

# CONTRIBUTION TO THE CYTOLOGY OF *TRIDAX PROCUMBENS* LINN.

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### *I. Introduction*

THIS is one of the commonest weeds of the plains of South India. The generic name is an old Greek name used by Dioscorides meaning 'Summerating'—alluding to a plant that was a summer vegetable. There are about 27 Tropical American species. This species seems to have been introduced into this country from South America. There is no other species known in India.

The family *Compositæ* has been widely investigated by Cytologists and Geneticists. Though a number of genera have come in for a general or a casual investigation, a few like *Crepis*, *Dahlia* or *Chrysanthemum* have been very intensively worked upon. A number of workers have investigated almost all the aspects of *Crepis* and almost a complete phylogenetic history of the genus is now known. It has been established for instance that the genus *Crepis* is made up of three sub-genera, *Catonia*, *Eucrepis* and *Barkhausia*. They are characterised by progressively greater specialization and reduction in the length of the life-cycle. Diploid chromosome number 10 is considered to be the most primitive, although the most prevalent diploid number is eight. Secondary numbers ranging from 6-40 are known to have originated by interspecific hybridization and amphidiploidy, and polyploidy. Some process like reciprocal translocation must have led to reduction in the number from 10 to 8 and from 8 to 6. Segmental interchange between non-homologous chromosomes followed by meiotic irregularities must have been responsible for the elimination of one or more pairs of chromosomes.

All this was based upon intensive cytogenetical work of several years on more than a hundred species, obtained in a living condition from all parts of the world. Navashin (1929), Collins, Hollingshead and Avery (1929), Babcock and Clausen (1929), Babcock and Navashin (1930), Babcock and Cameron (1934) were some of the important contributors who were responsible for the correct understanding of the evolution in the genus *Crepis*. Similarly Lawrence (1929) has investigated the *Dahlia*s and Shimo-tomai (1933 and 1937), the genus *Chrysanthemum*.

It is however curious, that in spite of their wide distribution in Tropical America, the genus *Tridax* should have escaped the attention of the American Cytologists. So far as we can make out, not even the chromosome number of any of the species has been determined. Our attention was attracted to this monospecific Indian representative, mainly on account of the scant attention that it has received at the hands of Cytologists; and in the present communication, the somatic and meiotic chromosome numbers have been reported for *Tridax procumbens* Linn. for the first time. Meiosis has been investigated in some detail, and a variety of abnormalities recorded. The development of the female gametophyte and of the embryo about which also there are no details available, will form the topic of another communication.

The heads in *Tridax procumbens* Linn. are heterogamous and are borne on long peduncles which may sometimes reach a length of even 2 ft. The ray florets are female with ligulate corolla. It is trifid and invariably pale

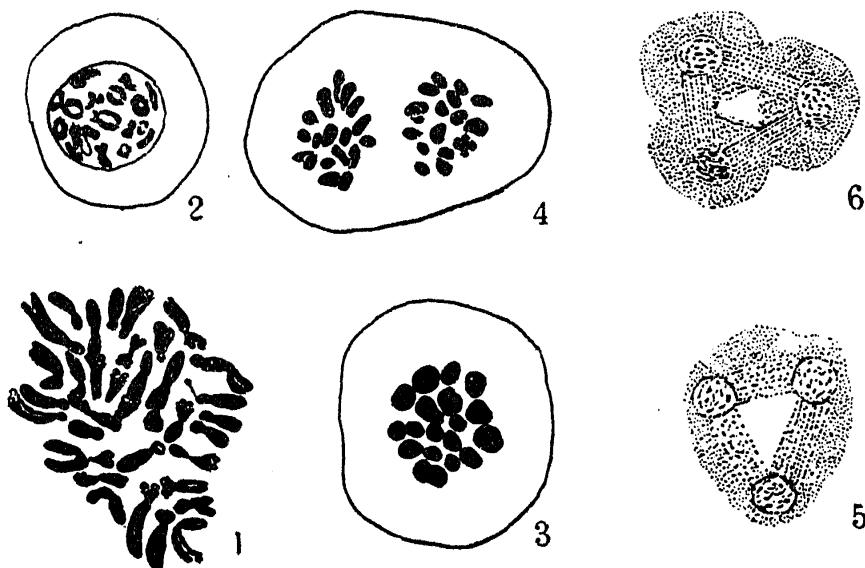
yellow in colour. However we were able to isolate from a natural population just half a dozen plants which possess ligulate flowers of a bright yellow colour. The cytology of this strain is being investigated. A number of heads from different localities in the University were examined to find out whether there is any constancy with regard to the number of ray and disc florets in individual heads. It was found that the range of variation in the ray-florets was from 4-7 while in the disc florets, it was 35-60.

## II. Materials and Methods

The required plants were isolated from a wild population and grown in pots in the University Botanic Garden. Anthers were examined in aceto-carmine and then fixed in Navashin's fluid, after a pre-fixation in Carnoy's fluid. The usual paraffin method with chloroform as the solvent was followed. Sections which were cut at 10-18 microns were stained with Iodine-Gentian Violet. The same procedure was adopted with regard to the root tips also. Pre-fixation was found necessary and the most favourable time proved to be between 10 A.M. and 11 A.M.

## III. Somatic Chromosomes

The diploid chromosome number is 36 (Text-fig. 1). On account of the large number and of the lack of prominent difference in the size of the chromosomes of the complement, no attempt was made at a quantitative idiogram analysis. All the chromosomes, however, possess centromeres of either the sub-terminal or of the median type. None has the terminal



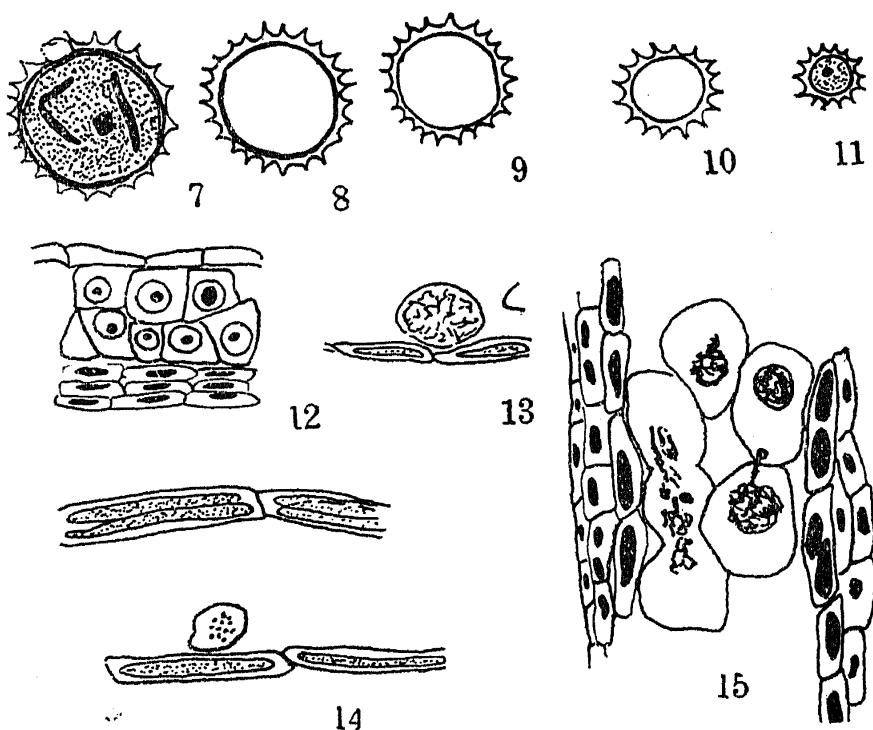
constriction. Only four pairs have the median type of centromere, the rest sub-terminal. There is a gradation in size in each of these two chromosome types. The longest median constricted and the longest sub-terminal type are of approximately the same length. So also with reference to the

shortest. Though a number of somatic plates were examined, it was found that only one sub-terminal chromosome possessed a satellite with a long trabant at its proximal end. The absence of satellite in its homologue cannot be easily explained. Whether it is a case of loss of the satellite brought about by elimination or only of the persistent non-showing of the other cannot be stated definitely.

