Cytotaxonomy of Piperaceae

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Of the two genera of Piperaceae (*Piper* and *Peperomia*), the former may be more primitive (Bentham and Hooker 1880, De Dalla Torre and Harms 1900–1907, Hutchinson 1969). According to Rousseau (1927), *Piper* is the basic group from which *Chloranthus* and *Saururus* (commonly placed in distinct families) are derived by minor modification, while *Peperomia* has evolved by major changes. Anatomical study (Metcalfe and Chalk 1950) suggest *Piper* to be most specialized.

Study of karyotype is an important field of research for understanding interrelations and evolutionary trends (Darlington 1937, White 1945, Babcock, Stebbins and Jenkins 1937, Love 1946, Kapoor and Love 1970, Asana and Adatia 1945, Bowden 1945, Tobgy 1943, Stebbins 1964, 1966, Sharma 1960). A few authors (Smith 1966, Mathew 1958, Sharma and Bhattacharyya 1959, Blot 1960) have studied the karyology of the family with special attention to phylogeny. Solution of phylogenetic problems requires further comparative study of chromosome morphology.

Material and methods

For the study of somatic chromosomes, root tips, collected from cuttings, planted in the experimental garden of the University, were pre-treated in aesculin (Sharma and Sarkar 1955) for one to three hours, fixed in acetic alcohol (1: 1) for 45 minutes to one hour, treated in acetic acid (45%) for 15 minutes and squashed in aceto-orcein technique. Proper plates (about five for each species) were selected and drawn with camera lucida, and oil immersion lens at a projected magnification of 2600. Species include:

Name	*Collector, Source*
Piper betle L. cultivar. *jhal pan*	Sen 1 (Pi), CU
P. betle L. cultivar. *mitha pan*	Sen 10 (Pi), CU
P. cubeba L.f.	Sen 2 (Pi), CU
P. longum L.	Datta 3 (Pi), CU
P. magnificum Trel.	Sen 4 (Pi), CU
P. nigrum L. (Agartala variety)	Datta 6 (Pi), CU
P. nigrum L. (South India)	Sen 5 (Pi), CU
Peperomia argyria E. Morr.	Sen 12 (Pep), CU
Pep. metallica Lindl. Rodig.	Sen 8 (Pep), CU
Pep. obtusifolia A. Dietr.	Sen 7 (Pep), CU
Pep. pellucida H.B.K.	Sen 9 (Pep), CU

Observations

On the basis of positions of constrictions and lengths, the chromosomes may be classified into the following types (Fig. 1):

Type A (2.3–3.7 μ) Two constrictions forming three segments, the middle being longest.

Type B (1.5–2.7 μ) Two constrictions forming three segments, all equal.

Type C (1.9–2.7 μ) Two constrictions forming three segments, one of the terminals being shortest, others equal.

Type D (1.9–2.3 μ) Two constrictions forming three segments, the middle being shortest.

Type E (0.77–2.7 μ) A submedian constriction.

Type F (0.77–2.3 μ) A median constriction.

On the basis of the above description of chromosome types, the karyotype of the different species may be described in the following way:

Piper betle L. *jhal pan* (2n=64). Length of the chromosome varies from 0.77 to 1.5 μ. Karyotype is represented as 4B, 56E and 4F, of which the two pairs of B bear secondary constrictions (Fig. 2).

P. betle L. *mitha pan* (2n=64). Length of the chromosome varies from 0.77 to 1.2 μ. Karyotype is represented as 6B, 36E and 22F, of which three pairs of B bear secondary constrictions (Fig. 3).

Piper cubeba Linn. f. (2n=24). Length of chromosomes varies from 0.77 to 2.5 μ. The karyotype is represented as 6B, 14E, 4F, of which three pairs of B bear secondary constriction (Fig. 4).

Piper longum L. (2n=48). Length of the chromosomes varies from 0.77 to 1.5 μ. The karyotype may be represented as 2B, 36E and 10F. Of these, one pair of B bears secondary constriction (Fig. 5).

Piper magnificum Trelease. (2n=24). Length of the chromosome varies from 1.2 to 3.7 μ. The karyotype consists of 4A, 2C and 18E, of which, two pairs of A and one pair of C bear secondary constriction (Fig. 6).

P. nigrum L. Agartala (2n=36). Lengths of the chromosome varies from 0.77 to 1.9 μ. The karyopype is represented as 2A, 4B, 26E and 4F. Of these, one pair of A and two pairs of B bear secondary constriction (Fig. 7).

