CYTOGENETICAL EVOLUTION WITHIN THE *SISYMBRIUM IRIO* COMPLEX

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SIGNIFICANT results have been obtained in the field of cytogenetical evolution in the angiosperms in the United States of America, Europe, Japan, Canada and lately also in Australia and New Zealand. Out of these, the work of the Californian group is synthetic in approach and is the most illuminating. It has become apparent that chromosomes are a valuable guide to the understanding of the evolutionary processes, and it is natural that their study has received the maximum attention in recent years. Few integrated studies of this type have been made in India, particularly on the wild angiosperms. Considering the richness of the flora and the diversity in eco-geography and geology of the Indian subcontinent, such studies on our plants should prove to be particularly rewarding. We are using such an approach for the study of the angiospermic flora of our region, hoping ultimately to monograph it with an evolutionary bias. We are at present concentrating on some polymorphic taxa which are taxonomically "problem-species". A short account of the cytogenetical mechanism underlying the evolution within one such species, *Sisymbrium irio* Linn. (Cruciferae), is summarized here (*cf.* Khoshoo, 1959 *e*, 1960, 1963 *b* and unpublished).

A combined genecological and cytogenetical attack on a large number of selections gathered from diverse sources and covering almost all variations seen in Nature in the Punjab, revealed the occurrence of diploid, tetraploid, hexaploid and octoploid races, and stray triploid hybrid individuals. Furthermore, the tetraploid itself contains three morphologically and ecologically distinct types. These are *caulis*, *subcaulis*, and *acaulis*, suited respectively to wet, dry and moist-grazed habitats. Their morphological and physiological characters appear to have a definite adaptive value, because these are in tune with the requirement of the three types of habitats which they occupy (*cf.* Khoshoo, 1957 *a*, 1958 *c*). The cytological characteristics of each of the races (*cf.* Khoshoo, 1955, 1957 *b*, 1959 *a*) may be summarized as below.

CYTOLOGY OF THE RACES

Diploid.—It possesses 14 chromosomes in the leaf cells and corresponding with this number, 7 bivalent were noted at diakinesis. Meiosis is normal, resulting in perfect pollen and seed fertility.

Autotetraploid.—These plants were raised by the colchicine treatment of the diploid and possess 28 chromosomes in leaf cells. During diakinesis 7 quadrivalents are most commonly formed. The meiosis is on the whole normal. Pollen fertility is about 98% but percentage of good seeds is lower being 83–94.

Tetraploid.—All the three types were investigated and showed uniformly 28 chromosomes in the leaf cells. Fourteen bivalents were persistently seen during diakinesis. The pollen and seed fertility is ordinarily normal.

Polyhaploid.—One plant of this type was discovered which resembled the tetraploid *subcaulis* qualitatively. The 14 chromosomes found in its somatic tissue generally formed 14 univalents during diakinesis. Average number of bivalents per cell was 0·4. The plant was totally pollen and seed sterile.

Triploid.—These hybrids possess $2n = 21$ and the most predominant association during meiosis is 7 bivalents and 7 univalents. The bivalents are of rod type and often loosely attached, and during anaphase I they often form bridges with or without fragments. Anaphase II is also not normal. Trivalents are conspicuous by their virtual absence (mean number 0·02 per cell). Pollen and seed fertilities are 0·005% and 0·0001–0·002%, respectively. The progeny of triploids, if viable, is always hexaploid.

Hexaploid.—Always 42 chromosomes were counted in leaf cells with 21 bivalents as the most preponderant association during meiosis. However, in some cells 1 or at the most 2 quadrivalents were observed during diakinesis. The average number of quadrivalents was only 0·5 per cell. Fertility of pollen is 75–93% and that of seeds 68-89%.

Octoploid.—Somatic chromosome number is 56 and during diakinesis there are 13–5 quadrivalents + 2–18 bivalents. However, mean number of quadrivalents is only 6·70 per cell. Anaphase I and II are generally clean. The percentages of pollen and seed fertilities are 92–100 and 70–96, respectively.

Synthesis of Higher Polyploids

It has been demonstrated that the triploid individuals arise every year by the crossing between diploid and tetraploid *caulis* (Khoshoo, 1959 *b*). Both parents occupy the same habitat and grow indiscriminately mixed together. The hexaploid race has been raised as the progeny of the triploids, and has also been synthesized by doubling the chromosome number of the triploids by colchicine.