#### IV. Meiosis

In the main, meiosis is regular. At diakinesis, eighteen bivalents are found, synapsis being complete (Text-fig. 2). A number of *p.m.c*'s at this stage of development were examined and it was found that the majority of the bivalents were of the ring type. In a few cases as many as 12 rings and the rest rods, were observed. Text-fig. 3, and Plate V, Fig. 14, show first metaphase plates showing eighteen gemini. Text-fig. 4 shows on M II plate with the 18/18 distribution. Cross walls are formed only after the second division. This appears to take place by a process of furrowing, described further down.

The microspores that are rounded off by the process of furrowing are uni-nucleate to start with (Pl. V, Fig. 26). This divides into two, the tube nucleus and the generative nucleus (Pl. V, Fig. 21). Around the latter a distinct hyaloplasm is organised leaving no doubt of the fact that it is a cell and not merely a nucleus. The tube cell thus differentiated comes to envelope the generative cell (Pl. V, Fig. 22). The generative cell divides into two male cells. In Pl. V, Fig. 23, the two nuclei of the male cells are seen just below the tube nucleus whose nucleolus is seen very prominently. The male cells which are at first rounded become elongated very soon. This is the condition at the time of shedding (Pl. V, Fig. 24 and Text-fig. 7).



In spite of the prevailing regularity in meiosis, the pollen grains formed are of different sizes. Text-figs. 7-11 show their range in size. Notwithstanding this discrepancy they all appeared to be viable. Effort to germinate them as described by Wulff and Raghavan (1937) was not successful. But in a rough way viability can be ascertained by ordinary acetocarmine staining. When a pollen grain smear is made and stained with acetocarmine, only those which are viable take up the stain while there are others which do not take up the stain however much the staining may be prolonged. These are usually shrivelled and do not show out the conspicuous spinous wall (Pl. V, Fig. 27). Hence these may be regarded as sterile or inviable. Such an examination was made of a number of flowers and it was found that in addition to what may be tentatively regarded on the basis of their taking up the stain as viable grains, there were a variable number which would not take up the stain and which on this account, must be regarded as sterile. The following table gives an idea of the proportion of the fertile and sterile grains:

TABLE I

Plant No.	Flower No. on the same head	Total No. of pollen grains	Fertile grains	Sterile grains	Percentage of fertile grains
1	<i>a</i>	239	159	80	66
	<i>b</i>	239	146	93	61
2	<i>a</i>	207	147	60	71
	<i>b</i>	179	96	83	53
3	<i>a</i>	375	225	150	60
4	<i>a</i>	205	165	40	80
	<i>b</i>	261	161	100	54
5	<i>a</i>	239	159	80	66
	<i>b</i>	239	146	93	61

It can be seen from the above table that the percentage of viable pollen grains is between 50-80 and such wide fluctuation is found not only in flowers belonging to different plants but also in the flowers borne on the same head. This is the case in 2 *a* and *b* and 4 *a* and *b*. The interesting thing about *Tridax* appears to be that the fertile grains or at any rate the

grains possessing normal visual viability are of different sizes. Text-figs. 7-11 show them all under the same magnification.

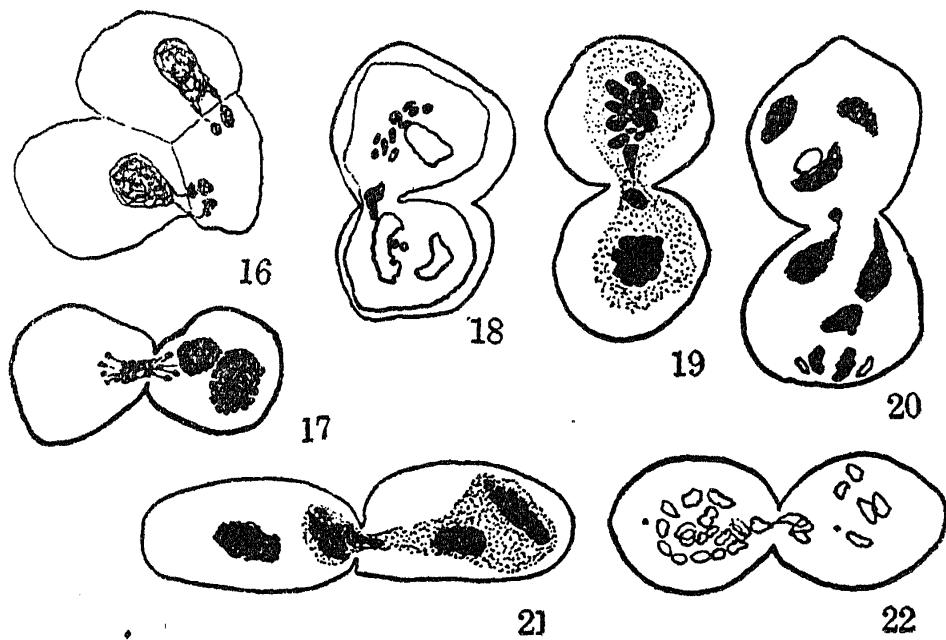
The pollen grains are rounded off by the process of furrowing and not by the regular formation of cell plate. Text-fig. 6 shows the three-furrowed state. Wodehouse (1921) considers that the prevailingly three-furrowed (tricolpate) type of pollen grain is directly associated with the tetrahedral arrangement of the four microspores in the *p.m.c.*, which is the usual arrangement in the Dicotyledons and that other numerical configurations of furrows are associated with other arrangements in the tetrad. This prevailingly tetrahedral arrangement of pollen tetrads is due to the ever present tendency among cells to assume least surface configurations.

The first division offers nothing unusual. As the daughter nuclei enter upon the telophase and reorganize, the fibres become numerous. These initial stages in phragmoplast activity persists for some time. The fibres show some thickening midway between the two daughter nuclei as if about to form a cell plate, but ultimately the phragmoplast disappears without completing cell division.

In this species the second division usually takes place in such a manner that their axes lie in planes at right angles to each other. As the chromosomes separate and move towards the poles of the spindles, the connecting fibres are straight and about this time four further spindles appear between the four nuclei (there could only be seen three nuclei and three spindles due to the tetrahedral arrangement) of the second division. Thus the *Tridax* tetrad is provided with six connecting spindles (Text-fig. 5). Each nucleus including the one which cannot be seen in a focus with the upper three comes to possess ultimately three radiating spindles.

