Cytologia **44**: 467–477, 1979

Chromosome Evolution in Certain Genera of Brassiceae

Kumkum Sikka and A. K. Sharma

Chromosome Research Centre, Department of Botany, University of Calcutta,
35, Ballygunge Circular Road, Calcutta-700019, India

Received September 28,1977

In the tribe Brassiceae (Cruciferae), the genus *Brassica* including many cultivated and economic forms has been subjected to cytological analysis and extensive hybridisation. Comparatively little work has been done on the allied genera and species of the tribe. Investigation into these species is necessary in order to work out the role of chromosomal changes in the evolution of the different taxa. The possibility of an exchange of gene material between the related wild and cultivated forms in this group by plant breeders in order to expand their genetic resources has been pointed by Harberd (1972).

The present paper deals with a study of the structure and behaviour of chromosomes in a number of genera of the tribe Brassiceae, with special reference to an analysis of karyotype details.

Materials

Seed samples of 28 species and varieties were investigated, of which 16 genera were obtained through the courtesy of Hortus Botanicus Hauniensis, Copenhagen, Denmark and two others, namely, *Erucaria* and *Schouwia* from the Botanical Garden of the Hebrew University, Jerusalem, Israel. The detailed list of the taxa is given in Table 2. All the seeds were germinated and some of the seedlings grew till the flowering and fruiting stages.

Methods

Studies on somatic chromosomes were made from squash preparations of root tips. In a majority of species, a saturated solution of alpha-bromonaphthalene for a time interval ranging from 1/2 hour to 2 hours at 12°–14°C, whereas in others a saturated solution of paradichlorobenzene and aesculine mixture (1 :1) at 10°–12°C for 3–4½ hours gave best results. The usual propiono-orcein schedule was followed and the optimum period for staining was 6–10 hours. For meiosis, propiono-carmine method was followed.

The figures were drawn at a table magnification of approximately ×2400. In the somatic figures the chromosomes with secondary constrictions were drawn in outline only.

Observation

The somatic chromosome number ranges from 14–60.

The morphology of the somatic chromosomes in all the genera studied shows a homogeneity with regard to size, position of primary constrictions and the number of pairs with secondary constrictions. The position of the primary constriction in each pair of chromosome is determined by calculating the centromeric index as in Table 1.

Table 1. Determination of the centromeric position by the centromeric index or F%
(F%=Short arm length/Total length × 100)

F%	50	37.5–49.9	25.1–37.4	25	18.6–24.9	12.6–18.5	12.5
Centromeric position	median	nearly median	nearly submedian	submedian	nearly submedian	nearly subterminal	subterminal

In all, six chromosome types are recognised and these are present in different combinations in the different taxa. They are presented as idiograms in Fig. 1 and described as follows:

Type A—Comparatively medium sized chromosomes (2.9 μ) with submedian to median primary constriction and satellite at the distal end of the shorter arm and occasionally at the distal end of the longer arm (e. g., *Hirschfeldia incana*).

Type B—Comparatively medium sized chromosomes (3.3 μ-1.4 μ) with primary and secondary constrictions, both nearly submedian to median in position. The three segments may be equal or one end segment longer then the others. Occasionally one constriction may be nearly subterminal (e.g., *Erucastrum canariense*).

Type C—Comparatively medium sized chromosomes (3.1 μ-2.9 μ) with submedian to subterminal primary constrictions. Only one pair is seen in *Brassicella erucastrum, Raphanus raphanistrum, Crambe hispanica* and *Erucaria myagroides*).

