^2 values, and composition of the groups is given in Table 2. Among

TABLE 2: Composition of the clusters of the accessions of *B. monnieri* based on D^2 analysis.

Cluster No.	No. of accessions	Accessions
I	7	Bm 10, Bm 26, Bm 28, Bm 33, Bm 34, Bm 51, Bm 5
II	17	Bm 2, Bm 8, Bm 15, Bm 17, Bm 18, Bm 21, Bm 22, Bm 23, Bm 25, Bm 38, Bm 39, Bm 40, Bm 43, Bm 44, Bm 45, Bm 50, Bm 60
III	19	Bm 1, Bm 5, Bm 6, Bm 7, Bm 12, Bm 13, Bm 16, Bm 19, Bm 24, Bm 29, Bm 30, Bm 31, Bm 36, Bm 37, Bm 41, Bm 56, Bm 57, Bm 58, Bm 59
IV	5	Bm 32, Bm 47, Bm 48, Bm 49, Bm 53
V	3	Bm 27, Bm 35, Bm 9
VI	2	Bm 20, Bm 42
VII	2	Bm 11, Bm 52
VIII	1	Bm 3
IX	1	Bm 4
X	1	Bm 14
XI	1	Bm 46
XII	1	Bm 54

the 12 clusters, cluster III is the largest cluster having 19 accessions, followed by the cluster II, possessing 17 accessions, cluster IV (5 nos.), cluster V (3 nos.), cluster VI and cluster VII (2 nos. each) and the clusters VIII, IX, X, XI and XII were single member clusters. Inter- and intracluster distances of the 12 groups are furnished in Table 3. Highest intercluster distance

TABLE 3: Inter- and intracluster distance of the clusters of the accessions of *B. monnieri*.

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I	**109.2**											
II	268.9	**151.3**										
III	747.5	490.5	**109.5**									
IV	227.8	514.6	1478.2	**93.3**								
V	258.7	264.6	263.7	826	**53.8**							
VI	660.2	301.8	300.4	1291.3	395	**88.5**						
VII	1896.2	1106.9	702.4	2668.1	1024.8	572.8	**154.11**					
VIII	243.8	378.3	891.2	332.8	356	723	1990	**0**				
IX	615.6	475.9	226.5	1188.6	216.5	427	1059.5	592.1	**0**			
X	2325.7	1481	587	3378.6	1856	770.5	638	2423.9	999.3	**0**		
XI	301.2	596.7	1590.8	180	944.5	1170	2984	500.5	1447	3281	**0**	
XII	248.5	328.7	291	679.2	225.9	443.5	1754.2	260	2524.1	1415	822.1	**0**

TABLE 4: Cluster means of the eight agrobotanic characters in respect of the clusters of the accessions of *B. monnieri*.

Sl. No.	Characters	Clusters											
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
1	Internode length (cm)	1.581	1.508	1.410	1.884	1.663	1.929	1.439	2.656	1.618	1.248	1.514	1.446
2	Leaf area (mm^2)	1112.9	103.4	81.6	126.2	94.9	88.9	69.4	110.8	87.4	56.6	62.4	75.2
3	Leaf thickness (mm)	0.053	0.041	0.0414	0.0408	0.042	0.038	0.041	0.038	0.036	0.044	0.032	0.04
4	Stem thickness (mm)	0.169	0.165	0.155	0.173	0.155	0.184	0.159	0.152	0.14	0.146	0.182	0.16
5	Biomass yield (%)	7.603	8.459	8.647	7.830	7.196	9.695	8.868	7.796	7.784	9.71	9.338	8.048
6	Bacoside A (%)	2.37	3.49	2.46	2.63	2.58	3.91	5.47	2.45	2.23	3.57	2.74	1.31
7	Bacopaside I (%)	0.82	0.76	0.82	0.68	0.95	0.58	1.27	0.69	0.83	0.58	0.31	0.61
8	Total bacosides (%)	3.19	4.25	3.27	3.38	3.81	4.49	6.74	3.14	3.05	4.14	3.06	1.92

(3378.6) was observed between the clusters IV and X and the lowest (180) between the clusters IV and IX. Cluster VII registered maximum intracluster distance (154.11) and the lowest (58.5) by the cluster VI. In the present study, the clusters possessed high intercluster distance and low intracluster distance, and this indicates good accuracy of the grouping. The cluster means of the eight quantitative characters are given in Table 4.

DISCUSSION

The divergence between the clusters is reflected in the number of cluster means having significant variation with respect to the eight agrobotanic characters. Since the clusters, IV and X are having the highest intercluster distance, they possess the most divergent genotypes of the species. Hence, ideally, the parents for hybridization programmes may be selected from these two clusters for exploiting hybrid vigour.

Mathur et al. (2003) carried out D^2 analysis on 27 accessions of *B. monnieri* collected from ecologically divergent locations in India. Their study grouped the 27 accessions into seven groups, based on principal component analysis and revealed high genetic divergence in the sample gene pool. The present study on a sample gene pool of *B. monnieri* from Kerala also reveals the existence of notable genetic divergence of the species in the State of Kerala and also points to the fact that many of the accessions (Bm 3, Bm 4, Bm 14, Bm 46 and Bm 54) are unique in their genetic makeup, which demand special attention for ensuring their conservation.

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REFERENCES

CHOWDHURI D K, PARMAR D, KAKKAR P, SHUKLA R, SETH P K & SRIMAL R C 2002 Antistress effects of bacosides of *Bacopa monnieri*: modulation of Hsp70 expression superoxide dismutase and cytochrome P450 activity in rat brain *Phytother Res* **16** 639–645

DAHANUKAR S A & THATTE U M 1997 Current status of Ayurveda in phytomedicine *Phytomedicine* **4** 359–368

ELANGOVAN V, GOVINDASAMY S, RAMAMOORTHY N & BALASUBRAMANIAN K 1995 In vitro studies on the anticancer activity of *Bacopa monniera Fitoterapia* **66** 211–215

MAHALANOBIS P C 1936 On the generalized distance in statistics *Proc Natl Inst Sci* **2** 49–55

MATHUR S, SHARMA S, GUPTA M M & KUMAR S 2003 Evaluation of an Indian germplasm collection of the medicinal plant *Bacopa monnieri* (L) Pennell by use of multivariate approaches *Euphytica* **133** 255–265

MUKHERJEE G D & DEY C D 1966 Clinical trial on Brahmi *Indian J Exp Med Sci* **10** 5

NARASIMHAYYA G & RAO V C 1974 Classification of the varieties of cigarette tobacco using D^2 statistics *Indian J Agric Sci* **44** 660–664

RAI D, BHATIA G, PALIT G, PAL R, SINGH S & SINGH H K 2003 Adaptogenic effect of *Bacopa monniera* (Brahmi) *Pharmacol Biochem Be* **75** 823–830

RASTOGI S, PAL R & KULSHRESHTHA D K 1994 Bacoside A (3): A triterpenoid saponin from *Bacopa monniera* *Phytochemistry* **36** 133–137

SINGH R H & SINGH L 1980 Studies on the anti-anxiety effect of the medyha rasayana drug Brahmi (*Bacopa monniera* Wettst) *Res Ayur Siddha* **1** 133–148

TRIPATHI Y B, CHAURASIA S, TRIPATHI E, UPADHYAY A & DUBEY G P 1996 *Bacopa monniera* Linn as an antioxidant: mechanism of action *Ind J Exp Biol* **34** 523–526

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CYTOLOGY IN THE SYSTEMATICS, PHYLOGENY AND EVOLUTION IN PLANTS II. GYMNOSPERMS

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SUMMARY

The major findings emerged from the cytological study of gymnospermous taxa (Cycads, Conifers, *Gnetum* and *Ephedra*) made earlier since 1960 in the Kerala University are highlighted and reviewed in relation to their systematics, phylogeny and evolution. The basic chromosome numbers in the genera of cycads studied are, x = 11 (*Cycas*), x = 8 (*Zamia*), x = 9 (*Dioon* and *Encephalartos*). The karyotypes of species of *Cycas* are marginally asymmetrical (2A), and those of the others more asymmetrical (2B). The basic numbers x = 8, 9, and 11 in the cycads are considered secondary ones originated from an ancestral x = 10, evolved by descending (x = 9, 8) and ascending (x = 11) aneuploidy. The chromosome numerical and karyomorphological evidences appear to corroborate with the taxonomic treatment of Bierhorst's placing the *Cycas* and *Zamia* groups of genera separately in different families, the Cycadaceae and Zamiaceae. The Coniferales are cytologically a heterogeneous group with an array of basic chromosome numbers (x = 9–19) of which x = 10 abounding in many genera, and it is suggested that the entire lot of basic chromosome numbers may have originated from x = 10 by stepwise ascending and descending aneuploidy, which in turn evolved by secondary hybrid polyploidy/polyploidy from an ancestral x = 5 state. Of the prevailing various taxonomic groupings of the Coniferales, Li's treatment holding the entire conifer genera in 4 families (Araucariaceae, Podocarpaceae, Cephalotaxaceae and Taxaceae) in the order Coniferales appears consistent with the cytological data. The basic chromosome numbers of *Gnetum* and *Ephedra* studied are x = 11 (*Gnetum*) and x = 7/6 (*Ephedra*). The karyotype of *Gnetum* is moderately assymetrical (2B) and of *Ephedra* more specialized (2C/4A). The x = 11 in *Gnetum* is suggested to have originated by secondary hybrid polyploidy from ancestral taxa with n = 6 and 5. The sharp distinction of the chromosome architecture between *Gnetum* and *Ephedra* strongly supports their placement separately in different orders, the Gnetales and Ephedrales.

Keywords: Cytology, systematics, evolution, Cycadales, Coniferales, Genetales.

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INTRODUCTION

The gymnosperms are a primitive, but a fascinating group of seed plants constituting the dominant element of the forests in the temperate zones of the earth. The extant gymnosperms are considered representatives of an ancient group of plants, first recorded as fossils in the Upper Devonian (Biswas & Joshi 1997). They are a very important group being the source of valuable timber, wood pulp, resins, medicines etc. and also many cultivated as ornamental plants. The group is beset with much confusion concerning their systematic relationships, phylogeny and evolution. Although reliable chromosome information is important for dealing with systematic relationships, phylogeny and evolution of related plant groups, cytological reports on the group are sparse. Available reports are mostly on alien taxa, while Indian reports confined to a few genera produced from the Punjab University in the North, and from the school of cytology, Kerala University, Thiruvananthapuram in the South. Khoshoo (1961) has compiled a chromosome inventory recording the chromosome numbers of the then known gymnosperms, listed in 13 families under four orders, the Cycadales, Ginkgoales, Coniferales and Ephedrales. The present paper is a review highlighting the major cytological findings emerged from the study carried out in the Thiruvananthapuram school earlier, and it covers some genera of the Cycadaceae (*Cycas, Encephalartos, Dioon* and *Zamia*), Coniferales (*Araucaria, Podocarpus, Cryptomeria, Cupressus* and *Thuja*) and Gnetales (*Gnetum* and *Ephedra*), considered in relation to their systematics, phylogeny and evolution.

Cycadales

The order includes both living and extinct forms considered to have originated in the Upper Triassic period of the early Mesozoic era (Vashista 1995), and flourished during the Jurassic and Cretaceous periods. The living forms comprise only 10 genera with around 115 species, all dioecious and restricted to Australia, South Africa, Central America and eastern Asia including India. The order has been variously classified by many. Chromosome numbers of species of all the genera are known, although in very limited numbers. The somatic chromosome numbers and basic numbers earlier studied in the Kerala University are, 2n = 22 on x = 11 in all the species of *Cycas* such as *C. pectinata* Grib. (Abraham & Mathew 1962), *C beddomii* Dyer, *C. circinalis* L. *C rumphii* Miq. and *C. revoluta* (Mathew unpublished); 2n = 16 on x = 8 in *Zamia pumila* (Abraham & Mathew 1971); 2n = 27 on x = 9 in *Encephalartos hildebrantii* Miq. (Abraham & Mathew 1966); 2n = 18 on x = 9 in *Dioon edule* L. (Mathew unpublished). The somatic chromosomes of species of all the genera were large sized; the karyotypes of the species of *Cycas* being predominated by m- and sm-type chromosomes (2A), while those of *Zamia, Encephalartos* and *Dioon*, fairly asymmetrical (2B).

