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Chromosome Study as an Aid in Tracing the Evolution in Cruciferae

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The family Cruciferae has attracted the attention of several workers because of the economic importance of several of its genera (Manton 1932, Alam 1936, Olson 1955, Mizushima 1968). The important genera in the family from economic stand point are *Brassica, Eruca* and *Raphanus*. Considerable amount of work informing both intergeneric and interspecific crosses have been carried out involving these genera (Karpechenko 1924, Richharia 1937, Sikka 1940 and Howard 1942). Extensive cytological works on different varieties and strains of *Brassica* and *Raphanus* have been done by Mukherjee (1971, 1972, 1973). Mukherjee suggested that the role of structural and numerical alteration of chromosomes in intra and interspecific level is the major factor in the evolution of different strains.

In addition to the above genera, quite a several number of other genera of Cruciferae grow not only in cultivation for agricultural purposes, but also are found to grow wild both in the plains as well as different ranges of the Himalayas. As the family has provided with data of fundamental cytological importance several of its constituent taxa, it was thought worthwhile to include them as far as practicable within the scope of present investigation. In the present work therefore, in addition to certain cultivated species of *Brassica*, several representatives of a few other genera have been studied with the purpose of throwing further light on their phylogeny, systematics and affinity.

Materials and methods

The materials for the present investigation have been selected from some of the cultivated and wild genera belonging to this family were collected from the Himalayan areas and Eastern India plains also. The following materials are included: 1) *Brassica alba* Boiss. 2) *B. campestris* L. 3) *B. juncea* Coss. 4) *B. nigra* Koch. 5) *B. oleracea* L. 6) *Eruca sativa* Mill. 7) *Raphanus sativus* L. 8) *Cardamine hirsuta* L. (collected from Shillong). 9) *C. hirsuta* L. (collected from Darjeeling). 10) *C. scutata* Thumb. (collected from Nepal). 11) *Capsella bursapastoris* Medic. (collected from Darjeeling). 12) *C. bursapastoris* (collected from Western Himalayan area). 13) *Erysimum pachycarpum* Hook. f. and Thomas. 14) *Nasturtium indicum* var. *benghalensis* type I. 15) *N. indicum* var. *benghalensis* type II. 16) *N. indicum* var. *benghalensis* type III. 17) *N. indicum* var. *benghalensis* type IV. 18) *N. montanum* Wall. 19) *N. officinale* R. Br. 20) *Senebiera pinnatifida* DC.

For somatic study, the healthy root-tips were selected and pretreated in aq.

aesculine solution 8° to 10°C (Sharma and Sarkar 1955) 2(1/2) to 3 hours and fixed in propionic alcohol (1: 2) for 45 minutes and next propiono orcein staining technique was followed. For meiotic study the flower buds were fixed in acetic acid ethyl alcohol (1: 2) and then smeared in 1% aceto carmine solution warming and covering with coverglass. Figures were drawn at a table magnification of ×3000 using a Zeiss compensating eye piece ×20 for somatic study and ×15 for meiotic study. Chromosomes with secondary constrictions have been drawn in outline in the somatic plates.