The exact manner in which these additional spindles arise does not seem to be very clear. Devisé (1921) suggests that they may be regarded as the reappearance of the heterotypic spindle split longitudinally into two halves. Soon after the appearance of the added spindles in the tetrad, quadripartition of the cell begins. This is accomplished by the process of simultaneous furrowing essentially as described by Farr (1916) for the tetrads of *Nicotiana*. It is as though each nucleus were a centre of attraction about which the cytoplasmic mass tends to round up. The furrows appear as two and three cornered depressions and growing inwardly they finally meet in the centre forming a three-cornered hole (vacuole) through the centre of the tetrad (Text-fig. 6). This leaves each nucleus in its own cell but connected by connection with its adjacent neighbour of the tetrad, and each of these channels encloses the remains of the poorly developed connecting spindle or

phragmoplast. At the same time the peripheral surface of the cell is cut by four furrows, one between each pair of nuclei and opposite to each of these corners of the central opening. By manipulating the focus of the microscope these furrows are found to be annular constrictions encircling the pit connections. These constrictions become deeper and ultimately cut through between the daughter nuclei and thus separate the daughter cells. Upon their separation, the daughter cells lose all traces of the pit connection and the four cells flatten slightly against each other tending to conform to the general curvature of the group as a whole. At this stage the spermocytes are smooth walled. Their walls soon begin to thicken and the spines appear as papillæ. They become more turgid and rounded and then the germinal furrows become evident. It was said that the viable (stain-taking) pollen grains ranged in size from the normal to the very small (Pl. V, Figs. 25 and 26). To our mind, while the small grains are formed in a manner described further down, the others showing only slight variation (Pl. V, Fig. 27) in size are to be considered to have been normally formed. The very nature of their origin by the method of furrowing is probably responsible for the slight difference in their diameter.



#### V. The Tapetum

The behaviour of the tapetal cells calls for some mention. There are usually three layers of parietal cells of which the first one beginning with the anther sac is the tapetum. Initially all the three layers are of equal size and form (Text-fig. 12), *i.e.*, when the microsporogenous cells have not yet entered upon their meiotic phase. As the latter progresses, the tapetal cells show a slight enlargement. There is however a very marked and almost sudden growth between zygotene and M I (*cf.*, Text-figs. 13 and 14). This growth

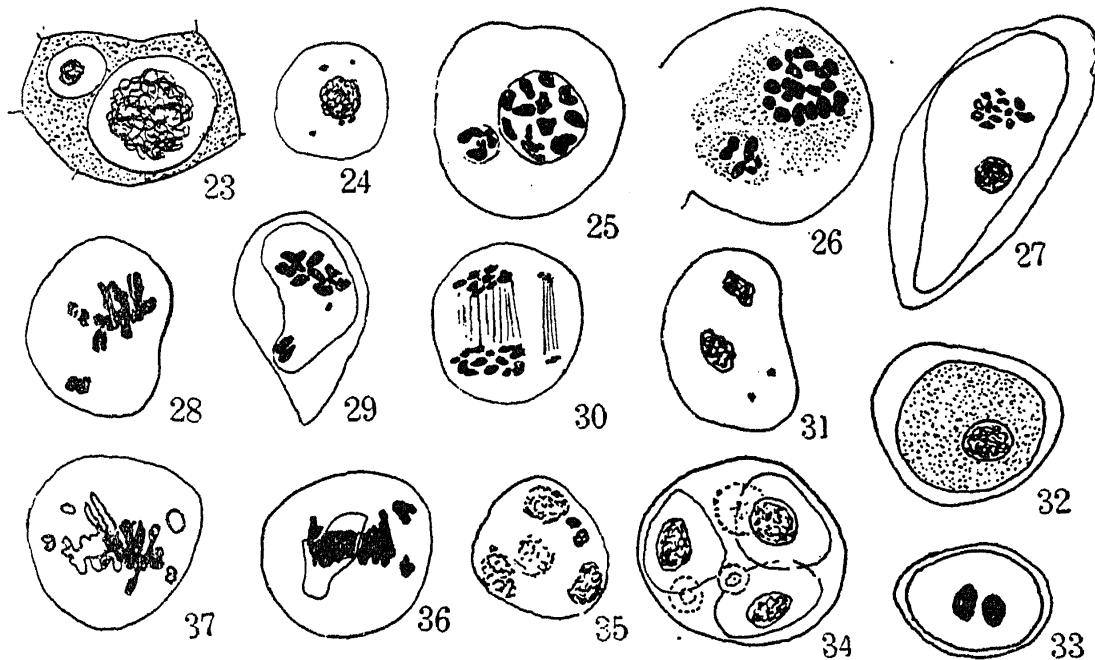
period is marked by an intense mitotic activity in the tapetal cells. Tapetal cells containing one nucleus change this condition and come to possess two and four nuclei (Text-fig. 15). But seldom do they remain separate, fusion taking place immediately. But more frequently, and this is almost the prevailing condition, the division of the tapetal nucleus does not result in the organization of a definite daughter nucleus as such, but there is a multiplication of the chromosomes without any anaphasic separation. The result is, long cylindrical nuclei are formed many times the length and diameter of the *p.m.c.* (Pl. IV, Figs. 5 and 6). Compared to the growth of the tapetal nuclei those of the *p.m.c.* is insignificant. In some two such long nuclei are seen and in others only one. The original single nucleus without attaining the binucleate condition goes on in some cases, dividing mitotically without chromosomal separation and thus result in single long tapetal nuclei. In others it would appear this is resorted to after the two tapetal nuclei have been organized (Text-fig. 14). There would appear, however, no correlation between such a behaviour and the position of the tapetal cells relative to that of the anther sac. But it was found that the position of the tapetum beginning with the inner side of the sac, showed more frequently the presence of the two long nuclei. The tapetal nuclei are discharged into the cavity and the disintegration of the nuclear matter takes place in the usual manner.

#### VI. *Meiotic Aberrations*

(a) *Cytomyxis*.—Though meiosis was in the main normal there were frequent cases of abnormalities of many kinds. These occurred promiscuously and bear no relation, so far as our observations went, to any particular location of the anther sac or of the flower on the head. It is therefore impossible to explain these on physiological grounds.

The most frequent of these, was the phenomenon of Cytomyxis and the most interesting fact about this is that it occurs in almost all stages of meiosis. It is very wide-spread in occurrence also. There was not a loculus but exhibited cytomyxis at some stage or other of the *p.m.c.* Text-figs. 16 to 22 show cytomyxis at different stages. Fig. 16 in prophase; Figs. 22 and 19 in diakinesis and prometaphase respectively. The transference of nuclear matter may be whole or partial, more frequently it is of the latter type. In Text-fig. 17 there is evidence of entire migration of prophase nucleus while in Fig. 19 there is only partial migration. In Pl. IV, Fig. 4, there is complete migration of early prophase nucleus. In Fig. 18 we see a portion of A I passing into the adjacent cell while in Fig. 21 there is complete migration of an entire nucleus. In Fig. 20 there are shown two *p.m.c.*'s with an opening between them through which partial cytomyxis is taking

place. The two *p.m.c.*'s are in the second telophase; but in the lower cell, one of the telophase groups has divided further to form groups of their own so that in that cell instead of four telophase groups there are seven.



(b) *Binucleate pollen mother cells*.—Normally *p.m.c.* is uninucleate (Text-fig. 32), but very commonly pollen mother cells were observed which contained either two large normal sized nuclei (Text-fig. 33) or one large and one small nucleus (Text-fig. 23). The latter condition however was more common. Evidence at later stages of meiosis reveals that in both types of syndiploidy, *i.e.*, (1) the occurrence of normal nuclei in pollen mother cell and (2) the occurrence of one normal and one small, the nuclei behave independently throughout meiosis. Figs. 23-37 show all stages of meiosis undergone by the two nuclei of the *p.m.c.*, one big and one small, just as if they were two independent *p.m.c.*'s. Pl. IV, Figs. 1-3, show photo-micrographs of this simultaneous division of the big and small nuclei at resting stage, diakinesis and M II respectively. It will be seen from these figures that in the nuclei, the division stages are entered upon simultaneously. This simultaneous independent behaviour of the two nuclei, though almost the rule is sometimes departed from, when we find that as for instance in Text-fig. 27 and Pl. V, Fig. 15, while the bigger nucleus is in M I, the smaller nucleus is in its resting condition.