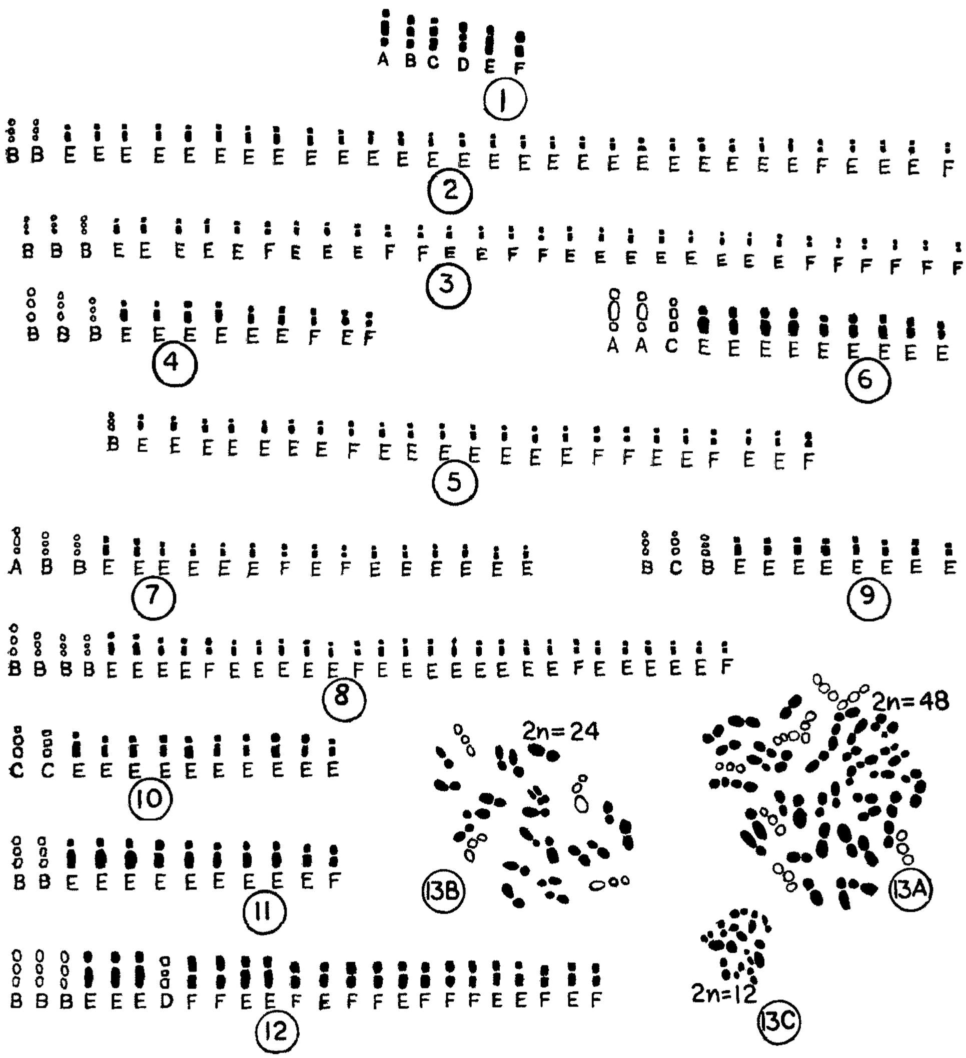
P. nigrum L. South India (2n=60). Length of the chromosomes varies from 0.77 to 2.3 μ. The karyotype may be represented as 8B, 44E and 8F. Of these types, four pairs of B bear secondary constriction (Fig. 8).

Peperomia argyreia E. Morr. (2n=22). Length of chromosomes varies from 0.77 to 1.9 μ. The karyotype has 4B, 2C and 16E chromosomes. Two pairs of B and one pair of C bear secondary constrictions (Fig. 9).

Peperomia metallica Lindl. Rodig. (2n=24). Length of the chromosomes varies from 1.1 to 2.3 μ. The karyotype has 4C and 20E chromosomes. Of these two pairs of C bear secondary constriction (Fig. 10).

P. obtusifolia C. DC. (2n=24). Length of the chromosomes varies from 1.5 to 2.6 μ. The karyotype may be represented as 4B, 18E and 2F. Of these two pairs of B bear secondary constriction (Fig. 11).

