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Structure and Behaviour of Chromosomes in *Piper* and *Peperomia* (Family Piperaceae)

Joseph Jose and Arun Kumar Sharma

Centre for Advanced Study in Cell and Chromosome Research,
Department of Botany, University of Calcutta,
35 Ballygunj Circular Road, Calcutta 700019, India

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The name Piperaceae seems to have been introduced by Rich (1815) in Humboldt, Bonpland and Kunth's *Nova genera et species plantarum* (Yuncker 1958). It is widely distributed in the tropics and subtropics with major concentrations in Latin America and Malaya Peninsula. Linnaeus (1753) recognized 17 species comprising the genus *Piper* while Ruiz and Pavon (1794) introduced *Peperomia* as the second genus. Additional genera were included by various authors during the subsequent century. Miquel (1843–44) subdivided the family into two tribes *Peperomeae* with 5 genera and 209 species and *Pipereae* with 15 genera and 304 species. De Candolle (1869) recognized a little over 1000 species among the two genera *Piper* and *Peperomia*. According to Rendle (1956), this family comprises two larger genera, *Piper* with over 700 species and *Peperomia* with more than 600 species in addition to seven smaller genera. However, according to Lawrence (1957), the family consists of 10–12 genera including the two larger ones *Piper* and *Peperomia*. Burger (1977) has also arrived at a similar conclusion. Thus the grouping of various genera and species under the family even today remains inconclusive.

The family Piperaceae stands close to the monocotyledonous boundary (Burger 1977) with certain unique features among dicotyledonous plants having both an inner and an outer rings of separate vascular bundles arranged variously in the axis (Metcalfe and Chalk 1950).

For the most part, *Piper* and *Peperomia* occupy more or less similar distributional patterns. However, there are certain areas where *Peperomia* abounds but where *Piper* is lacking. In India species of *Piper* predominate over those of *Peperomia* almost to the tune of 5: 1. *Peperomia* differs from other members of the family in having a constant number of two stamens which is considered to be an advanced character, while the distinct unicarpellate pistil points to a primitive simple flower (Tucker 1980). In addition, *Peperomia* stands apart within the family on the basis of anatomy (Datta and Dasgupta 1977a, 1977b, 1977c, 1979, Novak 1954, Rousseau 1927); embryology (Maheshwari and Kapil 1966); palynology (Erdtman 1952) and pharmaco-anatomy (Masao and Miyagawa 1976). Several authors (Airy Shaw 1973, Smith 1972) have given *Peperomia* the rank of family, based on the overall totality of characters. Burger (1981, personal communication) also favours such a view. None of these authors, however, have used cytological or phytochemical data in arriving at this conclusion. Further no systematic and detailed research effort has so far been directed towards the study of chromosome constitution of the two genera *Piper* and *Peperomia* with the aid of improved techniques, thus keeping an important lacuna in planning of any significant conclusion of the disputed systematic position of the two genera. In recent years the importance of study of cytology is being more and more appreciated in evolving a natural and phylogenetic system of classification (Babcock *et al.* 1937, Chaudhuri and Sharma 1978, 1979, Sharma 1960, 1967, Sharma and Bal 1956, Sharma and Bhattacharyya 1956).

Paucity of cytological data in the family Piperaceae may be largely due to the extremely small size but high number of chromosomes, interclonal variations and very heavy cytoplasmic contents making critical analysis difficult and time consuming (Dasgupta and Datta 1976, Jose 1981, Mathew 1958, Sharma and Bhattacharyya 1959). The present study aims to bridge the gap through a comprehensive investigation on the somatic chromosomes of fifty three taxa, including eight species of *Piper* and nine species of *Peperomia*. Improved techniques have been adopted (Sharma and Sharma 1980) with a view to understand the number, structure and behaviour of their chromosomes in relation to the systematic position of *Peperomia* within the family.

Materials and methods

Fifty three taxa of the genera *Piper* and *Peperomia*, bleonging to the family Piperaceae, have been investigated (Fig. 1).

For cytological study temporary aceto-orcein squash preparations were used. Actively growing young root tips from pot grown plants were pretreated in a mixture of saturated solution of para-dichlorobenzene and aesculin (1:1) with a pinch of saponin at 0–5°C for 3–5 minutes and then at 14–15°C for 3½ hours. They were fixed in acetic acid—ethyl alcohol (1:2) for 1 to 2 hours, then treated with acidulated alcohol (1:3) for 5 minutes. However in the genus *Peperomia* pretreatment has been done with saturated mixture of paradichlorobenzene and 0.002 M oxyquinoline solutions (1:1) for 3 hours. They were fixed in acetic acid–ethyl alcohol (1:2) for 1 to 2 hours. The usual schedule for aceto-orcein staining was followed.