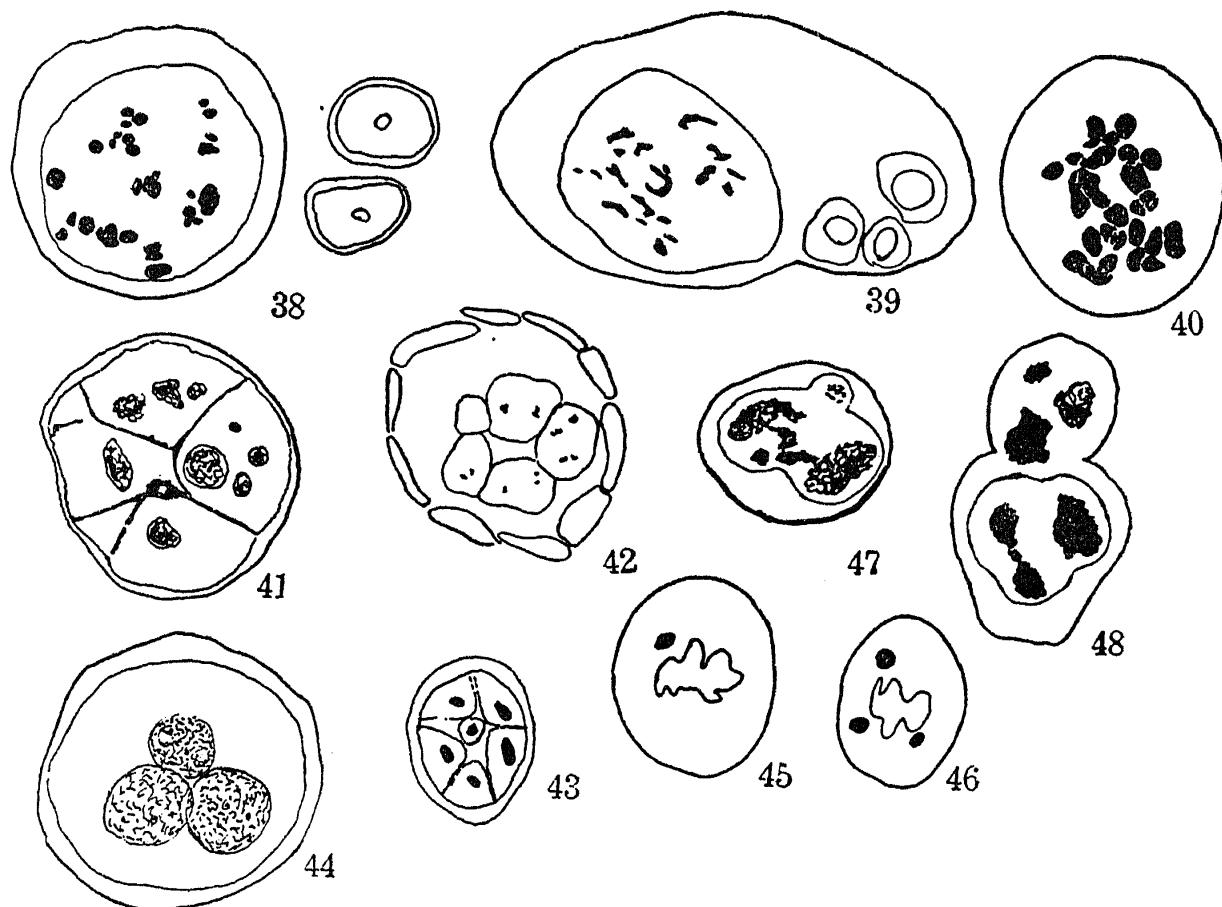
This independent behaviour normally results in the formation of four haploid and four sub-haploid pollen grains. These are the small grains referred to previously, and which by taking up the stain showed that they were viable. In the case of the other type, the occurrence of two normal-sized nuclei, eight haploid grains would result. The most common

condition in the former type is the formation of four haploid grains and only two sub-haploid grains (Text-figs. 34 and 35). This means that in such types, the second division of the smaller nuclei is suppressed. In Pl. V, Figs. 25 and 26, are seen some of these sub-haploid pollen grains arisen in this manner. They are not shrivelled as the others formed by eliminated chromosomes are.

The *p.m.c.*'s containing two normal nuclei have presumably originated by suppression of wall formation at the premeiotic mitosis. But the origin of the small nuclei in the *p.m.c.*'s is not quite clear. Two explanations may be offered; the first is that it may be a case of elimination of chromosomes. Such an extrusion is quite common in hybrids, triploids and also in X-rayed progeny. But in all these micro-nuclei or micro-cells, as described by us (Raghavan and Venkatasubban, 1940 *a*) in triploid *Urginea* result, and these seldom round off as pollen grains. Both the nuclei will be deficient and it is no wonder that neither of them functioned. Nor is it common to find these eliminated chromosomes undergoing the full round of meiotic process, right from the resting stage, to the organization of the grains; this would imply not the elimination of one or more chromosomes but the abstraction of a portion of the resting stage nucleus of the *p.m.c.* Normally extrusion takes place at M I, A I, and M II, and the eliminated chromosomes constitute themselves into one or more micro-nuclei and there they end. Such an elimination also occurs and it is these that constitute the shrivelled up bodies intermixed with the pollen grains. But the totally different behaviour of these small nuclei described herein, shows that these are not to be regarded as cases of eliminated chromosomes. Abnormal spindle behaviour at premeiotic mitosis of the *p.m.c.* must therefore be considered as the second and the more likely cause of the origin of the small nuclei. It may be that there was a failure of co-ordination of one of the spindles at the premeiotic mitosis. These abnormalities are due more to somatic instability than to any defect in the process of meiosis itself.

(c) *Fusion of pollen mother cells.*—*P.m.c.*'s showed a strong tendency to fuse with one another. Four or more *p.m.c.*'s aggregated and fused, the cross walls were absorbed and each loculus came to possess frequently only one such fusion product (synpollen mother cell), if such an expression could be applied. Text-figs. 42 and 43 show five and six *p.m.c.*'s coming very close together in an anther loculus. The next stage of fusion is shown in Fig. 41, and Pl. IV, Fig. 9. Frequently the nuclei of the fusin *p.m.c.*'s show early signs of degeneration, by breaking into unequal bits of chromatin masses (Text-figs. 41 and 42). But sometimes they seemed to enter upon further meiotic activity. In Text-figs. 38 and 39, and Pl. IV, Figs. 7 and 10

we see the resolution of the chromosome like bodies. Undoubtedly all the chromosomes are not present, as at least some of the fusing nuclei must have degenerated. This disparity in size between a synpollen mother cell which may now be regarded as a giant pollen grain in the making, and the grains formed out of normal meiosis is shown in Text-figs. 38 and 39. But these synpollen mother cells never succeed in forming a giant grain. They degenerate sooner or later.



