FURTHER INVESTIGATIONS ON CYTOLOGY OF MEMBERS OF COMMELINACEAE WITH SPECIAL REFERENCE TO THE ROLE OF POLYPLOIDY AND THE ORIGIN OF ECOTYPES

By ARUN KUMAR SHARMA and ARCHANA SHARMA

Cytogenetics Laboratory, Botany Department, Calcutta University, Calcutta-19, India

(*Received 14 March, 1957*)

Introduction

Previous records have shown the possibility of the occurrence of natural cytological variants in several species of the family Commelinaceae. In an earlier communication by the author, the investigations were carried out chiefly on materials collected from the plains. Hence a study of the different ecotypes within the same species could not be made.

The reports of certain rare cases and the unusual cytological data, presented formerly by the senior author (Sharma ,1955) distinctly indicated the scope of cytological study of ecotypes within this family. In Indian species too, the role of polyploidy and aneuploidy in the evolution of different forms was clearly indicated. The most interesting fact emerging from this paper was the evidence of the presence of individuals with different chromosome numbers in the same species.

Encouraged by these results, the different ecotypes, as well as other species, occurring in the temperate and sub-temperate zone of the Himalayas, were collected. The aim in view was to make a thorough study of the detailed cytology of these different ecotypes and species. The project was materially helped by the invention of a number of improved techniques, recently devised from this Laboratory, critical enough to bring out even the minute chromosomal details.

In the present report, cytological data of eleven different species and ecotypes have been incorporated. This study clearly demonstrates the role of polyploidy in bringing about the diversification of the members of this family.

Materials

The following plants have been used for the present investigation :

1. *Aneilema herbaceum* Wall. $2n = 40$;
2. *Aneilema spiratum* R.Br. $2n = 20$;
3. *Aneilema vaginatum* R.Br. $2n = 40$;
4. *Cyanotis barbata* Don. $2n = 24$;
5. *Commelina nudiflora* L., Type I $2n = 28$;
6. *Commelina nudiflora* L., Type II $2n = 56$;

7. *Commelina sikkimensis* Clarke — $2n = 60$;
8. *Commelina obliqua* Ham., Type I — $2n = 45$;
9. *Commelina obliqua* Ham., Type II — $2n = 60$;
10. *Commelina obliqua* Ham., Type III — $2n = 60$;
11. *Streptolirion volubile* Edgew. — $2n = 12$.

Of these, some plants were collected from the forests of Khumani and Rongo, at the base of the Himalayas, at altitudes of 2000 to 5000 feet above sea-level. The remaining plants were collected from the forests near Darjeeling and Sinchal, at altitudes ranging from 6000 to 7000 feet. In general, though differing in preference of habitat, they grow in slightly cool areas.

The plants were identified with the help of the authorities at the Herbarium, Lloyd Botanical Gardens, Darjeeling.

Methods

For a study of somatic division, healthy root-tips were collected from the nodes of the plants. The root-tips were fixed in Lewitsky's fluid (Chromic-formalin 1 : 2) for the preparation of paraffin blocks. Sections were cut 16μ thick and Newton's crystal violet staining schedule was followed with a mordanting treatment in 1% Chromic acid overnight prior to staining.

In certain cases, aceto-orcein squashes of the root-tips were made following paradichlorobenzene treatment (Sharma & Mookerjea, 1955), to obtain well-scattered metaphase plates.

For a study of meiosis, flower-buds were fixed in Nawaschin's fluid after pretreatment in Semmen's Carnoy's fluid. Paraffin sections 16μ thick were cut and stained following Newton's Crystal violet schedule.

The drawings were made at a Leitz Ortholux microscope with a 1·3 N.A. Fluorite objective and X12 compensating eyepiece at a table magnification ×2500 approximately.

In the drawings, chromosomes, bearing secondary constrictions or satellites, have been drawn in outline.

Observations

Genus—*Aneilema*

Three species belonging to this genus were worked out. The chromosome numbers are twenty in one and forty in the other two. The chromosomes, on an average, are short in size. The complement of *Aneilema vaginatum* is different from the other two in that it shows marked and abrupt size difference amongst its chromosomes.

1. *Aneilema herbaceum* Wall. ($2n = 40$)

Description. Stem erect, stout; leaves large with rounded base, blue flowers borne in stout panicles with branches spreading and ascending. The plants were collected from areas near Rongo, at 4000 to 5000 feet.

The somatic cells of the species show the presence of forty chromosomes in the complement, as studied from a number of metaphase plates.

The chromosomes, in general, are short in size. Size difference amongst the chromosomes is present, though not very marked, ranging from 1·2μ to 3·2μ.

The chromosomes can be divided into three general groups on the basis of their size :

1. Six pairs of comparatively long chromosomes,
2. Twelve pairs of comparatively medium-sized chromosomes, and
3. Two pairs of short chromosomes.

Of these, eight chromosomes are seen to bear secondary constrictions.

A detailed study of the morphology divides the chromosomes into the following types (Figs. 1 and 1a) :

1. Two pairs of comparatively long chromosomes, each having two constrictions, primary and secondary, one nearly median in position and the other located nearly at the middle of the slightly longer arm (A).
2. Two pairs of comparatively long chromosomes, each having a nearly median primary constriction and a satellite at the end of one of the arms (B).
3. Two pairs of comparatively long chromosomes with nearly submedian primary constrictions (C).
4. Twelve pairs of comparatively medium-sized chromosomes, with median primary constrictions (D).
5. Two pairs of short chromosomes with median primary constrictions (E).

2. *A spiratum* R. Br. ($2n = 20$)

Description : Dwarf prostrate plants; branches short with small sessile leaves; small blue flowers borne in terminal or axillary cymes. The plants were collected from Darjeeling at 6000 ft.

The normal somatic complement is found to contain twenty chromosomes. However, cells having varying numbers are also on record.

