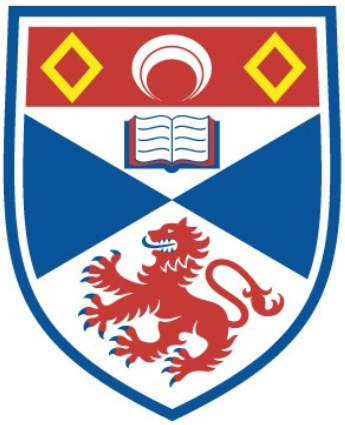
CYTOLOGY AND FERTILITY OF HYBRIDS

Nwe Nwe Tun

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1961

C Y T O L O G Y AND F E R T I L I T Y

OF

H Y B R I D S

by

Nwe Nwe Tun, B.Sc (Agric)

A Thesis submitted to the University of St. Andrews for the degree of Doctor of Philosophy.

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April, 1961.

ms 5476

DECLARATION

I hereby declare that this Thesis records the work done by me, that it is a composition of my own and that it has not been previously presented for a Higher Degree.

CERTIFICATE

I certify that Nwe Nwe Tun has spent nine terms of Research work under my direction, and that she has fulfilled the conditions of Ordinances No. 16 and 61 (St. Andrews) and that she is qualified to submit the accompanying Thesis in application for the degree of Doctor of Philosophy.

CAREER

I was graduated from the University of Rangoon in March, 1958, with a degree of B.Sc (Agric) when I also won U Tun Yee's General Proficiency Prize (Gold Medal).

I was then registered as a Research Student in the University of St, Andrews in October 1958 under Ordinances No. 16 and 61. The research was carried out in the Department of Botany at St. Salvator's College of the University of St. Andrews, and at the Scottish Horticultural Research Institute, Mylnefield, Invergowrie, under the direction of Professor J.H.Burnett.

The period during which this research was carried out lasted from October 1958 till April 1961.

ACKNOWLEDGEMENTS

I would like to acknowledge my heartfelt thanks to Professor J.H.Burnett, my supervisor, for his kind and constant interest and guidance throughout my work; Dr.T.Swarbrick, director of the Scottish Horticultural Research Institute, and other members of staff there for allowing me to make use of the facilities in the place; Dr.G.Haskell, head of the Genetics Department at the Institute and in whose department all my work was carried out, for his kind interest and useful advice; the late Mr.Patrick of the Botany Department, St. Salvators College, St.Andrews, for teaching me the techniques of photography; and Mr.J.Sunderland, the Institute photographer, for his help in photography.

I am also very grateful for the Colombo Plan Traineeship Grant awarded me during the period of my research and to all the members of the British Council concerned,for their help in various ways during my stay in this country.

C O N T E N T S

INTRODUCTION & LITERATURE REVIEW

The choice of material in the present research was determined by time and location. The limited period of time resulted in the choice of plant material with a fairly short life cycle. In the second year of research which was carried out at the Scottish Horticultural Research Institute, Dundee, work was done on the material available at the place. Consequently this thesis is divided into 2 parts viz:

Part I. On species hybrids of tomatoes in which cytology of both vegetative and reproductive cells were studied.

Part II. On species hybrids of Ribes and Rubus in which only somatic cytology was done.

The cytology of tomatoes, both diploids and tetraploids (auto- and allotetraploids) of different origins has been studied by several earlier workers (Afify 1933; Humphrey 1934; Jørgensen 1928; Lesley and Lesley 1930, 1935; Lindstrom and Humphrey 1933; Lindstrom and Koos 1931 and Upcott 1935). These workers studied the post-pachytene stages in meiosis.

Those few who studied the pachytene behaviour in tetraploids (Lindstrom and Humphrey 1933; Humphrey 1934 and Upcott 1935) did not agree in their observations especially on the nature of synapsis of the chromosomes. The method of squashing which is more efficient for chromosome study has superceded the older method of sectioning. It was, therefore, the main aim of this research, besides studying the general behaviour of hybrids, to determine as accurately as possible the chromosome behaviour with emphasis on the pachytene stage in diploid and polyploid F_1 species-hybrids. In addition, the cytology of octoploids described here is the first record of the cytology of octoploid tomatoes.

The species L.esculentum and L.pimpinellifolium used in the present experiments belong to the sub-genus Eulycopersicon (Red Fruited) of the genus Lycopersicon (Luckwill, 1943).

The basic chromosome number of this genus is 12, as first discovered by Winkler (1909). The somatic number is therefore 24. The two species cross readily and chromosome separation is very regular in the diploid F_1 hybrids with a high fertility (Jørgensen 1928; Lindstrom and Koos 1931; Lindstrom and Humphrey 1933). Irregularities were very rare and occurred as an occasional lagging of chromosomes at anaphase (Afify 1933).