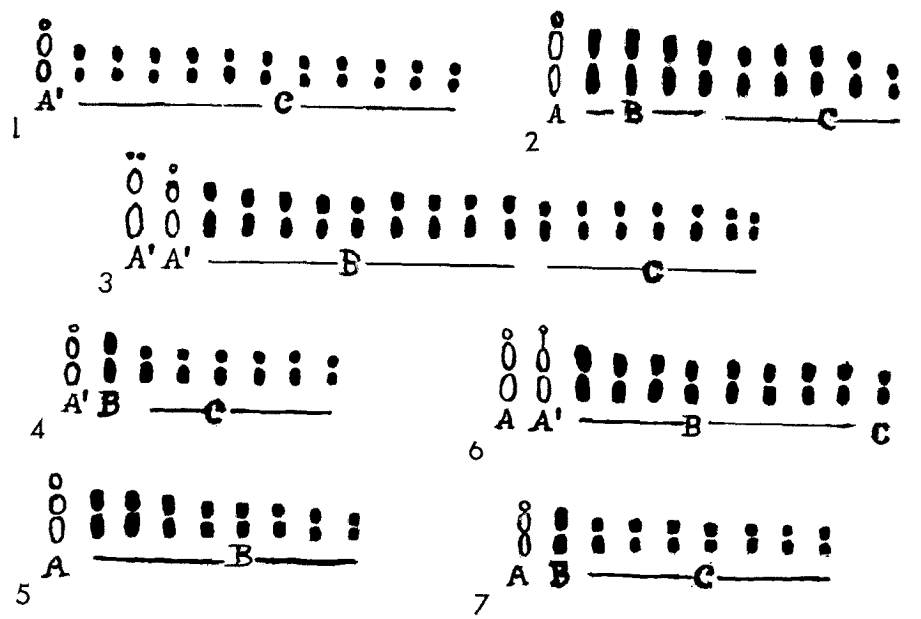
Observations

The somatic number of several species of the tribe Brassiceae show a varying range from 2n=16, 18, 20, 24 to 36. On the basis of their gross morphological character, a number of chromosomes seem to be common to all of them. A critical analysis shows that they differ from one another in minor alterations in the representatives of the types and the different combinations of these types as well. A general description of the types is given below:

Type A —Long chromosome with two constrictions, primary and secondary, one nearly median and the other nearly subterminal at the distal end of one of the arms.

Type A′—Long to medium sized chromosome with nearly median primary constriction and a satellite at the distal end of one of the arms.