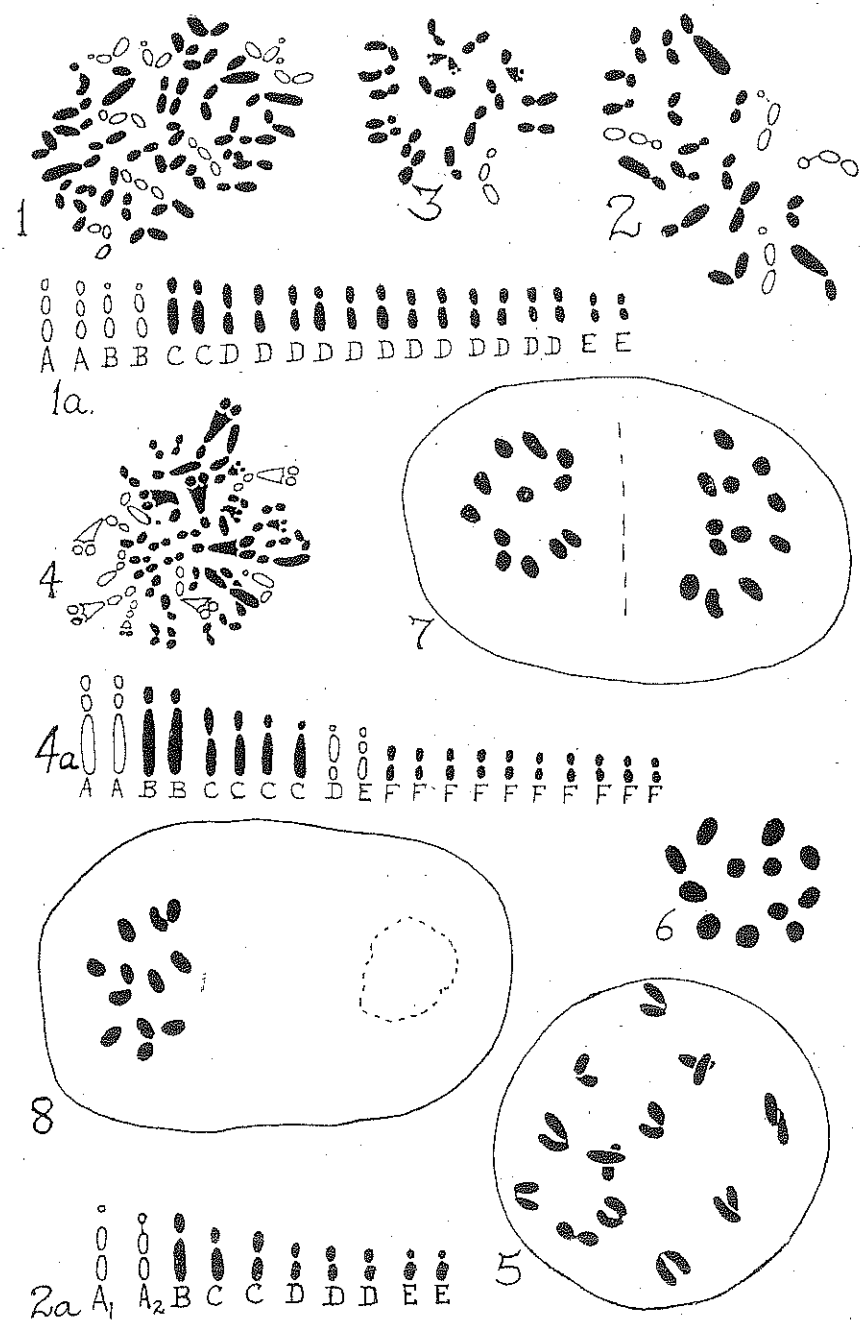
The chromosomes, in general, are short in size. Size difference among them is quite marked ranging from 1·2μ to 3·6μ. On its basis they can be divided into the following groups :

1. Three pairs of comparatively long chromosomes.
2. Two pairs of comparatively medium-sized chromosomes, and
3. Five pairs of short chromosomes.

Four chromosomes are seen to bear secondary constrictions.

A study of the positions of the constrictions, together with the size, divides the chromosomes into the following types (Figs. 2 and 2a) :

1. Two pairs of long chromosomes, each having a nearly median primary constriction and a satellite at the end of one of the arms. In one pair (A_2) the satellites are attached by satellite threads, while in the other (A_1), the threads are absent.
2. A pair of long chromosomes with nearly submedian primary constrictions (B).
3. Two pairs of medium-sized chromosomes with median to submedian primary constrictions (C).
4. Three pairs of short chromosomes with median primary constrictions (D).
5. Two pairs of short chromosomes with nearly submedian primary constrictions (E).



Figs. 1 and 1a. *Aneilema herbaceum* normal somatic metaphase ($2n = 40$) and idiogram respectively.

Fig. 2, 2a and 3. *A. spiratum*, normal somatic metaphase ($2n = 20$), idiogram and variation metaphase with 18 chromosomes respectively.

Figs. 4–4a. *A. vaginatum* normal somatic metaphase ($2n = 40$) idiogram respectively.

Figs. 5–8. *Cyanotis barbata* ($2n = 24$)—meiotic stages (vide text).

In addition to the normal complement, somatic nuclei having eighteen chromosomes are also on record (Fig. 3). Here only one chromosome of type A is present. This set has probably arisen from the normal one through the loss of two satellited chromosomes by non-disjunction and amphiplasty of another satellited member.

3. *Aneilema vaginatum* R. Br.

Description: Long slender stem with suberect branches; leaves long, linear; blue flowers with pedicels twice jointed in the middle, in axils of erect lanceolate bracts.

The plants were collected from wet localities near Khumani, about 1000 to 2000 feet above sea-level.

Forty chromosomes are present in the normal somatic complement of the species. The chromosomes are medium to short in size in general.

Size difference among the chromosomes is very marked, ranging from 1·2μ to 5·3μ. On its basis the chromosomes can be divided into the following three distinct groups :

1. Four pairs of comparatively long chromosomes.
2. Six pairs of comparatively medium-sized chromosomes, and,
3. Ten pairs of short chromosomes. Eight of these are seen to bear secondary constrictions.

A detailed study of the morphology, together with the relative size, divides the chromosomes into the following types (Figs. 4 and 4a) :

1. Two pairs of comparatively long chromosomes, each having two constrictions, primary and secondary, one nearly submedian in position and the other located in the middle of the shorter arm (A).
2. Two pairs of comparatively long chromosomes with submedian primary constrictions (B).
3. Four pairs of comparatively medium-sized chromosomes with median to submedian primary constrictions (C).
4. A pair of comparatively medium-sized chromosomes, each with a submedian primary constriction and a satellite at the end of the longer arm (D).
5. A pair of comparatively medium-sized chromosomes, each having two constrictions, primary and secondary, one median and the other submedian in position (E).
6. Ten pairs of comparatively short chromosomes with median primary constrictions (F).

GENUS—*Cyanotis*

Only one species could be studied.

4. *Cyanotis barbata* Don.

Description: Stems slender, branched, creeping; leaves sessile, ciliate, cobwebby beneath; bracts much longer than the scorpioid cymes.

The plant was collected near Rongo at an altitude of 4000 feet.

The somatic complement could not be studied due to the scarcity of healthy root-tips. Meiotic studies showed the $2n$ number to be twenty-four. Meiosis was found to be

regular with the formation of twelve bivalents at diakinesis and first metaphase (Figs. 5 & 6) and clear twelve and twelve segregation at second metaphase (Figs. 7 & 8).

GENUS—*Commelina*

Six distinct types, distributed amongst three species, of this genus were collected from different localities.

The chromosomes, on an average, are of medium size.

a. *Commelina nudiflora* L.

Description : Creeping herbs with long leaves having acute apices; blue flowers borne in cymose inflorescence enclosed in funnel-shaped spathe.

5. *C. nudiflora* L., Type I ($3n = 28$)

These plants were collected from wet localities near the forests of Khumani at an altitude of 1000 to 2000 feet.

The somatic complement of the species is composed of twenty-eight chromosomes. The chromosomes, on an average, are of medium size. Size difference is not very marked, the length of the chromosomes ranging from $3{\cdot}2\mu$ to $0{\cdot}6\mu$. Secondary constrictions could not be brought out in the preparations.

The chromosomes of this type can be classified as follows (Fig. 9) :

1. Two pairs of comparatively long chromosomes with nearly submedian primary constrictions.
2. Two pairs of comparatively long chromosomes with median primary constrictions.
3. Ten pairs of chromosomes, grading from medium-sized to short, with median to submedian primary constrictions.

6. *C. nudiflora* L., Type II ($2n = 56$)

These plants were collected from near Kurseong hills, at an altitude of nearly 5000 feet above sea-level. The individuals show markedly longer leaves and larger flowers than those of Type I.

The normal somatic cells are found to bear fifty-six chromosomes. However, the number of twenty-eight has also been recorded.