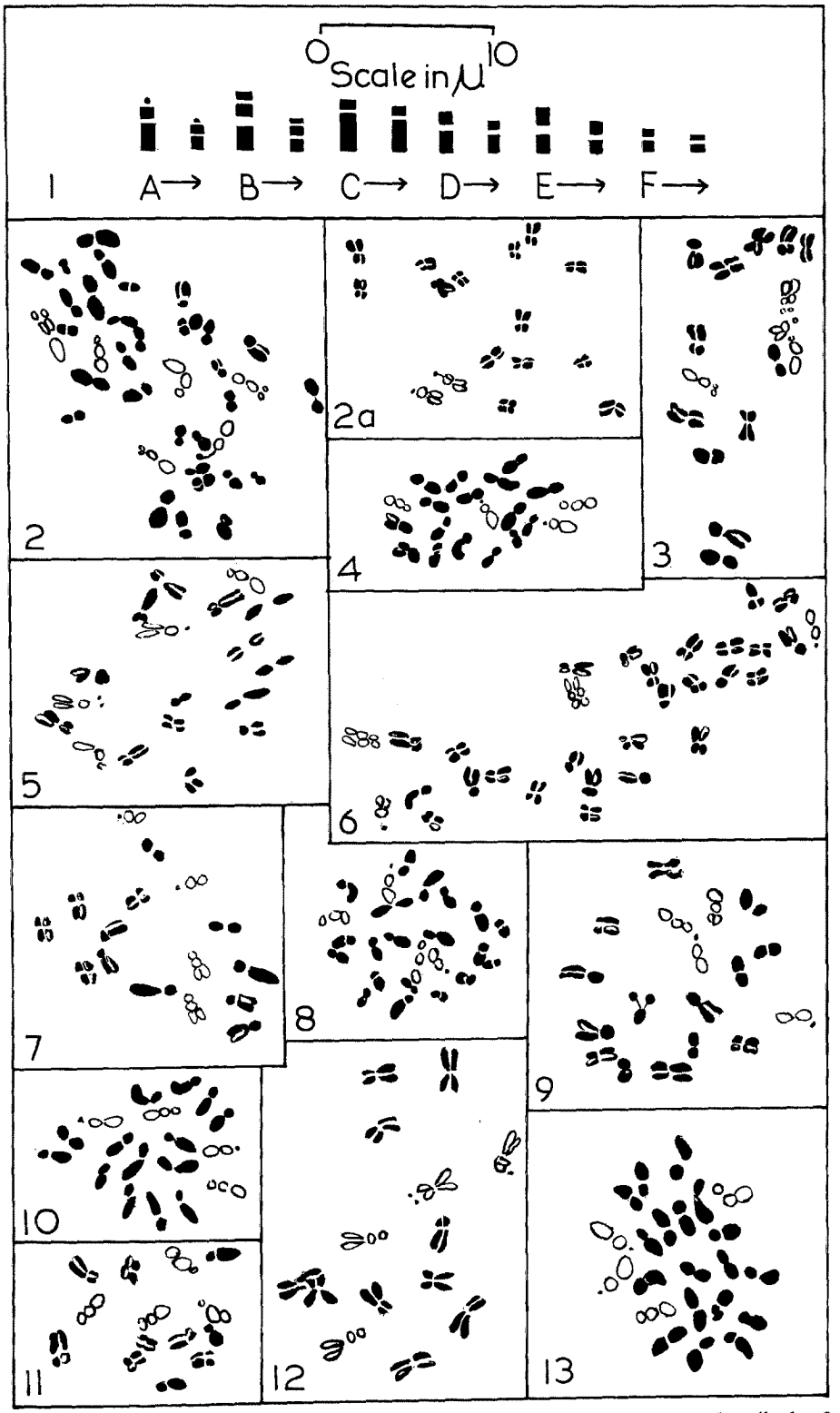
Type D—Comparatively medium sized chromosomes (2.5 μ–1.6 μ) grading in size with nearly submedian primary constrictions.

Type E—Comparatively medium sized chromosomes (2.9 μ–1.8 μ) with median primary constrictions (e.g., *Erucastrum canariense, Sinapis arvensis* var. *orientalis, Eruca sativa, Raphanus raphanistrum* and *Erucaria myagroides*).