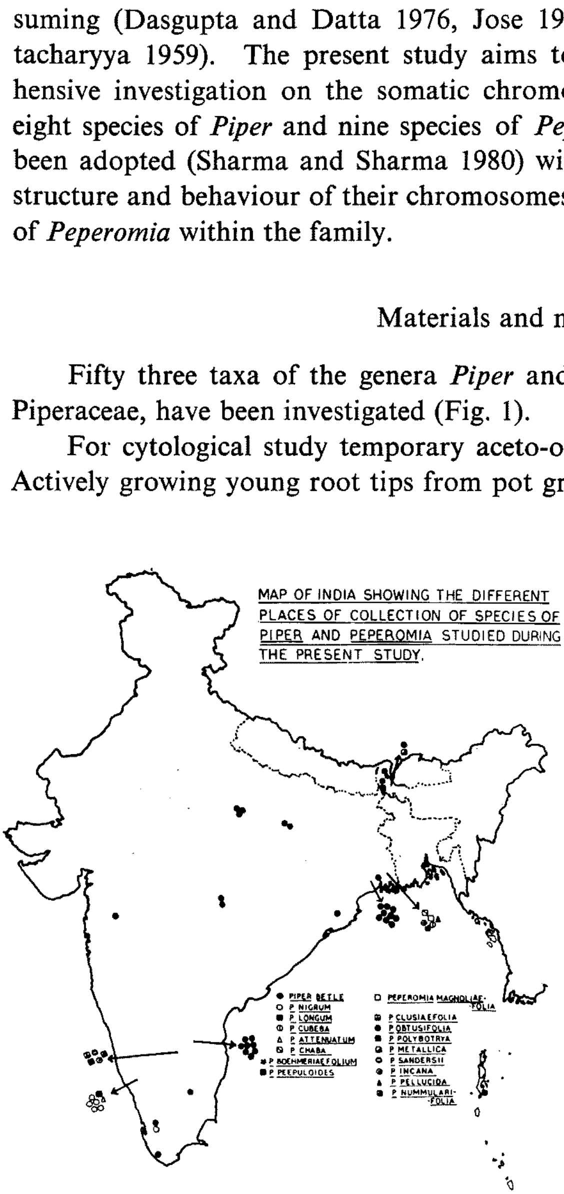

Fig. 1. Map of India showing the different places of collection of species and varieties of *Piper* and *Peperomia* studied during the present investigation.

Observations

In the present investigation 53 taxa including eight species of

Piper and nine species of *Peperomia* have been studied. The normal somatic chromosome number ranges from 2n=24 to 195 in *Piper*, whereas in *Peperomia* it ranges from 2n=22 to 44. Except for *Piper cubeba* L. f. (3.33 μ to 1.40 μ) the morphology of the chromosome in all the species of *Piper* shows a homogeneity in the extremely short size (2.41 μ to 0.56 μ). The chromosomes decrease in size progressively and bear nearly median or nearly submedian primary constriction. However, in *Peperomia* the chromosomes are longer (6.67 μ to 1.30 μ) with almost nearly median to nearly submedian primary constrictions. In karyotypes, ratio of the short arm to the total length of chromosome in percentage or F% was calculated to classify the nature of primary constrictions, given as follows:

Table 1. Determination of the centromeric position by the centromeric index or F%

$$\left(F\% = \frac{\text{Short arm length}}{\text{Total chromosome length}} \times 100\right)$$

F%	50	37.5–49.9	25.1–37.4	25.0	18.6–24.9	12.6–18.5	12.5
Centromeric position	Median	Nearly median	Nearly submedian	Sub-median	Nearly submedian	Nearly sub-terminal	Sub-terminal