The octoploid individuals arise from the "off-season" plants of the tetraploid race. Due to "modificative asynapsis" in the "off-season" plants there are produced octoploid individuals in their progeny, in all probability as the result of fertilization between unreduced spores. The colchicine-induced octoploids from tetraploid confirm

such an origin. The artificial hexa- and octoploids show higher multivalent frequency than their natural counterparts (Khoshoo, 1959 *b*).

CYTOLOGY OF THE HYBRIDS

¶ The various races including the colchi-autotetraploid from the diploid race and synthesized hexa- and octoploid types were crossed amongst one another in all directions. From such crossing only the following types of hybrids have been secured whose meiotic details are given below (*cf*. Khoshoo and Sharma, 1959; Khoshoo, 1959 *d*).

Hybrids within the tetraploid race.—The six reciprocal hybrids involving the three types of tetraploid always show 14 bivalents as in their parents. Hybrids are fully fertile and segregate in F_2. The genetics of the morphological and physiological differences that enable them to have distinct habitat preferences is under study.

Hybrids within the octoploid race.—The two types of octoploid have also been reciprocally crossed. The meiosis and fertility of the hybrids resemble their parents.

Diploid × *tetraploid and reciprocal.*—The progeny in both the cases was triploid and meiosis resembles the natural triploid plants.

Autotetraploid × *tetraploid* (*Hybrid-tetraploid*).—The diakinesis in this complex is conspicuous by the presence of 7 bivalents + 14 univalents. The mean number of trivalents per cell is only 0·2. Anaphase is characterized by being highly abnormal because of the presence of univalents. Though the hybrid was tetraploid in constitution, yet it esembled neither of its parents in the nature of its meiosis.

Natural × *synthesized hexaploids and octoploids.*—These hybrids did not reveal any aberrant behaviour up to metaphase I, but at anaphase I and II bridges were noted with and without fragments, thereby indicating that structural differences exist between the chromosomes of the raw and natural counterparts.

GENOME RELATIONS

The following conclusions emerge after taking together the foregoing cytological analysis of the various races, polyhaploid plant and natural and artificial hybrids (Khoshoo, 1960):

1. The tetraploid can be regarded as a genomic allotetraploid for two reasons. Firstly, it shows only bivalents, while autotetraploid (from the diploid) shows quadrivalents, even though both have the same chromosome number and size. Secondly, the polyhaploid plant from the tetraploid has 14 univalents which is further strengthened by the presence of 14 univalents even in the hybrid-tetraploid. In the latter the 7 bivalents are formed by the 14 chromosomes of the auto-tetraploid while the 14 univalents by the chromosomes of the natural tetraploid. In short, the latter has arisen after an intercenospecific cross and is a perfect allotetraploid.

