Cytogenetics of Polyploids

II. Cytology of polyploid *Artemisia maritima* Linn

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Artemisia maritima is a shrubby white woolly plant with a stout root stock with many segmented bipinnatesect alternate leaves and possesses a homogamous disciform yellowish head. It is characteristic of deep inner valleys of Himalayas to which moonsoon does not penetrate. It covers the hill sides replacing grass and the herbaceous vegetation. In Gurez (Kashmir) it grows on dry rocky exposed places at low levels but as we ascend up, of the lower vegetation it becomes dominant one. At higher altitudes it is predominantly found on all the slopes, regardless of the nature and position of the slope. Its foliage has an aromatic smell and possesses santonine, its roots and branches are used as a fuel by the natives of Gurez. In fact, it not only occurs in India but is also distributed from the coasts of Europe and extends throughout the saline tracts in N. Asia (Hooker, 1879). This species has been reported as a diploid from Europe by Polyla (1948) and hexaploid from Japan by Suzuka and Koriba (See Darlington and Wylie). Seeds of the plant imported from Japan have been grown at Tangmarg (Kashmir) and are hexaploid. Khoshoo and Sobti (1958) reported tetraploidy in the material. In all the clones examined, present author found $2n=36$ (Fig. 14). The failure of viable seed formation in this polyploid aroused an interest to study the meiotic behaviour of chromosomes at the tetraploid level to aid in the study of nature and probable phylogeny of the material. The present study has these objects also, viz., a. the interrelationship of aberrant chromosome movement, spindle organization, microsporocyte division and their relation to the changed meiotic rates, b. determination of the possible limits of deviation from the normal patterns of structure and function which provides some information concerning the limits of variability. Since, the fundamental processes by which the chromosome reappointment and cell division take place are still unknown, an objective remains the description of aberrant details of meiosis from the routined normal meiosis of diploids

Materials and methods

Most of the material used in the present study was obtained from 3 clones that were got from Gurez (Kashmir), along with their rootstocks in

1962. Since the plant was found to propagate vegetatively a genetic homogeneity of the material is visualised. Flower buds from immature heads were fixed in 3:1 mixture of 95% ethanol and glacial acetic acid for 48 hours and then washed and stored under refrigeration in 70% ethanol.

Observations

Efforts to homologize chromosome behaviour and stages of meiosis in the present material encounter many difficulties. Major problems in the interpretation of meiosis in the material arise from irregularities and absence of a uniform and regular sequence of events and from the wide ranges of stages found randomly distributed among the microsporocytes in a single anther. The nuclear upset may be manifested even during prophase I, in some microsporocytes. Densely staining chromocenters, precluding usual chromosome behaviour and resulting in death of such cells were also observed. In some sporocytes which were not completely arrested such disturbances caused stickiness, with members of the complement fused to one another. Coenocytes were also found. The nuclei of some of these appeared tetraploids, but in others chromosome complement was numbered several hundred. Many other coenocytes possessed both very small nuclei consisting of a few chromosomes and in others very large. Precocious separation of chromosomes sometimes occurred at or before diakinesis (Figs. 1 and 10). At metaphase I, a plate of several irregularly oriented chromosomes was sometimes formed with other members of the complement scattered throughout the cell (Fig. 5). Some chromosomes were already at the poles while others were just becoming oriented at the plate (Fig. 5). In other sporocytes precocious separation (Fig. 1) and faulty spindle formation resulted in a complete absence of orientation of chromosomes (Fig. 3). Congression to the plate was often irregular. Multiple association of chromosomes in many sporocytes and bridges unaccompanied by fragments were the result of stickiness and not due to polyploidy involved (Fig. 5). Failure of association of homologous chromosomes at diakinesis and metaphase I was also observed. The degree of asynapsis differed in different cells (Figs. 1-4). Metaphase I was characterized by varying number of scattered univalents located near or far away from the clumped bivalents on the metaphase plate (Fig. 5). The two homologous chromosomes comprising a bivalent at diakinesis and metaphase I appeared to be associated terminally. In Fig. 3, some chromosomes comprising a bivalent seem to be associated by a lightly staining chromatin and bear a resemblance to several of the bivalents in Brown's (1949) photomicrographs of diakinesis in *Lycospermum esculantum*. As pointed out by Brown (1949), interstitial chiasmata occurring within distally located lightly staining regions may be difficult to distinguish from terminal chiasmata. In some cells, at diakinesis the well rounded univalents tend to be distributed at random throughout

Figs. 1-9. 1, precocious separation of chromosomes and their random distribution. 2, early diakinesis-bivalent formation. 3, early metaphase-non orientation of chromosomes. 4, reverse movement of chromosomes from poles to centre. Note the secretion of a thick wall by P.M.C., giving the appearance of a pollen grain. 5, metaphase plates with irregularly oriented chromosomes; chromosome stickiness and scattered univalents. 6, anaphase I: Unequal distribution of dyads and laggards. 7, anaphase II with loose grouping of chromosomes, and many independent plates (Complement fractionation). 8-9, tetrads with additional micronuclei. Magnification: Figs. 1-9 $\times 1900$.