The basic chromosome constitution of the entire cytologically known genera of the Cycadaceae except *Cycas* are x = 8 or 9, and that of *Cycas* exclusively x = 11. According to the concept of Stebbins (1971), the basic numbers higher than x = 9 and 10 could be secondary/tertiary having originated from

lower ancestral ones; and on such a consideration, x = 9 in most of the known cycads and x = 11 in *Cycas* should be secondary ones, possibly originated from an x = 10 ancestor, the x = 9 and 8 by stepwise descending aneuploidy, and x = 11 by ascending aneuploidy on x = 10. It may be noted that the x = 10 constitution is common in many taxa of gymnosperms, especially among the Coniferales (Khoshoo 1961). Polyploid taxa are noticeably absent in the entire Cycadaceae, except a lone triploid taxon of *Encephalartos hildebrandtii* with 2n = 27, the karyotype of which comprised two distinct genomes, a diploid set of nine pairs and one haploid set of nine chromosomes, suggestive of allotriploidy arisen by natural mixing of unreduced and reduced gametes of two related diploid 2n = 18 species (Abraham & Mathew 1966). The rarity of polyploids in cycads may be attributed to the very large size of their chromosomes.

There is a great deal of discord concerning the taxonomic treatment of the Cycadales. Pilger (1926) included all the living genera in a single family Cycadaceae, dividing them into five subfamilies. Sporne (1965) treated the entire lot of cycads (*Cycas, Zamia, Encephalartos, Dioon* etc.) together in his family Cycadaceae, while Bierhorst (1971) treated them separately, *Cycas* in his Cycadaceae, *Dioon*, *Zamia* etc. in another family, Zamiaceae. Exomorphologically, *Cycas* and the *Zamia* group of genera are clearly different, the latter possessing more advanced features including the compact female cone, distinctly different from that of *Cycas*. The more specialized karyotype features of the *Zamia* group of genera characterized by more evolved lower basic numbers (x = 9 and 8) and more specialized karyotypes than *Cycas* with x = 11 and less specialized karyotypes, appear to lend cytological support to Bierhorst's treatment.

Coniferales

These are mostly distributed in the southern and northern temperate zones (Coulter & Chamberlain 1964), and poorly represented in the Indian flora. Their main centre of distribution in India is extrapeninsular Himalayas and the connected Sub-Himalayan ranges. The group is sparsely represented in the South, barring a very few species of *Araucaria* and *Podocarpus*. However, many taxa occur here as exotics, domesticated and grown as garden plants. Since the pioneering work of Sax (Sax & Sax 1933), several papers on the cytology of the Coniferales are known, including a comprehensive review of chromosome data compiled by Khoshoo (1961) based on the then known chromosome number data, of mainly alien taxa. Karyomorphological data are too sparse, notable ones being in *Araucaria* (Cardemi at al. 1984, Hansen 2001), *Podocarpus* (Hizume et al. 1998) and *Cupressus* (Toda 1980). Mathew et al. (2014a) studied the karyomorphology of 7 species in 5 conifer genera such as *Araucaria, Podocarpus, Cryptomeria, Cupresses* and *Thuja*. The basic chromosome numbers in them are, x = 13 in *Araucaria*, x = 19 in *Podocarpus*, x = 11 in *Cupressus*, *Cryptomeria* and *Thuja*. The existing basic chromosome numbers in the entire Coniferales (Khoshoo 1961) are, x = 13 in Araucariaceae, x = 9, 10, 11, 12, 13, 14, 15, 17, 18, 19 in Podocarpaceae, x = 11 and 12 in Taxaceae, x = 12 in Pinaceae, x = 10 in Sciadopityaceae, x = 11 in Taxodiaceae and Cupressaceae. It appears from the data that the fairly common basic numbers

in the order are x = 11 and 12. In the entire Coniferales, the conventional processes which effect changes in basic numbers apparently have been most operative in *Podocarpus* which is cytologically heterogeneous and dynamic and polybasic with an array of basic numbers (x = 9 to 15 and 17 to 19). The n = 19 in the South Indian taxon (*P. chinensis*) could be a polyploid derivative from an ancestral n = 10 to n = 20, followed by descending aneuploidy at the tetraploid level.

The available reports show that polyploids are very rare in Coniferales. Khoshoo (1959), who evaluated the role of polyploidy in the coniferales, contended that rarity of polyploids in the group may be due to the ecospecific differentiation between polyploids resulting from hybrids involving morphologically distinct species consequent on their being unable to establish and diverge into perfect alloploids, because polyploids with this sort of properties would have only little chance of survival. Another major factor limiting polyploids in conifers may be the large size of their chromosomes. In many angiosperms with very small chromosomes, polyploids are remarkably frequent as in the genus *Piper* (Mathew & Mathew 1999). This is in conformity with the concept of Mikshe & Hotta (1973) that very small chromosomes and smaller quantity of less repetitive DNA bring about more dynamic and evolutionary versatility and better avenues of cyclic diversication. Another contributing factor for the rarity of polyploids may be the woody habit and temperate habitats of the conifer genera.

The chromosomes, being the carriers of the genetic material, changes in them, both numerical and structural, bear direct relationship to the genetic-evolutionary processes than do any other changes (Stebbins 1971). The karyomorphological information can help understand the systematic relationships of plants at different taxonomic levels. Mathew et al. (2014a) provided karyotype data of several species of Coniferales studied from South India. The karyotypes of nearly all of them, except that of *P. chinensis* were characteristically symmetrical predominated overwhelmingly by chromosomes with median centromeres, which was particularly so in species of *Cupressus* and *Araucaria*. The karyotype categories recognized in most of the coniferous taxa studied are 1A/2A with fairly high TF% value and graded karyotypes. The karyotype of the species of *Podocarpus*, however, was notably different, predominated by sm- and st-type chromosomes, and the karyotype asymmetrical (3C) and specialized. The increased chromosome number in the taxon and its chromosome structural repatterning appear to have occurred both concomitantly. Khoshoo (1959) who evaluated the role of karyotype changes in evolution and differentiation of gymnosperms, particularly of the Coniferales, has contended that the processes which bring about chromosome structural alterations have been without significant changes in their karyotype architecture in most families of the Coniferales. He has also found that in the Coniferales in general, the cytological data followed the taxonomic grouping. It appears that in various conifer families and genera, despite their stable single basic chromosome constitution, speciation and evolution have occurred unabated and unaverse to polyploidy and perceptible degree of chromosome structural repatterning, and it may be that species diversification and evolution in them must have mainly occurred through other means, possibly cryptic structural changes in chromosomes and gene mutations. It may be noted that x = 10 is present in many conifer genera, which abound in a few

(*Dicrydium, Podocarpus*) along with other basic numbers such as x = 8, 9, 11, 12, 13 and 15. This appears to be suggestive that x = 10 could be the earlier evolved constitution in the entire Coniferales from which the others derived by stepwise aneuploid reduction (x = 9, 8), and ascending aneuploidy (x = 11, 12, 13). It is also possible that the x = 10 itself could be a secondary basic number evolved from an ancestral x = 5. The presence of species of *Dicrydium* with n = 15 appears to lend credence to the possibility because the n = 15 should be the derivative from progenitor taxa with n = 10 and 5 by secondary hybrid polyploidy. However, it is found that polyploidy has not crossed the 4x level in the entire conifers. The overall chromosome data of the Coniferales is suggestive that chromosome evolution at the basic chromosome level by secondary hybrid polyploidy followed by both ascending and descending aneuploidy was profoundly operative in the diversification and speciation of the group. The Coniferales are a taxonomically complex, morphologically variable and cytologically heterogeneous group, and a line of classificatory treatments exist. Coulter & Chamberlain (1964) recognized only two families, Pinaceae and Taxacaceae. Pilger (1926) splitted Coniferales into seven families and Bierhorst (1971) recognized nine families, all based on morphological characters. In his cytological inventory, Khoshoo (1961) followed the arrangement of families of Coniferales after Li (1953), by which the Coniferales hold 4 families, Araucariaceae, Podocarpaceae, Cephalotaxaceae and Taxaceae, in which the cytological data known so far, by and large follow this taxonomic grouping.

Gnetales

Gnetales include two monotypic genera, *Gnetum* and *Ephedra* along with *Welwitschia* (Sporne 1965). The genus *Gnetum* includes 30–35 dioecious species of woody trees, shrubs or climbers, while *Ephedra* holding about 40 species all dioecious. The species of *Ephedra* are reed-like shrubs distributed in the arid regions of the tropics and subtropics of northern and southern hemisphere. In India, the genus is represented by six species mostly in the alpine Himalayas (Vashishta 1995). Chromosome reports are known for two species of *Gnetum* and over 20 species of *Ephedra* (Khoshoo 1961, Mathew et al. 2014b). The reported chromosome numbers in the species of *Gnetum* are 2n = 44, both tetraploids based on x = 11, while species of *Ephedra* are diploids and tetraploids based on x = 7, the two ploidy types almost evenly distributed; and the South Indian ones are both diploids, based on x = 7 and 6. The somatic chromosomes of *G. ula* are medium-sized, and the karyotype 2B category with fairly high TF%, and those of *Ephedra* are also medium-sized, the karyotype of one of which (*E. foliata*) 2A type, and that of the other (*E. californi*a) 3C in cytotype 1, and 4A in cytotype 2. Both the cytologically known species of *Gnetum* are polyploids on x = 11 (Mehra & Rai 1957, Mathew et al. 2014b) which possibly formed by secondary hybrid polyploidy involving two ancestral taxa with x = 6 and 5. The genus *Ephedra* is dibasic (x = 7 and 6) of which x = 7 is the most predominant one; and the x = 6 could be derivation from x = 7. Although polyploids are very rare in the main stream gymnosperms (Ahuja Raj & Neale 2005), both *Gnetum* and *Ephedra* are clear exceptions. The known ploidy levels in *Ephedra* are 4x and 6x on x = 7. Intraspecific polyploids and dysploids also occur (Delevoryas 1980).

There is a great deal of discord concerning the taxonomic treatment of *Gnetum* and *Ephedra*. (1) Eichler (1883) treated *Gnetum, Ephedra* and *Welwitschia* under the Gnetales under a single family, Gnetaceae; (2) *Gnetum* and *Ephedra* under different families (Gnetaceae and Ephedraceae) under a common order, the Gnetales (Sporne 1965); (3) under different families and under separate orders (Gnetales and Ephedrales) by Eames (1952), Pant (2002) and Maarten et al. (2011). Cytological data show remarkable degree of difference between the two in respect of both chromosome number and karyomorphology. Species of *Gnetum* are polyploids based on x = 11 and have moderately asymmetrical karyotype (2B), while species of *Ephedra* are very low chromosome-numbered, based on x = 7 and 6, and karyotypes highly specialized (4A/3C). The sharp cytological distinction between the two very much favours their placement under separate orders. Moreover, *Gnetum* shows certain degree of morphological, anatomical and embryological characters common with angiosperms. *Gnetum* also shows a mitochondrial sequence akin to angiosperms (Won & Renner 2003). There are quite a few hypotheses pinpointing phylogenetic relationship of *Gnetum* and angiosperms (Donoghue & Doyle 2000), and some even holding that the ancestors of angiosperms are like that of *Gnetum* itself. Although, the existing chromosomal evidences do not appear to favour any proposal of *Gnetum*-angiosperm relationship, *Gnetum* remains largely a phylogenetic puzzle.