2. The relationship between the diploid and the tetraploid is clarified by the meiosis in triploid and hexaploid. In triploid there are 7 rod bivalents and 7 univalents, while in hexaploid there are 21 bivalents. Therefore, the 7 bivalents of triploid are due to the pairing between homeologous chromosomes which is further supported by the presence of bridges with and without fragments formed by the bivalents of the triploid. Evidently, in triploid the chromosomes form bivalents because of the differential affinity between the homologous chromosomes, but on doubling (*i.e.*, in hexaploid) chromosomes again form bivalents because of the preferential pairing between the homologous chromosomes. Therefore, if the genomic constitution of the diploid is AA, then that of tetraploid is $2(A_1B)$, of triploid AA_1B and of hexaploid $2(AA_1B)$. The constitution of the hexaploid is auto-allohexaploid in which the autoploid portion $[2(AA_1)]$ is segmental-alloploid in nature.
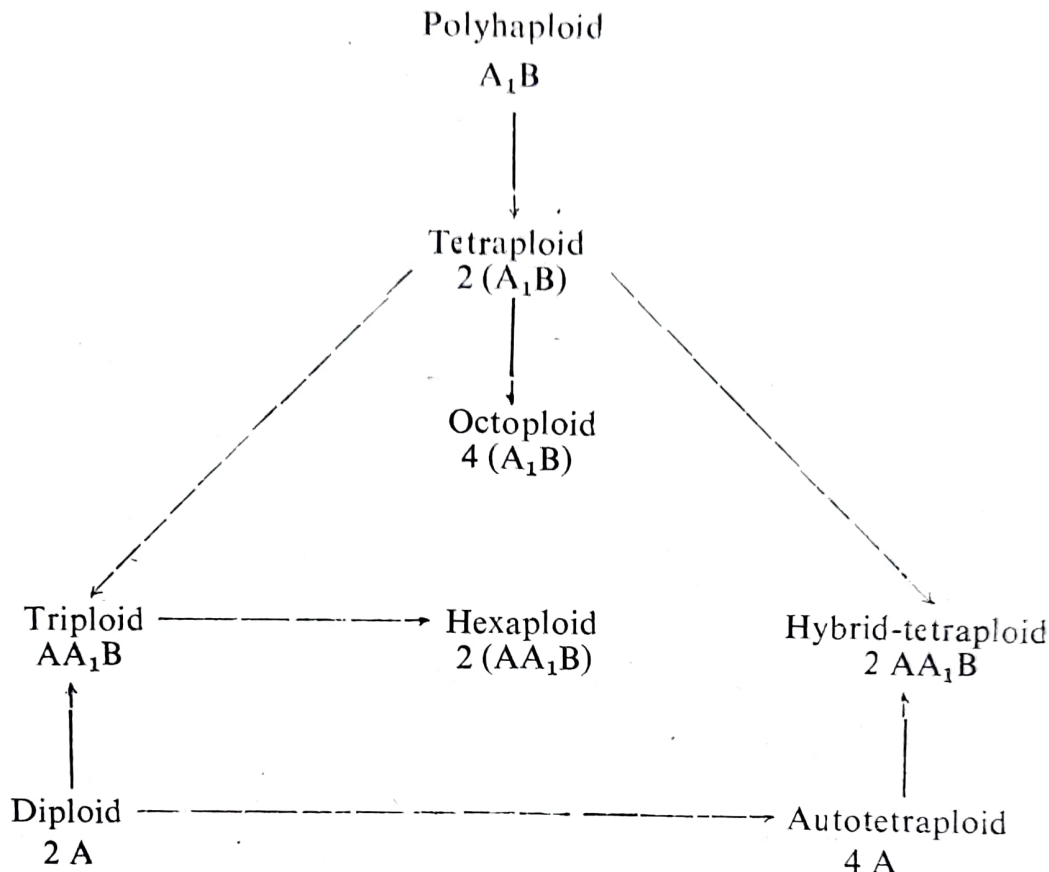
3. The constitution of the octoploid is simple to infer since it is only a duplication of the tetraploid. It is, therefore, $4(A_1B)$ and is auto-allo-octoploid.

4. The presence of higher quadrivalent frequency in the raw hexa- and octoploids than their natural counterparts is due to their being raw. As such, they contain more duplicated genic material. Furthermore, the raw types have not undergone selection for the reduction of quadrivalents. The hybrids between the natural and synthesized types show evidence of rearrangement of chromosomes. The reason for this is the possible difference in the chromosomes of the strains involved for the origin of these races in Nature and those utilized by the writer. Alternatively, it may be looked for in the difference in modes of their origin. The natural types arise through fertilization between unreduced spores, while the synthetic types were prepared by the somatic doubling by colchicine.

5. The three genomes, A, A_1 and B, are not entirely non-homologous. Of course, the degree of homology between A and A_1 is considerable; however, the intergenomic pairing seen in polyhaploid and also in triploid and hybrid tetraploid, though low, nevertheless, shows that the A and A_1 genomes on one hand, and B on the other, do contain some segments in common.

6. According to the present analysis the A genome in tetraploid is differentiated in comparison to the A genome of the diploid. It does not necessarily mean that the diploid is not one of the parents of the tetraploid. The latter is very widely distributed and appears to be an old taxon (*cf.* Khoshoo, 1961 *a*). It is possible that ever since the A genome of the diploid entered in association with the B genome, to form the present tetraploid, the two genomes have undergone a measure of structural rearrangement and genetical divergence in comparison to the original condition, one of which is still preserved in the present diploid (AA).