The interesting nature of the pachytene chromosomes which are structurally differentiated was first described by Lesley and Lesley (1935) followed by Brown (1949). These workers studied especially the nucleolar chromosomes as they were the most readily identifiable pair of chromosomes in the complement. Lesley and Lesley named it "Chromosome A" and showed that the size of satellite in this chromosome was variable in different varieties of tomatoes. Then the 12 pairs of chromosomes in the set were identified individually by Barton (1950) who numbered them according to their lengths. Chromosome 'A' of Lesley and Lesley then became chromosome 2, as it was the second longest in the complement.

Differentiated pachytene chromosomes, similar to those in the genus Lycopersicon, are also said to occur in Agapanthus and Kniphofia (Darlington 1933). But the differentiated condensation is less pronounced in Kniphofia than in Agapanthus. These chromosomes could be easily observed from pachytene to diakanesis. As in tomatoes, by metaphase only deep staining regions could be seen and terminalisation was complete.

Tomato tetraploids showed all the characteristic increase in vigour typical of tetraploids (Jørgensen 1928; Lindstrom

and Koos 1931; Lindstrom and Humphrey 1933, and Humphrey 1934); lower pollen fertility and lower number of seed set per fruit. In autotetraploids tetravalent association of chromosomes at metaphase ranged from none (Jørgensen 1928; Humphrey 1934) to a complete set of 12 quadrivalents (Lindstrom and Koos 1931) with variable number of multivalents per cell (Lesley and Lesley 1930; Upcott 1935, and Afify 1936). Since it was very difficult to study the nature of pairing in the greatly contracted metaphase chromosomes it was thought that pachytene chromosomes might offer the chance.

Hybrids between closely related species with regular chromosome pairing are known in several genera among which are Galeopsis (Müntzing 1930), Prunus (Darlington 1933), Paeonia (Stebbins 1938), Ribes (Meurman 1928), and others. These are all hybrids of high fertility though not as high as in pure species. Beasley (1943) studied similar hybrids in Gossypium which had more irregularities than the pure species.

Such hybrids with regular chromosome pairing and normal fertility retain their identity as species only by geographical or physiological isolation (Sax 1935). But in none of these has a thorough study been made of pachytene configurations of

hybrids or their descendents. Thus it seemed worthwhile, for this reason, to study especially pachytene chromosomes of tomato.

MATERIALS AND METHODS

METHODS

1. CULTURAL.

All the tomato plants studied were raised under green house conditions. In the first year, seeds were sown in vermiculite in the dark and were brought into light when they germinated. When the third true leaf appeared on the seedlings they were transplanted each into a 1½ inch pot. When about 3 inches high, the seedlings were again transplanted into 3 inch pots and then finally into 4 inch pots when they were tall enough to be staked.

Throughout their life, the plants were grown in vermiculite and fed with Pfeffer's solution (MacLean & Cook 1941). For the first few weeks half strength solution was used, but when mature full strength solution was fed every alternate day, tap water being used in between.

In raising the F_1 progeny of the first year plants, seeds were sown in boxes in a propagating house (80° - 90° F) and when they were about 3 inches high they were transplanted into 4 inch pots and were transferred into the green house (50° - 70°F). Finally the plants were transplanted into

6 inch pots when they were staked. In this case soil was used through all stages of the plants' growth.

The difference in the method of raising the plants between the first and second years was determined by the methods adopted at the two separate places at which this research was done.

2. EMASCULATING & CROSSING.

About 48 hours before anthesis the flower buds of the tomatoe were emasculated by the gentle removal of the anthers by means of a pair of fine forceps (sterilised), and the flowers were then bagged in 1½" by 1" cellophane bags. When the stigmas of the bagged flowers were ready to receive pollen the bags were removed and ripe pollen from the anther of the required plant was transferred on to the stigma by means of forceps. The flowers were bagged again. Pollen grains readily adhere onto a ripe stigma. A successful fertilisation was confirmed after about 10 days by the development of the ovary. Sometimes, however, the ovary swelled about a month or more after fertilisation, but in this case it was found to contain no seeds. Although no actual fertilisation took place, parthenocarpous fruit was formed.

Soon after the fruits were formed the cellophane bags were removed.