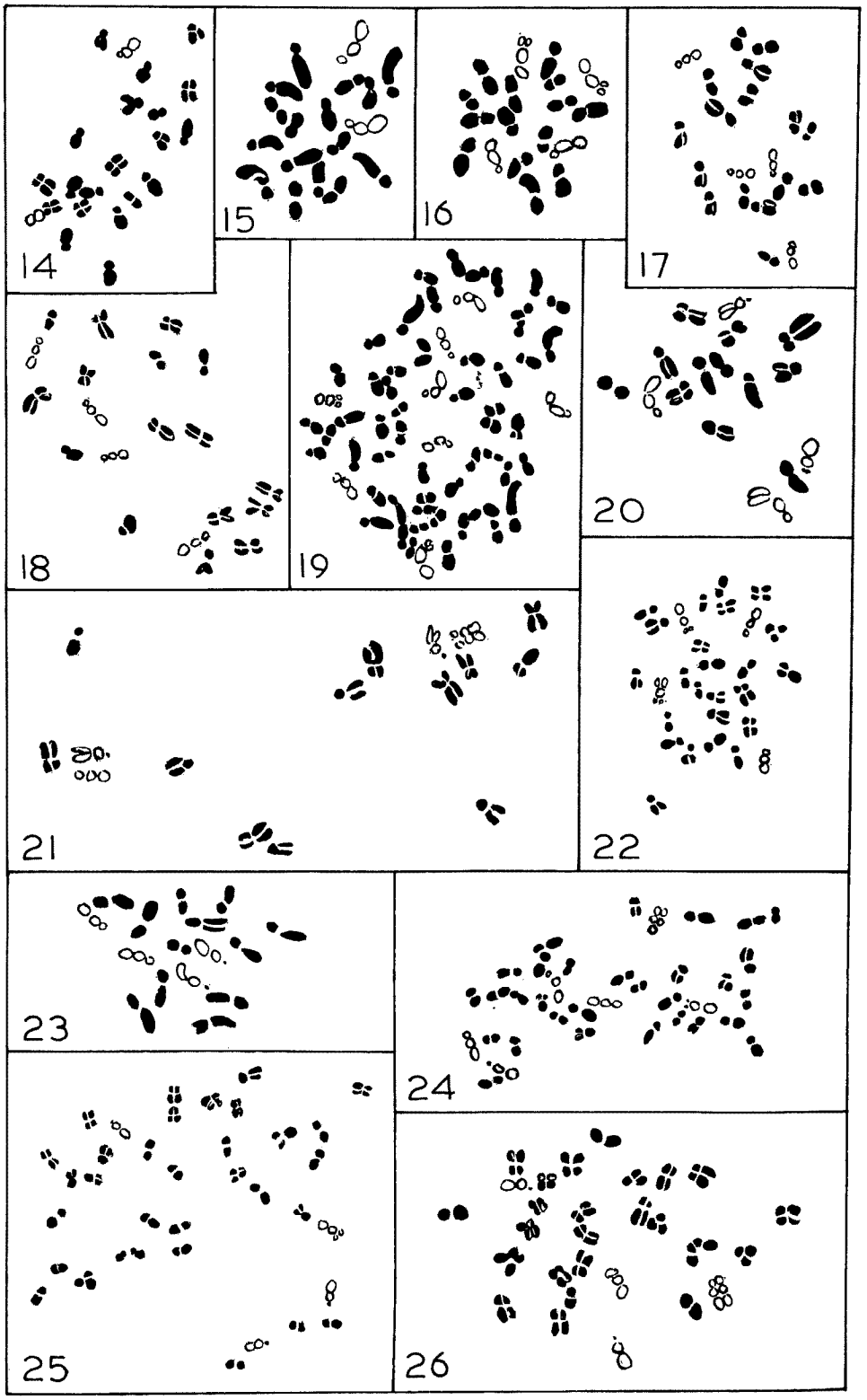
Type F—Comparatively short chromosomes (1.4 μ–0.7 μ) with submedian to median primary constrictions.

The karyotype, which is graded in all, is represented in the following table:

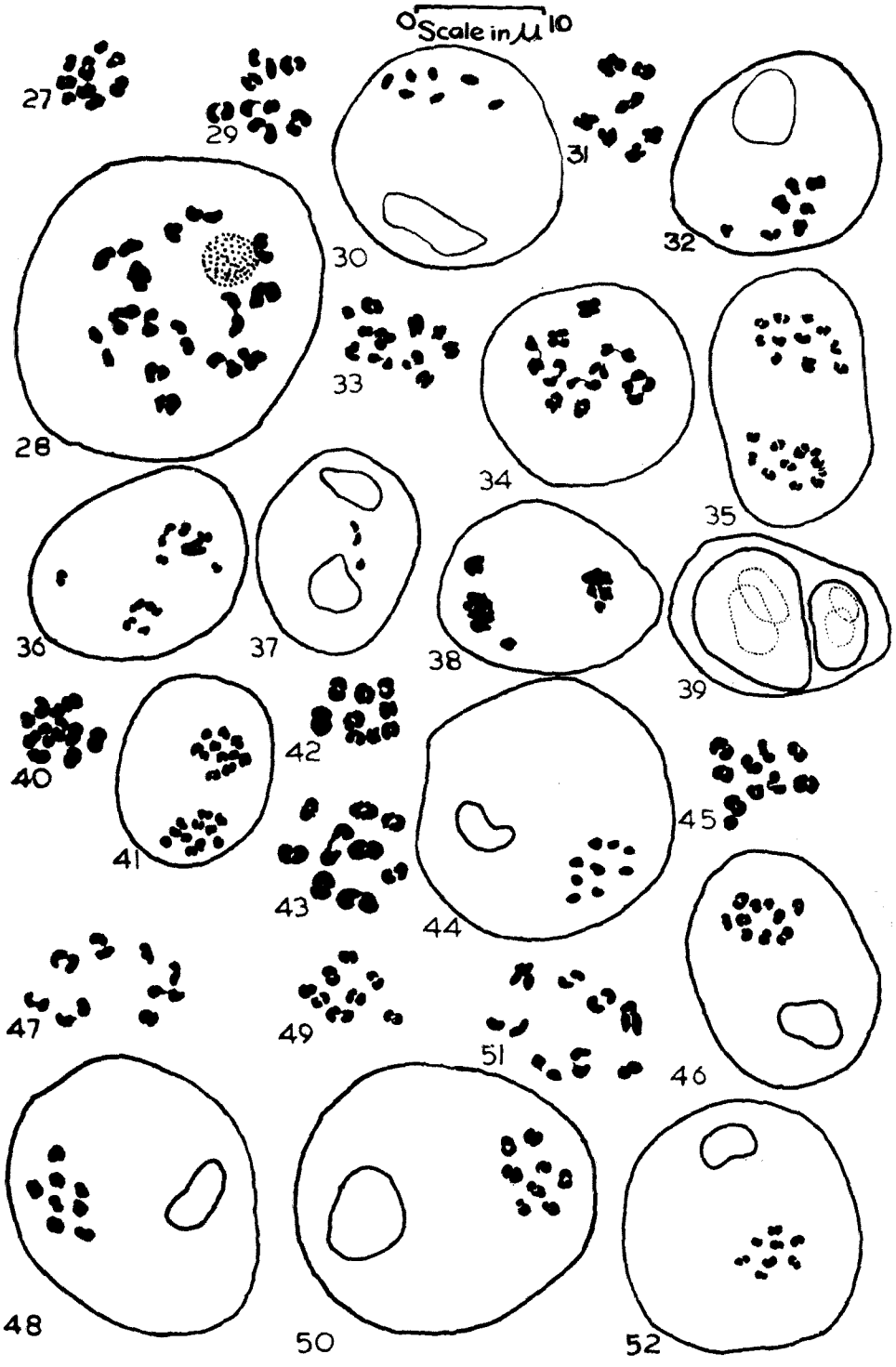
Somatic metaphases ×2400. 2 and 2a, *Brassica integrifolia* (2n=36) and variant nucleus (2n=16). 3, *Brassica integrifolia* var. *carinata* (2n=16). 4, *Sinapidendron angustifolium* (2n=20). 5, *Erucastrum canariense* (2n=18). 6, *E. gallicum* (2n=30). 7, *Brassicella erucastrum* (2n=16). 8, *Sinapis alba* (2n=24). 9, *S. arvensis* var. *orientalis* (2n=18). 10, *S. arvensis* subvar. *schkuriana* (2n=18). 11, *Hirschfeldia incana* (2n=14). 12, *Diplotaxis erucoides* (2n=14). 13, *Eruca sativa* (2n=22).



Figs. 1–13. 1, idiograms and range in size of the main chromosome types described. 2–13.



Figs. 14–26. Somatic metaphases. ×2400. 14, *Eruca sativa* var. *oblongifolia* (2n=22). 15, *Raphanus raphanistum* (2n=18). 16, *Raphanus sativus* (2n=18). 17, *Enarthocarpus lyratus* (2n=20). 18, *E. strangulatus* (2n=20). 19, *Crambe hispanica* (2n=60). 20, *Erucaria myagroides* (2n=16). 21, *Erucaria uncata* (2n=16). 22, *Rytidocarpus moricandioides* (2n=28). 23, *Carrichtera annua* (2n=16). 24, *Succowia barlearica* (2n=36). 25, *Schouwia thebaica* (2n=36). 26, *Moricandia arvensis* (2n=28).