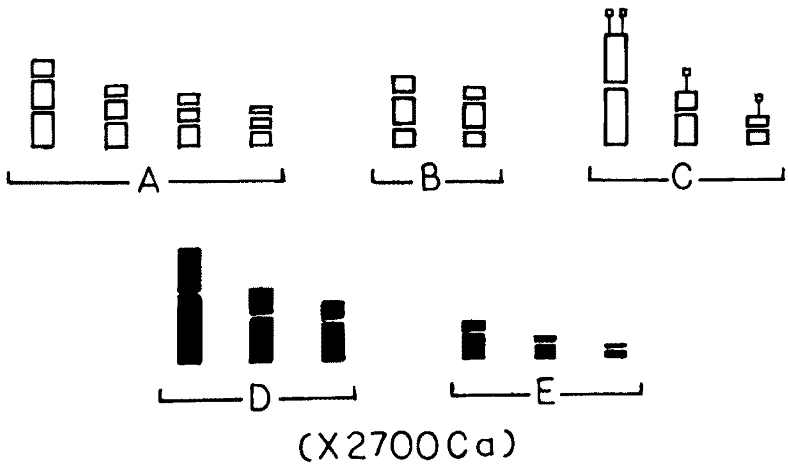

Fig.2. Diagrammatic representation of different chromosome types in different species and varieties of *Piper* and *Peperomia* investigated during the present study.

A general description of the chromosome types is given below, followed by a karyotype description for individual species and varieties (Fig. 2).

Type A—Comparatively long chromosome with two constrictions, one median and the other nearly median in position (4.81 μ to 1.48 μ).

Type B—Comparatively long chromosome with two constrictions, primary and secondary, nearly submedian at the opposite ends of chromosome dividing it into two outer short and one middle larger segments (3.33 μ to 2.41 μ).

Type C—Comparatively long chromosome with nearly median to nearly submedian primary constriction and a satellite at the distal end of shorter arm, joined by a SAT thread (6.67 μ to 1.48 μ).

Table 2. Comparison of the somatic chromosomes of the species and varieties of *Piper* and *Peperomia* investigated

Name	2n	Somatic variation nuclei (2)	Karyotype formula	Chromosome pairs with secondary constriction	Range of chromosome length (μ)	Total chromosome length (μ)	Total short arm length (μ)	T.F. value (%)
(1)	(1a)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Piper betle Linn.								
〃 var. *Jhol Bangla*	78	42, 52, 70, 72, 76, 143	$A_6C_2E_{70}$	4	1.85–0.55	78.70	28.15	35.76
〃 var. *Pullykody*	78	—	A_6E_{72}	3	1.67–0.56	77.96	27.40	35.15
〃 var. *Sanchipan*	78	29	A_4E_{74}	2	1.85–0.56	79.62	29.26	36.74
〃 var. *Ranipan*	58	26, 33, 52	A_4E_{54}	2	2.04–0.56	57.40	20.56	35.81
〃 var. *Karpurkanthi*	42	46, 48	A_4E_{38}	2	2.22–0.56	49.62	17.96	36.19
〃 var. *Metha* cum *Bangla*	78	—	A_6E_{72}	3	2.22–0.74	85.92	30.93	35.99
〃 var. *Metha Thackpala*	78	—	A_6E_{72}	3	2.04–0.56	73.34	27.22	37.12
〃 var. *Metha Gathpala*	78	54, 58, 64, 68, 72, 76	A_6E_{72}	3	1.85–0.74	92.96	34.81	37.45
〃 var. *Bangla*	78	26, 52	A_4E_{74}	2	2.41–0.74	86.30	31.30	36.27
〃 var. *Gonogathe*	78	64	A_4E_{74}	2	2.22–0.74	86.30	31.67	36.70
〃 var. *Hajurikiamali*	78	—	A_4E_{74}	2	2.04–0.74	80.74	29.81	36.93
〃 var. *Desi-Mahoba*	78	66	A_4E_{74}	2	2.04–0.74	79.62	29.44	36.98
〃 var. *Desi-Bangla*	78	—	A_4E_{74}	2	1.67–0.56	68.88	25.93	37.63
〃 var. *Kali Bangla*	78	—	A_8E_{70}	4	1.85–0.74	74.82	27.03	36.14
〃 var. *Sanchi Banaras*	78	52, 58	A_4E_{74}	2	1.85–0.65	72.40	27.22	37.60
〃 var. *Sadadohl*	78	64	A_4E_{74}	2	1.85–0.74	75.92	28.15	37.10
〃 var. *Belhari*	78	—	A_4E_{74}	2	1.85–0.74	80.00	30.00	37.50
〃 var. *Gachpan*	78	54, 64, 72, 76	A_4E_{74}	2	1.85–0.56	75.92	28.33	37.32
〃 var. *Green*	78	66	A_6E_{72}	3	1.85–0.74	76.66	28.15	36.71
〃 var. *Nagoli*	78	—	A_8E_{70}	4	1.85–0.74	80.74	29.26	36.24
〃 var. *Kare*	78	—	A_6E_{72}	3	2.41–0.74	84.82	29.81	35.15
〃 var. *Ramtek Bangla*	78	—	A_4E_{74}	2	1.85–0.74	85.56	31.67	37.01
〃 var. *Kuljedu*	78	72	A_4E_{74}	2	2.04–0.74	74.82	27.60	36.88
〃 var. *Kapoori-Pune*	78	—	A_4E_{74}	2	2.04–0.74	77.78	28.89	37.14