P. pellucida H.B.K. (2n=48). Length of the chromosomes varies from 1.9 to 2.7 μ. The karyotype is represented as 6B, 2D, 20E and 20F. Of these three pairs of B and one pair of D bear secondary constrictions (Fig. 12). In addition to this common pattern, variation plates showing 24 and 12 chromosomes, were encountered (Fig. 13).



Figs. 1–13. 1, types of chromosomes found in Piperaceae. 2–12, idiogram of *Piper betle* (*jhal pan*), *P. betle* (*mitha pan*), *P. cubeba*, *P. longum*, *P. magnificum*, *P. nigrum* (Agartala), *P. nigrum* (South India), *Peperomia argyreia*, *Pep. metallica*, *Pep. obtusifolia* and *Pep. pellucida*. 13A,B,C, somatic metaphase plates of *Peperomia pellucida* showing variation of chromosome number.

Table 1 represents the details of the karyotype of the species. The total chromosome length (haploids) of each species has been shown in the table and in Fig. 14.

Table 2 represents an upto date record of reported chromosome numbers of the family, for comparison.

Table 1. Details of karyotype study

Species	*Piper betle Jhal pan*	*Piper betle* var. *Mitha pan*	*Piper cubeba*	*Piper longum*	*Piper magnificum*	*Piper nigrum* (Agar. var.)	*Piper nigrum* (S. Ind. var.)	*Peperomia argyreia*	*Peperomia metallica*	*Peperomia obtusifolia*	*Peperomia pellucida*
Chromosome number	2n=64	2n=64	2n=24	2n=48	2n=24	2n=36	2n=60	2n=22	2n=24	2n=24	2n=48
Chromosome types	4B, 56E, 4F	6B, 22F, 36E	6B, 14E, 4F	2B, 36E, 10F	4A, 18E, 2C	2A, 4B, 26E, 4F	8B, 44E, 8F	4B, 16E, 2C	4C, 20E	4B, 18E, 2F	6B, 20E, 2D,20F
Length groups	4 long, 18 M. 42 Short	10 long, 4 M. 50 Short	6 long, 8 M. 8 S.M. 2 Short	4 M. 44 Short	6 long, 8 S.M. 8 M. 2 Short	6 long, 4 M. 24 Short 2 S.T.	12 long, 38 M. 12 Short	6 long, 12 M. 4 Short	6 long, 16 M. 2 Short	10 long, 14 M.	22 long, 26 M.
Total chromosome length (n) μ	30	39	18	29	24	14	36	16	19	24	49
Mean length per chromosome	0.905	1.21	1.50	1.20	2.0	0.77	1.20	1.45	1.57	2.0	2.04
Position of primary constriction	4 S.T. 56 S.M. 4 M.	6 S.T. 22 M. 36 S.M.	14 S.M. 4 M. 4 S.T.	2 S.T. 36 S.M. 10 M.	18 S.M. 6 S.T.	6 S.T. 2 M. 28 S.M.	8 S.T. 44 S.M. 8 M.	16 S.M. 6 S.T.	4 S.T. 20 S.M.	18 S.M. 2 M. 4 S.T.	20 M. 20 S.M. 8 S.T.
Range of chromosome length, μ	1.5–0.77	1.1–0.77	2.3–0.77	1.5–0.77	3.1–1.2	1.9–0.77	2.3–0.77	1.99–0.77	2.3–1.1	2.6–1.5	2.7–1.9
No. of secondary constriction	4	6	6	2	6	6	8	6	4	4	8
Length of chromosome types, μ — A	—	—	—	—	3.1	1.9	—	—	—	—	—
B	1.5	1.1	2.5–2.3	1.5	—	1.5	2.3–1.9	1.9–1.5	—	2.6	2.7
C	—	—	—	—	2.3	—	—	1.9	2.3–1.9	—	—
D	—	—	—	—	—	—	—	—	—	—	2.3
E	1.1–0.77	1.1–0.77	1.5–1.1	1.1	1.9, 1.5, 1.2	1.5–0.77	1.9–0.77	1.5–0.77	1.9–1.1	2.6–1.9	2.7, 2.3, 1.9, 2.3
F	0.77	0.77	0.77	1.1	—	0.77	1.1–0.77	—	—	1.9	2.3, 1.9, 1.5