Figs. 27–52. Meiotic plates. ×1700. 27, *Erucastrum canariense* (n=9), metaphase I. 28, *E. gallicum* (n=15), diakinesis. 29 and 30, *Hirshfeldia incana* (n=7), metaphase I and II. 31 and 32, *Diplotaxis erucoides* (n=7), metaphase I and II. 33–39. *Eruca sativa* (n=11). 33, metaphase I. 34, metaphase I with a quadrivalent. 35, metaphase II. 36, 37 and 38, metaphase II with laggards. 39, unequal pollen formation. 40 and 41, *E. sativa* var. *oblongifolia* (n=11), metaphase I and II. 42, *Raphanus raphanistrum* (n=9) metaphase I. 43 and 44, *R. sativus* (n=9), metaphase I and II. 45 and 46, *Enarthocarpus lyratus* (n=10), metaphase I and II. 47 and 48, *Rapistrum hispanicum* (n=8), metaphase I and II. 49 and 50, *R. rugosum* (n=8) metaphase *orientale* (n=8), metaphase I and II.

Table 2

Name of the taxa	Chromosome number		Karyotype formula	Length in μ	Total chromatin length (2n) in μ	Fig. no.
	n	2n				
Subtribe: Brassicinae		36	2A+4B+16D+14F	3.3–1.04	66.4	2,2a
Brassica integrifola (West) 0. E. Schulz						
B. integrifola (West) O. E. Schulz var. *carinata*		16	2A+2B+10D+2E	2.5–1.6	32	3
Sinapidendron angustifolium (DC.) Lowe		20	2A+2B+14D+2F	2.08–1.2	33.6	4
Erucastrum canariense Webb et Berth	9	18	2A+2B+6D+4E+4F	2.9–2.08	39.8	5,27
E. gallicum (Willd.) O.E. Schulz	15	30	2A+2B+14D+12F	2.08–1.25	47	6,28
Brassicella erucastrum (L.) O. E. Schulz		16	2A+2B+2C+4D+2E+4F	2.9–1.4	29	7
Sinapis alba L.		24	2A+2B+6D+14F	2.3–1.1	37.6	8
S. arvensis L. var. *orientalis*		18	2A+2B+12D+2E	2.5–1.8	37.2	9
S. arvensis L. subvar. *schkuriana*		18	2A+2B+14D	2.5–1.6	39.2	10
Hirschfeldia incana (L.) Lag. -Foss.	7	14	2A+2B+6D+4F	2.5–1.2	25.2	11,29,30
Diplotaxis erucoides (L.) DC.	7	14	2A+2B+10D	2.4–1.8	29.2	12,31,32
Eruca sativa Mill.	11	22	2A+2B+14D+4E	2.9–1.8	52.8	13,33–39
E. sativa Mill. var. *oblongifolia*	11	22	2B+2D+18F	1.6–1.2	29.4	14,40,41
Subtribe: Raphaninae						
Raphanus raphanistrum L.	9	18	2B+6C+6D+4E	3.1–1.6	42	15,42
R. sativus L.	9	18	4B+8D+2E+4F	2.7–1.2	38.2	16,43,44
Enarthocarpus lyratus DC.	10	20	4B+12D+4F	1.8–1.2	31.6	17,45,46
E. strangulatus Boiss.		20	4B+12D+4F	2.08–1.2	33.4	18
Crambe hispanica L.		60	8B+2C+18D+32F	2.5–0.41	94.4	19
Rapisturum hispanicum (L.) Crantz	8					47,48
R. rugosum (L.) All.	8					49,50
R. rugosum (L.) All. var. *orientale*	8					51,52
Subtribe: Cakilinae						
Erucaria myagroides (L.) Halac.		16	2A+2B+2C+10D	2.9–1.8	37	20
E. uncata (Boiss). Asch. et Schw.		16	2A+2B+12D	2.03–1.4	26.4	21
Subtribe: Vellinae						
Rytidocarpus moricandioides Coss.		28	4B+6D+18F	2.08–1.2	40.4	22
Carrichtera anna L.		16	2A+2B+12D	2.5–1.6	38.4	23
Succowia barlearia (L.) Medic.		36	2A+2B+32F	1.4–0.7	35.2	24
Schouwia thebaica Webb ex Parl.		36	2A+4B+8D+22F	1.6–0.9	44.8	25
Subtribe: Moricandinae						
Moricandia arvensis DC.		28	2A+2B+18D+6F	2.08–1.04	43.8	26

Discussion

The classification of Cruciferae has been extensively dealt with by Schulz (1919, 1936). Manton (1932) dealt with a cytological survey of the family and traced the lines of evolution. She noted aneuploid relationships between the genera, especially in Brassicinae and Brassiceae. Polyploidy, according to her, is associated with multiplication of forms.