The chromosomes are, on an average, shorter than those of Type I, presumably due to polyploidy. Size difference among the chromosomes is present, though not marked, ranging from $3{\cdot}6\mu$ to $1{\cdot}5\mu$. On the basis of the size, they can be divided into the following general groups :

1. Four pairs of comparatively long chromosomes.
2. Twenty-two pairs of comparatively medium-sized chromosomes, and
3. Two pairs of short chromosomes.

Of these, eight are seen to bear secondary constrictions.

A detailed study of the morphology, taken together with the relative size, divides the chromosomes into the following types (Figs. 10 and 10a);

1. A pair of comparatively long chromosomes, each having two constrictions, primary and secondary, located at submedian positions at opposite ends of the chromosome (A).

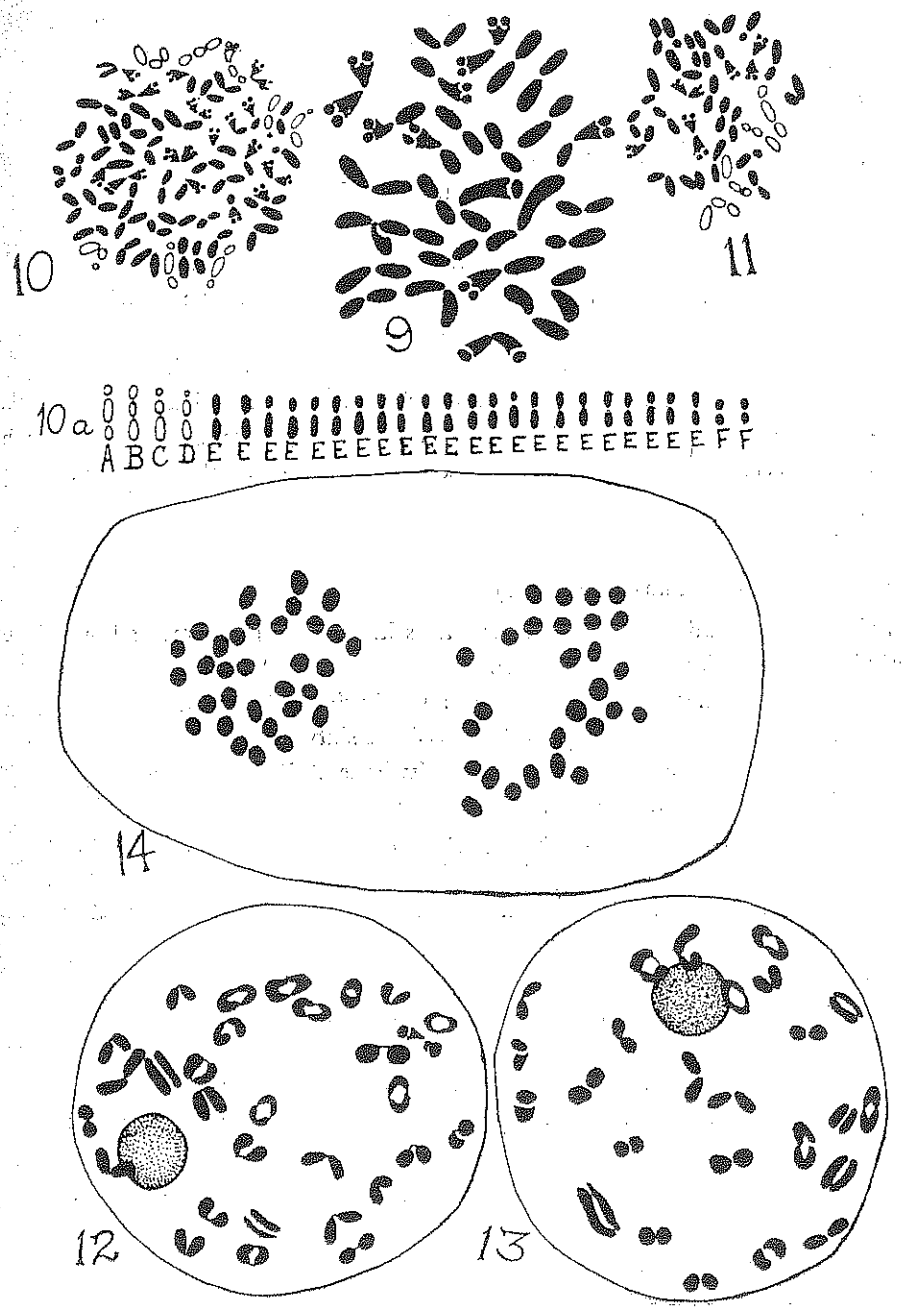


Fig. 9. *Commelina nudiflora*. Type I—normal somatic metaphase showing 28 chromosomes.

Figs. 10, 10a and 11. *C. nudiflora*, Type II—normal somatic metaphase ($2n = 56$), idiogram and variation nucleus with 28 chromosomes respectively.

Figs. 12–14. *C. sikkimensis* ($2n = 60$)—Meiotic stages showing thirty and twenty-five bivalents at diakinesis and 28 and 32 chromosomes at daughter cells in Metaphase II, respectively.

2. A pair of comparatively long chromosomes, each having two constrictions, primary and secondary, one nearly median in position and the other located at the middle of the slightly *longer* arm (B).
3. A pair of comparatively long chromosomes, each having two constrictions, primary and secondary, one nearly median in position and the other located in a nearly submedian position at the distal end of the slightly *shorter* arm (C).
4. A pair of comparatively long chromosomes, each having a nearly median primary constriction and a satellite at the end of one of the arms (D).
5. Twenty-two pairs of comparatively medium-sized chromosomes with median to submedian primary constrictions (E).
6. Two pairs of short chromosomes with median primary constrictions (F).

In addition to the normal complement described above, a variant metaphase with twenty-eight chromosomes (Fig. 11) is also on record. The chromosomes, four of which bear secondary constrictions, possibly represent the haploid set of the normal complement.

7. *Commelina sikkimensis* Clarke ($2n = 60$)

Description : Slender, creeping stem; leaves lanceolate; blue flowers borne in bifid cymes with peduncled, acuminate spathe.

The plants were collected near Rongo, at an altitude of 4500 feet.

The normal chromosome number of the species is sixty.

Meiotic studies show the presence of thirty bivalents at diakinesis (Fig. 12). Abnormal behaviour is occasionally recorded in the formation of twenty-five bivalents during diakinesis in certain pollen mother cells (Fig. 13). Such abnormality in number possibly arises from disturbances in premeiotic mitosis. Irregularity in segregation is seen during Metaphase II, such as separation into thirty-two and twenty-eight chromosomes (Fig. 14).

b. *Commelina obliqua* Ham

Description : Tall branching herbs with stems 2′ to 3′ high; blue flowers borne in tufted cymes, covered by funnel-shaped spathe; leaves broader and longer than other members of the same genus.

8. *C. obliqua* Ham., Type I ($2n = 45$)

The plants were collected near Sinchal, at an altitude of about 7500 feet above sea-level. The leaves of the plants were thicker in texture than those found in the plains.

This type shows forty-five chromosomes in the pollen-mother cells. Meiotic behaviour in the first division was regular. During anaphase, segregation leading to two different numbers in two poles could be observed. In second meiotic metaphase, two daughter nuclei having twenty-one and twenty-four chromosomes in them (Fig. 15) could be seen. This leads to the production of gametes having unequal numbers of chromosomes.