3. COLCHICINE TREATMENT.

Tomato seeds were grown in petri-dishes lined with filter paper soaked in a 0.25% aqueous solution of colchicine and the dishes were kept in the incubator at 20°C. More colchicine solution was introduced in order to keep the filter paper perpetually moist. After leaving the seeds to germinate in this way for 10 days they were changed to new petri-dishes, this time lined with filter paper soaked in distilled water. After three days they were planted out in soil in the propagating house and any necessary transplantings made as the seedlings grew.

4. CLEANING OF TOMATO SEEDS.

Ripe fruits were opened diameter-wise and the pulp together with the seeds were put into small beakers. A small quantity of concentrated hydrochloric acid was added to the beakers and left for about 5 to 10 minutes after which the seeds were washed on a sieve under running water. Cleaned seeds were dried on blotting paper. (Method used in S.H.R.I., Dundee).

5. MEASUREMENT OF STOMATA.

For the study of stomata on the leaves of tomato, the method of North (1956) was adopted, using cellulose acetate film.

The cellulose acetate solution was painted on the lower epidermis of the leaves with a camel hair brush which was kept in acetone when not in use. When the film dried, within a few seconds, the painted area was flooded with a strong solution of detergent. Then the film was easily lifted, mounted on a slide with the surface which faced the plant uppermost, and dried by dabbing with blotting paper. It was viewed under a microscope using a green filter. Measurements of the stomatas were made by an ocular grid using a high power objective.

6. CYTOLOGICAL.

For chromosome counts in tomato young root-tips were fixed in 1:3 acetic-alcohol solution after pre-treating in para-dichlorobenzene (saturated aqueous solution) for 2 - 3 hours at room temperature. The fixed root-tips were then heated in 1 part N.HCl and 9 parts of 2% orcein in 45% acetic acid for a few minutes and then left for 20 - 30 minutes. Squashing was done in fresh 1% aceto-orcein (Sharma and Sharma 1957).

For study of PMCs flower buds of favourable size (e.g. in diploids, buds of about 2.5 mm in length contain pachytene stage) were taken, sepals and petals were removed and then

fixed in 1:3 acetic-alcohol for 12 to 36 hours at room temperature. After fixation they were either squashed immediately or stored in 70% alcohol. The staining method used was that of Dr. M.S.Walters as described by Barton (1950). Fixed or fixed and stored buds were washed in distilled water for 30 minutes changing the water several times; mordanted in 4% iron alum for 30 - 45 minutes and then washed in distilled water again for half an hour. The washed buds were put in 0.5% aceto-carmine stain and squashed in the same. It was found that buds could be left in the stain for as long as two or three days after which the cytoplasm tended to darken.

In fact the best preparations were obtained from squashes of buds left in the stain for more than a day. Heating the slides over a steam bath (Barton 1950) gave better differentiation of the chromosomes. This method of staining is also applicable to young petals for the study of somatic chromosomes but pre-treatment with 0.002M solution of oxyquinoline or para-dichlorobenzene (saturated solution) is required to enhance spreading of the chromosomes.

Squashed preparations were best sealed in paraffin wax and then stored in a refrigerator. Staining was found to be at optimum after one or two days of sqashing.

In FMC squashes temporarily sealed preparations were made permanent according to the method of Celarier (1956) using tertiary butyl alcohol. After scraping off the sealing wax from the cover glass of the slide, it was put in a mixture of equal parts of tertiary butyl alcohol and glacial acetic acid. When the cover glass had loosened (after 10 - 20 minutes), the separated slide and cover glass were left there for about 5 - 10 minutes and then transferred to 1 part glacial acetic acid and 3 parts tertiary butyl alcohol. After 15 - 30 minutes in the second mixture they were transferred to pure tertiary butyl alcohol and left there for 30 minutes for complete dehydration. Then the slides and cover glasses were mounted preferably in Euparal although Canada Balsam could be used.

For squashes in orcein, permanent preparations were made by separating the slide and cover glass in 1:3 acetic alcohol and two changes of absolute alcohol, 2 minutes in each, and then mounted either in Euparal or Balsam (Darlington and La Cour 1950).

7. PHOTOGRAPHY.

Microphotographs were taken on half tone panchromatic plates (slow process) using an oil immersion lens of N.A. 1.32, an oil immersion condenser of N.A 1.3 and x6 compensating eye

piece. Camera lucida drawings were made at the stage level. Temporary preparations were used both in taking microphotographs and in making camera lucida drawings.

For the production of photodrawings, the original photograph of chromosomes printed on matt-surface paper was inked over with Indian ink. Then the photographic image was bleached out in 10 per cent potassium ferricyanide and 20 per cent plain hypo solution so that the drawing alone remained. The bleached photograph was then passed through acid hypo and thoroughly washed before being dried (Manton 1950).