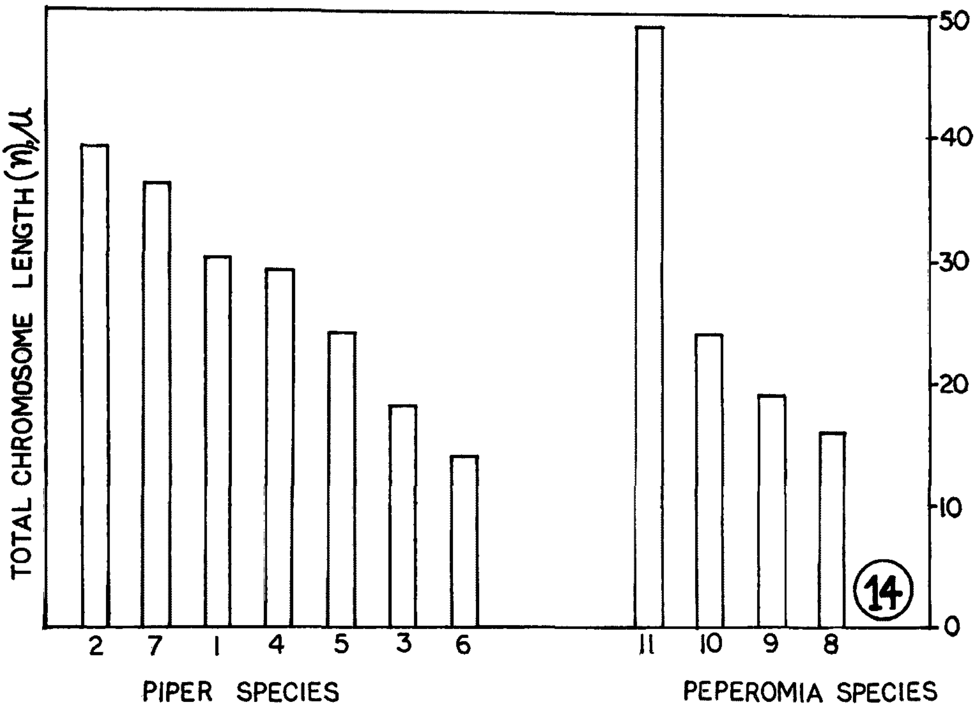


Fig. 14. Histogram showing total chromosome length (haploid) of each species (numbered). 1, *Piper betle* (*jhal pan*). 2, *P. betle* (*mitha pan*). 3, *P. cubeba*. 4, *P. longum*. 5, *P. magnificum*. 6, *P. nigrum* (Agartala). 7, *P. nigrum* (South India). 8, *Pep. argyreia*. 9, *Pep. metallica*. 10, *Pep. obtusifolia*. 11, *Peperomia pellucida*.

Table 2. Reported chromosome number

Species	*n*	2*n*	Author	Date
1. *Piper betle* Linn.		64	Sharma & Bhattacharyya	1956
2. *Piper betle* Linn.		78	Mathew	1958
3. *Piper betle* Linn.		32	Johnson	1910
4. *Piper betle* Linn.		64	Present report	1970
5. *P. cubeba*		24	Janaki-Ammal	1955
6. *P. cubeba*		24	Present report	1970
7. *P. futokazura* Sub. et Zuce.		24	Yoshida	1960
8. *Piper geniculatum*		28	Maugini	1950
9. *P. grissico-argenta* Yunku		22	Smith	1966
10. *P. longum*		24	Tjio	1948
		24,48,96	Sharma & Bhattacharyya	1959
		48	Present report	1970
	26	52	Mathew	1958
11. *P. medium*		28	Maugini	1953
12. *P. magnificum* Trelease		26	Smith	1966
		24	Present report	1970
13. *P. nigrum* Linn.		C. 128	Janaki Ammal	1955
		48	Sharma & Bhattacharyya	1959
	26	52, 104	Mathew	1958
		36, 60	Present report	1970

Table 2 (Cont'd)