the nucleus (Fig. 1 and 10). The chromosomes pass from diakinesis to a scattered arrangement in relation to the first division spindle with only occasional cells showing any pronounced grouping of the chromosomes near the equator. The extent of movement, of intact chromosomes towards the spindle poles, varies greatly and appears in part to be a function of timing relations. In many cells, the initial movement of the chromosomes in the direction of the poles (Fig. 11) is followed by a movement to the equatorial region of the spindle (Figs. 12-13). This reverse movement often continues until most, but rarely all of the chromosomes occupy a position at the equator (Figs. 12-13). This reversal of the chromosome movement has also been observed by Snyder (1961) in *Paspalum secans*. Advanced stages in the separation of chromosomes into two distinct groups were never observed, but many cells were commonly seen in which various stages of this division were in the process of breaking down to form restitution nuclei. No further division or movement took place in these chromosomes,

Figs. 10-14. 10, diakinesis showing scattered univalents. 11, 1st. anaphasic separation of chromosomes in two groups. 12-13, stages in the movement of chromosomes back to the equator. 14, somatic metaphase; $2n=36$. Magnification: Figs. 10-13, $\times 1900$. Fig. 14. $\times 2400$.

instead the pollen mother cells containing these chromosomes assumed a young pollen grain like appearance (Fig. 4). A thick wall was secreted around this parent cell. At metaphase I common abnormalities observed were a poor spindle development (Fig. 5), absence of a complete bivalent formation in many pollen mother cells (Figs. 1 and 10), nonorientation of most of the chromosomes and clumping to the equatorial plate (Fig. 5). A common feature of metaphase I is that chromosomes get oriented at many equatorial plates but still many are left behind (Fig. 5). These plates represent separate spindles and function independently during the latter stages of meiosis. The resulted scattered chromosomal groups are not synchronized. These independently existing metaphase plates may be parallel, distant, adjacent or even at right angles to each other, a case, as was observed by the author in an autotetraploid *Artemisia vulgaris* (Koul, 1964a and b). The configurations of 50 metaphase plates were analysed. A range in association from 18 bivalents in some cells (Fig. 2) to a complete disassociation of

bivalents with 36 univalents were observed (Fig. 1). The average number of univalents per cell was 13.7. With the exception of some cells having a few bivalents and the remainder complement disassociated (Fig. 3), the metaphase I configuration was clumped (Fig. 5); so that a precise analysis of the number of bivalents and univalents in an individual cell was not possible. An average of 17.8 univalents occurred in the cells showing asynapsis. The average number of univalents per asynaptic cell and the percent cells with univalents could not be very accurately estimated, since cells with additional univalents were present but undetected in the crowded metaphase configurations. Lagging chromosomes and an unequal distribution of dyads (Fig. 6), absence of an active polar movement, first division restitution nucleus (Fig. 4) were evident at anaphase I. This division was not exactly a multiple of the haploid number but varied from cell to cell and was random and unequal (Fig. 6).

Chromosome behaviour during the post restitution division is generally regular in contrast to that observed in pre-restitution stages. Interkinesis is a stage of short duration during which the chromosomes rapidly condense and get distributed throughout the nucleus. In prophase II, in a few cells, presence of a varying number of nucleoli, chromosome clumping, lagging chromosome forming micronuclei and similar other configurations were seen. The irregularities in the metaphase cells are very few and result from the unequal and independent separation of chromosomes at MI.

At anaphase II, a loose grouping of the chromosomes and phenomena like non-disjunction and complement-fractionation were seen (Fig. 7), as have been also found in *A. vulgaris* by the present author (Koul 1964b). Due to these aforementioned abnormalities, resulting in an unbalance in chromosome number, triads, pentads and polyads were observed (Fig. 8-9). The microspores produced were highly irregular. Micronuclei were evident in many of the spores (Figs. 8-9) and the occurrence of small supernumerary spores along with the large spores were also found frequently (Fig. 8-9). In view of this abnormal meiosis especially the occurrence of the phenomena like asynapsis, restitution nuclei, non-disjunction and complement fractionation, the microspore nucleus is bound to contain varying chromosome numbers. So a pollen study was undertaken. Many of the young uninucleate microspores were shrunk. At dehiscence pollen grains were deformed in shape and did not take carmine stain. The pollen sterility ranges between 87-92%.