REFERENCES

ABRAHAM A & MATHEW P M 1962 Cytological studies in *Cycas* – Sex chromosomes in *Cycas Ann Bot* **26** 261–221

ABRAHAM A & MATHEW P M 1966 Cytology of *Encephalartos hildebrandtii Ann Bot* **30** 239–226

ABRAHAM A & MATHEW P M 1971 Cytology of Cycads – Karyomorphological studies in *Zamia* and a possible sex chromosome mechanisms *J Indian Bot Soc Golden Jubilee Vol* **50A** 297–302

AHUJA RAJ M & NEALE D B 2005 Evolution of genome size in Conifers *Silve Genet* **54** 126–137

BIERHORST D W 1971 *Morphology of vascular plants* McMillan Co New York

BISWAS C & JOSHI B M 1997 *The gymnosperms* Narosa Publishing House New Delhi

CARDEMI L, SALAS E & GODOY M 1984 Comparative study of the karyotypes of South American *Araucaria J Heredity* **75** 121–125

COULTER M & CHAMBERLAIN C J 1964 *Morphology of gymnosperms* Central Book Depot Allahabad

DELEVORYAS T 1980 *Polyploidy in gymnosperms In* Lewis W H (ed) *Polyploidy – Biological Relevance* Plenum Press New York pp 215–218

DONOGHUE M & DOYLE J A 2000 Seed plant phylogeny: demise of the anthophyte hypothesis *Curr Biol* **10** 106–109

EAMES A J 1952 Relationship of *Ephedra Phytomorphology* **2** 79–100

EICHLER A W 1883 *Syllabus der vorlesungen Uber Speciella und medicinalis pharmaceutische botanic* Lepzig

HANSEN L 2001 Chromosome number karyotype and DNA C – value of the Wollemi Pine (Araucariaceae) *Bot J Linn Soc* **135** 271–274

HIZUME M, SHIRAISHI H & TANAKA A 1998 Cytological study of *Podocarpus macrophyllus* with special reference to sex chromosome *Jap J Genet* **63** 413–423

KHOSHOO T N 1959 Polyploidy in gymnosperms *Evolution* **13** 24–39

KHOSHOO T N 1961 Chromosome numbers in gymnosperms *Silvae Genetica* **10** 1–9

LI H L 1953 *Amentotaxus J Arnold Arbor* **33** 192–198

MAARTEN J M, REVEAL J L, GARDNER M F, MILL R R & CHASE M W 2011 A new classification and linear sequence of extant gymnosperms *Phytotaxa* **19** 55–70

MATHEW P J & MATHEW P M 1999 Trends of chromosome evolution in Piperaceae *J Cytol Genet* **34** 119–127

MATHEW P M, MATHEW P J, CINTHYA CHRISTOPHER & HARIDAS P 2014a Karyomorphological studies in Conifers *J Cytol Getet* **15** (NS) 107–121

MATHEW P M, MATHEW P J, CINTHYA CHRISTOPHER & HARIDAS P 2014b Cytological study with emphasis to karyomorphology of *Gnetum* and *Ephedra J Cytol Genet* **15** (NS) 123–134

MEHRA P N & RAI K S 1957 Cytology of *Gnetum ula J Genet* **55** 394–396

MIKSHE J P & HOTTA Y 1973 DNA base composition and repetitive DNA in several conifers *Chromosoma* **41** 26–36

PANT D D 2002 *An introduction to Gymnosperms Cycas and Cycadales* Birbal Sahni Institute of Palaeobotany Lucknow

PILGER R 1926 Gymnospermae *In Engler A and Prantl K Die Natulischen Pflazenfamilien* Wilhelm Engelmann Verlag Leipzig

SAX K & SAX H J 1933 Chromosome number morphology in the Conifers *J Arnold Arbor* **14** 356–375

SPORNE K R 1965 *The morphology of gymnosperms* Hutchinson London

STEBBINS G L 1971 *Chomosome evolution in higher plants* Edward Arnold London

TODA Y 1980 On the karyotype of *Cryptomeria japonica J Jap For Soc* **62** 264–269

VASHISHTA P C 1995 *Gymnosperms* S Chand & Co New Delhi

WON H & RENNER S S 2003 Horizontal gene transfer from flowering plants to *Gnetum Proc Natl Acad Sci USA* **100** 10824–10839

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CYTOLOGY IN THE SYSTEMATICS, PHYLOGENY AND EVOLUTION IN PLANTS III. LILIALES

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SUMMARY

Extensive cytological study was accomplished in over 20 angiosperm families of both dicots and monocots by the senior author in collaboration with the doctoral students since 1970. This paper provides a brief review of the major findings emerged from the study in the Liliales sensu Hutchinson which comprise the Liliaceae, Smilacaceae, Ruscaceae, Amaryllidaceae and Agavaceae. The cytological evolution of the constituent families has been discussed vis-a-vis the implication of cytology in their systematics, phylogeny and evolution. Most of the tribes of Liliaceae indigenous to South India are polybasic with x = 10 constitution predominating which is postulated as the earlier evolved constitution, originated from a primary x = 5 by polyploidy/secondary hybrid polyploidy. Polyploidy is fairly prevalent in the family, which together with aneuploidy and structural repatterning of chromosomes are suggested to have played a major role in speciation and evolution. The predominant basic chromosome numbers in the other families of the order are, x = 16 in Smilacaceae, x = 10 in Ruscaceae, x = 11 in Amaryllidaceae and x = 30 (15) in Agavaceae. The systematic relationships and affinities of the lilialean families are discussed in the light of cytological data. Hutchinson's reorientation of the group of families in the order gains appreciable cytological corroboration such as assignment of family status to *Smilax* (Smilacaceae), *Ruscus* (Ruscaceae), treatment of *Crinum* under a separate tribe Crineae etc. However, there are also certain incongruities as well e.g., transfer of Allieae from Liliaceae to Amaryllidaceae, questionable solidarity of his Agavaceae due to inclusion of *Dracaena* which is chromosomally a misfit in the Agavaceae etc.

Keywords: Cytology, systematics, phylogeny, evolution, Liliales.

INTRODUCTION

The Liliales (sensu Hutchinson) constitute a large group of monocotyledonous families (Liliaceae, Smilacaceae, Ruscaceae, Amaryllidaceae and Agavaceae) chiefly distributed in the tropical and subtropical regions. The plants are mostly herbs, and very few woody. The group has been

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subjected to a variety of taxonomic treatments by a line of classical and modern taxonomists; and there exists a great deal of discord in respect of the composition, interrelationships and affinities. Reliable chromosome information on a large number of taxa of a group from a single geographical region is helpful for tackling taxonomic problems and for elucidating systematic relationships and phylogeny of related plant groups. Chromosome reports on a large number of taxa of the Liliales were reported globally, and these are mostly mere chromosome number reports of taxa largely confined to the temperate regions. Detailed chromosome study, including karyomorphology of a very large number of taxa of the Liliales (sensu Hutchinson) from the South Indian region was made in the Botany Department of Kerala University since 1970, which concerns families such as Liliaceae (52 species in 17 genera and 12 tribes), Smilacaceae (7 species of the type genus *Smilax*), Ruscaceae (2 species of *Ruscus*), Amaryllidaceae (33 species in 12 genera and 8 tribes) and Agavaceae (24 species in 6 genera and 4 tribes), and the results were published in a series of papers (Mathew & Thomas 1974, Vijayavalli & Mathew 1986, 1987, 1988a, d, 1989a,b, 1990a–e, 1991, 1992a–d). This paper aims at providing a brief review highlighting the major findings emerged from the study in relation to the cytological evolution vis-a-vis the issues of systematics, phylogeny and evolution of the group.

Cytological evolution

For meaningful discussion of cytological evolution, it is important to make use of valid data of relevant cytological parameters such as (1) behavior of chromosomes at meiosis, (2) the number and morphology of somatic chromosomes, (3) the basic chromosome constitution and (4) the modes and magnitude of factors which bring about numerical and structural changes in chromosomes at different taxonomic levels. The factor of basic chromosome numbers at the level of genera, tribes and families has played a prominent role in shaping the prevailing concept of evolution. This has been one of the widely used parameters in formulating phylogenetic speculations (Jones 1970) and also considered as a dependable and stable marker of the direction of evolution. Stebbins (1950) has suggested diminution of basic numbers as playing significant role in the process of evolution in plants. Polyploidy has played a prominent role and also influenced evolution of basic numbers, mostly of the higher order either by ‘polyploid drop’ (Darlington 1956) or by ‘polyploid lift’ (Jones 1970), often producing new unrelated basic chromosome numbers in a group.

Liliaceae

Chromosome number

From the list of basic chromosome numbers known for different tribes of the Liliaceae studied from South India (Table 1) it may be noted that a wide range of basic numbers exist in a few tribes, e.g., Asphodeleae (x = 6, 7, 8, 9, 10, 11, 13), Hemerocallideae (x = 7, 8, 10, 11), Polygonatae (x = 6, 7, 8, 9, 10, 11), Tulipeae (x = 6, 7, 8, 9, 10) and Scilleae (x = 3, 4, 5, 6, 7, 8, 9, 10, 11). It is significant that x = 10 occurs in almost all the South Indian tribes. A few of the tribes are notably

TABLE 1: Known basic chromosome numbers in the families of South Indian Liliales.

Family	Tribe	Basic number
Liliaceae	Asphodeleae	6, 7, 8, 9, 10, 11, 13
	Ophiopogoneae	9
	Kniphofeae	6, 9
	Hemerocallideae	7, 8, 10
	Aloineae	7
	Aspidistreae	7, 8, 9, 10
	Polygonateae	6, 7. 8. 9, 10, 11,
	Dianellieae	8. 9
	Uvularieae	7, 9, 11
	Asparageae	10
	Tulipeae	6, 7, 8, 9, 10
	Acilleae	3, 4, 5, 6, 7, 8, 9, 10, 11,
Smilacaceae	*Smilax* (genus)	13, 14, 15, 16
Ruscaceae	*Ruscus* (genus)	10
Amaryllidaceae	Agapantheae	5, 6
	Allieae	5, 6, 7, 8, 9, 10, 16, 17
	Amaryllidaceae	6, 8, 9, 11
	Crineae	11
	Zephyrantheae	6, 7
	Haemantheae	6, 8, 9, 11
	Eucharideae	10, 11, 12, 23
	Hippeastreae	8, 9, 10, 11
Agavaceae	Yucceae	8, 9, 10, 11
	Dracaeneae	30 (15)
	Agaveae	19, 20, 21
	Polyantheae	30 (15)

monobasic with a single stable basic chromosome constitution e.g., Ophiopogoneae (x = 9), Aloineae (x = 7) and Asparageae (x = 10). Since taxa with x = 10 occur in almost all tribes, coexisting with lower and higher ones, it is reasonable to consider that x = 10 could be the earlier evolved basic constitution in such tribes, and more so in the entire family, from which the lower constitutions derived by descending (x = 9, 8, 7, 6), and higher ones by ascending aneuploidy (x = 11, 12, 13). Stebbins (1971) and Grant (1982) have held a strong view that the basic chromosomes of the magnitude x = 10 and 9 could be secondary ones having arisen from lower ones, and according to this contention, x = 10 in the Liliaceae is to be reckoned as the product of polyploidy/secondary hybrid polyploidy from ancestral taxa with n = 5. The highest basic number present in the South Indian tribes is x = 13. But

still higher constitutions are known in another tribe, Convallarieae (x = 16, 17, 18, 19) not represented in the South Indian study. The x = 19 in this tribe could be a derivation from x = 20, and the lower ones derived by descending aneuploidy from x = 20, which itself in turn be a polyploid derivative of x = 10. The basic chromosome scenario in the tribe Scilleae showing a regular descending series from x = 10 down to x = 5, 4, 3 appears to provide a clue for the possibility of x = 5 as the primary basic constitution of the entire family. However, the uninterrupted descending series from x = 10 down to x = 3 in the herbaceous tribe apparently cast a question also as to the probability of such extent of descending aneuploidy. There is an alternative possibility that the basic numbers lower than x = 5 (x = 4, 3) in this tribe could be the products of descending aneuploidy directly from x = 5 or even of their being other independent primary constitutions. On such a consideration, the x = 8 and 6 in this tribe could also be polyploid derivations from x = 4 and 3. However, it is to be reckoned that, the Liliaceae being a monocot family of ancient origin with very complex chromosome constitutions exhibited in various tribes, determination of its probable primary ancestral basic number with absolute certainty is difficult. However, the possibility of x = 10 to be the earlier evolved basic number in great bulk of the tribes is a strong possibility, which in turn originated from the primary x = 5.