Harberd (1972) studied 85 species of *Brassica* and closely allied genera. He included different species having the same chromosome number which could be hybridised resulting into interfertile plants, into distinct cytodemes. A total of 45 cytodemes were classified of which 35 were diploids with chromosome numbers ranging from 7–13.

Subtribe: Brassicinae

Under the genus *Brassica, B. integrifolia* with 2n=36 chromosomes and *B. integrifolia* var. *carinata* with 2n=16 chromosomes have been studied. Harberd (1.c.) on the basis of hybridisation studies included *B. integrifolia* var. *carinata* in a different cytodeme, namely, *B. carinata* cytodeme. *B. carinata* cytodeme arose as an allotetraploid between *B. oleracea* and *B. nigra* (Morinaga 1934). Harberd reported n=17 in *B. carinata.* His material, according to him, is also an allotetraploid between *B. oleracea* and *B. nigra* cytodemes but could be a separate synthesis from *B. carinata* proper. *B. integrifolia,* in the other hand, belongs to the *B. juncea* cytodeme which arose an allotetraploid between *B. campestris* and *B. nigra.* The present record of 2n=16 chromosomes in *B. integrifolia* var. *carinata* is rather interesting. The number is rather common for the species of *B. nigra.* On the basis of the chromosome number as well as the origin of *B. carinata* cytodeme as reported earlier, this material deserves a separate status. It is rather significant that in *B. integrifolia* variant nuclei with 2n=16 chromosomes have been recorded. Unfortunately no meiotic studies have been made yet which is necessary for maintaining the correct status.

Brassicella erucastrum shows 2n=16 chromosomes in the somatic nuclei. In all populations of this material n=12 chromosomes have been reported (Wright 1936, Favarger 1965, Harberd 1.c.), excepting in one population studied by Lorenzo-Andreu and Garcia Sanz (1950) where the number is 8. It may be that in *B. erucastrum* at least two distinct complexes are present, one showing n=8 and the other n=12. The relationship between these complexes can only be ascertained after thorough meiotic analysis and hybridisation.

Erucastrum canariense (2n=18) and *E. gallicum* (2n=30) have both been included in *Erucastrum canariense* cytodeme by Harberd. The genus *Erucastrum* as a whole shows a high degree of aneuploidy within its species and n=8, 9 and 15 are rather common (Manton 1932, Mulligan 1957 and Larsen 1960).

In one species of *Sinapiendron* viz. *S. angustifolium,* 2n=20 chromosomes have been recorded confirming the previous report of 10 bivalents in the species.

Three species of *Sinapis* viz. *S. alba* (2n=24), *S. arvensis* var. *orientalis* (2n=18), *S. arvensis* subvar. *schkuriana* (2n=18) have been studied of which the former

belongs to the *S. alba* cytodeme and the latter two to the *S. arvensis* cytodeme. In *Hirshfeldia incana* the chromosomes are slightly longer and the number is n=7 (Baez-Major 1934, Heiser and Whitaker 1948) similar to that in *Diplotaxis erucoides* (Manton 1932, Ibarra and La Porte 1947, Lübbert, 1951 and Harberd l.c.).

Both *Eruca sativa* and *Eruca sativa* var. *oblongifolia* contain 2n=22 and n=11 chromosomes in the somatic nuclei and pollen mother cells respectively. The chromosome study on *E. sativa* var. *oblongifolia* is done for the first time. Constancy in the chromosome number is a remarkable feature of the genus (Will 1966, Harberd 1972, Kaul *et al.* 1974).

As regards the karyotype, the chromosomes of *E. sativa* are comparatively longer than those of *E. sativa* var. *oblongifolia* and differ in the number of chromosome pairs bearing secondary constrictions. Meiotic behaviour is different in the two varieties. In *E. sativa* many abnormalities in meiosis have been recorded. Quadrivalents have also been seen indicating the presence of duplicated chromosomes. The meiotic behaviour of *E. sativa* var. *oblongifolia* is comparatively normal. In the two varieties, similarity in external morphological characters is distinct. It is likely that within this species, extensive karyotype changes have been effective in evolution at an interspecific level.