Fusion of *p.m.c.*'s is one of the important methods in the formation of polyploid gametes. No viable polyploid gametes were however found in this case. Text-fig. 44 (Pl. IV, Fig. 8) is hard to interpret. It may be a case of fusion of three *p.m.c.*'s at the resting condition of their nuclei. The fusion has taken place so completely that not even vestiges of the *p.m.c.* walls are visible as in the other cases (*cf.* with Figs. 41 and 42) or it may be something like the binucleate *p.m.c.* described previously. Just as we get two normal-sized nuclei in a *p.m.c.* owing to failure of wall formation at the premeiotic mitosis, we may also get the trinucleate condition where one of the nuclei has again divided unaccompanied by wall formation. Or it may be a case of cytomyxis; but the relatively large size of the cell precludes the latter possibility. The first method seems to be the most likely in the matter of its

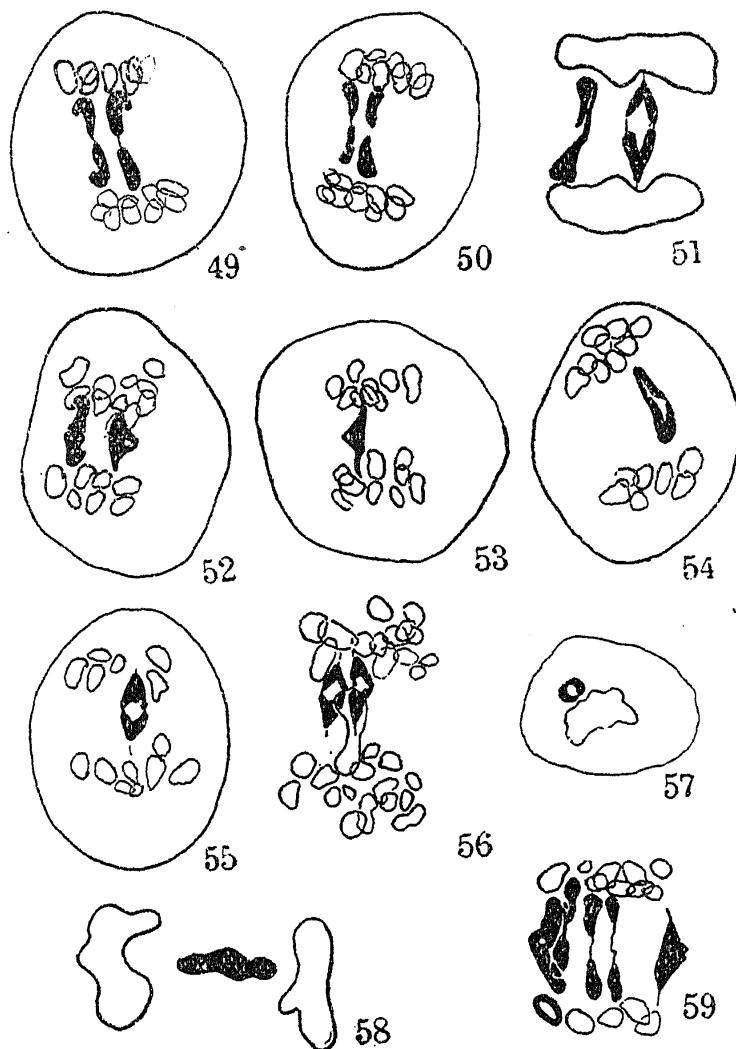
formation. All these three methods would result in the ultimate formation of polyploid microspores.

(d) *Semiheterotypic division*.—Frequently chromosomes at first division are widely scattered over the spindle (Text-fig. 40). At a stage corresponding to the anaphase the chromosomes were still widely scattered on the spindle as laggards. These were so numerous that the nuclear membranes normally laid down at the poles instead of cutting through the spindle-figure inclosed the entire chromosome complement in a single nuclear membrane. The single nucleus thus formed was often dumbbell-shaped and gave the appearance of an amitotic division. In many instances however the nucleus was rounded out to a normal shape although it contained the somatic number of chromosomes. These may be regarded as restitution nuclei. Those of normal shape must be expected to undergo normal division resulting in the formation of a dyad of nuclei each of which is diploid. Such a behaviour was designated semi-heterotypic division by Rosenberg (1917). The semiheterotypic division is of wide-spread occurrence in known and suspected hybrids.

(e) *Extrusion, non-disjunction, Bridge formation, etc.*—Cases of extrusion of chromosomes either univalents or bivalents were also met with. In Text-fig. 57, Pl. V, Fig. 13, a bivalent is seen off the first metaphase spindle. In Text-figs. 45 and 46 we see one and three univalents respectively extruded off the spindle. This means that pairing was not complete. Though in the majority of cases complete synapsis was the rule, in some a few chromosomes would not pair. These univalents were extruded. How far this is due to hybridity must for the present remain an open question. Text-figs. 54-56 are clear cases of non-disjunction. The bivalents in these are seen to reach the pole without disjoining. These cases are peculiar in that the bivalents in question act as laggards. They do not seem to reach the poles earlier than the rest as the other case (Text-fig. 57 and Pl. V, Fig. 13) of the extruded bivalent would indicate. The bivalents may not be included in the daughter nuclei in which case like the eliminated univalents they may also organise micronuclei. If however they are included in the pole they would bring about a disparity in the number of chromosomes at each end. Such a disparity was not infrequent.

Frequently chromatin masses suffered fragmentation into bits of unequal size. Soon after the first division, the telophase nuclei so organized, showed signs of division in this what may be regarded as an amitotic manner. Text-figs. 47 and 48 and Pl. V, Figs. 11 and 16, show such telophase masses. These of course, are symptoms of degeneration.

Another frequently occurring phenomenon is that of the stretching of the chromosomes along the spindle during the first division to form bridge-like configurations. These are not considered to be inversion bridges mainly



because of the absence of fragments associated therewith. Most of these are due to delayed disjunction (Text-figs. 49-51). In others there is delayed terminalization of the chiasmata and the bivalents lie on the spindle (Text-figs. 52 and 53). Text-fig. 58 and Pl. V, Figs. 17 and 18, are presumably trivalents lagging on the spindle. In Text-fig. 59 and Pl. V, Fig. 19, a number of bridges are stretched along the spindle.

### VII. Discussion

(a) *The balance system of meiosis*.—In a recent paper, Gentcheff and Gustafsson (1940) have recorded what may be regarded as meiotic abnormalities in some apomictic species of *Hieracium*. Not only is it a related genus to *Tridax*, but some of the abnormalities, recorded therein, conform to those described in this paper. It may be useful to discuss whether or not their interpretation is applicable to this case. Gentcheff (1937) first found

a peculiar mode of tapetum development in several apomicts of *Hieracium*. This kind of tapetum development and degeneration was considered by him peculiar to apomicts. Since pollen production in most apomictic Archieracium biotypes is very low, and tapetum degeneration differs from the behaviour in sexual types, it was suspected that some causal connection existed between these. This led to a detailed re-examination of tapetum development, and meiosis in *Hieracium robustum*.

This is an apomict, never producing pollen; the meiotic irregularities are classified into three broad types and all these three are connected in some way with the tapetum development.

In division type 1, a sort of a semi-heterotypic division (Rosenberg, 1927) is seen. There is no pairing. Univalents are scattered over the metaphase spindle. Second division is also common. Some of these have also been recorded here. Anaphases are irregular leading to the origin of poly-nuclear pollen mother cells. In this type, division starts when tapetal cells are uninucleate and very small, and pollen mother cells are very small also. Prophase is remarkably precocious. In some cases metaphases contain mitosis-like chromosomes and more or less pseudohomotypic (Gustafsson, 1935) in character with no chromosome pairing and univalents gathering in the equatorial plane and dividing at first division and with no second division.