〃 var. *Bangla Banarasi*	78	—	A_4E_{74}	2	2.04–0.74	75.92	28.33	37.32
〃 var. *Bhabna*	78	72	A_4E_{74}	2	2.22–0.74	79.26	29.44	37.15
〃 var. *Ramtek Kapoori*	52	58, 72	A_4E_{48}	2	1.85–0.56	48.52	17.78	36.64
〃 var. *Malvi*	78	—	A_6E_{72}	3	2.22–0.74	88.14	32.03	36.34
〃 var. *Karpuri*	78	—	A_6E_{72}	3	2.04–0.74	84.44	30.37	35.96
〃 var. *Deswari*	78	—	A_6E_{72}	3	2.04–0.74	91.12	32.78	35.98
〃 var. *Andaman-wild*	52	—	A_4E_{48}	2	1.85–0.74	53.70	19.44	36.21
〃 var. *Gottipan-wild*	52	39, 54	A_4E_{48}	2	1.67–0.56	46.30	16.85	36.40
〃 var. *Chava-wild*	195	52	A_4E_{191}	2	1.48–0.56	173.88	65.02	37.38
〃 var. *Panniyur*-1	52	32, 39, 44, 53	A_4E_{48}	2	2.22–0.74	67.04	24.26	36.19
〃 var. *Cheriakaniakadan*	52	—	A_2E_{50}	1	1.67–0.74	51.48	18.89	36.69
〃 var. *Karimunda*	52	26	A_4E_{48}	2	2.04–0.74	56.66	20.74	36.60
Piper nigrum Linn.								
〃 var. *Wil*	104	17, 26, 33, 44	A_4E_{100}	2	1.85–0.56	103.34	38.89	37.63
P. longum Linn.	52	26	A_4E_{48}	2	1.85–0.74	55.56	20.19	36.33
P. cubeba L.f.	24	—	$B_8D_2E_{14}$	4	3.33–1.48	52.60	15.37	29.23
P. attenuatum Ham.	52	—	$A_4C_2E_{46}$	3	1.90–0.74	66.02	23.39	35.43
P. chaba Hunter.	104	26, 52, 64, 76	A_4E_{100}	2	1.48–0.56	96.66	36.11	37.36
P. boehmeriaefolium Wall.	52	46	$A_2C_2E_{48}$	2	2.04–0.74	61.48	22.22	36.14
P. peepuloides Roxb.	156	44, 130, 143	A_4E_{152}	2	1.67–0.74	151.86	57.03	37.56
Peperomia magnoliaefolia A. Dietr.	22	—	$A_2D_{12}E_8$	1	3.33–1.85	50.00	17.96	35.93
P. clusiaefolia Hook.	22	—	$A_2D_{18}E_2$	1	4.10–2.04	61.12	23.34	38.18
P. obtusifolia A. Dietr.	22	—	A_4D_{18}	2	3.70–2.41	65.92	25.19	38.20
P. obtusifolia variegata A. Dietr.	22	6	$A_2C_2D_{14}E_4$	2	3.15–1.85	55.92	20.19	36.09
P. polybotrya H.B.K.	22	—	$A_4C_2D_8E_8$	3	3.52–1.30	52.22	17.41	33.33
P. metallica Linden & Rodig.	22	44	$A_4D_{10}E_8$	2	2.96–1.67	48.14	18.15	37.69
P. sandersii C. DC.	22	—	$C_2A_4D_4E_{12}$	3	2.96–1.48	46.30	15.56	33.60
P. incana A. Dietr.	22	—	A_4E_{18}	2	2.96–1.48	41.12	15.19	36.94
P. nummularifolia H.B.K.	23	46	$C_2A_2D_{19}$	2	6.67–2.59	96.30	30.19	31.35
P. pellucida H.B.K.	44	24, 29, 33, 38, 48, 77	A_4D_{40}	2	4.81–2.22	139.26	52.96	38.03

Type D—Relatively long chromosome (5.74 μ to 2.22 μ) with nearly median to nearly submedian primary constrictions.