Subtribe: *Raphaninae*

Raphanus is a stable genus with n=9 chromosomes. Extensive cultivation and selection have led to the stabilization of several cultivated varieties. *Raphanus sativus* (2n=18) and *R. raphanistrum* (2n=18) both have medium sized chromosomes but differ in the details of the morphology. Both belong to the *Raphanus* cytodeme of Harberd (1.c.). *Raphanus raphanistrum* is cross fertile with *R. sativus* but F_1 is partially sterile (Trouard-Riolle 1914, Panetsos and Baker 1968). The difference in chromosome morphology as mentioned above may account for partial sterility.

Under the genus *Enarthocarpus,* the two species *E. lyratus* and *E. strangulatus* show 2n=20 chromosomes in the somatic nuclei and belong to the *Enarthocarpus* cytodeme of Harberd.

Three varieties of *Rapisturum* viz., *R. hispanicum, R. rugosum* and *R. rugosum* var. *orientale* have n=8 chromosomes confirming the previous reports of Manton (1932), Baez-Major (1934), Ibarra and La Porte (1948), Harberd (1972) and Queiros (1974).

Crambe hispanica with 2n=60 chromosomes is the species with the highest chromosome number in the present investigation and confirms the previous report of Manton (1932) and White (1975) for the species. The genus *Crambe* as a whole is characterised by high chromosome numbers, the lowest being 15. The high base number 15 might have been derived from a combination of 6 and 9 instead directly from 5 which is not found in this genus.

Subtribes: Cakilinae and Moricandiinae

Two species of *Erucaria, E. myagroides* and *E. uncata* under the subtribe *Cakilinae* have similar karyotypes with 2n=16 chromosomes.

Four genera under the subtribe Vellinae have been investigated. The report for *Rytidocarpus moricandioides* with 2n=28 chromosomes is a new one. *Carricht-*

era annua with 2n=16 chromosomes has been observed by Jaretzky (1932) and Manton (1932).

The present report of 2n=36 chromosomes in *Succowia barlearica* agrees with those of Jaretzky (1932), Manton (1932) and Harberd (1972).

One species under the genus *Schouwia* viz., *Schouwia thebaica* has been reported for the first time. The karyotype shows 36 chromosomes and the morphology is similar to *Succowia barlearica*.

Under the subtribe Moricandiinae, *Moricandia arvensis* with 2n=28 chromosomes has been investigated confirming the reports of Manton (1932) and Reese (1957).

Conclusion

A general assessment of the karyotypes shows the presence of a series of base numbers within the tribe Brassiceae as a whole even extending to the generic level. Aneuploidy has been a major factor in the evolution of the different species within this group. The different base numbers have obviously been derived one from the other through numerical alterations as intraspecific variations too are on record. It is difficult to state as which of the several base numbers viz., x=6, 7, 8, 9 and 10 is the most primitive one. Some of them are more frequent for a particular taxon than the others. Evidently these numbers have been stabilised for that particular taxon (species or genus) during their long antecedent period of evolution. Polyploidy has been observed in several cases, but aneuploidy is much more frequent and has a definite role in the evolution of species.

Structural alteration of chromosomes is present as shown by minor differences in chromosome morphology between different species. It has a relatively much less important role in the diversification of genera since the overall patterns of karyotypes are remarkably similar. During evolution, diminution of chromosome size has accompanied aneuploidy or polyploidy as has been observed in many other families as well (Sharma 1974). The role of gene mutation from previous records is rather significant in that it has resulted as well in the formation of distinct interbreeding complexes (cytodemes) within a species through the establishment of compatibility barriers.

Summary

Chromosome studies were carried out on 28 species and varieties belonging to 18 genera of Brassiceae after evolving special pretreatment techniques. In general the tribe is characterised by a graded karyotype with medium to short chromosomes and constrictions mainly median to submedian in position.

In the subtribe Brassicinae, eight genera with chromosomes ranging from 14 to 36 in the somatic nuclei have been studied. Different base numbers have been observed and the same genus often shows more than one number. Intraspecific variations are on record. Four genera under the subtribe Raphaninae investigated have chromosome numbers ranging from 2n=16 to 60. In the subtribe

Cakilinae, 2n=16 chromosomes are present in two species of a genus investigated.

In the subtribes Vellinae and Moricandiinae 2n=16, 28 and 36 chromosomes have been observed.