Polyploidy is known to be the most widespread cytogenetic process which has greatly contributed to speciation and evolution in higher plants (Stebbins 1971). This is mainly due to the ability of the polyploids to increase the chance of fertilization by breaking reproductive barriers, and also to their invasive potential and high tolerance to adverse environmental conditions. The phenomenon is known to play significant role in initiating discontinuity, both within and between species. Polyploid taxa are fairly prevalent in the South Indian group (50%) occurring at different levels, the more prevalent being tetraploids (Vijayavalli & Mathew 1990e). According to Stebbins (1971), plant growth habit is the crucial factor influencing polyploids in angiosperms, the highest frequency in perennial herbs and lowest in annuals. Although the Liliaceae mostly comprise perennial herbs, polyploidy is more frequent in the herbaceous perennials, as in taxa of *Chlorophytum* (Mathew & Thomas 1974), *Ophiopogon, Scilla, Ornithogalum* etc. (Vijayavalli & Mathew 1990e). In the genus *Chlorophytum*, a polyploid series was evident (4x, 6x, 8x, 12x), all displaying normal meiosis suggesting allopolyploidy, and a few (8x/12x) suspected autoallopolyploidy.

The genus *Aloe* presents a striking instance of very little polyploidy (Vijayavalli & Mathew 1991). Karyomorphologically also the genus displays profound homogeneity, and it appears that neither numerical nor structural changes of chromosomes have been operative in the genus. Cryptic structural changes of chromosomes and gene mutation could have been the main agents of variation leading to the evolution of enormous number of species of the genus.

The occurrence of more than one euploid type within a previously recognized taxonomic species has been known in many plant groups; and such differences of chromosome numbers in species complexes are often correlated with notable difference in plant morphology, and when they do so the phenomenon can bring about speciation (Vijayavalli & Mathew 1990e).There are a few such species

complexes detected in the South Indian group. In the genus *Chlorophytum*, there are a few species complexes (Mathew & Thomas 1974). *C. orchidastrum* occurring in different polyploid levels (2x, 4x, 6x, 8x), and also other species like *C. attenuatum, C. nepalens* and *C. laxum*. Sheriff & Chennaveeraiah (1975) reported three cytotypes in *C. attenuatum*, which are phenotypically different, and they suggested a breakup of the species complex. Three varieties of the species of *Ophiopogon* studied from South India were high polyploids (2n = 108) (Vijayavalli & Mathew 1990c). *Scilla indica* which is widely distributed in Central India is highly polymorphic (Vijayavalli & Mathew 1990e).

Aneuploidy in angiosperms is known to occur most often at the diploid level, either ascending or descending, brought about by unequal apportionment of chromosome halves between daughter cells at mitosis, and by nondisjunction of chromosomes of bivalents during meiosis. The data from South Indian study show aneuploid changes at diploid levels resulting in deviations of basic chromosome numbers of the lower category relatively less frequently. However, this has played a significant role in some genera of tribes such as Asphodeleae (*Anthurium*), Polygonatae (*Polygonatum, Disporum*). Tulipae and Scilleae (*Scilla, Ornithogalum, Dipcadi* etc.) (Vijayavalli & Mathew 1990e). In most cases, the change was descending from x = 10. Aneuploidy at polyploid levels is evident in genera of the tribe Convallarieae (x = 16, 17, 18, 19). However, aneuploidy resulting in change of basic numbers in the South Indian group was rare in a few genera (*Hemerocallis, Aloe and Gloriosa*), while profuse and frequent in certain others such as *Disporum* (x = 6, 7, 8, 9, 11), *Scilla* (x = 4, 5, 6, 7, 8, 9, 10, 11), *Ornithogalum* (x = 4, 6, 7, 8, 9, 10, 11) and *Dipcadi* (x = 4, 6, 7, 8, 9, 10, 11). Intraspecific aneuploidy was widespread, eg. *S. indica* (2n = 58, 60, 68) (Vijayavalli & Mathew 1990e), *O. caudatum* (2n = 54, 54), *Lillium neilgehrrens* (2n = 23, 23), *D. eradii* (2n = 22, 21). In all cases, aneuploid cytotypes showed little or no plant morphological difference.

Karyomorphology

The chromosomes being the carriers of the genetic material, changes in them bear direct relationship to the genetic-evolutionary process than do any other type of changes. Karyomorphological information is helpful for better understanding of systematic relationships, in addition to being a useful tool in tracing the trend and direction of evolution. Stebbins (1971) has outlined certain karyotype characteristics formulated on the basis of intrakaryotypic chromosome size difference and differences in the position of centromeres. Karyotypes with chromosomes mostly of the same size and having median and submedian centromeres considered symmetrical, and those with increased frequency of st- or acrocentric chromosomes and with appreciable intrakaryotypic size difference of chromosomes asymmetrical. Increasing asymmetry of karyotypes and other characters such as chromosome number, plant habit and morphology have been relied upon for tracing the direction of evolution. In the Liliaceae, the tribes Asphodeleae and Ophiopogoneae, whose members having symmetrical karyotypes occupy a primitive position in Hutchinson's grouping of the family, while the Scilleae with highly asymmetrical karyotypes placed at an advanced position; and this implies that there is a remarkable association between karyotype specialization and phylogenetic advancement.

However, there is also an opposing trend as in the case of *Aloe, Haworthia* and *Hosta*, all of which occupy relatively primitive position in the family while possessing highly specialized karyotypes (4B, 4C).

Change of basic chromosome number and processes involved in karyotype specialization are two major factors contributing to karyotype evolution. Stebbins (1971) has recognised a few patterns of variation such as (1) increasing asymmetry/increasing chromosome number/increasing plant morphological specialization, (2) different basic numbers/little difference in karyotype symmetry and (3) constant basic numbers/variation of karyotype symmetry. *Chlorophytum* (Mathew & Thomas 1974) and *Aloe* (Vijayavalli & Mathew 1991) are the two genera in which detailed karyotype analysis made involving a large number of species. The x = 7 group of species of *Chlorophytum* showed profound degree of karyotype evolution in the polyploid species implying that polyploidy and chromosome structural repatterning have concomitantly operated in the genus. However, in the genus *Aloe*, the factors which alter the karyotype framework have been little operative in the process of diversification and evolution of innumerable species in the genus.

Smilacaceae

This is a small family with only four genera (*Ripogonum, Smilax, Pseudosmilax, Heterosmilax*) of which the type genus forms the bulk, which is characterized by leaves with reticulate venation, flowers small and dioecious. Cytology of seven taxa belonging to five species were studied from South India (Vijayavalli & Mathew 1987) of which four species (*S. aspera, S. zeylanica, S. bracteata, S. wightii*) are diploids with n = 16 and 2n = 32 and karyotype 3B, while the other species (*S. ovalifolia*) exists here in three polyploid cytotypic forms with 2n = 64 (4x), 96 (6x) and 128 (8x), all based on x = 16, and with 3B karyotypes (Vijayavalli & Mathew 19989a). The available chromosome reports show that the genus is polybasic with x = 13, 14, 15 and16 of which x = 16 the most frequent, and concentrated among the more woody taxa, and considered as the earlier evolved basic constitution from which the lower ones derived by descending aneuploidy (Vijayavalli & Mathew 1987). Polyploid species are very rare in the genus, the known exceptions being species with 2n = 60 based on x = 15 reported from China (Sato 1942) and the South Indian species, *S. ovalifolia* which exists in three intraspecific polyploid cytotypic forms. The plants of the different cytotypes show certain recognizable plant morphological features differing in regard to the size, shape and texture of leaves and nature of the stem. The karyotypes of all the species are predominated by st- and t-types of chromosomes, and their karyotypes strikingly asymmetrical. In view of this distinct plant morphological difference among the three cytotypes associated with remarkable ploidy difference, the species complex poses a taxonomic problem, invoking further study.

Ruscaceae

This is a very small family with only three genera, of which chromosome reports known only for two, *Danae* with 2n = 40 (Sato 1942) and *Ruscus* also with 2n = 40, detected from the South Indian

study of two species both tetraploids based on x = 10. The karyotypes of the two species are of medium symmetry (2B, 2C) (Vijayavalli & Mathew 1990e).

Amaryllidaceae

Thirty three species representing 12 genera in 8 tribes were studied from South India (Vijayavalli & Mathew 1990e). A wide variety of basic constitutions occur (Table 1) in some, especially the Allieae (x = 5–10, 16,17) followed by Amaryllideae (x = 6, 8, 9, 11), Eucharideae (x = 10, 11,12, 23), and very few in Agapantheae (x = 5, 6) and Zephyrantheae (x = 6, 7) and monobasic in Crineae (x = 11), showing often the lowest basic number in Allieae (x = 5) and highest in Eucharideae (x = 23). There are conflicting views concerning the original basic number in the family, Raven (1975) holding x = 12, and Flory (1977) sharply opposing this and considering x = 11 as the most probable basic constitution, especially as this occurs in most of the tribes along with its descending series (x = 10, 9, 8) in most tribes. The tribe Crineae is monobasic with x = 11. It is most probable that the x = 11 here is a secondary one derived from ancestral x = 6 and 5 by secondary hybrid polyploidy. The occurrence of species based on x = 6 and 5 in some tribes appears to favour this possibility. Considering the frequent occurrence of x = 11 in the closely related family, Liliaceae, Flory (1977) even inclined to suggest that some liliaceous taxa with 2n = 22 could be ancestral stock to the original amaryllidaceous group.

Polyploidy and aneuploidy occur in a number of species and genera of the Amaryllidaceae globally, although relatively low in the South Indian group (Vijayavalli & Mathew 1990e). Among the five taxa of *Allium* studied from South India, four are diploids and only one tetraploid (2n = 32). Intraspecific polyploidy is prevalent in the genus. The amaryllidaceous species, *A. belledone* studied from South India is a tetraploid, occurring in three cytotypes (2n = 22, 33, 44) based on x = 11. The genus *Crinum* is chromosomally stable based on x = 11. Widespread discontinuity occurs in the genus with the species occurring in a polyploid series (2x, 4x, 6x, 8x). The variant numbers, 2n = 21 and 20, noticed in *C. moorei* (Vijayavalli & Mathew 1990e) must be the result of descending aneuploidy on 2n = 22. In the type genus *Zephyranthes* of the tribe Zephyrantheae, many species occur in a euploid series on x = 6, ranging from 2n = 12 to 120. This is a profoundly dynamic and heterogeneous tribe, constituting a long line of polyploid series from 2x–20x, on x = 6. Four genera (*Pancratium, Hymenocallis, Eucharis, Eurycles*) of Eucharideae are representd in the South Indian study (Vijayavalli & Mathew 1990e). The basic numbers in them are x = 11 in *Pancratium* and *Hymenocallis*, x = 23 in *Eucharis* and x = 10 in *Eurycles*. The type genus, *Hippeastrum* of Hippeastreae shows three basic numbers, x = 8, 9 and 11 of which x = 11 shows considerable polyploid evolution (2n = 22, 44, 66 and 77). In *H. vittatum*, several variant numbers exist (2n = 41, 43, 45 and 46) indicating dynamic chromosome evolution. Karyomorphology of a large number of taxa of the family is known, and the data show predominance of karyotypes with medium asymmetry (2C, 3B) in many genera, while the more evolved 3C karyotype occurs in *Haemanthes*, and less specialized and asymmetrical (1A, 2A, 2B) in the taxa of *Allium*, *Tulbagha* and *Agapanthes*.