Type E—Medium to short chromosome (2.04 μ to 0.56 μ) with nearly median to nearly submedian primary constrictions.

Using this classification the relevant karyomorphological data are summarized in Table 2.

Discussion

i) *Chromosome numbers in the genera studied*

In the present study two different genera have been investigated including eight species of *Piper* and nine of *Peperomia*. In the genus *Piper*, thirty three varieties of *Piper betle* Linn. and four varieties of *Piper nigrum* Linn. have been included within the scope of the present investigation. In *P. betle* Linn. majority of the varieties show the chromosome number of 2n=78 (Table 2). In general *P. betle* Linn. represents a polyploid species of which certain varieties show higher or lower levels of polyploid series with a basic set of 13 chromosomes. It is remarkable that, inspite of vegetative reproduction, there is constancy in the chromosome number in majority of the varieties. However numerical variations within the same individual have been noted in several varieties. Moreover, the occurrence of certain varieties with the number other than 78 may be attributed to such somatic variants playing an effective role in the origin of new genetic strains through their participation of the formation of daughter shoots. As far as other species of *Piper* are concerned excepting *P. cubeba* L.f. where the chromosome number is 2n=24, the rest show multiples of 13 chromosomes (Table 2). Therefore, taken as a whole, the genus *Piper* is a homogeneous assemblage in which the number n=13 is a deep-seated one and it has been maintained through human selection from forms which have arisen by cryptic genic changes or minor structural alteration of chromosomes, propagated through vegetative means. However, in *P. cubeba* L.f. the haploid chromosome number is 12. Evidences, however, show that *P. cubeba* L. f. n=12 may represent a comparatively primitive number from which n=13 might have been evolved.

In the genus *Peperomia* the chromosome number 2n=22 has been noted in 8 taxa. *P. pellucida* H.B.K., the common weed, is a tetraploid with 2n=44 as the normal somatic chromosome number. Moreover, numerical variations within the same individual have been recorded in *P. pellucida* H.B.K. *P. nummularifolia* H.B.K. on the other hand, has 2n=23 chromosomes, evidently a derivative of 2n=22. The constancy of the chromosome number in the genus *Peperomia* may be taken as an index of the homogeneity in which n=11 chromosomes is deep-seated.

ii) *Structural patterns of chromosomes in Piper and Peperomia*

As far as the chromosome structure is concerned all species and varieties of *Piper* except *P. cubeba* L.f., show a gross uniformity in the karyotype. The chromosomes, in general, are medium sized to small in a graded karyotype. The number of chromosomes with secondary constriction varies from 1 to 4 pairs. It appears that the genus *Piper* as a whole, more or less, represents a homogeneous assemblage where gene mutation or imperceptible chromosome changes, if any, have affected the evolution both at interstrain and interspecific levels. Occasional polyploidy and structural alteration involving principally chromosomes with secondary constriction have taken place.

However, in *Piper cubeba* L.f. not only there is a difference in their chromosome number n=12, but there has been an overall increase in the chromosome size also. The total chromatin length with 2n=24 chromosome complement is almost equal to *P. nigrum* Linn. or other species and varieties of *Piper* showing 2n=52 chromosomes. The number of chromosomes with secondary constriction too is four pairs, though the morphology is more or less identical with other species of *Piper*. It appears, from both chromosome size and number taken in conjunction with the karyotype, that *P. cubeba* L.f. may represent a primitive species of *Piper*.

The karyotype of *Peperomia* is characterised by relatively larger chromosomes than the genus *Piper* and the karyotype is more or less symmetrical. The number of secondary constrictions or satellite varies from 2 to 6. Even at the diploid level the species differ in minute details of karyotype. This may suggest that the genus *Peperomia* is homogeneous (Table 2), yet minute structural alterations affecting the chromosomes with secondary constrictions have been effective in evolution.

iii) *Systematic status of the two genera*

The present inverstigation therefore shows that the basic chromosome number, the karyotype and the chromosome size of the genus *Peperomia* are quite different from the genus *Piper*. There are contradictory opinions with regard to the systematic positions of the two genera. Rendle (1956) placed them under one family. The same stand has been taken by Hutchinson (1973). Smith (1972) as well as Airy Shaw (1973), on the other hand, placed *Piper* and *Peperomia* under two different families as Piperaceae and Peperomiaceae under the Order Piperales.