In the tribe Brassiceae, various base numbers namely, 6, 7, 9 and 10 have been observed but it is difficult to determine which one is more primitive. In evolution, polyploidy has been encountered in many cases. Aneuploidy is even more frequent. Gene mutation has been responsible for stabilisation and diversification of genera. The role of structural alteration of chromosomes is comparatively less in the diversification of major taxonomic units.

Acknowledgement

The authors are thankful to the University Grants Commission (Special Assistance Programme) for the award of a fellowship to one of them (K. S.) during the tenure of which this work was done.

References

Baez-Major, A. B. 1934. Estudio cariológico de algunas crucifers y su interpretatión en la sistemática. Cavanillesia **6**: 59–103.

Favarger, C. 1965. Notes de caryologie alpine IV. Bull. Soc. newchâtel. Sci. Nat. (Sér 3) **88**: 5–60.

Harberd, D. J. 1972. A contribution to the cytotaxonomy of *Brassica* (Cruciferae) and its allies. Bot. Linn. Soc. **65**: 1–23.

Heiser, C. B. and Whitaker, T. W. 1948. Chromosome number, polyploidy and growth habit in California weeds. Am. J. Bot. **35**: 179–186.

Ibarra, E. and La Porte, J. 1947. Las Cruciferas del genero *Diplotaxis* adventicias en la Argentina. Rev. Argen. Agron. **14**: 261–272.

— and — 1948. Las Cruciferas del género *Rapistrum* adventicias en la Argentina. Rev. Argen. Agron. **15**: 81–89.

Jaretzky, R. 1932. Beziehungen zwischen Chromosomenzahl und Systematik bei den Cruciferen. Jb. wiss. Bot. **76**: 485–527.

Kaul, M. K., Bhat, B. K. and Bakshi, S. K. 1974. IOPB Chromosome number reports XLIII. Taxon **23**: 193–196.

Larsen, K. 1963. Contribution to the cytology of the endemic Canarian element II. Bot. Not. **116**: 409–424.

Lorenzo-Andreu, A. and Garcia-Sanz, P. 1950. Chromosomas somáticos de plantas espontaneas de Aragon II. An. Estac. exp. Aula Dei **2**: 12–20.

Lubbert, G. 1951. Vergleichende cytologische, morphologische und physiologische Untersuchungen innerhalb der Gattung *Diplotaxis*. Beitr. Biol. Pfl. **28**: 254–295.

Manton, I. 1932. Introduction to the general cytology of Cruciferae. Ann. Bot. **46**: 506–556.

Morinaga, T. 1934. Interspecific hybridisation in *Brassica* VI. the cytology of F_1 hybrids *B. juncea* and *B. nigra*. Cytologia **6**: 62–76.

Mulligan, G. A. 1957. Chromosome numbers on Canadian weeds I. Canad. J. Bot. **35**: 779–789.

Panetsos, C. A. and Baker, H. G. 1968. The origin of variation in "wild" *Raphanus sativus* (Cruciferae) in California. Genetica **38**: 243–274.

Queiros, M. 1974. Contribution to the knowledge of the cytotaxonomy of spermatophyta of Portugal IX. Cruciferae. Bull. Soc. Broteriana **47**: 315–335.

Reese, G. 1957. Über die Polyploidiespektren in der norsaharischen Wüstenpflanzen. Flora **144**: 598–634.

Schulz, O. E. 1919. Cruciferae-Brassiceae, Part I. Brassicinae and Raphaninae. In A. Engler, Das Pflanzenreich IV, 105. Leipzig: Engelmann.

— 1936. Cruciferae. In A. Engler, und P. Prantl. Die natürlichen Pflanzenfamilien. 2nd ed. **17b**: 227–658. Leipzig: Engelmann.

Sharma, A. K. 1974. Plant cytogenetics. The Cell Nucleus. **2**: 263–291.

Trouard-Riolle, Y. 1914. Recherches morphologiques et biologiques sur les radis cultivees. Annls. Sci. agron. fr. **31**: 346–550.

White, G. A. 1975. Distinguishing characteristics of *Crambe abyssinica* and *C. hispanica*. Crop. Sco. **15**: 91–93.

Wills, A. B. 1966. Meiotic behaviour in the Brassiceae. Caryologia **19**: 103–116.

Wright, F. R. E. 1936. The Lundy *Brassica* with some additions. J. Bot. Lond. **74**; 1–8.