Type B —Comparatively long to medium sized chromosome with median to subme-

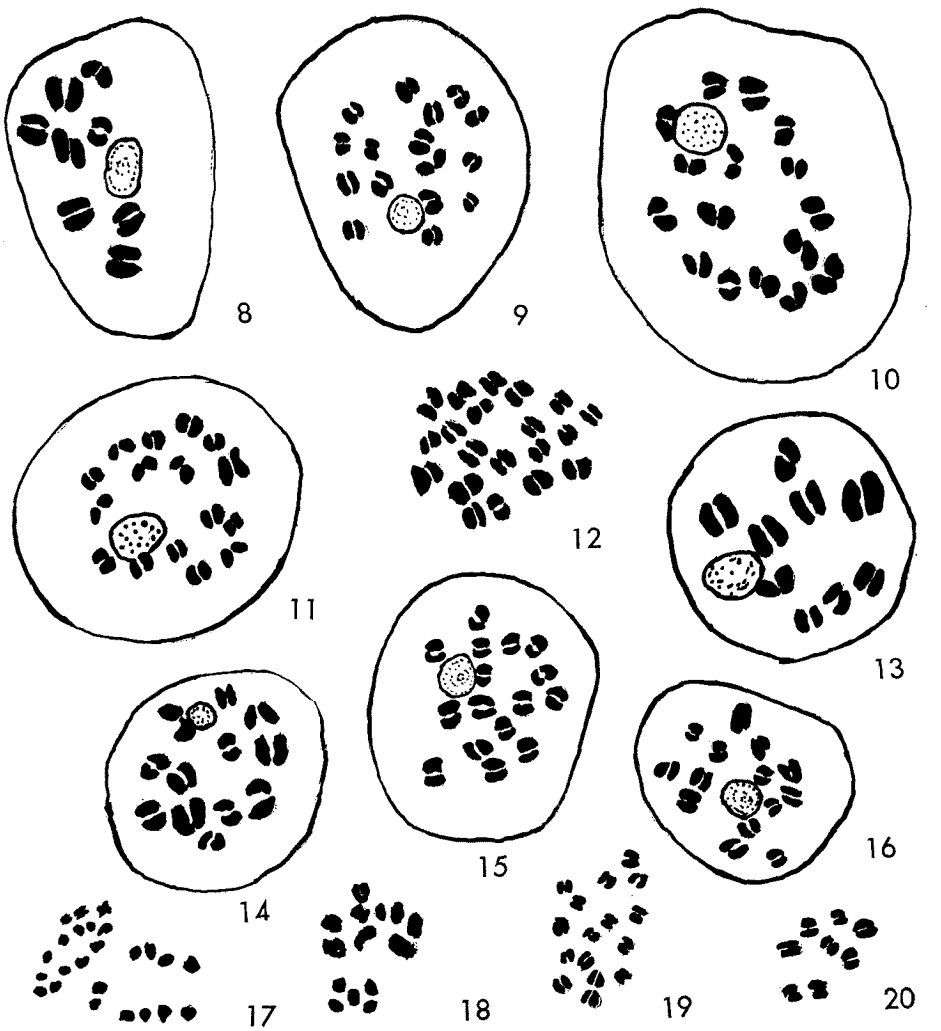


Figs. 1-7. Idiograms of *Brassica alba*, *B. campestris*, *B. juncea*, *B. nigra*, *B. oleracea*, *Eruca sativa* and *Raphanus sativus* showing 2n=24, 20, 36, 16, 18, 22 and 18 respectively. ×3000.

dian primary constriction.

Type C —Short chromosome with median to submedian primary constriction.

The following species of *Brassica*, *Eruca* and *Raphanus* show the different combinations of the above mentioned types:



Figs. 8–20. 8, *Cardamine hirsuta* (Shillong). Diakinesis with n=8 II. 9, *C. hirsuta* (Darjeeling). Diakinesis with n=16 II. 10, *C. scutata*. Diakinesis with n=16 II. 11, *Capsella bursapastoris* (Darjeeling). Diakinesis with n=16 II. 12, *C. bursapastoris* (Western Himalayan areas). Metaphase I with n=20 II. 13, *Erysimum pachycarpum*. Diakinesis with n=9 II. 14, *Nasturtium indicum* var. *benghalensis* Type I. Diakinesis showing n=12 II. 15, *N. indicum* var. *benghalensis* Type II. Diakinesis with n=16 II. 16, *N. indicum* var. *benghalensis* Type III. Diakinesis with n=14 II. 17, *N. indicum* var. *benghalensis* Type IV. Metaphase I showing n=24 II. 18, *Nasturtium montanum*. Metaphase I showing n=14 II. 19, *N. officinale*. Metaphase I showing n=16 II. 20, *Senebiera pinnatifida*. Metaphase I with n=8 II. All figures, ×2000.

1) *Brassica alba* Boiss. $2n=24=A_2+C_{22}=3\ \mu$–$1.5\ \mu$ (Fig. 1).
Meiotic analysis reveals twelve bivalents in diakinesis.
2) *B. campestris* L. $2n=20=A_2+B_8+C_{10}=3.2\ \mu$–$1.5\ \mu$ (Fig. 2).
3) *B. juncea* Coss. $2n=36=A_4'+B_{18}+C_{14}=3.8\ \mu$–$1.8\ \mu$ (Fig. 3).
4) *B. nigra* Koch. $2n=16=A_2'+B_2+C_{12}=3.2\ \mu$–$1.6\ \mu$ (Fig. 4).
5) *B. oleracea* L. $2n=18=A_2+B_{16}=3\ \mu$–$1.8\ \mu$ (Fig. 5).
6) *Eruca sativa* Mill. $2n=22=A_2+A_2'+B_{16}+C_2=3.6\ \mu$–$1.6\ \mu$ (Fig. 6).
7) *Raphanus sativus* L. $2n=18=A_2+B_2+C_{14}=2.3\ \mu$–$1.6\ \mu$ (Fig. 7).
Meiotic studies of several species and varieties of the different genera show the following haploid number.
8) *Cardamine hirsuta* L. (Shillong) (n=8).
Meiosis shows eight bivalents in diakinesis (Fig. 8).
9) *C. hirsuta* L. (Darjeeling) (n=16).
Meiotic analysis reveals sixteen bivalents in diakinesis (Fig. 9).
10) *C. scutata* Thumb. (Nepal) (n=16).
Meiosis shows sixteen bivalents in diakinesis (Fig. 10).
11) *Capsella bursapastoris* Medic. (Darjeeling) (n=16).
Meiosis shows sixteen bivalents in diakinesis (Fig. 11).
12) *C. bursapastoris* (Western Himalayan areas) (n=20).
Meiotic study shows twenty bivalents in metaphase I (Fig. 12).
13) *Erysimum pachycarpum* Hook. f. and Thomas (n=9).
Nine bivalents are observed in diakinesis (Fig. 13).
14) *Nasturtium indicum* DC. var. *benghalensis* Type I (n=12).
Meiosis shows twelve bivalents in diakinesis (Fig. 14).