The foregoing analysis has been summarized in the following diagram.

$$\text{Polyhaploid} \atop A_1B$$

$$\downarrow$$

$$\text{Tetraploid} \atop 2(A_1B)$$

$$\downarrow$$

$$\text{Octoploid} \atop 4(A_1B)$$

Triploid $\longrightarrow$ Hexaploid   Hybrid-tetraploid
AA_1B $2(AA_1B)$ $2\,AA_1B$

$\uparrow$ $\uparrow$

Diploid $\longrightarrow$ Autotetraploid
$2\,A$ $4\,A$

Extent and Nature of Barriers to Gene Exchange

Isolating mechanisms, though negative factors, are nevertheless almost an integral part of the evolutionary processes. There are four types of barriers to crossability in this complex, namely, ecological preferences, autogamy, seed incompatibility resulting from the matings between parents with different ploidy levels and, lastly, chromosomal sterility (Khoshoo, 1959 *e*, 1963 *b*).

On the basis of the habitat preferences the complex can be classified into three categories, accordingly as the races occupy dry (tetraploid *subcaulis* and octoploid), moist-sun or shade (diploid, tetraploid *caulis* and hexaploid), and moist-grazed (tetraploid *acaulis*) situations. Ordinarily there is no chance of hybridization between members of the three different categories. On the other hand, one can expect hybrids to arise between races occupying one and the same type of habitat. However, such crossing is prevented due to more or less obligate autogamy. Autogamy is augmented by the fact that there is decrease in length of the ovary in the higher ploids (Khoshoo, 1959 *c*). The only hybrids possible are with the protogynous flowers of the diploid as the female parent. Due to seed failure, not all the expected types

of hybrids arise. An embryological examination has shown that a serious endosperm malfunction results in crosses involving parents of different ploidy levels (Khoshoo and Sharma, 1959; Khoshoo and Raj, 1960). These three types of isolation barriers are often superimposed upon one another. In Nature, due to their interplay, there results only one type of hybrid, *i.e.*, triploid.

It may be emphasized that even this hybrid does not break the isolation between its parents, *i.e.*, diploid and tetraploid. This is because of the chromosomal sterility (*cf.* Stebbins, 1950) resulting in total sterility. No seed is produced except for the rare occurrence $(0 \cdot 0001 - 0 \cdot 002\%)$ of hexaploid seeds resulting from the chance fusion between unreduced spores. Such seeds, as indicated earlier, result in hexaploid individuals which again are isolated. The net result is that the diploid and the tetraploid though bridged by the triploid and hexaploid, remain, nevertheless, isolated and there is no gene exchange between the parents and their amphiploid, *i.e.*, hexaploid.

There is, however, no seed-incompatibility between races with the same chromoscme number, whether they are genetically akin or not. This is apparent from the vigorous hybrids that result from the matings among the three tetraploid types, two octoploid types and, lastly, what is more significant, between autotetraploid $(4 A)$ and tetraploid $(2 A_1 2B)$. Furthermore, it is apparent that the extent of natural hybridization is very limited primarily because of the autogamy. So far the only natural hybrids seen were the triploid individuals; however, recently I have discovered, what may be looked as inter-ecotypic (between *caulis* and *subcaulis* types of tetraploid) hybrid swarms in the intermediate habitats. These are being analysed at present.

The foregoing discussion has been summarized in the following diagram.

Hexaploid————————Octoploid
Diploid————————Tetraploid

———— Cross successful   ------ Cross unsuccessful
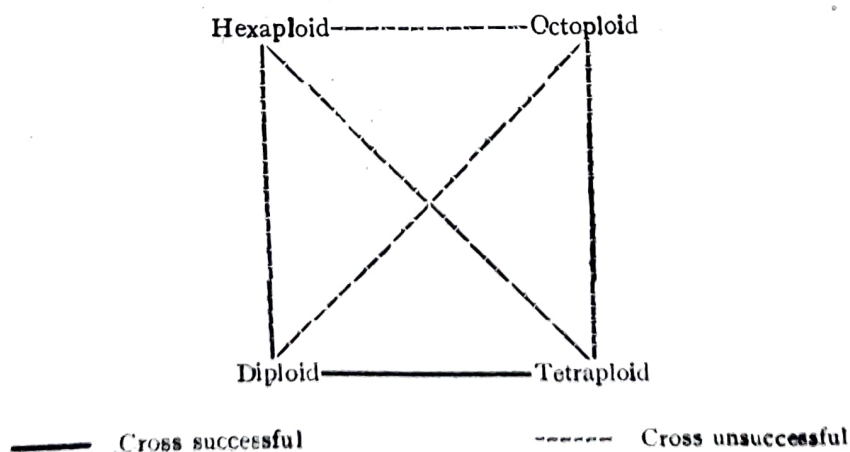
GENERAL REMARKS AND CONCLUSIONS