	Species	*n*	2*n*	Author	Date
		26	52	Martin & Greg.	1962
14.	*P. ornatum* N.E.Br.		80	Sharma & Bhattacharyya	1959
15.	*P. subpeltatum* (Umbellatum)		24	Johansen	1931
			Peperomia		
1.	*Peperomia argyroneura* E. Morr.		22	Sharma & Bhattacharyya	1959
2.	*P. argyreia* E. Morr.		22	Smith	1966
3.	*Peperomia argyreia*		22	Present report	1972
4.	*P. arifolia* Miq.		24	Blot	1960
5.	*P. blanda* H.B.K.		C. 32	Blot	1960
6.	*P. baronii* Baker		44	Smith	1966
7.	*P. camptotucha* Miq.		24	Blot	1960
8.	*P. caperata* Yuncker		33	Smith	1966
9.	*P. clusiifolia* (Jacq.) Hooker		22	Smith	1966
10.	*P. dolaberiformis* H.B.K.		22	Smith	1966
11.	*P. eekana* C. DC.		36	Skottsberg	1955
12.	*P. erythroclada* C. DC.		28	Skottsberg	1955
13.	*P. elrunea* Sodiro		24	Blot	1960
14.	*P. hedraefolia* Hort.		24	Blot	1960
15.	*P. hawiiensis* C. DC.		42, 46	Skottsberg	1955
16.	*P. hesperomanii* Waura		48, 66	Skottsberg	1955
17.	*P. hilli* Trel n=36		24	Mathew	1958
18.	*P. incana* A. Dietr.		24	Blot	1960
19.	*P. incana*		22	Smith	1966
20.	*P. japonica* Makino		44	Smith	1966
21.	*P. lilifolia* C. DC.		42	Skottsberg	1955
22.	*P. maculosa*		44	Martinoli	1948
23.	*P. maculosa* L. (Hooker)		22	Smith	1966
24.	*P. metallica*		22	Sharma & Bhattacharyya	1959
25.	*P. metallica*		22	Present report	1970
26.	*P. metallica* Lindl. and Rodig.		32	Blot	1960
27.	*P. magnoliaefolia* (Jacq.) Dietr.		12	Blot	1960
28.	*P. nivalis* Miq.		22	Smith	1966
29.	*P. obtusifolia* C. DC.		24	Blot	1960
30.	*P. obtusifolia* (L.) A. Dietr.		22	Smith	1966
31.	*P. obtusifolia*		24	Present report	1970
32.	*P. pellucida*		C. 24	Johnson	1914
33.	*P. pellucida* H.B.K.		44	Harvey	1966
34.	*P. pellucida*		24, 48	Present report	1970
35.	*P. resediflora*		24	Hauser	1916
36.	*P. reflexa* A. Dietr.		C. 40	Blot	1960
37.	*P. rubescens* C. DC.	11	22	Blot	1960
38.	*P. rhombea* Rez and Pav.		44	Smith	1966
39.	*P. rotundifolia* (L.) H.B.K.		22	Smith	1966
40.	*P. sandersii* (arifolia)		24	Sugiura	1936
41.	*P. sandersii*	11	22	Mathew	1958
42.	*P. scandens* R. and Par.		C. 60	Blot	1960
43.	*P. sintenisii*		16	Brown	1908
44.	*P. urocarpa* Fisch and Mey		44	Smith	1966
45.	*Peperomia nerschaffeltii*		24	Abele	1923
46.	*P. verticillata* (L.) Dietr.				

Table 2 (Cont'd)

Species	*n*	2*n*	Author	Date
=(*P. pulchella*) Dietr.	12	24	Blot	1960
47. *P. velutina* Lind. and Andre		22	Smith	1966
48. *P. nictoriana* C. DC.		22	Smith	1966
49. *P. zalioides* H.B.K.		22	Huynh	1965

Discussion

Trends of karyological specialization

From a glance on the chromosome numbers in Piperaceae (Table 2) it is evident that the number is very variable in this family. Similar numbers occur in both the genera and their numerical distinction is difficult. Though nearly 2000 species are in records, the chromosome numbers of only 64 species, including the present reports, are in literature. The numbers occur as multiples of 8, 11, 12, 13 and 14, of which multiples of 11 (42.06%) and 12 (40.47%) are most frequent. *Piper* contains multiples of 12 and *Peperomia* multiples of 11 in most of their species.

According to Mathew (1958) and Sharma and Bhattacharyya (1959), 11 is the basic number for *Peperomia*. But for the occurrence of other numbers, Smith (1966) suggests the possibility of more than one basic number. From the primary basic number 11, secondary basic numbers of the genus can evolve. Numbers 12 and 14 may arise by addition and 8 by loss of certain chromosomes through aneuploidy. Sharma and Bhattacharyya (1959) suggest 12 as the basic number in *Piper*. Mathew (1958) and Smith (1966), on the contrary, recorded $x=13$ as the basic number for the genus. The table shows that nearly 47.3% of the species of *Piper* have $x=12$ and only 19.04% have $x=13$. Thus it is more possible that from 12, the other basic numbers have evolved.