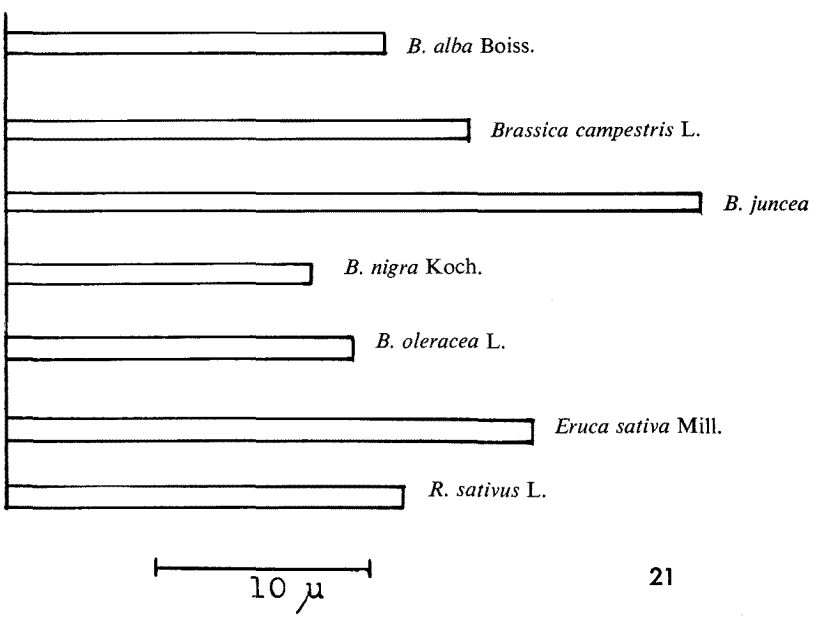


Fig. 21. Histogram showing total amount of chromatin matter in length of the haploid complements of different species of certain genera of the tribe Brassiceae.

15) *N. indicum* var. *benghalensis* Type II (n=16).
Meiotic study reveals sixteen bivalents in diakinesis (Fig. 15).
16) *N. indicum* var. *benghalensis* Type III (n=14).
Meiosis shows fourteen bivalents in diakinesis (Fig. 16).
17) *N. indicum* var. *benghalensis* Type IV (n=24).
Meiosis shows twentyfour bivalents in metaphase I (Fig. 17).
18) *N. montanum* Wall (Shillong) (n=14).
Meiotic study reveals fourteen bivalents in metaphase I (Fig. 18).
19) *N. officinale* R. Br. (n=16).
Meiosis shows sixteen bivalents in metaphase I (Fig. 19).
20) *Senebiera pinnatifida* DC. (n=8).
Meiosis shows eight bivalents in metaphase I (Fig. 20).