In division type 2, double reproduction of chromosomes takes place. In this the cells and nuclei are reported to have grown intensely. In response to the changed proportion of cell and nucleus volume, the chromosomes are forced to reproduce twice.

Division type 3 is associated with bivalent and fragmentation phenomena. Prophases and metaphases of this type of division occur at very advanced tapetum stages, even more advanced than in type 2. Four-fused and eight-fused tapetal cells are very common. The number of bivalents is variable. Anaphase separation is irregular, due to the high number of univalents. Inversion bridges are frequent at I and II divisions. In the same plate three or four bridges may be found indicating a high degree of structural differences. The most conspicuous feature in the case of bivalent formation is the extreme fragmentation of chromosomes.

From these and other data it is concluded that bivalent formation occurs exclusively when a proper balance exists between tapetal growth and activity, pollen mother cell growth, prophase onset and chromosome reproduction. They emphasise the view that meiosis is greatly under the physiological influence from or *via* the tapetum cells. This would appear to be especially note-

worthy in organisms where pollen mother cells form one cell-row only, covered with tapetum cells on all sides. For example, in a *Crepis* apomict Stebbins and Jenkins (1939) found a correlation between the mitotically inactive tapetal cells and the undeveloped pollen mother cells. Bivalent formation occurs exclusively when a proper balance exists between tapetal growth and pollen mother cell growth.

For all practical purposes, *Tridax* is a sexual diploid. Whether it is a secondary polyploid, as it may well turn out to be, can be ascertained only after we have some knowledge of the basic chromosome number. Of this we have no data at present. The first point to be noted is that some of the meiotic aberrations, like semi-heterotypic division, pseudo-homotypic division, which have been known to occur in apomicts, are common in sexual species also. As far as our observations go, no exact connection exists between the occurrence of the aberrations and the position of the flowers on the head. Their occurrence is more or less at random, and apparently not governed by any physiological factor, as determined by a particular location on the inflorescence. Intermixed in the same loculus with pollen mother cells showing regular meiosis, the ones showing semi-heterotypic division also occur. The behaviour of the tapetum as described in the text is more or less uniform, and obviously on account of the very nature of the distribution of the abnormal pollen mother cells, no correlation could subsist between the abnormalities and the behaviour of the tapetal cells.

Of the three metaphase types of meiosis, type I was found here and there. We were not able to find any case of double reproduction of chromosomes, giving rise to an increase in the chromosome number at interphase. Of the third, there were cases of bridge-formation. But so far as our observations went most of them were due to delayed disjunction and not inversion bridges. No fragments could be seen. Gentcheff and Gustafsson (1940) described a variety of methods of fragmentation but no evidence of fragmentation existed in our material. Fig. 38, 39 may at first suggest the presence of fragments but the cell-size and the other stages described show unmistakably that it is the fusion product of a few pollen mother cells in which the chromatin masses have undergone contraction and partial regeneration.

The interesting feature is that in some details the meiotic behaviour of the pollen mother cells in the present material is identical with that of a apomictic forms. It is mainly upon a study of these apomictic species that the correlation was established between the pollen mother cell behaviour and that of the tapetal cells. If that is so, either of two alternative inferences

seem justifiable. That no such definite correlation is tenable at least so far as this genus is concerned or since these are almost characteristic of apomictic forms, *Tridax* must be regarded as an apomict. Seed production however is quite copious and that is about the only method of propagation known in this species. That the meiotic peculiarity could in any way be connected with any abnormality in seed formation—whether it could be a case of agamospermy—seems quite unlikely.

(b) *Cytomyxis*.—This phenomenon was first described by Gates (1911) in *Oenothera gigas* when he applied this term to the process of extrusion of chromatin from the nucleus of one pollen mother cell to that of an adjacent cell. Since then the phenomenon has been reported in a number of genera. It has been noted especially in cytological description of grasses (Church, 1929 and 1936). In the grasses different types of cytomyxis have been reported. In the first type, the phenomenon was seen only in the spireme stage, but with resultant extrusions persisting in later stages. In the second type the process was manifested in diakinesis. It was also observed that the greatest amount of cytomyxis is correlated with the greatest amount of irregularities in the maturation divisions. In the third type chromatin transference was observed during interkinesis.

In the present material cytomyxis has been observed in almost all stages of meiosis and of all the meiotic abnormalities this is the most frequent so far as this material is concerned. If one made an analytical study of the plants in which phenomenon has been recorded so far, two facts will stand out prominently. Firstly that in all plants there is a certain amount of sterility, so that it is almost certain that cytomyxis is responsible for the degeneration of the pollen mother cells and even of whole anther sacs. To this is presumably due the not insignificant proportion of sterility that is so common a feature of the pollen grains of this species (*cf.* the table giving the percentage of sterility). Secondly cytomyxis recognised as an abnormality has been reported in several definitely known hybrids. At spireme stage it has been seen in *Oenothera Rubrinervis* (Gates, 1905). A hybrid form of *Typha angustifolia* showed the phenomenon at diakinesis (Roscoe, 1927). Kattermann (1933) described it in the *p.m.c.*'s of *Triticum*  $\times$  *Secale* hybrids and Percival (1930) in the hybrids of *Aegilops* sp.  $\times$  *Wheats*. So rather than regard it as an artefact as Sinoto (1922) would have us believe, its occurrence is undoubtedly associated with hybrids. In the investigation of Church (1929), he found that while the occurrence of this phenomenon was very striking in connection with hybrids the examination of considerable material of *Phalaris arundinacea*, a normal diploid, revealed no cytomyxis.

(c) *Non-disjunction*.—Cases of non-disjunction in the heterotypic division have also been met with as illustrated by figures. This is the particular type of unequal chromatin distribution in which one or two bivalents may approach the equatorial plate, but are pulled undivided to one of the poles. Consequently instead of the usual 18/18 segregation, 20/16 and 22/14 in anaphase were often seen.

Non-disjunction has been reported frequently in hybrids. *Oenothera lamarckiana* may show an anaphase segregation of 8/6 or 9/5 instead of the usual 7/7 (Sinoto, 1922). In the tetraploid species of *Datura*, segregates of 23/25 instead of the 24/24 are quite regularly found. The same situation has been reported in *Nicotiana alata* (Ruttle, 1927). Unequal segregation leading to the formation of pollen grains of varying number of chromosomes (17-29) instead of 23, has been reported in *Scilla indica* (Raghavan and Venkatasubban, 1939).

When gametes of different numerical chromosome equipment mature as a result of unequal distribution or non-disjunction, it is obvious that they are functional in producing dysploid offspring. This particular type of polyploidy has been seen in a striking manner in the dysploid series of *Carex* (Heilborn, 1924).