Sisymbrium irio is not only very widely distributed, but also occupies a geologically, edaphically and climatically diversified area (*cf.*

Khoshoo, 1963 *a, b*). It is, therefore, understandable that the species is polymorphic. The polymorphicity owes its origin to the phenotypic modifications induced by the varying environments on one hand, and the genetic diversity on the other (Khoshoo, 1958 *b*, 1963 *b*). The former is not polymorphicity in the real sense, because it vanishes as soon as the plants are cultivated under uniform environmental conditions. It is completely reversible. However, capacity of the various genotypes to modify in relation to the environments is a distinct advantage, because it enables them to grow in a variety of habitats, from adverse to favourable.

The genetic diversity is primarily due to the auto- and/or allopolyploidy, and secondarily to the gene mutations and structural alterations in chromosomes. These phenomena constitute the cytogenetic mechanisms that underly evolution in this complex.

At least two (A and B) and at the most three (A, A$_1$ and B) genomes have been interwoven by auto- and allopolyploidy to give rise to one phylogenetic unit. In this unit there occur three distinct polyploid races (4x, 6x and 8x) and stray triploid hybrid individuals. Furthermore, at the tetraploid level there have resulted three distinct ecotypes due to genic differentiation. These types seem to have arisen due to the selective action of the edaphic and biotic factors. The hybrids between the raw and the synthesized polyploids have revealed the role played by the structural alterations in chromosomes.

One of the important features of evolution in the complex is the simultaneous and abrupt origin of the barriers to crossability of the various races, since polyploidy *per se* acts as an effective barrier. Its action is supplemented by habitat preferences, autogamy and chromosomal sterility. For their perpetuation and further spread these taxa have to be effectively isolated from one another, particularly when some of them occupy the same habitat. The various races do not show very strong morphological differences, but with the strong isolating mechanisms in operation, these are likely to evolve independently and may, in course of time, attain the requisite morphological distinctness. Here, therefore, we are faced with a situation where the various races have a taxonomic status of not more than varieties, but a cytogenetic status of distinct and mature species.

In conclusion, it may be said that the present results reveal the combined role of polyploidy, hybridization, gene mutations and structural changes in the evolution and variation of a single species.

It is of interest to point out that there appears to be a correlation between the annual habit, self-pollination and polyploidy in this complex (*cf.* Grant, 1956; Khoshoo, 1958 *a*; Baker, 1959). This is because of the fact that all the polyploid races of this complex have arisen as the result of fertilization between unreduced spores, and chances of production of polyploids in this manner are greater with

self-pollination than with the cross-pollination. Furthermore, such a mode of origin assumes importance because the species has an annual habit, which, so to say, acts as the limiting factor.

The species complex is able to maintain its far-flung boundaries (*cf.* Khoshoo, 1963 *a*) because of its excellent genetic system which promotes weedy tendencies. Some of its traits are: extreme aggressiveness, capacity for extreme phenotypic modifications (that arise in response to the diverse environmental conditions in which it can grow), obligate autogamy, high reproductive potential and auto- and allo-polyploidy accompanied by efficient isolating mechanisms. The autogamy is a distinct advantage under the habitat conditions which this complex exploits, because it causes these races and ecotypes to be ordinarily inbred and, therefore, these are true-breeding. Furthermore, it also ensures high seed production and high viability.

Similar type of data are being obtained for the various taxa of the angiosperms of this region and some interesting results have been already obtained. It is hoped that in course of time it will be possible to present an integrated picture of evolutionary corspectus of the angiospermic element of the flora of this region. This in turn would throw light on the composition, evolutionary status, affinity and migration of the flora of this region in relation to past geographical and geological events.

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