Discussion

In the family Cruciferae, the range of chromosome numbers has been found to be very wide between n=4 to 15. According to Schulz (1936) there are 17 tribes. On the basis of chromosome number, it is rather difficult to characterise the different tribes excepting a few. Most of the tribes individually show several haploid numbers. In the genus *Brassica*, the chromosome numbers according to the most of the authors fall into six different types, the haploid number noted so far are 8, 9, 10 and 11 as well as 17, 18, 19 with an also further possible derivation noted in the present observation mentioned in the paper. In the present observation *B. campestris* show the chromosome number 2n=20 confirming the previous report (Morinaga 1934). In *B. juncea* 2n=36 chromosomes have been observed within this amphidiploid species (Alam 1936). *B. nigra* studied show the chromosome number as 2n=16 confirming the previous report (Morinaga 1934). For *B. alba* 2n=24 chromosomes have been reported here which is not only the first record in this species but also the number so far not found in the genus *Brassica*. Here 12 bivalents are observed in diakinesis. This is quite interesting in view of the fact that in the genus *Brassica* 6 chromosome types form the basic set, an allopolyploidy has been established to be an important factor in the evolution. *B. oleracea* studied here, the chromosome number have been observed as 2n=18 confirming the previous report of n=9 chromosomes in this species (Howard 1939).

That the different species, though having different chromosome numbers are yet being derived from a common genome is also indicated in the idiogram studied here (Figs. 1 to 5). The general similarity in chromosome morphology, consisting mainly of medium sized chromosomes which are mostly medianly constricted with one or two pairs of secondary constrictions, indicates that all the different species are allied to each other. Of the five different species of *Brassica* studied here, it has been noticed that inspite of general similarity in the karyotype, *B. campestris* has chromosome complements longer than the rest which has also been borne out in the histogram (Fig. 21). Within the genus *Brassica*, it would not be reasonable to assume that *B. campestris* in view of slightly longer chromosomes, may represent a comparatively primitive status in relation to the rest of the species studied. Diminution in

chromosome size has been found to be an associate feature in the evolution of most of the angiosperm (Stebbins 1950, Sharma and Sharma 1959). In the case of *B. juncea*, the histogram shows as an increase in length which is due to its expolyploid constitution.

Another two genera of the tribe Brassiceae, included here are *Raphanus* and *Eruca*. In these genera, the chromosome number of *R. sativus* is observed as 2n=18 confirming the previous report by Karpechenko (1924) and in another genus *Eruca* of which *E. sativa* has shown the chromosome number as 2n=22. This is in confirmation of the number so far reported by the previous authors (Alam 1936). As the number is n=11, the chromosomes fall in pattern with other number of Brassiceae. Therefore, in Brassiceae as a whole, taken in conjunction with the previous and the present it may be noted that several chromosome numbers exist within the group. But inspite of the difference in chromosome number, there is a relationship between different genera, is quite obvious as evidence from the karyotype. Intergeneric crosses reported by several previous authors also bear testimony to this assumption (Mizushima 1968). The basic chromosome number for *Brassica*, which has been assumed to be six, is probable and an intricate reticulate relationship between the different genomes of this group has been suggested.

Of the tribe Lepideae, the genera *Capsella* and *Senebiera* have been studied. The chromosome numbers have been observed as n=16 and 20 collected from Eastern and Western regions of the Himalayas respectively. The number n=16 chromosomes confirmed the previous report recorded from other parts of the world. The chromosome number so far recorded from other parts of the world (Mulligan 1957, Löve and Löve 1961, and Easterly 1963). The aneuploid number n=20 has been suggested as arising out of duplication of the two chromosomes at the basic level followed by polyploidy. Formation of 20 bivalents indicates structural homology due to the homozygosity of structural alterations. The new chromosome number of *Senebiera pinnatifida* has been noted as n=8 which falls in the general pattern of the tribe Lepideae.