Agavaceae

This is a small family of about 500 species in 19 genera and 6 tribes (Hutchinson 1973) widely distributed in the tropics and subtropics, of which the South Indian study (Vijayavalli & Mathew 1992b) revealed 24 species in 6 genera and 4 tribes (Yucceae, Dracaeneae, Agaveae, Polyantheae). The known basic chromosome constitutions in the genera of the Yucceae, Agaveae and Polyantheae are on x = 30, while the tribe Dracaeneae stands out with a different cohort of basic numbers, x = 19, 20 and 21. All the genera of Dracaeneae studied from South India show striking similarity in regard to their chromosome constitution and karyotype architecture (graded). The x = 20 in *Dracaena* is considered as the earlier evolved condition, possibly derived from an ancestral x = 10; and the x = 19 and 21 arose by descending and ascending aneuploidy from x = 20. The x = 30 in *Yucca* and *Polyanthes* could be a derivation from the ancestral n = 20 and n = 10, resulting in x = 15 from which by polyploidy arose the x = 30. An extensive literature exists on the cytology of the type genus *Agave*, and the reported chromosome numbers constitute a polyploid series (2n = 60, 90, 120 and 180) based on x = 30 which could be a derivation from an ancestral x = 15. The available data suggest remarkable degree of polyploid evolution taken place in the genus. The distinct bimodality and asymmetrical karyotypes of species of *Yucca* and *Polyanthes* are suggestive of large scale chromosome structural repatterning involving remarkable degree of intrakaryotypic chromosome size diminution.

Systematics

The families of the Liliales (Liliaceae, Smilacaceae, Ruscaceae, Amaryllidaceae, Agavaceae) have been subjected to a variety of treatments in respect of their composition and affinities in the different classifications – classical (Bentham & Hooker 1883), semimodern (Hutchinson 1973, Takhtajan 1980 etc.) and more modern (Dahlgren et al. 1985, APG III 2009 and APG IV 2016) systems. Hutchinson has removed the woody liliaceous tribe Yucceae and woody amaryllidaceous tribe Agaveae along with certain other woody genera like *Dracaena, Cordyline* and *Phormium* from the Liliaceae, and *Polyanthea* from the Amaryllidaceae etc. into a newly erected family, Agavaceae, placing it under Agavales. This together with separation of *Smilax* and *Ruscus* resulted in substantial reduction of Bentham & Hooker's Liliaceae. Although Hutchinson's reorientation of the group of families has, by and large, gained appreciable cytological corroboration, there are incongruities also in a few instances. The glaring merits and demerits of Hutchinson's treatment are highlighted. His inclusion of *Hosta* (x = 10, small chromosomes, bimodal karyotypes) and *Hymenocallis* (x = 11, large chromosomes) in the same tribe Hymenocallideae, appears unsound on chromosomal grounds, because *Hosta* resembles the agavaceous genera, while *Hymenocallis* the *Amaryllis* type. In chromosome number (x = 16) and karyomorphology (very small chromosomes, asymmetrical karyotype), the genus *Smilax* stands out from the general Liliaceae. This together with the unique exomorphological features (climbing habit, reticulate leaf, dioecious flowers) and phytochemical attributes, support Hutchinson's removal of *Smilax* from the Liliaceae, assigning separate family status. The propriety of placement of *Asparagus* in the Liliaceae is questionable from the cytological perspective in view of the notably distinct

small-sized chromosomes which are uncommon in the typical Liliaceae. The APG III (2009) and APG IV (2016) systems create even an order, Asparagales around this, in the former including the Agavaceae, Asparagaceae etc. Although *Ruscus* resembles *Asparagus* in chromosome number (x = 10) and size, Hutchinson has assigned this a separate family status. Hutchinson's Amaryllidaceae constitutes a fairly homogeneous assemblage chromosomally. He has effected a major reshuffle of the Liliaceae and Amaryllidaceae by disregarding the importance of the position of the ovary (superior/ inferior), and instead attaching importance to the type of inflorescence (umbellate with involucres bract), thereby transferring a few of Bentham and Hooker's Liliaceae (Agapantheae, Allieae) to the Amaryllidaceae. Since members of Allieae are distinctly different from the typical Amaryllidaceae in chromosomal and phytochemical attributes, the transfer of Allieae based on just the inflorescence character is superfluous and untenable. Regarding the grouping of *Crinum*, *Pancratium*, *Hymenocallis*, *Eucharis* and *Eurycles*, there are varying proposals. The chromosome features appear to favour Hutchinson's treatment of *Crinum* under a separate tribe Crineae, with all the other four in another tribe, Eucharideae, and this is consistent with cytological evidence. Although Hutchinson's agavaceous tribes, Yucceae, Dracaeneae, Agaveae and Polyantheae show exomorphic likeness, cytologically they do not constitute a homogeneous group. In chromosome number (x = 19, 20, 21) and karyomorphology (graded karyotype), Drcaeneae stand out from the *Yucca, Agave and Polyanthes* group (x = 30, sharply bimodal karyotype), and hence Dracaeneae appears a misfit in Hutchinson's Agavaceae(Jyothilekshmi at al. 2015). Based on chromosomal likeness, Sharma & Choudhuri (1964) suggested inclusion of *Sansevieria, Ophiopogon* and *Dracaena* together in one family, far removed from Agavaceae. Patil & Pai (2011) have suggested a polyphyletic affinity for many genera of Hutchinson's Agavaceae. Their evolutionarily distinctive karyotype features demands an in depth karyotype study which may reveal their putative affinities facilitating possible realignment of them.

According to the latest APG IV (2016) treatment, the Liliales comprise 10 families, and it includes only two Hutchinson's lilialean families such as Smilacaceae anf Liliaceae, but excludes the rest (Ruscaceae, Amaryllidaceae, Agavaceae). The genus Asparagus which had only a tribal status (Asparageae) in Hutchinson's Liliaceae, is given a separate ordinal status, Asparagales comprisesing 14 families which includes Asparagaceae and Amaryllidaceae, but excludes Agavaceae. The earlier APG III (2009) system which also assigned an ordinal status for Asparageae, however, had included Agavaceae in it. The higher taxonomic status assigned to *Asparagus* (Asparagales) is cytologically tenable and appropriate in view of the chromosome architecture and vegetative feature (phylloclade) distinctly different from the rest of the Liliales. The Agavaceae finds only a subfamily status (Agavoideae) in the APG IV.

REFERENCES

APG III 2009 A phyologenetic classification of the land plants to accompany APG III *Bot J Linn Soc* **161** 122–127

APG IV 2016 An update of the Angiosperm Phylogeny Group classification for the order and families of flowering plants APG IV *Bot J Linn Soc* **181** 1–20

BENTHAM G & HOOKER J D 1883 *Genera Plantarum* Vol **III** Reeve London

DAHLGREN R M T, CLIFFORD H T & YEO P F 1985 *The families of the Monocotyledons Structure evolution and taxonomy* Springer Verlag Berlin Heidelberg New York

DARLINGTON C D 1956 *Chromosome botany* George Allen and Unwin London

FLORY W S 1977 Overview of chromosome evolution in the Amaryllidaceae *Nucleus* **20** 70–88

GRANT V 1982 Periodicities in the chromosome numbers of the angiosperms *Bot Gaz* **143** 379–389

HUTCHINSON J 1973 *The families of flowering plants* 3rd ed Clarendon Press Oxford

JONES K 1970 Chromosome changes in plant evolution *Taxon* **19** 172–179

JYOTHILEKSHMI P, MATHEW PM & VIJAYAVALLI B 2015 Cytology and its systematic bearing on certain agavaceous taxa from South India *J Cytol Genet* **16** (NS) 7–16

MATHEW P M & THOMAS K J 1974 Cytological studies on *Chlorophytum New Botanist* **1** 34–45

PATIL D A & PAI R M 2011 The Agavaceae Taxonomic and phylogenetic appraisal *J Exp Sci* **2** 20–24

RAVEN P N 1975 The bases of angiosperm phylogeny cytology *Ann Missouri Bot Gard* **62** 724–764

SATO D 1942 Karyotype alternation and phylogeny in Liliaceae and allied families *Jap J Bot* **12** 57–161

SHARMA A K & CHAUDHURI M 1964 Cytological studies as an aid in assessing the status of *Sansevieria Ophiopogon* and *Curculigo Nucleus* **7** 43–58

SHERIFF A & CHENNAVEERAIAH M S 1975 Cytological studies in *Chlorophytum attenuatum* complex and cytotaxonomic consideration *Cytologia* **40** 401–408

TAKHTAJAN A 1980 Outline of the classification of flowering plants *Bot Rev* **46** 225–354

STEBBINS G L 1950 *Variation and evolution in plants* Columbia University Press New York

STEBBINS G L 1971 *Chromosomal evolution in higher plants* Edward Arnold London

VIJAYAVALLI B & MATHEW P M 1986 New report of polyploid cytotype in *Smilax ovalifolia Curr Sci* **55** 66

VIJAYAVALLI B & MATHEW P M 1987 Cytology of five species of *Smilax* from South India *Nucleus* **30** 57–60

VIJAYAVALLI B & MATHEW P M 1988a Cytology of species of *Hymenocallis* Salisb *II All Ind Conf Cytol Genet* 28–30 December 1988 held at Warangal p 38 (Abstr)

VIJAYAVALLI B & MATHEW P M 1988b Cytology of South Indian Senicioneae *II All Ind Conf Cytol Genet* 28–30 December 1988 held at Warangal p 39 (Abstr)

VIJAYAVALLI B & MATHEW P M 1988c Cytology of species of *Hosta* and *Hemerocallis* Salisb *II All Ind Conf Cytol Genet* 28–30 December 1988 held at Warangal p 92 (Abstr)

VIJAYAVALLI B & MATHEW P M 1988d Studies on South Indian Liliaceae I Cytology of *Scilla indica* Linn *J Cytol Genet* **23** 161–165

VIJAYAVALLI B & MATHEW P M 1989a Karyomorphology of five species of *Smilax Cytologia* **54** 65–72

VIJAYAVALLI B & MATHEW P M 1989b Cytology of species of *Cordyline* and *Dracaena* from South India *Cytologia* **54** 573–579

VIJAYAVALLI B & MATHEW P M 1990a Karyomorphology of a few morphotypes of *Gloriosa superba* L *Cytologia* **55** 531–533

VIJAYAVALLI B & MATHEW P M 1990b Cytology of species of Hemerocallideae from South India *Cytologia* **55** 335–358

VIJAYAVALLI B & MATHEW P M 1990c Cytology of *Ophiopogon infermedius New Botanist* **17** 27–31

VIJAYAVALLI B & MATHEW P M 1990d Cytological studies in the South Indian Eucharideae *New Botanist* **17** 175–181

VIJAYAVALLI B & MATHEW P M 1990e *Cytotaxonomy of the Liliaceae and Allied Families* Continental Publishers Trivandrum

VIJAYAVALLI B & MATHEW P M 1991 Cytology of twelve species of *Aloe New Botanist* **17** 255–266

VIJAYAVALLI B & MATHEW P M 1992a Cytological studies in *Gloriosa superba J Indian Bot Soc* **71** 29–32

VIJAYAVALLI B & MATHEW P M 1992b Cytology of a few Agavaceae from South India *New Botanist* **18** 121–129

VIJAYAVALLI B & MATHEW P M 1992c Cytology of species of *Gloriosa Nucleus* **35** 55–58

VIJAYAVALLI B & MATHEW P M 1992d Karyomorphology of three species of *Crinum Cytologia* **57** 27–32

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GC-MS PROFILE AND HPTLC ANALYSIS OF BARK EXTRACT OF *CASSIA AURICULATA* LINN.