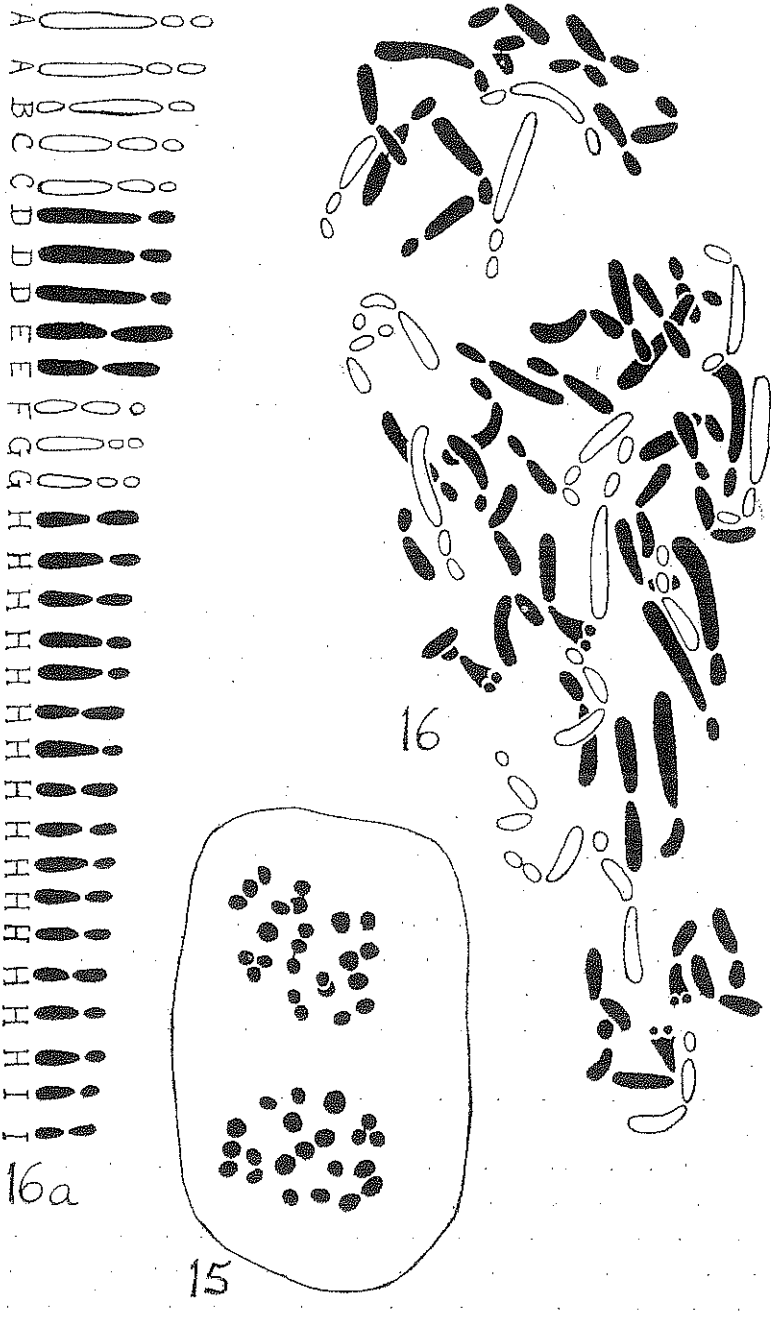


Fig. 15. *Commelina obliqua*, Type I ($2n = 45$)—Second meiotic metaphase with 21 and 24 chromosomes in daughter nuclei.

Figs. 16–16a. *C. obliqua*, Type II ($2n = 60$)—Normal somatic metaphase and idiogram respectively.

9. *C. obliqua* Ham., Type II ($2n = 60$)

This type was collected from the forests near Khumani, at an altitude of about 2000 feet above sea-level.

The normal somatic cells of the plants are seen to contain sixty chromosomes in the set. However, cells having numbers varying from the normal are also on record.

The chromosomes range in size from nearly long to nearly short. Size difference between them is quite marked, ranging from $3{\cdot}2\mu$ to $8{\cdot}9\mu$. On the basis of their size, they can be classified into the following general groups :

1. Ten pairs of long chromosomes.
2. Eighteen pairs of medium-sized chromosomes, and
3. Two pairs of short chromosomes.

Of these, sixteen are seen to bear secondary constrictions.

A detailed study of the morphology, taken together with the relative size, divides the chromosomes into the following types (Figs. 16 and 16a) :

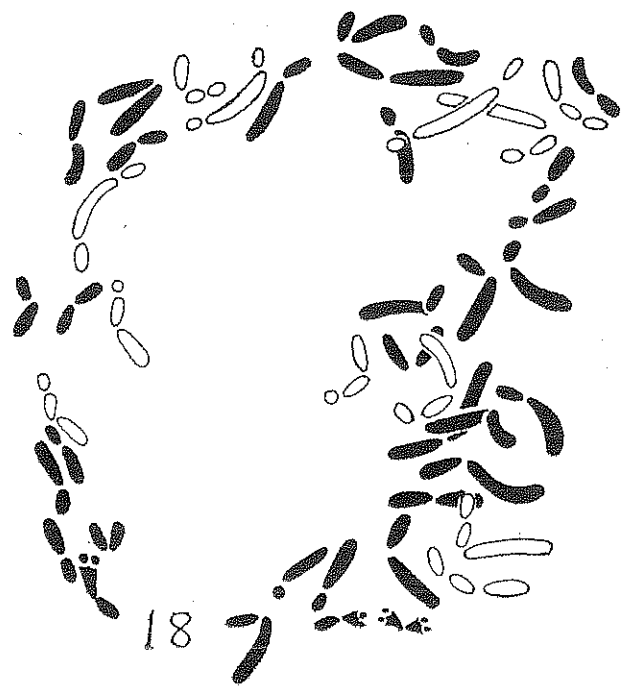
1. Two pairs of long chromosomes, each having two contrictions, primary and secondary, one nearly submedian in position and the other located in the middle of the shorter arm (A).
2. A pair of long chromosomes, each having two constrictions, primary and secondary, located at the opposite distal ends of the chromosome (B).
3. Two pairs of comparatively longer chromosomes, each having two constrictions, primary and secondary, one nearly median in position and the other nearly submedian, at the distal end of the slightly shorter arm (C).
4. Three pairs of long chromosomes, with submedian to nearly subterminal primary constrictions (D).
5. Two pairs of long chromosomes with median primary constrictions (E).
6. A pair of medium-sized chromosomes, each having two constrictions, primary and secondary, one nearly median in position and the other nearly subterminal at the end of one of the arms (F).
7. Two pairs of medium-sized chromosomes, each having two constrictions, primary and secondary, one nearly median in position and the other located in the middle of the slightly shorter arm (G).
8. Fifteen pairs of medium-sized chromosomes with median to submedian primary constrictions (H).
9. Two pairs of short chromosomes with median to submedian primary constrictions (I).

In addition to the normal complement described above, nuclei having thirty and forty-five chromosomes are also on record.

Fig. 17 shows a plate with thirty chromosomes, eight of which bear secondary constrictions. One chromosome of A, one of B, two of C, one of F and one of G are present. Two new chromosomes with secondary constrictions, resembling B, but much smaller in size, are seen. This set seems to have arisen from the normal one through structural alterations and non-disjunction.

Fig. 18 shows a cellowith forty-five chromosomes, twelve of which bear secondary

constrictions. Two chromosomes of A type, three of B, two of C, two of F and three of G are present. This set is possibly a derivative from the normal one through non-disjunction.