Of the tribe Arabideae two genera have been studied viz. *Cardamine* and *Nasturtium*. In the genus *Cardamine* extensive works so far have been done in different species and n=8 chromosome types have been found to be the lowest so far reported in the genus (Löve and Löve 1961, Packer 1964). Diploidy and tetraploidy have been found in several of the species viz. *C. parviflora* (Rollins 1966), *C. pratensis* (Zukova 1960). In the present investigation two populations of *Cardamine* have been studied viz. *C. hirsuta* collected from Darjeeling (Eastern Himalayan region altitude 7000 ft) as well as *C. hirsuta* collected from Shillong (Khasi hills altitude 4500 ft). The eastern Himalayan population is tetraploid and shows n=16 chromosomes whereas the population collected from Shillong shows n=8 chromosomes. Though both the populations belonging to the Eastern Himalayan sector, the altitudinal preferences from the two distinct cytotypes are remarkable. Many such cases have been extensively discussed by Löve and Löve (1953). Therefore an interesting correlation between diploid and polyploid species along with their ecology has been observed in *C. hirsuta* collected from different altitudes of the Himalayas. Another species of *Cardamine* viz. *C. scutata* collected from Central

Himalayas, Nepal (7000 ft.) has shown the tetraploid number n=16 and it is a new report. A particular type of cytotype has been observed in *Nasturtium indicum* var. *benghalensis* with n=12, 14, 16 and 24 chromosomes collected from different localities. The presence of 12 chromosomes in the genus *Nasturtium* brought forward in the present investigation may suggest that in this tribe too, the basic should be 6 as in Brassiceae.

In *Erysimum pachycarpum* of the tribe Hesperedeae also an aneuploid number as n=9 chromosomes have been observed. The present investigation shows multiple of 18 chromosomes in addition to 7 and 8 chromosomes. The occurrence of 9 chromosomes is the first of such reports in this genus. In this genus too, it is rather difficult to ascertain the exact basic set, but the multiple of 9 chromosomes are rather wide spread.

On the basis of the previous and the present observations it has been claimed that aneuploidy has been one of the frequent featurea in the evolution of different species of Cruciferae. As the family indicates quite a homogeneous grouping specially in chromosome morphology, the subdivision of the tribe as done by Schulz may be kept for identification but need not be considered as indicating phylogeny. That diminution in chromosome size has played a significant role in evolution is found in the present investigation as well. But such a diminution must have occurred within each of the tribes independently evolving from a common basic type. Such a basic type might have 6 chromosomes as specially noted in Arabideae. The lower or higher numbers might have had their origin from such a basic set. All these evidences suggest that the diversification in number and structure of chromosomes must have evolved in parallel lines from a common basic ancestor.

Summary

A detailed cytological analysis of different genera of the family Cruciferae has been carried out in order to find out the role of structural differences in the phylogeny of *Brassica* and the allied genera. The genera studied grow wild and cultivated throughout India. The present study reveals that several chromosome numbers exist within the group of *Brassica*. But inspite of difference in chromosome number that there is a relationship between different genera, is quite obvious as evidence from the karyotype. The basic chromosome number for *Brassica* which has been assumed to be 6 is probable and intricate relationship between different genomes of this group has been suggested. The new chromosome number of *Senebiera pinnatifida* has been noted as n=8. An interesting correlation between diploid and polyploid species along with their ecology has been observed in *Cardamine hirsuta* collected from different altitude of the Himalayas has been discussed. A particular type of cytotype has been observed in *Nasturtium indicum* with n=12 to 24 chromosomes. The presence of 12 chromosomes in the genus *Nasturtium* may suggest that in the tribe Arabideae too, the basic type should be 6 as in Brassiceae.

On the basis of previous and present observation, it has been claimed that aneuploidy and diminution in chromosome size have played a significant role in evolution. But such diminution must have occurred within each of the tribes independently

evolving from a common basic type. The lower or higher numbers might have had their origin from such a basic set and the diversification in number and structure of chromosomes must have evolved in parallel lines from a common basic ancestor.

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