(d) *Irregularities in Nuclear Divisions—their causes*.—Meiotic irregularities are, as shown already, widely prevalent in species hybrids. They can also be caused by physical or chemical agents. According to Kostoff (1930) one has to take into consideration bio-physical and bio-chemical phenomena involved in the cells elsewhere accompanied by irregular nuclear division. The direct activity of the physical or chemical agents is expressed by change in the viscosity of the cytoplasm (CV). An increase or a decrease might lead to a reversible or irreversible coagulation. The latter means death to the cell. The determination of the relative CV has been made by Brownian movement, cytoplasmic streaming, centrifuging and plasmolytic methods. Various agencies have been found to affect the CV. By wounding, coagulation and an increase of CV occurs in the neighbouring tissue around the wound. A slight increase of CV offers favourable conditions for bringing about cell divisions. Apparently such a phenomenon occurs in galls, tumours, etc. The foreign substances penetrating into the dividing tissue increase the CV and this is followed by intensive cell division. Even in normal cell division at the beginning of the anaphase, there is according to Chambers (1919), a decrease of CV. The occurrence of this diminution is prevented in galls, tumours, tapetum, etc., by a continuous activity of the foreign agents. If the CV is held relatively high by these, chromosome division occurs,

separation being inhibited by the high CV, and somatic polyploidy is the result. Multinucleate cells originate when the difference between the normal CV during the anaphase and the one maintained by the activity of these foreign bodies is not very great, and only a slight separation of the chromosomes takes place. If this difference is relatively small non-disjunctions occur and chromosomes lag on the spindle. Other agencies that cause a similar change in CV and consequent dislocation of nuclear divisions are X-rays, Radium rays, Ultra Violet rays of  $280 \mu$  wave-length and various chemicals. In a previous communication (Raghavan and Venkatasubban, 1940 b) we have described some of the meiotic abnormalities induced by X-radiation and also by colchicine.

The first question that arises from the preceding discussion is whether the irregularities of nuclear and cell division in species hybrids are controlled by causes similar to those controlling irregularities springing from the above mentioned causes. This is answered by Kostoff (1930) in the affirmative. The germ plasm of one species of the hybrid contributes something foreign to that of the other. That which is foreign causes physical and chemical changes in the protoplasmic properties of  $F_1$  including the CV. The lagging chromosomes and the occurrence of somatic polyploidy so common in hybrids are a result of increase in CV. The effect on *p.m.c.*'s is even greater. In a similar manner X-rays cause an increase in CV and chromosomal aberrants are common among the progenies of X-rayed plants, undoubtedly as a result of disturbance in the chromosome distribution in the X-rayed plants.

The second question is to find an explanation of the peculiar behaviour of the tapetal cells. In the light of the preceding discussion the morphology of the tapetum appears to be an inevitable result of the effect of special agents coming from the *p.m.c.* These agents may be considered foreign for the soma and raise the CV of the tapetal cells so that a continuous chromosome division without separation takes place. Plurinucleate tapetal cells would appear to be widespread among plant species. The binucleate condition prior to disorganization is almost the rule. Uninucleate tapetal cells at the time of their disintegration is the least common. A natural question is, are all cases of multinucleate tapetal cells to be regarded as species hybrids, even though they may not exhibit any other visible hybrid characters? Such an inference would appear warranted if we accepted *in toto* the principles underlying the preceding discussion. To our mind it seems that it must await further correlated study, establishing a definite relationship between the tapetal behaviour and the hybrid nature. Multinucleate tapetal cells and somatic polyploidy appear to be common in species hybrids

but such a behaviour is also exhibited by species which have not yet been proved to be hybrids or whose hybrid nature is not yet established beyond doubt. How far this is applicable to cases of structural hybridity must for the present remain unanswered. The other alternative is, that whether a species is a hybrid or not, the special agents that come from the *p.m.c.* act as foreign bodies and raise the CV of the tapetal cells, and this leads to the consequential mitotic aberrations. If we accept the first alternative then it follows that inasmuch as in hybrids meiotic aberrations such as those described are very common, the tapetal behaviour and these meiotic irregularities must be considered as correlated. In other words multinucleate tapetal cells, somatic polyploidy of the tapetum, etc., must be expected only in such species which being species hybrids would also show aberrations in the meiotic divisions of the pollen mother cells.

This however is not substantiated by facts, for such a tapetal behaviour as is said to be universal in hybrids, occurs unassociated with any meiotic irregularity in many cases. We have found recently (Raghavan and Venkatasubban, 1940 c) in a species of *Crescentia* a similar plurinucleate condition of the tapetum but there is absolutely nothing abnormal in respect of the meiosis of the *p.m.c.*

It seems probable therefore that if the tapetal behaviour must be explained on the basis of the increased CV, then the second alternative must be regarded as being more tenable. So far as the species under investigation is concerned, it seems safe to say that the occasional prevalence of the meiotic abnormalities may be taken as a fair indication of the contribution of some amount of 'foreign blood' in the make up of the species. This is in a way supported, as has already been mentioned, by the widespread occurrence of the phenomenon of cytomyxis. The suggestion made in the earlier part of the discussion that there appears to be no correlation between meiotic abnormality such as semi-heterotypic division, etc., and the peculiar tapetal behaviour, seems to be justified from this point of view also.

(e) *Tridax procumbens*—a true breeding hybrid?—From the foregoing description of the meiotic process in the species and of the behaviour of the cells of the tapetum, one thing stands out very prominently. And that is, it exhibits a number of meiotic features which are usually associated with hybrids and most of these what may be regarded as meiotic abnormalities have to do with the ultimate production of polyploid gametes. Such are, fusion of *p.m.c.*'s cytomyxis, suppression of wall formation at premeiotic mitosis, restitution nucleus formation, etc. But the interesting thing is

that though these phenomena are exhibited in their earlier stages, the respective processes are not carried through to their ultimate end, namely to the formation of polyploid grains. Degeneration sets in sooner or later. These facts set one thinking whether *Tridax* is a true breeding hybrid. Hybridity associated with polyploidy undoubtedly plays an important part, as recent investigations have shown, in the evolution of plant species. Polyploid gametes have been produced as a result of hybridization. These gametes have proved viable in various combinations and have resulted in the production of polyploid hybrid forms. These hybrids intercrossed or back-crossed have led to the establishment of stable types. Such stable types exhibit usually a different chromosome number from either of the parents and the reduction divisions are regular or nearly so. Certain of these polyploid forms are known to be even more fertile than the diploid forms. Having these facts in our mind, it may occur to one that in the evolution of this species, some of these phenomena have been involved. The occurrence of the meiotic aberrations usually associated with hybrids, but which do not either lead to the ultimate formation of polyploid gametes or affect in the least the fertility and seed-production of the species, seem to imply to our mind that hybridity has played an important part in the formation of this species. We are not unaware, however, of the difficulties lying in the way of such a concept, especially when it is known that *Tridax procumbens* is an exotic species and that in India it is monospecific.

### VIII. Summary

The diploid and haploid chromosome numbers of *Tridax procumbens* Linn., have been determined for the first time to be 36 and 18 respectively.

Meiosis has been described in detail.

A number of phenomena in the nature of (a) fusion of *p.m.c.*'s, (b) semi-heterotypic division, (c) cytomyxis, (d) binucleate *p.m.c.*'s are exhibited which instead of culminating as they should, in the ultimate formation of polyploid gametes, abort somewhere near the end of the respective processes.

A number of other things like delayed-separation-bridges, non-disjunction, amitotic division of the T I nuclei are also of frequent occurrences.

It is suggested that in the evolution of this species, hybridization has played an important part.

The behaviour of the tapetal nuclei is described and found to be somewhat like that in Apomicts and hybrids. The view that meiotic aberrations such as occur in these forms are correlated to the peculiar behaviour of the tapetal cells is discussed and not accepted.

The bio-physical and bio-chemical phenomena involved in irregularities of nuclear and cell division is discussed and the tapetal behaviour explained on this basis.

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#### EXPLANATION OF PLATES AND TEXT-FIGURES

Plates IV and V are photomicrographs.