Figs. 17–18. *Commelina obliqua*, Type II—Variation somatic metaphases with 30 and 45 chromosomes respectively.

10. *C. obliqua* Ham., Type II ($2n = 60$)

This type was collected from the forests near Khumani, at an altitude of 2000 feet. It is distinguished from Type II by having the peduncle with the flowers rather elongated and raised out of the sheathing spathe.

The normal somatic cells are seen to bear sixty chromosomes. Of these, twelve are seen to bear secondary constrictions. The chromosomes are shorter than those of Type II, ranging from 16μ to $5{\cdot}2\mu$. The karyotype of this plant differs from Type II in the number of the different types of chromosomes present.

As compared with the idiogram of Type II, the idiogram of Type III shows in its set (Figs. 19 and 19a) :

1. Two pairs of chromosomes of B type,
2. One pair of C type,
3. Three pairs of D type,
4. One pair of E type,
5. Three pairs of F type, and
6. Twenty pairs of H type.

GENUS—*Streptolirion*

Only one species was studied.

11. *Streptolirion volubile* Edgew., ($2n = 12$)

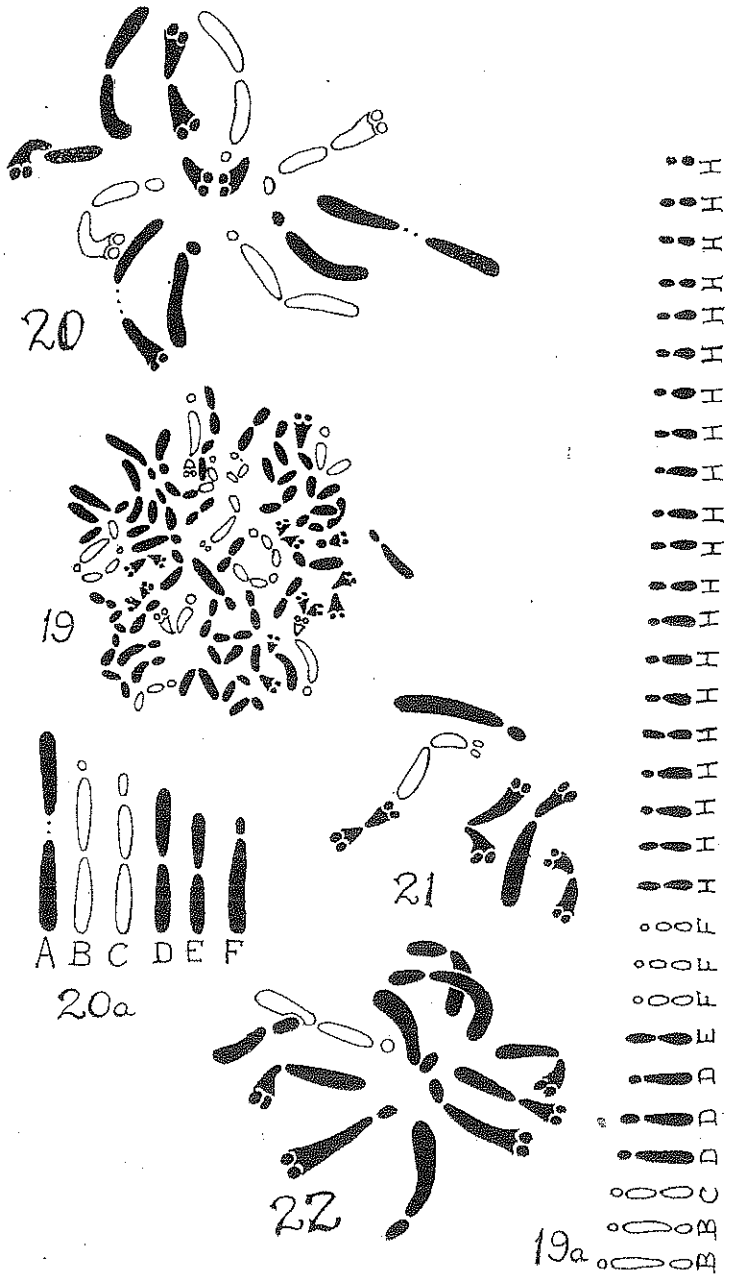
Description.—Climbing, flaccid herb; leaves with long petiole and deeply two-lobed base; white flowers borne in scorpioid cymes with short sheath.

The plants were collected near Darjeeling, at an altitude of about 6500 feet.

The normal somatic chromosome number is found to be twelve. Cells having varying numbers of chromosomes are also present. The chromosomes are long in size, as compared to the other plants of the family studied. Size difference amongst the chromosomes is present, though not marked, ranging from 6μ to $10{\cdot}4\mu$. The chromosomes form a uniformly graded series from the longest to the shortest.

A detailed study of the morphology, together with the relative size, divides the chromosomes into the following types (Figs. 20 & 20a) :

1. A pair of very long chromosomes with median primary constrictions, the constriction gap being rather pronounced (A).
2. A pair of long chromosomes, each with a median primary constrictions and a satellite at the end of one of the arms (B).
3. A pair of comparatively medium-sized chromosomes, each having two constrictions, primary and secondary, one median in position and the other nearly submedian at the distal end of one of the arms (C).
4. A pair of comparatively medium-sized chromosomes with median primary constrictions (D).
5. A pair of comparatively short chromosomes with median primary constrictions (E).
6. A pair of comparatively short chromosomes with nearly subterminal primary constrictions $(F)_0$



Figs. 19–19a. *Commelina obliqua*, Type III ($2n = 60$)—normal somatic metaphase and idiogram respectively.

Figs. 20, 20a and 22. *Streptolirion volubile* ($2n = 12$)—normal somatic metaphase, idiogram and variation metaphase with six and eleven chromosomes respectively.

In addition to the normal complement, variant nuclei with six and eleven chromosomes have also been recorded.

Fig. 21 shows a plate with six chromosomes, one of which is satellited. One chromosome of A type, one of B, one of D, two of E and one of F are present. Its origin may be assumed to be from the normal set through non-disjunction.

Fig. 22 shows a cell having eleven chromosomes only, one of which bears secondary constrictions. This set is also possibly a derivative from the normal one through minor structural changes, including amphiplasty, and non-disjunction.

Discussion

i. *Analysis of the present data in relation to the previous ones*

A discussion of the observations presented in the text makes their interpretation necessary in the light of the previous data. Four different genera have been worked out here. Except *Streptolirion*, a number of other species of *Commelina*, *Aneilema* and *Cyanotis* have been the subject of cytological study at different times.

Three different species of *Commelina* have been dealt with in their paper. The two different types of *Commelina nudiflora* reported here, show the 2*n* number to be twenty-eight and fifty-six respectively. The former type was collected from the plains and the latter from the temperate areas. The individuals found in the plains, of the same species, investigated in our previous report (Sharma, *l.c.*,) showed the somatic complement to be composed of thirty chromosomes. Anderson & Sax (1936) reported the presence of types with fifty-six chromosomes in the tropics.