Change of chromosome number in evolution seems very natural in these two genera, for occurrence of chromosomal biotypes in many species (for example—*Peperomia hawiiensis, P. hesperomannii, Piper longum, P. nigrum, Peperomia pellucida*, etc., Table 1). Numerical change in chromosomes may arise from aneusomatic cells (Sharma 1956, Sharma and Datta 1960, Datta 1971) or meiotic aneuploidy or parthenogenesis. Variation of somatic chromosome number is common in the root tips of *Peperomia pellucida* (vide text), a sexually reproducing species. Whether such variation in premeiotic cells, in reproductive organs, occur, is worth examination.

Mean lengths of chromosomes in individual species of *Peperomia* vary insignificantly. The total chromosome length is related to polyploidy. The trend of evolution in this genus is thus probably related to the numerical change of chromosomes and constrictions. If $x=11$ is regarded as the basic stock, *Pep. obtusifolia* and *Pep. metallica* have evolved by formation of a new basic number ($x=12$). *Pep. pellucida* shows the same basic number ($x=12$); but for its tetraploidy, the species appears as further evolved. The longest chromosome (mean length), generally median primary constrictions and the shortest range of variation in chromosome length in *Pep. pellucida*, indicate that the species is a primitive one. Increase in chromosome lengths is of course, often related to the evolutionary process (Sharma and Bhattacharyya 1959). The highest number of secondary constrictions in *Pep. pellucida* (8) is actually the exact double of the two diploid species, *Pep. metallica* and *Pep. obtusifolia*, and is unrelated to structural alteration, which is more marked in *Pep. argyreia*. Probably, the genus has two trends, one of multiplication (as in *Pep. pellucida*) and the other of structural alteration, evidenced by increased number of secondary constrictions and decrease of chromosome length (as in *Pep. argyreia* of the basic stock).

In the genus *Piper*, polyploidy (vide Table 2) is associated with a marked diminution in chromosome length (Table 1), while diploidy is related to longer chromosomes. *Piper cubeba* (2n=24) and *P. magnificum* (2n=24) seem to be primitive for their lowest chromosome number and longest individual chromosomes (mean length 1.5 and 2.0 μ, respectively). *P. longum*, though tetraploid, is also a less specialized species than others for moderately long chromosomes, only a single pair of secondary constrictions, and mostly median primary constrictions. Probably, *P. cubeba, P. magnificum* and *P. longum* (all having x=12) from a common stalk, more primitive than others. Two varieties of *Piper nigrum* (2n=36, 60) are comparatively advanced for shorter chromosomes, higher number of secondary constriction and higher degree of polyploidy (triploidy and pentaploidy), probably balanced secondarily. *P. betle* (2n=64) seems to represent a different line of evolution having x=8 or 16. Both appear as more specialized than the basic group of *P. cubeba*, etc., for shorter chromosomes, fewer long chromosomes per genome and high degree of polyploidy. From the chromosome length, and number of long chromosomes and median constrictions, the variety *mitha pan* seems to be more primitive than the *jhal pan*. The decrease in the number of secondary constriction in *jhal pan* may be related to deletion of chromosome ends, resulting in diminution of length.

In the whole family, majority of the recorded chromosome numbers represent x=11 (42.06%), next being x=12 (40.47%). The remaining 17.47% include other basic numbers. But the frequency of 11 and 12 are so near, that it is impossible to derive one from the other. Slightly higher frequency of the basic number 11, supports, though weakly, the conclusion of Rousseau (1927), that *Peperomia* is more primitive than *Piper*, but not that of Engler (1894) and Bentham and Hooker (1880).

Summary

From a comparison of the chromosome numbers of different species, it appears that 11 is the basic number of *Peperomia* and 12 of *Piper*. Variation in the reports of chromosome number of the same species may be due to occurrence of chromosomal biotypes.

The trend of evolution in the two genera (*Peperomia* and *Piper*) appear different, numerical and structural change in the former, and polyploidy in the latter. The basic number (x=11) is represented by *Peperomia argyreia*, where structural alteration is clear. x×12 is represented by *Pep. metallica, Pep. obtusifolia* and

Pep. pellucida, polyploidy occurring only in the last one.

Piper cubeba (2n=24), *P. magnificum* (2n=24) and *P. longum* (2n=48), probably form a basic group of *Piper*. *P. nigrum* (2n=36, 60) is more advanced in chromosome structure. *P. betle* (2n=64) represents a different line having x=8 or 16.

Records of chromosome number suggest that *Peperomia* is more primitive than *Piper*.

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