The Text-figures were drawn at table level with an Abbe drawing apparatus at bench level; their approximate magnifications are given against each.

#### PLATE IV

FIGS. 1-3. *P.M.C.* with one large and one small nuclei, showing simultaneous division stages, early prophase, diakinesis, M I and M II.

FIG. 4. Complete cytomixis at prophase; same as Text-fig. 17.

FIGS. 5, 6. Tapetal nuclei, greatly elongated, due to repeated division unaccompanied by separation.

FIGS. 7, 10. Syn-*p.m.c.* simulating to enter upon division stages.

FIG. 8. Trinucleate *p.m.c.*; same as Text-fig. 44.

,, 9. An earlier stage in the fusion of the *p.m.c.* Note the remains of the walls.

PLATE V

FIG. 11. Amitotic division of the telophase nucleus (same as Text-fig. 48).  
 .. 12. Bridge in the spindle.  
 .. 13. Bivalent off the spindle.  
 .. 14. P.M.C. M I, 18 bivalents.  
 .. 15. Binucleate p.m.c.—non-simultaneous behaviour.  
 .. 16. An earlier stage than Fig. 11.  
 FIGS. 17, 18. Trivalent lagging on the spindle.  
 FIG. 19. Multiple bridge—delayed disjunction.  
 .. 20. Non-disjunction.  
 .. 21. Binucleate pollen grain.  
 .. 22. Generative cell organised.  
 .. 23. Generative cell divided into two male cells.  
 .. 24. The male cells assume an elongated form (same as Text-fig. 7).  
 FIGS. 25, 26. Pollen grains, showing the very small ones intermixed with the normal ones. These appear viable and have arisen by the independent behaviour of the small nucleus of the binucleate p.m.c. Note also the presence of shrivelled grains.  
 FIG. 27. Pollen grains of slightly different sizes due to the "furrowing origin".

LEGEND TO TEXT-FIGURES

FIGS. 1-6

FIGS. 1 and 4  $\times$  3,600. FIGS. 2 and 3  $\times$  2,700. FIGS. 5 and 6  $\times$  1800.

FIG. 1. Somatic metaphase plate;  $2n$  36. Note the satellited chromosome  
 .. 2. P.M.C. in diakinesis.  
 .. 3. P.M.C. M I.  
 .. 4. P.M.C. M II. Showing 18/18 distribution.  
 FIGS. 5, 6. Furrowed origin of the pollen grains. ca. 180

FIGS. 7-15.  $\times$  ca 900

FIGS. 7-11. Pollen grains of different sizes; 11 has arisen from the small nuclei of the binucleate P.M.C.  
 .. 12-15. Tapetal behaviour at different stages of the p.m.c.

FIGS. 16-22.  $\times$  ca 1800

Cytomyxis at different stages of meiosis.

FIGS. 23-37.  $\times$  ca 1800, except FIG. 27 which is  $\times$  ca 900

FIG. 23. Binucleate p.m.c. nuclei of different sizes; same as Plate VI, Fig. 1.  
 FIGS. 24-26, 28-31, 34-37. Showing different stages of the simultaneous division of the two nuclei. In 34 and 35 we see 4 haploid big and 2 sub-haploid small nuclei formed. 27. Non-simultaneous behaviour of the two nuclei of the binucleate p.m.c., same as Plate V, Fig. 15. 32. Uninucleate p.m.c. 33. Binucleate p.m.c. both the nuclei equal in size.

Figs. 38-48.  $\times$  ca 1,800; except Figs. 40  $\times$  ca 2,700; FIG. 42  $\times$  ca 380;  
 FIG. 43  $\times$  ca 900

Figs. 38-39. Synpollen mother cells showing an attempt at division; compare them with the normal pollen grain. 38 same as Plate IV, Fig. 7.

FIG. 40. Semi-heterotypic division.

Figs. 41-43. Stages in the fusion of the *p.m.c.*'s; 41 same as Plate IV, Fig. 9.

FIG. 44. Trinucleate *p.m.c.* same as Plate IV, Fig. 8.

Figs. 45-46. Extrusion of one and three univalents.

,, 47-48. Amitotic division of the T I nuclei.

Figs. 49-59. FIGS. 49, 50, 52, 53, 54, and 55  $\times$  ca 2,700; FIGS. 51, 56,  
 58 and 59  $\times$  ca 3,600; FIG. 57  $\times$  ca 1,800

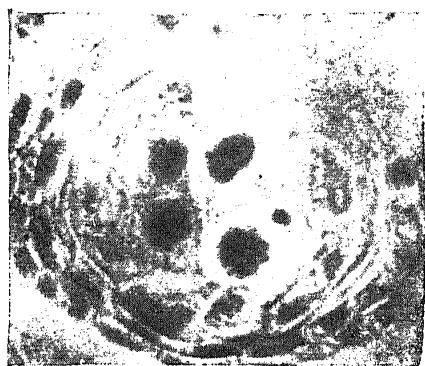
Figs. 49-51. Bridge configuration due to delayed disjunction.

,, 52-53. Bridge configuration due to the persistence of the interstitial chiasmata.

,, 54-57. Non-disjunction.

FIG. 58. Trivalent lagging on the spindle. Same as Plate V, Fig. 17.

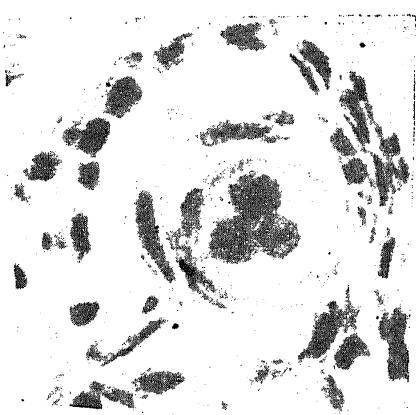
,, 59. Multiple bridge formation due to delayed disjunction and delayed terminalisation; same as Plate V, Fig. 19.



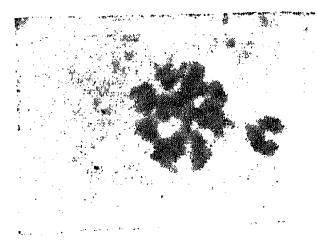
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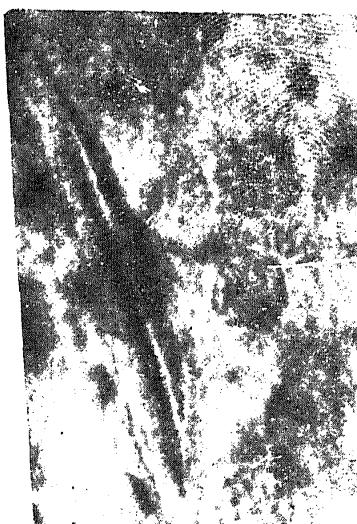
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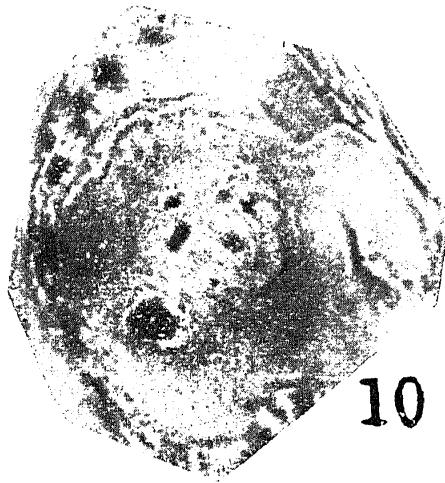
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7



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10