The importance of polyploidy in the differentiation of various ecotypes within this species is apparent. In the external morphology of the plant, the individuals collected from the hills differ from those occurring in the plains. Gigantism of the leaf and flower characters in the former is specially remarkable. The origin of forms having thirty chromosomes from those with twenty-eight or *vice versa*, is easily explained on the basis of aneuploidy. As the basic number in this genus is much lower than the existing haploid number, no difficulty in survival is experienced by these aneuploid plants. They originate, in all probability, initially through non-disjunction. So far as this particular species is concerned, a careful scrutiny of the individuals occurring in the plains, but in different localities, always reveals minute morphological differences between them. Evidently, this is also true for the two types with twenty-eight and thirty chromosomes reported by us. An intensive taxonomic study of the different ecotypes of *Commelina nudiflora*, followed by their cytological investigation, may reveal the presence of numerous chromosomal biotypes in nature.

Three different types of *Commelina obliqua* have been cytologically investigated here. The one collected from the temperate areas shows forty-five chromosomes, whereas the other two of the sub-temperate areas, though differing in their karyotypes, show sixty chromosomes in their somatic complement. Different individuals from the plains, collected from different areas, show one hundred and one hundred and fifty chromosomes in their somatic sets respectively (Sharma, *l.c*). In this particular species, the role of increase in chromosome number, mainly polyploidy, in the differentiation of the

ecotypes found at different altitudes, is very marked. This species serves as a very good example of the correlation of climatic changes with polyploidy (Cain, 1944). Löve & Löve (1949) in their excellent treatise on the geo-botanical significance of polyploidy, cite many similar cases.

Commelina sikkimensis, reported here for the first time, also shows sixty chromosomes. Though the number sixty is common for both *C. obliqua* and *C. sikkimensis*, the external morphology of the two is markedly different. This fact justifies fully its independent specific status. However, as the number indicates, *C. obliqua* may be related directly or indirectly to this species.

Aneilema spiratum, *A. herbaceum* and *A. vaginatum* are the three species studied here. Of these, the two latter are new records. This *A. spiratum* was collected from the Temperate Himalayas and showed twenty chromosomes in the somatic set. Murthy (1934) reported forty chromosomes in *A. spiratum* of the plains. In the same species, here too, the occurrence of polyploidy in different climatic types is evident. Both *A. vaginatum* and *A. herbaceum* show forty chromosomes in the somatic cells, suggesting their polyploid nature. Both of them are natives of the tropical to sub-tropical areas, though the individuals of *A. herbaceum*, studied by us, were collected from cultivation at the Botanical Gardens of Darjeeling. Mitsukuri (1947) reported thirty chromosomes in *A. keisak*. All the available data, therefore, clearly indicate that duplication of the chromosome set is responsible, to a large extent, for diversification in the genus *Aneilema*, including even the various types within the same species, occurring at different altitudes.

Of *Cyanotis*, only one species, *C. barbata*, has been worked out here. This shows twenty-four to be the normal $2n$ number. The number is also found in *C. cristata*, reported previously (Islam & Baten, 1952; Sharma, *l.c.*). In view of previous records in other species of *Cyanotis* (Anderson & Sax, *l.c.*), it seems obvious that aneuploidy, instead of polyploidy, is possibly responsible for the evolution of different species in this genus, since the attainment of its generic status. It may be mentioned here that taking into account the cytological and morphological data, a separate generic status for *Cyanotis axillaris* was suggested (Sharma, *l.c*).

Streptolirion volubile is interesting for its chromosome number and also for its karyotype. The $2n$ number being twelve, as observed here, apparently places it in the *Tradescantia* line. But, it differs so markedly in its morphology from the well-known complex of *Tradescantia*, that it is obvious that the relationship is not a close one. It is likely that though possessing the same ancestry, the two genera have diverged rather early in the course of evolution. A thorough cytological investigation of the different species of *Streptolirion* may reveal facts of fundamental cytological importance, similar to *Tradescantia* as noted by other authors (Darlington, 1929; Anderson & Sax, *l.c.*; Bhaduri, 1942 etc.).

ii. *Relative Importance of the role of Auto-, Allo- and Aneuploidy in the evolution of different species*

Members of this family exhibit different degrees of Polyploidy in their varying forms. In order to estimate their relative role in the origin of new forms, it is preferable to discuss them separately.

As far as direct duplication of chromosomes is concerned, it has no doubt occurred within the family to a large extent. But the evidences of its direct responsibility in the origin of new types seem to be meagre. Cases where polyploid series can be noted between different species of the same genus, may be set aside for the sake of discussion, as they may easily involve allopolyploidy, evidences for which are present. Occurrence of autopolyploidy is marked between different ecotypes of the same species, such as *Commelina obliqua* and *C. nudiflora*. It may be recollected that chromosome numbers like twenty-eight and fifty-six were recorded in the latter and forty-five, sixty, one hundred, and one hundred & fifty were noted in the former. The absence of a large number of multivalents may be due to the short size of the chromosomes, resulting in low chiasma frequency. But even then the existence of structural differences of chromosomes is apparent between lower and higher polyploid types. A comparison of the karyotypes reveals that all the chromosomes are not present in multiples, as expected from the degree of polyploidy attained. Diminution of chromatin matter is clearly noticed with rising degrees of polyploidy. Furthermore, structural differences of chromosomes between different polyploid ecotypes of the same species, clearly indicate that in addition to duplication of chromosomes, alterations in chromosome morphology contribute to their origin. The authors agree with the view recently endorsed by Stebbins (1947) that autopolyploidy alone cannot account for the origin of new forms. An increase in gene dosage may cause merely gigantism in some cases, but does not provide any scope for the addition of new characters. It gives an impetus to the stabilisation of structurally altered types, which may originate before or after the attainment of polyploidy.

The role of allopolyploidy, in increasing the number of species of the family, is evident. In the genera investigated by Anderson & Sax too, the role of allopolyploidy was found to be marked. Practically all the genera belonging to this family show different levels of polyploidy. The absence of multivalents in most of these cases clearly indicates that different sets of chromosomes are involved in their origin. Even in *C. sikkimensis* ($2n=60$), investigated here, not a single multivalent could be recorded in meiosis. The size of the chromosomes of course stands in the way of the formation of multivalents. But the fact that a number of long chromosomes are also present in the complement, over-rules the possibility of their being autopolyploids. These records clearly demonstrate the significant role played by allopolyploidy in the differentiation of species.

Lastly, the aneuploid series in the family have to be discussed. The previous literature taken in conjunction with the present data, shows the occurrence of aneuploid numbers in species of *Commelina* and *Cyanotis*. They show multiples of eleven, twelve, fourteen and fifteen chromosomes in various species as well as in different types of the same species. For example, in *Commelina benghalensis*, the numbers reported are twenty-two, sixty-eight and forty-eight, in different types (Ganguly, 1946; Sharma, *l.c.*; Darlington, *l.c.*; Anderson & Sax, *l.c.*). In *C. communis*, they are forty-eight and ninety (Mitsukuri, 1947). In *C. nudiflora*, forms with twenty-eight and thirty chromosomes have been observed (Sharma, *l.c.*, and present data). In different species, chromosome numbers like thirty, ninety, fifty-two, fifty-six, fifty-eight, seventy-five etc., are recorded. In *Cyanotis cristata* and *C. barbata*, the $2n$ number is twenty-four, as mentioned before,

whereas in *C. somaliensis* it is twenty-eight (Anderson & Sax, *l.c.*). These are the indications of the role of aneuploidy in speciation within the family. It is noticeable, however, that minute structural changes of chromosomes are mostly associated with this process.