Discussion

The occurrence of a high univalent frequencies, restitution nuclei and such abnormalities like cell cleavage that is out of phase with chromosome behavior have also been reported in many hybrid microsporogenesis. Walters (1958) observed these irregularities in a study of three intersectional

hybrids in *Bromus*. In the present material it seems that the univalent chromosomes have arisen in two ways. It is either a chromosome which has never undergone pairing at zygotene or it is one which paired to form a bivalent but whose two component chromosomes separated at diplotene for no chiasma was formed between them. Asynapsis here has been utilized to designate failure of association of chromosomes during the first division. But in the present material zygotene and pachytene stages are not amenable for detailed studies. Consequently, it was not possible to determine whether exactly the occurrence of univalents at diakinesis and metaphase I is due to the initial absence of synapsis or due to the subsequent disassociation following synapsis. Li *et al.* (1945) utilized the term asynapsis for failure of homologues to remain associated. Unpaired strands could be observed in some pachytene configurations. However, the tangled nature of pachytene and other early prophase stages eliminated comparison of the frequency of pairing during early prophase I with the frequency of univalents at metaphase I. Thus, as with most reports of asynapsis, it was not possible to determine whether the univalents detected at the diakinesis and metaphase I were the result of initial failure or desynapsis following pairing of homologous chromosomes. Accordingly a more general term asynapsis has been utilized. In the present material the univalents do not appear to be the main cause for the formation of restitution nucleus as was proposed by Darlington (1930) in *Prunus*, but it seems that the formation of restitution nucleus is due to the failure of a well organized spindle mechanism. Although the daughter bivalents separate, yet they are unable to move to the poles and all of them ultimately get included in a single membrane, in many P. M. C. cells.

As Walters (1958) has indicated, the type of complex pre-restitutional chromosome movement described above, poses certain problems for a hypothesis of metaphase orientation of the chromosomes and the subsequent anaphase behaviour such as proposed by Östergren (1951). According to the mechanistic concept of Östergren (1951), the orientation of kinetic granules, of which there is one per chromosome, determines the intact poleward movement or equatorial position of the univalent chromosome. The affinity between a given chromosome (univalent) and a given spindle pole is viewed as a matter of orientation of the "kinetic side" of the centromere without reference to distance. It is assumed that univalent chromosomes adopt equatorial positions, after lying at random over the spindle, if the centromeric granules of two chromatids become oriented towards two poles. As applied to the present material the centromeric granules would have to shift orientation from one pole, when the univalents being directed towards that pole, to the opposite pole at a somewhat later stage. In studies on meiosis in certain insects, Hughes-Schrader (1947) has described a phenomenon which she refers to as "Prometaphase stretch".

This behaviour results from an early movement of the centromeres of a bivalent towards opposite poles and is followed by the contraction of the bivalent and their movement to the equator. In the material under investigation, the bivalents were found to behave in an asynchronous manner with respect to these movements. Östergren (1951) concluded that such chromosome movement are a usual phenomena. The general movement of the univalents, shows perhaps precocious and "protracted" prometaphase behaviour as has also been suggested by Snyder (1961) in *Paspalum secans*. The complex movement of the chromosomes, the highly variable onset of meiosis among the microsporocytes and irregular cleavage of the microsporocytes, all suggest a decided upset in timing relations in the material.

The formation and organization of the spindle and its relation to the chromosomes, are problems that have received attention from many workers working with the aberrant materials. The occurrence of multiple spindles or of spindles with multiple poles or with non-convergent poles has been regarded as an evidence for the existence of such entities as "pole determinants" (Darlington and Thomas, 1937; Swanson and Nelson, 1942) or "spindle organizers" (Walters, 1958). In the present material, single chromosome or small groups of chromosomes that were not included in the restitution nucleus were observed to be associated with spindle development, also underwent post-restitution division. Similar behaviour of small groups of chromosomes has been observed by Walters (1958). These observations indicate that spindle formation is associated with isolated chromosome groups down to the level of single chromosome. The present material as also most of the angiospermous plants and animals, show no evidence of defined mitotic centers. The presumed function of the mitotic center is in the organization of the spindle, possibly as a source, along with the centromere, of an agent responsible for secondary bonding between the protein chains comprising the spindle fibres (Mazia, 1958). Although the presence of such centers has been variously inferred in higher plants, the simplest hypothesis consistent with the observations on spindle formation and organization in plants such as *A. maritima* appear to be that these functions relate directly to the chromosomal material.

Finally, the nature and extent of meiotic irregularities, the presence of varying number of univalents and their complex behaviour and the probable basis of these irregularities, all suggest that the polyploid has originated as a result of wide hybridization. The variation in the percentage of univalents might be expected on the basis of comparative homology that exhibited between the chromosomes of the parent species and also probably on the time interval that might have elapsed since the hybridization took place. It is well known that nature always tends towards stability. Besides a lack of homology, environmental factors might also be effecting the conjugation of

chromosomes, thus causing a variation of univalency to a certain extent.

Summary

Artemisia maritima growing in Gurez (Kashmir) has been studied cytologically and is a tetraploid with $2n=36$. The microspore meiosis is characterised by asynapsis, complement fractionation, nondisjunction of chromosomes, univalency and occurrence of restitution nuclei. There is an upset in timing relations of meiosis resulting in an aberrant meiosis which has been described fully. The initial movement of the chromosomes towards poles at AI is reversed towards the equator and a restitution nucleus is formed. A wall is secreted by such a P. M. C. and this assumes the appearance of a pollen grain. Although interesting irregularities were observed in 1st division of meiosis, the post-restitution division is comparatively regular. It is suggested that this tetraploid has originated as a result of wide hybridization and is thus an allopolyploid.

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