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SUMMARY

The qualitative phytochemical analysis of the bark extract of *Cassia auriaculata* showed the presence of active phytoconstituents like carbohydrates, glycosides, alkaloids, phenols, flavonoids, tannins, steroids, volatile oils, gums and mucilage in both methanol and chloroform extracts. However, oils, gums and mucilage were found to be absent in petroleum ether extract. For characterization of individual compound, the bark extract was subjected to HPTLC analysis and the presence of caffeic acid in the bark extract is suggestive of the antioxidant potential of this species. The methanolic bark extract of *C. auriculata* showed the presence of 18 compounds of medicinal value.

Keywords: *Cassia auriculata*, bark extract, phytoconstituents, HPTLC, caffeic acid, GC-MS.

INTRODUCTION

Medicinal plants are indispensable for the modern medicine with numerous plant-derived therapeutic agents. They are the main ingredients of local medicines and are of vital importance in traditional healthcare. Many plants contain a variety of phytochemicals useful in the fields of agriculture, human and veterinary medicines (Raj et al. 2012). In India, nearly 7000 medicinal plant species are widely used by the ethnic communities for various ailments (Sirajuddin 2006). The phytochemicals in plants have protective effect against diseases and disorders (Gurudeeban 2013). Therefore, researchers focused their interest in isolation and quantification of active metabolites present in ethnomedicinal plants which are important for evaluatiom of therapeutic action and commercial value.

Cassia auriculata (Caesalpiniaceae) is an ethnobotanically important shrub with attractive yellow flowers and commonly called ‘tanners senna’ which is abundantly found in hot deciduous forests of India and holds a very prestigious place in Siddha and Ayurveda systems of medicine. This species has been reported to contain antipyretic, hepatoprotective, antidiabetic, antiperoxidative, antihyperglyceamic and microbicidal activity (Raj et al. 2012). A structure based molecular docking studies was carried out by Rajkumar et al. in 2016 and the analysis revealed the presence of eight compounds. Studies on chemical composition and characterization of *C. auriculata* flower extract was carried out by Ponnusamy & Soundharajan (2014). They noticed the occurrence of flavonoids and phenols in CAFMEt and three fractions were collected from column chromatography and concluded that the refractive index by GC-MS analysis showed alkanes, alcohols, esters and hydrocarbons. A

study of in vitro antioxidant activity and HPTLC fingerprint of quercetin in *C. auriculata* was performed by Jyothi & Somashekaraiah (2013) and HPTLC analysis of methanolic extract of twigs, leaves and flower buds indicated the presence of the most abundant dietary flavonol, quercetin. Methanol extracts of *C. fistula* was used for phytochemical analysis and totally 10 different phytoconstituents were identified from GC-MS analysis (Kulkarni et al. 2015). Their studies indicated two compounds (citronellol and linoleic acid) out of ten have already shown as anticancer agents. Till date no work has been done on the bark extract of *C. auriculata* on the quantification and identification of chemical compounds and the GC-MS profile. Therefore, the present study is aimed at isolation and characterization of phytochemicals using HPTLC and GC-MS analyses respectively.

MATERIAL AND METHODS

Plant material

The twigs of *C. auriculata* were collected from in and around Jnanajyothinagar near Bangalore University campus during the month of February-March 2018 and washed with running tap water to remove the dust and other soil particles present on the surface of the bark. Then the bark was peeled off and dried under shade for 10–12 d and ground into fine powder for further use.

Preparation of bark extract

10 g of powdered material was extracted with 50 ml of methanol and kept in water bath for 4 h at 50° C and filtered after cooling with Whatmann filter paper. The filtrate containing crude extract was transferred to Eppendorf's vials.

HPTLC analysis

Sample preparation: 1 g of bark extract of *C. auriculata* was mixed with 30 ml HPTLC grade methanol and kept in water bath at 70–80° C for 30 min. After cooling to room temperature the mixture was filtered and concentrated to 5 ml before proceeding to spotting.

Standard preparation: 10 mg of caffeic acid reference standard mixed with 10 ml of methanol was kept in water bath for 10–15 min at 70–80° C then filtered and concentrated to 1 ml and proceed for spotting.

20 ml of mixture of chloroform:methanol:formic acid:acetic acid (8.5:1.5:0.9:0.9) was transferred to the chromatographic tank and allowed it to saturate for 30 min. Later 5 µl of sample(s) and 5 µl standard was applied on a HPTLC silica plate using a Linomat HPTLC applicator. The plate was allowed to be in fume hood to let the solvent to evaporate. Thereafter, the plate was placed in the tank as near vertical as possible ensuring that the line of application is well above the solvent level. The lid should be replaced tightly and allowed the solvent to ascent to 1.5 cm below the top of the plate. The plate was removed from the tank and let it to air dry in fume hood. Retention factor (Rf) values were calculated by using the following formula:

$$Rf = \frac{\text{Distance travelled by the solvent}}{\text{Distance travelled by the sample}}$$

HPTLC was performed on TLC plate pre-coated with silica gel 60 F254 of thickness 0.2 mm (Merck) of size 10 × 10 cm. Caffeic acid (1 ml) and samples (5 ml) were applied on the plate as band of 8.0 mm width using Hamilton syringe and CAMAG Linomat V sample applicator. The plate was developed to a distance of 8.0 cm in a CAMAG twin trough chamber previously saturated with mobile phase chloroform:ethyl acetate:formic acid:acetic acid in the ratio

8.5:1.5:0.9:0.9 for 30 min. After development, the plate was dried at room temperature and densitometric evaluation was performed at 366 nm in CAMAG TLC scanner 3 linked to WINCATS software. The image of the plate was captured at short range in UV chamber.

Gas chromatography and Mass spectroscopic analysis (GC-MS)

For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1 µl of methanolic extract was employed. The injector temperature was maintained at 260° C, the ion source temperature 200° C, the oven temperature was programmed for 80° C (isothermal for 4 min)with an increase of 10° C/min to 200° C, then 5° C/min to 280° C, ending with a 6 min isothermal at 280° C. Mass spectra were taken at 70eV; a scan interval of 0.5 sec. and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 50 min. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2. The relative retention times (Rt) and mass spectra of the extract components were compared with those of authentic samples. The spectra of the compounds are matched with WILEY 8.0 and NIST 11 libraries.

OBSERVATIONS

Qualitative phytochemical analysis revealed the presence of nine different phytoconstituents such as carbohydrates, glycosides, alkaloids, phenols, flavonoids, tannins, steroids, oils, gums and mucilage in the methanol and chloroform extracts. However, in petroleum ether extract, oils, gums and mucilage were found to be absent.

The result of the HPTLC analysis for the bark extract showed that caffeic acid in the extracts was quantified by comparing the total peak area of caffeic acid band in standard solution with that of the sample solution (Fig. 1).

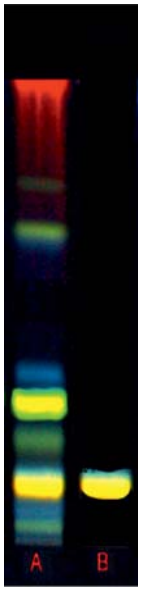

Fig. 1: HPTLC chromatogram of *C. auriculata.* (A. Sample; B. Caffeic acid)

TABLE 1: Chemical composition of methanolic bark extract of *C. auriculata.*

Sl. No.	Retention time (min)	Area	Area (%)	Height (mm)	Height (%)	Chemical constituents	Base m/z
1	10.75	56320	8.57	2029	1.36	Cryptophycin G	77.05
2	11.02	11196	1.71	6168	4.13	2-Propynenitrile	51.00
3	11.11	44192	6073	6103	4.09	Be,-o.ami,e//6-/40-ami,o-20-meth12-50-p1rimi3i,124-3-5e,zo12thio-5—orm12-4-meth12-5-aza-3-he6e,12 3ih13roge,ephosphate	51.00
4	14.50	71377	10.87	11094	7.43	3(2H)-Pyridazinone, 6-Methyl-	110.05
5	14.60	47500	7.24	11654	7.80	Methyl (2R*,3S*)-2,3-Dimethyl-3-Ethyl-2-(1(S*)-Hydoxyethyl)-4-Pentenoate	55.00
6	14.64	43675	6.65	10831	7.25	2H-Pyran-5-Acetic acid, 4-hydroxy-6-Methyl-2-Oxo-	69.00
7	14.74	29929	4.56	6228	4.17	9-/2-(Hydroxymethyl) Cyclopropyl4-1-Oxacyclonon-6-EN-2-One Trimethylsilylderivatives	110.05
8	21.38	26927	4.10	9117	6.10	10 C1c2ope,ta/c4p1ra,-4-car5o612ic aci3, 7-meth12-, meth12 ester	190.00
9	26.30	13893	2.12	4228	2.83	3-Methoxypropanal	59.95
10	26.50	41559	6.33	7134	4.78	Butanoic acid, 3-Methyl-	60.05
11	26.60	57508	8.76	8692	5.82	Cyclobutane, 1,3-Difluoro-1,3-Dimethyl-, Trans-	60.00
12	26.72	53466	8.15	19399	12.99	Do3eca,oic aci3, 2-pe,te,-1-12 ester	68.05
13	28.53	24545	3.74	12583	8.42	Nonanoic acid, Methyl ester	74.05
14	29.44	56313	8.58	16295	10.91	Decanoic acid	73.00
15	43.30	14350	2.19	5795	3.88	4,8-Dimethyl-3(E),7-Nonadienyl thioacetate	69.05
16	44.89	31985	4.87	5373	3.60	Bicyclo/1.1.14Pentane, Ethanone deriv.	123.00
17	45.04	16090	2.45	1375	0.92	2-Propyn-1-one, 1-(2,3-Dimethylbicyclo/2.2.14Hept-2-YL)-, (2-Exo 3-Endo)-(+-.)-	123.05
18	48.59	15659	2.39	5264	3.52	(+)-.Alpha.-Tocopherol	165.05
	TOTAL	656394	100.00	149362	100		

The GC-MS analysis of methanolic bark extract of *C. auriculata* revealed the presence of eighteen compounds (Fig. 2). The identified compounds are given in Table 1.