Erdtman (1952) recorded that pollen grains of *Peperomia* are non-aperturate and quite distinct from *Piper*. On the basis of anatomy, morphology, embryology and floral biology, the genera *Piper* and *Peperomia* are quite distinct. In the genus *Peperomia*, the constant reduction of the floral type, the presence of a single integument round the ovule, an apparently reduced stem structure, which also shows a remarkable departure from the normal course in the development of the female gametophyte, indicate its specialised status. The usual polarity in the embryo-sac is absent. The first four nuclei produced are large and arranged tetrahedrally. From these 16 parietal nuclei are formed, one of which at the micropylar end forms the egg, an adjoining one functions to form the primary endosperm nucleus. The remaining preserve their parietal position and are finally cut off by walls and do not in any way suggest antipodal cells (Rendle 1956).

Maheshwari and Kapil (1966) consider that *Peperomia* forms a connecting link between Saururaceae and Piperaceae. In the order Piperales, Smith (1972) recognized three families–Saururaceae, Piperaceae and Peperomiaceae. Burger (1977) indicated that *Peperomia* stands apart within the family on the basis of anatomy, embryology and palynology. Datta and Dasgupta (1977a) suggested that the lateral walls of metaxylem tracheary elements and distribution of bundles of *Peperomia* indicate its primitive status and distinct character, supporting the separation of Peperomiaceae from Piperacese as initially done by Novak (1954). The multi-circular medullary bundle is also another characteristic feature of *Peperomia*. Tucker (1980), however, suggests that the extensive variation of floral development in *Peperomia* may indicate genetic plasticity and its advanced nature. Burger (1981, personal communication) has also stated that *Peperomia* represents a primitive status and the separation of the two genera into two families is justified.

Evidences from anatomy, embryology, palynology, floral biology, etc. so far obtained, favour the separation of *Peperomia* from Piperaceae and give a family status as Peperomiaceae. They also show the primitiveness of *Peperomia*.

The cytological data obtained during the present investigation where the chromosomes have been worked out with the aid of improved techiques, are also quite relevant as regards the deep-seated number n=11 in *Peperomia*, less than that of *Piper*. Chromosomes are comparatively larger and more symmetrical than those of *Piper*. These evidences clearly indicate the distinctness of the two genera and comparatively primitive status of the former. These evidences taken in conjunction with the data obtained from anatomy, morphology, palynology, embryology and floral biology, indicate that the separation of the two families under Piperales as done by certain taxonomists is justified with *Peperomia* representing a primitive state in evolution.

Summary

Fifty three taxa have been studied during the present investigation, including eight species of *Piper* and nine of *Peperomia*. In *Piper* chromosome numbers ranging from 2n=24 to 195 have been found and polyploidy has been shown to be an important factor in evolution. The species of *Piper* studied here have multiples of 13, except *P. cubeba* L.f. which is deep-seated for the genus. However in *P. cubeba* L.f. n=12 chromosomes may represent the basic set from which n=13 might have been derived and this number became deep-seated in the species of *Piper*, possibly due to selective advantage. All the species of *Piper* except *P. cubeba* L.f. show a gross uniformity in the karyotype. However, differences in the number of nucleolar chromosomes have been recorded.

In *Peperomia*, eight taxa have shown 2n=22 chromosomes, whereas in *P. nummularifolia* H.B.K. and *P. pellucida* H.B.K. 2n=23 and 2n=44 chromosomes are seen respectively. In the genus *Peperomia* the basic set of n=11 chromosomes is pronounced. The karyotype is characterised by relatively larger chromosomes than that of the genus *Piper*. The species of *Peperomia* differ in minute details of karyotype, suggesting the role of minute structural alterations in evolution.

Cytologically the two genera *Peperomia* and *Piper* are quite distinct from each other. These evidences taken in conjunction with the data obtained from anatomy, morphology, palynology, embryology and floral biology, support the separation of the two genera into two different families under Piperales as Peperomiaceae and Piperaceae, as done by certain taxonomists, with *Peperomia* repre-

senting a primitive state in evolution.

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