Summarising this part of the discussion, it may be concluded that so far as speciation in this family is concerned, allo- and aneuploidy have played much more important roles than autopolyploidy.

iii. *Polyploidy in relation to distribution*

It is worth while to make an attempt to find out the correlation, if any, existing between polyploidy and distribution of the different species investigated here. A glance at the previous records clearly indicates the existence of conflicting ideas as regards this issue. Broadly they may be classified under two different categories. Some of the workers state that extreme climatic conditions are preferred by polyploid types (Flovik, 1940; Hagerup, 1928; Manton, 1937: Babcock & Stebbins, 1938). Wealth of data on this aspect is far from negligible. However, as the theory does not hold good in all cases, others (Cain, 1944) consider that polyploids merely occupy different areas from the diploids. This implies that if the centre of origin of a particular species lies in the temperate regions, the corresponding polyploids may be expected to occur in the tropics.

The different species of *Aneilema* so far investigated by the present and the previous authors show a number of polyploid forms. *A. spiratum*, reported by Murthy (*l.c.*) from the plains, contains forty chromosomes in the somatic set. The corresponding diploid form of the same species, reported in this paper, was collected from the Himalayas, at an altitude of nearly 7000 feet. It is evident that the original forms of *A. spiratum* occur in temperate areas whereas the derivative polyploids are found at lower altitudes. Such high chromosome number is shown by *A. vaginatum* of the tropical and Sub-tropical areas (present report). On the contrary, *A. herbaceum*, with same number, is found in the Himalayas (present report).

In the genus *Commelina*, similar polyploidy is also evident. Diploid forms of *C. nudiflora* ($2n=28$) occur in the tropics and polyploid types in the Himalayas ($2n=56$) (present report). It is significant that Anderson & Sax (*l.c.*) reported polyploid types of the same species from the tropics. This species, therefore, one can easily assume, has originated in the tropics and produced polyploid types there, which later on, because of their higher tolerance range, have migrated to the temperate areas. *C. sikkimensis* ($2n=60$), a polyploid type, also occurs in the Himalayas. A different picture is represented by the types of *C. obliqua* studied. Here, as the table shows, types with lower chromosome numbers occur in higher altitudes, and those with high numbers in the plains. This species, therefore, in contrast to *C. nudiflora*, must have originated in the temperate areas and higher polyploid types occurred in the plains. (Table I).

Streptolirion and *Cyanotis* may be omitted as polyploid series have not yet been discovered in them.

The above resumé clearly brings out the fact that, so far as these species are concerned,

Table I. *Showing chromosome number and habitat of different species and ecotypes of four genera investigated*

No.	Name of species	Chrom. No.	Name of worker	Locality where collected
1.	*Aneilema nudiflorum*	i. 20 ii. 20	Simmonds, 1954 Sharma, 1955	S. E. Asia Plains of W. Bengal, India
2.	*A. keisak*	30	Mitsukuri, 1947	Japan
3.	*A. spiratum*	i. 40 ii. 20	Murthy, 1934 Present authors	India, plains of S. India India, Darjeeling at 6000 feet
4.	*A. herbaceum*	40	Present authors	India, Rongo at 4000 to 5000 feet
5.	*A. vaginatum*	40	Present authors	India, Khumani, 1000—2000 feet.
6.	*Cyanotis axillaris*	i. 20 ii. 20	Islam & Baten, 1952 Sharma, 1955	Dacca plains, Pakistan Plains of W. Bengal, India
7.	*C. crista*	i. 24+0—1B ii. 24	Islam & Baten, 1952 Sharma, 1955	Dacca plains, Pakistan India, plains of W. Bengal
8.	*C. somaliensis*	28	Anderson & Sax, 1936	E. Africa
9.	*C. barbata*	24	Present authors	India, Rongo, 4000 feet to 45 feet.
10.	*Commelina benghalensis*	i. 22 ii. 68 iii. 22 iv. 48	Ganguly, 1946 Darlington, 1929 Sharma, 1955 Anderson & Sax	India, plains of Bengal O. W. Tropics India, plains of W. Bengal Tropics
11.	*C. diffusa*	30	Simmonds, 1954	Tropics
12.	*C. communis*	48, 90	Mitsukuri, 1947	China
13.	*C. elegans*	c. 52	Simmonds, 1954	S: U.S.A.
14.	*C. nudiflora*	i. 56 ii. 30 Type I iii. 28 Type II iv. 56	Anderson & Sax, 1936 Sharma, 1955 Present authors Present authors	Tropics India, plains of W. Bengal India, Khumani, 1000 feet India, Kurseong, 5000 feet
15.	*C. coelestis*	90	Anderson & Sax, 1936	Mexico
16.	*C. salicifolia*	75	Sharma, 1955	India, plains of Bengal
17.	*C. obliqua*	i. 100, 150 Type I ii. 45 Type II iii. 60 Type III iv. 60 (different karyotype)	Sharma, 1955 Present authors Present authors Present authors	India, plains of Bengal Sinchal, India, 7000 feet India, Khumani, 1000–2000 feet. India, Khumani, 1000–2000 feet.
18.	*C. sikkimensis*	60	Present authors	India, Rongo, 4000–5000 feet
19.	*C. hirtella*	*c.* 58	Bowden	E. U.S.A.
20.	*Streptolirion volubile*	12	Present authors	India, Darjeeling, 6500 feet

there is no absolute rule that polyploids must occur in the higher altitudes. Some of the species may originate in the temperate regions, whose corresponding polyploids will then occur in the tropics and *vice versa*. The only rule that governs the distribution is that the polyploids can occupy different and, in some cases, wider areas than the diploids, because of their higher tolerance capacity.

iv. *Importance of karyotypic changes in the evolution of species*

The role of structural alterations in speciation is well-established. All the species investigated in this paper give clear evidence in support of this idea. None of the species show absolute karyotypic similarities. Differences in chromosome morphology are noticeable between different types of the same species. The Type II and Type III of *Commelina obliqua* may be cited as examples in this connection. Both of them show sixty chromosomes in the somatic set, but the karyotypes of the two differ. As this process provides maximum scope for the addition of new characters, it may be concluded that the role of structural alterations of chromosomes is far more important in the evolution of species of Commelinaceae, than that of polyploidy. The latter mainly serves as a stabilizing process.

v. *Relationship between alterations in chromosome complement of somatic cells & speciation*

Considerable literature has accumulated within the last few years showing the role of chromosomal variations in somatic cells in the origin of species (vide Sharma & Sharma, 1955; Sharma, 1957). In plants where reproduction through sexual means is absent or rather is obsolete, this method of speciation is obligatory. In cases, also, where asexual reproduction is of wide-spread occurrence, this method though not obligatory, is still operative.

Of the species investigated here, a number show such somatic variations. For example, in *Commelina nudiflora*, Type II ($2n=56$) cells with twenty-eight chromosomes are found to occur along with the normal ones. The frequency of their occurrence is much lower than that of the normal type. In *C. obliqua*, Type II ($2n=60$) cells with thirty and forty-five chromosomes are also on record along with the normal ones in the same tissue. If a large number of root-tips can be collected in the other species too, it is not unlikely that they may reveal facts of a similar nature. It may be mentioned that in the previous communication also, similar observations in other species were recorded.

The origin of such variations may be through diverse means. In cases where only one or two chromosomes are involved, the process may be non-disjunction. When such abnormal numbers are half the normal ones or nearly so, it may involve somatic reduction. The occurrence of such spontaneous somatic reduction in plants is well demonstrated in recent years (Sharma & Bhattacharjee, 1953). The case of *Commelina obliqua* needs special comment. Here in Type II ($2n=60$) the variations in number noted are thirty and forty-five. The last mentioned numbers can no doubt arise through further reduction from the former.