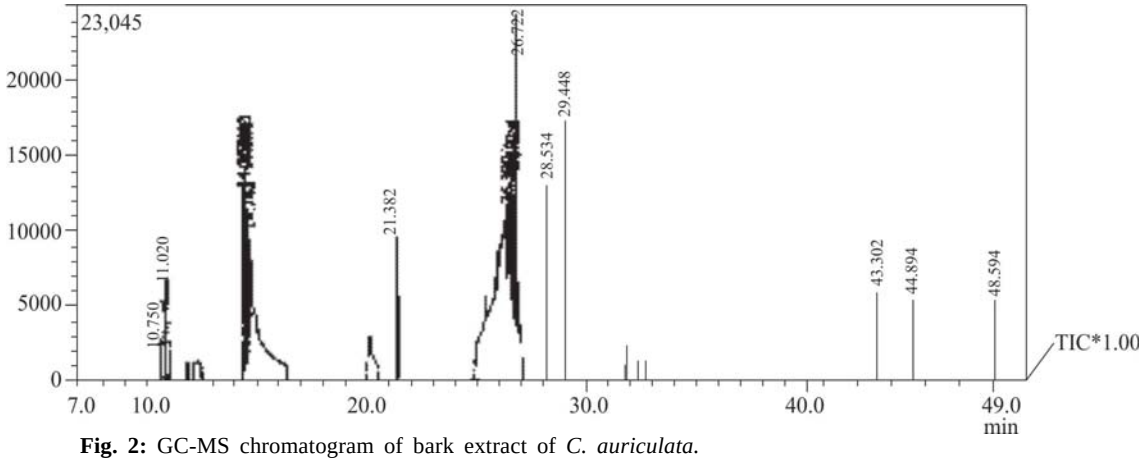

Fig. 2: GC-MS chromatogram of bark extract of *C. auriculata.*

DISCUSSION

The qualitative phytochemical analysis of the bark extract of *C. auriaculata* showed the presence of active phytoconstituents like carbohydrates, glycosides, alkaloids, phenols, flavonoids, tannins and steroids. Similar observations were made on *C. auriculata* by Jyothi & Somashekaraiah (2013). Chemical composition and characterization of floral extract of *C. auriculata* was performed by Ponnusamy & Soundharajan (2014) and their studies showed the presence of total flavonoids and phenol content in the methanolic extract. The present study also revealed the presence of flavonoids and phenols in the bark extract of *C. auriculata.* The plant's defense system for protection against harmful agents has been attributed to the presence of these compounds (Ponnusamy & Soundharajan 2014).

In the present investigation, for the characterization of individual compound, the bark extract was subjected to HPTLC analysis and the presence of caffeic acid was recorded in the bark extract of *C. auriculata* by using HPTLC analysis and this might enhance the effective antioxidant potential of this species.

Rajkumar et al. (2016) recorded eight compounds in ethanol extract of the flowers of *C. auriculata*. The present investigation is in conformity with that of Rajkumar et al. (2016). Kulkarni et al. (2015) recorded ten different phytoconstituents from the methanolic extract of *C. fistula* out of which two compounds are considered as anticancerous agents. In the present study, eighteen compounds were isolated and characterized through GC-MS analysis which would be further tested for antidiabetic and anticancerous properties.The identified compounds may have medicinal value and possess various pharmaceutical applications and need further research on toxicological aspects to develop safer drugs.This type of GC-MS analysis is the first step towards understanding the nature of active principles of medicinal plants. Such studies assist to uncover enigma of nature and facilitate to employ those principles for human welfare.

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REFERENCES

GURUDEEBAN S 2013 *Studies on type II diabetes and formulation of tablets from DNA bar-coded Rhizophora apiculata Blume derived glycosin on streptozotocin-induced diabetic rats: in vivo in silico and molecular approaches* Ph D Thesis Annamalai University

JYOTHI S GOWDA & SOMASHEKARAIAH B VEERABHADRAPPA 2013 Study of in vitro antioxidant activity and HPTLC fingerprint of quercetin in *Cassia auriculata* L *Asian J Plant Sci Res* **3** 162–169

KULKARNI A, GOVINDAPPA M, RAMACHANDRA Y L & KOKA P 2015 GC-MS analysis of methanol extract of *Cassia fistula* and its in vitro anticancer activity on human prostate cancer cell line *Indo Am J Pharm Res* **5** 937–944

PONNUSAMY R D & SOUNDHARAJAN S R 2014 Chemical composition and characterization studies of *Cassia auriculata* flower extract *Int J Pharm Sci* **6** 327–330

RAJ J Y, PETER M P J, & JOY V 2012 Chemical compounds investigation of *Cassia auriculata* seeds A potential folklore medicinal plant *Asian J Plant Sci Res* **2** 187–192

RAJKUMAR P, SELVARAJ S, SUGANYA R, VELMURUGAN D, & KUMARESAN S 2016 GC-MS characterization of the anti-diabetic compounds from the flowers of *Cassia auriculata* (AVARAM): a structure based molecular docking studies *Int J Inn Res Sci Eng Tech* **1** 85–93

SIRAJUDDIN S M 2006 *All India anthropometric survey: Lakshadweep Islands (No. 119)* Anthropological Survey of India Library of Congress New Delhi

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Short commuunication:

ESTIMATION OF GENETIC PARAMETERS OF NINE AGROBOTANIC TRAITS OF *ELEPHANTOPUS SCABER* L. FROM KERALA

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SUMMARY

Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2), genetic gain, genotypic correlation coefficient (r_G) and phenotypic correlation coefficient (r_p) in respect of nine agrobotanic traits of *Elephantopus scaber*, a medicinally important plant, were estimated based on data of 20 accessions of the species occurring in Kerala. Comparing the estimates of GCV and PCV for the nine traits, it was found that the PCV values are consistently dominated, suggesting substantial influence of environment on the expression of these characters. Difference between GCV and PCV is marginal in the case of a few traits such as anther length, ovary length and style length, which is suggestive of negligible influence of environment on them. Fairly high h^2 values were registered for most of the characters, which suggest less influence of the environment. The values of r_G of all the character pairs were higher than their r_ps, which is suggestive of little influence of environmental factors in inhibiting strong inherent relationships between such character pairs.

Keywords: *Elephantopus scaber*, Asteraceae, genetic parameters, GCV, PCV, correlation, heritability, genetic gain.

Elephantopus scaber, a scapigerous herbaceous species of the tribe Vernonieae (Asteraceae), is widely distributed in South India, growing in all ecological niches. In Indian traditional systems of medicine such as Ayurveda and Siddha, the medicinal attributes of this species has been known for a long time, and as per these systems, the roots of this species are used as antipyretic, cardiotonic, and antidiuretic (Sivarajan & Indira Balachandran 2006). The extracts of the whole plant have been used in the folk medicine for treatment of various human ailments. The plant also has antimicrobial and antifungal activities (Hiradeve & Rangari 2015). Phytochemical analyses have shown the presence of chemical constituents like flavonoids, terpenoids, flavonoid esters and sesquiterpene lactones in the plant (Das & Mukherjee 2015). Sesquiterpene lactones are most important due to their antitumor activity. On account of the profound medicinal value, the species is being indiscriminately collected from the wild leading to overexploitation and depletion of this valuable genetic resource. Despite being a priceless medicinal commodity, very little attempt has been made for evaluating the genetic variability of its agrobotanic traits, and estimation of genetic parameters such as genotypic coefficient

of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2), genetic gain, genotypic correlation coefficient (r_G) and phenotypic correlation coefficient (r_p) which are important prerequisites for genetic improvement of the plant. Selection of germplasm depends on discrete knowledge of genetic variability of various plant characters (Mishra et al. 1995). The r_G and r_p are the measures of the degree of closeness of the linear relationship between pairs of variables, of which the former describes the inherent relationship between pairs of variables, while the latter is a measure of modified genotypic expression due to the environment; h^2, in broad sense, is a measure of sets of genes exhibiting dominance, which is useful for judging the expression of a character. Genetic gain provides the degree of gain obtained in a character under a particular selection pressure. This paper concerns the estimation of the aforesaid genetic parameters in regard to nine agrobotanic traits of *E. scaber*.

The plants growing in different ecological niches in the state of Kerala are found to possess immense variability in many of its morphological characters, both qualitative and quantitative. Twenty accessions of the species, five each from four districts such as Thiruvananthapuram, Kollam, Pattanamthitta and Idukki were collected, and they were grown and maintained in uniform garden conditions. Nine quantitative agrobotanic traits such as leaf length, leaf breadth, leaf area, leaf perimeter, petiole length, anther length, ovary length, style length and fruit length were selected for the study (Table 1). The accessions were grown in Randomised Block Design with two replications, and simple random sampling was followed for collecting the data. Five observations were scored for each character from each plant. The GCV and PCV (Burton 1952), r_G and r_p (Snedecor & Cochran 1980), h^2 (Hanson et al. 1956) and genetic gain (Singh & Chaudhary 1977) of the traits were estimated.

The results of GCV, PCV, h^2 and genetic gain of nine agrobotanic traits are furnished in Table 1. The GCV was maximum for leaf area (83.56) and least for style length (31.73). PCV also was

TABLE 1: Genetic parameters of nine agrobotanic traits in 20 accessions of *E. scaber.*

Characters	GCV	PCV	h^2	Genetic gain
Leaf length	35.83	46.82	0.65	64.95
Leaf breadth	44.21	52.31	0.73	81.03
Leaf area	83.56	99.03	0.53	99.01
Leaf perimeter	34.26	43.28	0.62	63.07
Petiole length	46.78	51.28	0.87	97.81
Anther length	33.01	34.05	0.97	71.02
Ovary length	32.97	33.01	0.94	71.53
Style length	31.73	32.98	0.98	68.92
Achene length	34.36	36.01	0.96	70.19

maximum for leaf area (99.03) and minimum for style length (32.98). Genetic gain as per cent mean, was higher for leaf area (99.01) followed by petiole length (97.81), and minimum in leaf perimeter

(63.07). h^2 was higher for style length (0.98) and lower for leaf area (0.53). Comparing the estimates of GCV and PCV for the nine traits, it was found that the PCV value is consistently dominated. The r_G and r_P estimated between and among the nine character combinations, at 5% and 1% levels, are given in Table 2. Notable range exists for both genotypic and phenotypic characters. Among the various

TABLE 2: r_G and r_Ps among nine agrobotanic traits in 20 accessions of *E. scaber.*

Character		Leaf length	Leaf breadth	Leaf area	Leaf perimeter	Petiole length	Anther length	Ovary length	Style length	Achene length
Leaf length	r_G	1.00	0.77**	0.89**	0.98**	0.68**	0.09	0.05	0.47*	0.29
	r_P	1.00	0.74**	0.88**	0.96**	0.60**	0.07	0.04	0.43**	0.25
Leaf breadth	r_G		1.00	0.97**	0.88**	0.88**	0.29	-0.19	0.42*	0.06
	r_P		1.00	0.96**	0.85**	0.74**	0.22	-0.18	0.35*	0.05
Leaf area	r_G			1.00	0.98**	0.73**	0.18	-0.17	0.34	0.08
	r_P			1.00	0.95**	0.71**	0.17	-0.14	0.31*	0.07
Leaf perimeter	r_G				1.00	0.71**	0.18	0.01	0.46*	0.27
	r_P				1.00	0.64**	0.11	0.01	0.46**	0.21
Petiole length	r_G					1.00	0.29	-0.13	0.14	0.03
	r_P					1.00	0.24	-0.11	0.11	0.03
Anther length	r_G						1.00	0.14	0.46**	0.16
	r_P						1.00	0.11	0.43**	0.15
Ovary length	r_G							1.00	0.55**	0.78**
	r_P							1.00	0.54	0.71**
Style length	r_G								1.00	0.49**
	r_P								1.00	0.46**
Achene length	r_G									1.00
	r_P									1.00

r_G= Genotypic correlation coefficient; r_P= Phenotypic correlation coefficient. **1% level of significance; *5% level of significance.

pairs of characters, which exhibited significant positive r_G level (1%), the most significant was that between leaf length with leaf perimeter, and leaf area with leaf perimeter (r = 0.98), followed by leaf breadth with leaf area (r = 0.97). Most significant r_P was between leaf length and leaf perimeter and also between leaf breadth and leaf area (r = 0.96).

In the present study, both the GCV and PCV are maximum for leaf area as against the minimum for style length. For the entire assemblage of agrobotanic traits, the PCV registered higher values than the corresponding GCV, and this is suggestive of substantial influence of environment on the expression of these characters. Similar results were reported in several crop plants like *Piper nigrum* (Mathew et al. 1999), *Mucuna pruriens* (Haridas et al. 2013) and *Cucumis sativus* (Shet et al. 2018). The PCV value for leaf area (99.03) is noticeably higher than its GCV (83.56), which implies high

influence of environment in determining leaf area, while difference between GCV and PCV is marginal in the case of a few traits such as anther length, ovary length, style length and achene length which is suggestive of negligible influence of environment on them, and in these cases, simple selection would be highly effective for further improvement as suggested against similar results in cucumber (Shet et al. 2018). h^2, in broad sense, has important role in determining the heritable portion of variation. Knowledge of h^2 of a trait is an important measure in choosing suitable genotypes for crop improvement, and it gives an insight into the proportion of inherent variation, and it also plays an important role in assessing the relative value of selection (Veena et al. 2012), but Johnson et al. (1955) had shown that h^2 and genetic advance should be jointly considered for reliable conclusion. The present study revealed higher h^2 values for most of the characters, which indicates less influence of environment, and is governed by additive gene effects (Shet et al. 2018). Higher h^2 coupled with higher genetic advance indicated addictive gene action in the control of making these characters to respond better for selection. The r_G values of all the character pairs studied are consistently higher than their r_ps, which suggest little influence of environmental factors in inhibiting strong inherent relationships between such character pairs.

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REFERENCES

BURTON G W 1952 Quantitative inheritance in grasses *Proc of 6th Int grassland Cong* **11** 277–283

DAS M & MUKHERJEE A 2015 Preliminary phytochemical analysis of *Elephantopus scaber* L A wild medicinal plant *Int J Pharm Biosci* **6** 455–461

HANSON H F, ROBINSON H F & COMSTOCK R E 1956 Biometrical studies on yield in segregating population of Korean *Lepedoza Agron J* **48** 268–272

HARIDAS P, RENJITH N B, MATHEW P J, MATHEW P M & VIJAYA RAGHAVA KUMAR 2013 Estimation of genetic parameters of certain agrobotanical characters of *Mucuna pruriens* (L) DC *J Cytol Genet* **14** (NS) 109–113

HIRADEVE S M & RANGARI V D 2015 Antibacterial and antifungal activities of *Elephantopus scaber* L. *Int J Biomed Res* **6** 338–345

JOHNSON H W, ROBINSON J F & COMSTOCK R E 1955 Estimation of genetic and environmental variability in soya bean *Agron J* **7** 314–318

MATHEW P J, MATHEW P M & PUSHPANGADAN P 1999 Genotypic and phenotypic correlations and heritability of certain quantitative characters in *Piper nigrum* L *J Cytol Genet* **34** 87–90

MISHRA K K, RAI P W & JAISWAL H R 1995 Performance of popular clones under Tarai conditions of Uttar Pradesh *Indian Forester* **127** 1155–1157

SHET R M, SHANTAPPA T, ASHOK & GURUMURTHY S B 2018 Genetic variability and correlation studies for productivity traits in cucumber *Cucumis sativus* L *Int J Chem Stud* **6** 236–238

SINGH R K & CHAUDHARY B D 1977 *Biometrical methods in quantitative genetical analysis* Kalyani Publishers New Delhi

SIVARAJAN V V & INDIRA BALACHANDRAN 2006 *Ayurvedic drugs and their plant sources* Oxford and IBH Publishing Co New Delhi

SNEDECOR & COCHRAN W G 1980 *Statistical methods* 7th ed Iowa State University Press Ames

VEENA R, AMRIK SINGH, SIDHU PITCHAIMUTHU M & SOURAVI K 2012 Genetic evaluation of cucumber *Cucumis sativus* L genotypes for some yield and related traits *EJPB* **3** 945–948

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Short commuunication:

CHROMOSOME STUDIES ON TWO GALL FORMING APHID SPECIES INFESTING *PISTACIA INTEGERRIMA*

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SUMMARY

Chromosomes of two species of gall forming aphids namely, *Geoica utricularia* **(**Passerini**)** and *Forda hirsuta* Mordvilko infesting *Pistacia integerrima* J. L. Stewart from Mandi district of Himachal Pradesh were analysed. Both the species have revealed the diploid chromosome number of 18. The chromosomes were holocentric and their lengths were measured at metaphase and the total complement length as well as relative lengths of chromosomes were calculated. Idiograms were constructed based on relative length data.

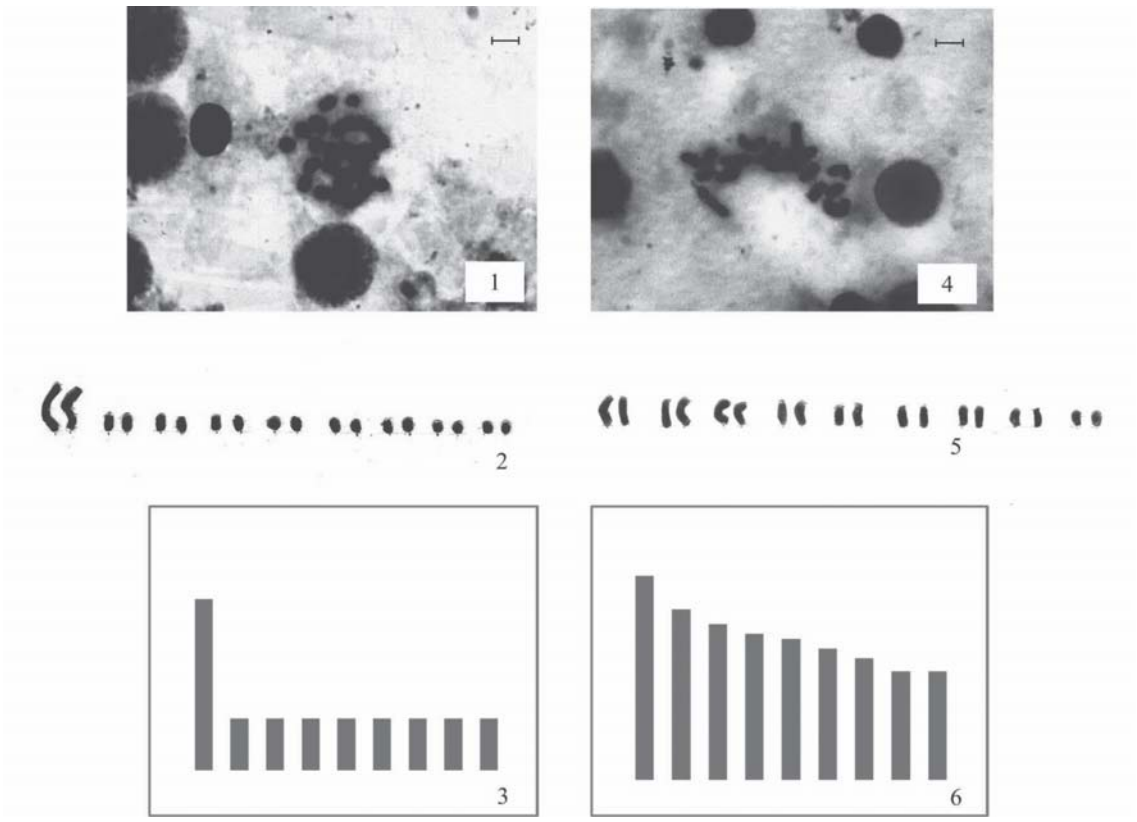
Keywords: Holocentric chromosomes, gall aphid, karyotype.

Aphids are soft bodied insects that cause damage to plants by sucking the plant sap from phloem and also by transmitting a number of plant virus diseases (Kennedy et al. 1962). At present, ca.5000 valid species of aphids are reported from all over the world (Favret 2015). Of these, about 700 form galls on coniferous and deciduous trees (Blackman & Eastop 1994). More than 250 aphid species are considered as serious pests of horticultural and agricultural crops the world over (Blackman & Eastop 1984). Ghosh et al. (1981) reported 27 gall forming aphid species of the subfamily Pemphiginae from India. *Pistacia integerrima* J. L. Stewart is infested by two species of gall forming aphids, *Geoica utricularia* **(**Passerini**)** and *Forda hirsuta* Mordvilko. We report here our findings on the chromosome number and morphology in these species.

The aphids were collected from galls on the leaves of *P. integerrima* a well known medicinal plant from Mandi district of Himachal Pradesh (31.10°N latitude and 77.17°E longitude). For chromosomal study, only apterous, parthenogenetic and viviparous females were used. The embryos were taken out by puncturing the posterior end of abdomen with the help of a needle. They were pretreated with 0.7% sodium citrate solution for 30 min and fixed in 1:3 acetic alcohol for about 15–20 min and placed on a glass slide in a drop of 45% acetic acid for 3–5 min. Staining was done in 2% Giemsa solution for 15–30 min. A cover slip was put on the material with one edge extended outside the slide and was tapped gently and pressed in between the folds of filter paper which absorbed excess of stain. Cover slip was dislodged off the slide with a sudden jerk using sharp edge of a razor blade.

Slide and cover slip were then washed with distilled water and dried at room temperature and made permanent after dipping in xylene and then mounting in DPX. Slides were observed under binocular research microscope and photomicrographs of well spread metaphase plates were taken and selected for measurements of chromosome length using ocular micrometer. From actual lengths, the total complement length (TCL) and relative lengths of chromosomes were calculated based on which the idiograms were constructed for each species.

In both the species studied here the chromosomes are holocentric and their diploid chromosome number was found to be 18 (Figs 1, 4). In *G. utricularia*, the mean length of chromosomes ranged from 1.27 µm to 4.02 µm with TCL being 28.33 µm. The relative lengths of chromosomes ranged from 4.42 to 14.67 (Figs 2, 3). In *F. hirsuta*, the mean actual length of chromosomes ranged from 1.64 µm to 3.13 µm with TCL being 39.37 µm. The relative lengths of chromosomes ranged from 4.21 to 7.92 (Figs 5, 6).



Figs 1–6: Cytology of aphids. 1–3. *G. utricularia*. 1. Metaphase plate. 2. Karyotype. 3. Idiogram. 4–6. *F. hirsuta*. 4. Metaphase plate. 5. Karyotype. 6. Idiogram. (Scale = 5µm)

Gall forming aphids have wide range of chromosome numbers from 2n = 6 to 38 (Blackman & Eastop 1994). Both the species included in the present study showed 2n = 18 confirming the earlier reports of Blackman (1980, 1987). However, Blackman (1980) also reported 2n = 16 and 17 in addition to 18 in *G. utricularia*.

Blackman & Eastop (1994) reported chromosome numbers in other gall forming species of *Forda*, *F. formacaria*, *F. marginata* and *F. riccobonii* whose diploid chromosome number ranging from 17 to 23. Whereas *F. formacaria* showed 2n = 18–23, *F. marginata* has diploid number ranging from 17 to 20. However, *F. riccobonii* has diploid chromosome number of 2n = 18. Thus, the earlier chromosome number reports in both the species reveal certain deviation from the present findings of 2n = 18 which is consistently seen in the two species examined here (Blackman 1980, Blackman & Eastop 1994).

REFFERENCES

BLACKMAN R L 1980 Chromosome number in the Aphididae and their taxonomic significance *Syst Entomol* **5** 7–25

BLACKMAN R L 1987 Chromosome of *Forda Genetica* 7**4** 81–88

BLACKMAN R L & EASTOP V F 1984 *Aphids on the World's Crops: An Identification and Information Guide* John Wiley and Sons Chichester London

BLACKMAN R L & EASTOP V F 1994 *Aphids on World's Trees: An Identification and Information Guide* CAB International Wallingford

FAVRET C 2015 Aphid species file version 5 0/5 0 http://Aphidspeciesfile.org

GHOSH A K, CHAKRABARTI S & BHATTACHARYA D K 1981 Galls of Pemphiginae (Homoptera : Aphidoidea) in the Indian region with description of a new species *Bull Zool Surv India* **4** 319–330

KENNEDY J S, DAY M F & EASTOP V F 1962 *A conspectus of aphids as vectors of plant viruses* Commonwealth Institute of Entomology London

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