All these species are propagated profusely through vegetative means, though of course, sexual reproduction is also operative. The occurrence of such varying numbers in the somatic tissue obviously provides scope for the origin of new types, through their presence in the growing point of daughter shoots. Experiments recently conducted in this laboratory reveal their occurrence in the shoot apex as well (Sharma & Sharma, 1957).

A rational evaluation of the role of sexual and asexual reproduction in speciation in this group suggests that the latter is more suited for the process than the former. Different polyploid and aneuploid (*C. nudiflora*—$2n=28$, 30 etc.), types may originate more conveniently through this means than through sexual reproduction. The same may be the case with different chromosome numbers reported in different individuals of *C. benghalensis* by different authors. The reason for this is the fact that in sexual reproduction, a number of conditions must be satisfied for the origin of such forms. These conditions include the occurrence of variations in both parents, their chance union, and the survival of the embryo. On the other hand, in propagation by asexual means, no such conditions are to be satisfied and a new form can originate through a single step in evolution. On the basis of these facts, it may be assumed that such somatic variations play a significant role in speciation within the family, as noted in a number of other plants too.

vi. *Different lines of evolution*

In the species investigated here, two lines of evolution are clearly evident, namely the *Commelina*, *Aneilema* and *Cyanotis* line, and the other represented by *Streptolirion*. The last genus, with its characteristic chromosome complex, is possibly a line by itself, though related to the *Tradescantia* group.

The three other genera, though different in their chromosome morphology, possess some gross similarities among themselves. They may have diverged quite early in evolution from a common ancestral stock. This supports the contention embodied in a previous communication (Sharma, *l.c.*).

Aneilema vaginatum, however, has a characteristic chromosome complex. Its chromosomes are unusual for the genus *Aneilema*. A number of them resemble those of other species of *Aneilema*, whereas the others are much longer and markedly different. In general, the chromosomes of the complement show abrupt size difference. It seems likely, therefore, that the ancestry of this species involves crosses between some species of *Aneilema* with a species of another genus. Probably the other parent is one related to *Cyanotis axillaris* investigated before.

Summary

1. Eight species under four genera of the family Commelinaceae have been studied cytologically in this paper. A number of ecotypes of two of the species have also been studied. The chromosome numbers determined are :

1. *Aneilema herbaceum* Wall	..	.. $2n = 40$
2. *A. spiratum* R.Br.	..	.. $2n = 20$

3. *A. vaginatum* R.Br.	..	.. $2n = 40$
4. *Cyanotis barbata* Don.	..	.. $2n = 24$
5. *Commelina nudiflora* L., Type I	..	.. $2n = 28$
6. *C. nudiflora* L., Type II	..	.. $2n = 56$
7. *C. sikkimensis* Clarke	..	.. $2n = 60$
8. *C. obliqua* Ham., Type I	..	.. $2n = 45$
9. *C. obliqua* Ham., Type II	..	.. $2n = 60$
10. *C. obliqua* Ham., Type III	..	.. $2n = 60$
11. *Streptolirion volubile* Edgew.	..	.. $2n = 12$

2. Investigations reveal the marked role of polyploidy and aneuploidy in the evolution of these species and ecotypes. The most interesting feature is that the chromosome complements of different ecotypes differ from each other. These phenomena have been shown to be associated with structural changes of chromosomes. Karyotypic studies indicate that alteration in chromosome complement is the principal factor in speciation in this group.

3. A discussion of the previous and present data on the cytology of members of this family collected from various localities, has been made. No correlation could be established between habitat and polyploidy. Polyploids have been found to occupy wider areas than diploids because of their tolerance range.

4. Two lines of evolution are observed in the members worked out. One is characterised by *Aneilema*, *Commelina* and *Cyanotis*, corroborating the previous assumption, and the other is represented by *Streptolirion*. The latter in its chromosome number shows similarity with the *Tradescantia* complex.

5. *Aneilema vaginatum* has been suggested to have arisen through intergeneric crosses, one of the parents being a species of *Aneilema* and the other related to *Cyanotis axillaris*.

6. On the basis of karyotypic variations met with in the somatic cells, it is suggested that this process plays a significant role in speciation through asexual means.

LITERATURE CITED

ANDERSON, E. & SAX, K. (1936). A cytological monograph of the American species of *Tradescantia*. *Bot. Gaz.*, **97**, 433.

BABCOCK, E. B. & STEBBINS, JR., G. L. (1938). The American species of *Crepis*. Their Interrelationships and distribution as affected by polyploidy and apomixis. *Carnegie Inst. Wash. Pub.*, **504**, 1-199.

BHADURI, P. N. (1942). Application of new technique to the cytogenetical re-investigation of the genus *Tradescantia*. *J. Genet.*, **44**, 87–127.

*BOWDEN, W. N. (1940). *Chron. Bot.*, **6**, 123.

CAIN, S. A. (1944). Foundations of Plant Geography. Harper & Bros., New York & London.

DARLINGTON, C. D. (1929). Chromosome behaviour and structural hybridity in the Tradescantieae. *J. Genet.*, **21**, 207–286.

FLOVIK, K. (1940). Chromosome numbers and polyploidy within the flora of Spitzbergen. *Hereditas*, **26**, 430–440.

GANGULY, J. K. (1946). The somatic and meiotic chromosomes of *Commelina benghalensis* Linn. *Curr. Sci.*, **15**, 112.

HAGERUP, O. (1928). Morphological and cytological studies of Bicornes. *Dansk. Bot. Arkiv.*, **6(1)**, 1–27.

ISLAM, A. S. & BATEN, A. (1952). Cytology of Cyanotis. *Nature*, **169,** 457.

LOVE, A. & LOVE D. (1949). The Geobotanical significance of Polyploidy. I. Polyploidy and Latitude. *Port. Acta. Biol. Ser. A. R. B. Goldschmidt.*, Vol. 273–352.

MANTON, I. (1937). The problem of *Biscutella laevigata*. L. II. The evidence from meiosis. *Ann. Bot.* (Lond.), *N.S. 1*, 439–462.

MURTHY, K. L. (1934). Gametogenesis and Embryogeny in some Commelinaceae. *Curr. Sci.*, **3,** 258–259.

SHARMA, A. K. (1955). Cytology of some of the members of Commelinaceae and its bearing on the interpretation of phylogeny. *Genetica*, **27,** 323–363.

SHARMA, A. K. (1957). A new concept of a means of speciation in plants. *Caryologia*, **9,** 93–130.

SHARMA, A. K. & (MISS) BHATTACHARJEE, D. (1953). Somatic reduction in untreated Leguminous plants. *Genetica*, **26,** 410–414.

SHARMA, A. K. & (MISS) MOOKERJEA, ARCHANA (1955). Paradichlorobenzene and other chemicals in chromosome work. *Stain Tech.*, **30,** 1–7.

SHARMA, A. K. & SHARMA, ARCHANA (née MOOKERJEA) (1956). Fixity in chromosome number of plants. *Nature*, **177,** 335–336.

SHARMA, A. K. & SHARMA, ARCHANA (née MOOKERJEA) (1957). A theory regarding the stability of chromosome complement in a species. *Naturwis.*, **44,** 1, 17.

STEBBINS, G. L. JR. (1947). Types of polyploids. Their classification and significance. *Advances in Genetics*, **1,** 403–427.

* Not consulted in original.