MATERIALS

A commercial stock of Lycopersicon esculentum var. "Early Red" and L.pimpinellifolium (SR 1234) were used as parental lines and in the second year the F_1 hybrids between these two were also used. "Kondine Red", a tetraploid variety of L.esculentum was used for the study of meiosis in autotetraploids.

MORPHOLOGICAL STUDIES

Initially five plants of Lycopersicon esculentum Mill. and six plants of Lycopersicon pimpinellifolium Mill. were raised as parental strains. Each was selfed and reciprocal crossings were made between them. In the following year the seeds so obtained were sown and 4 plants each of the parental lines and 6 plants each from the families of the reciprocal crosses were raised to maturity;

Hereafter the crosses L.esculentum x L.pimpinellifolium and L.pimpinellifolium x L.esculentum will be referred to as EP and PE respectively.

Among the F_1 hybrid seeds of the 2 families viz. EP_2 and PE_2, distinct variation in seed size was observed. Consequently two lots of seeds in each family were sown separately. The letter "l" was suffixed to the family number to denote that the plants were raised from large seeds e.g. EP_1; the letter "s" similarly denotes small seeds. In EP_2 none of the small seeds germinated.

Table 1 shows the number of plants in each family raised for experimentation.

Germination

Seeds of L.esculentum and the reciprocal hybrids all germinated on the same date, while those of L.pimpinellifolium were two days later in germinating. The percentages of germination may be compared in Table 1.

Table 1. Number of families, plants per family, their pedigree numbers and germination percentage

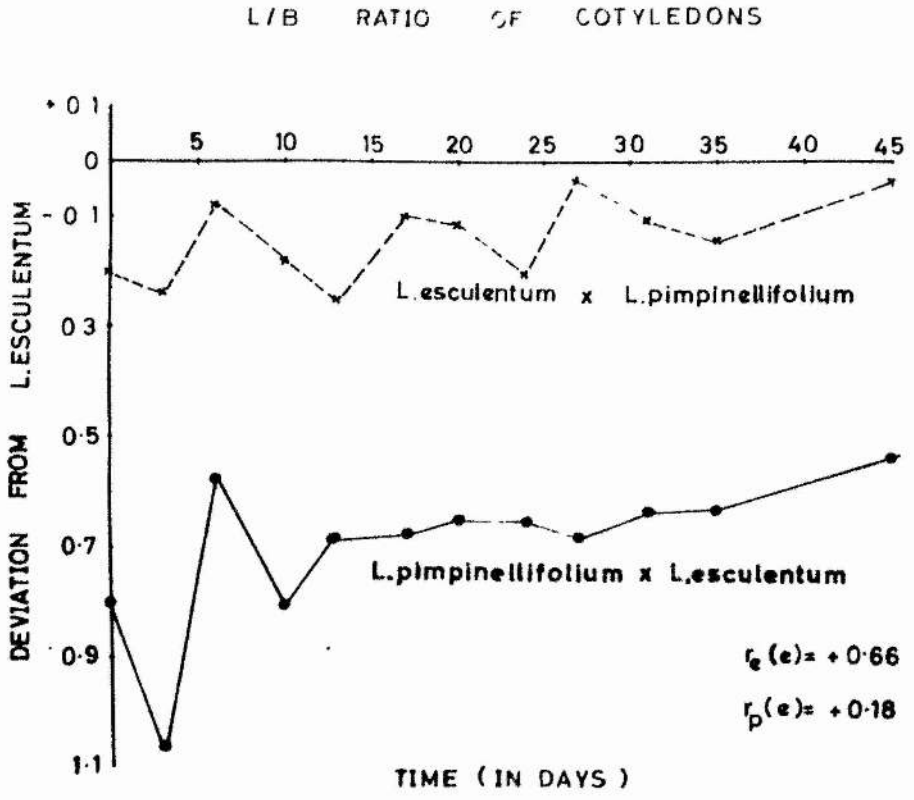
Strain	Family number	Number of plants raised	Pedigree numbers	Percentage Germination
L. esculentum	E_2	4	E21/60...E24/60	81.8
L.pimpinellifolium	P_5	4	P51/60...P54/60	100.0
L.esculentum x				
L.pimpinellifolium	EP_5	6	EP51/60..EP56/60	77.5
"	EP_12	6	$EP_121/60..EP_126/60$	
L.pimpinellifolium x				
L.esculentum	PE 1	6	PE 11/60..PE 16/60	
"	PE_12	6	$PE_121/60..PE_126/60$	98.1
"	PE_s2	6	$PE_s21/60..PE_s26/60$	

Cotyledons

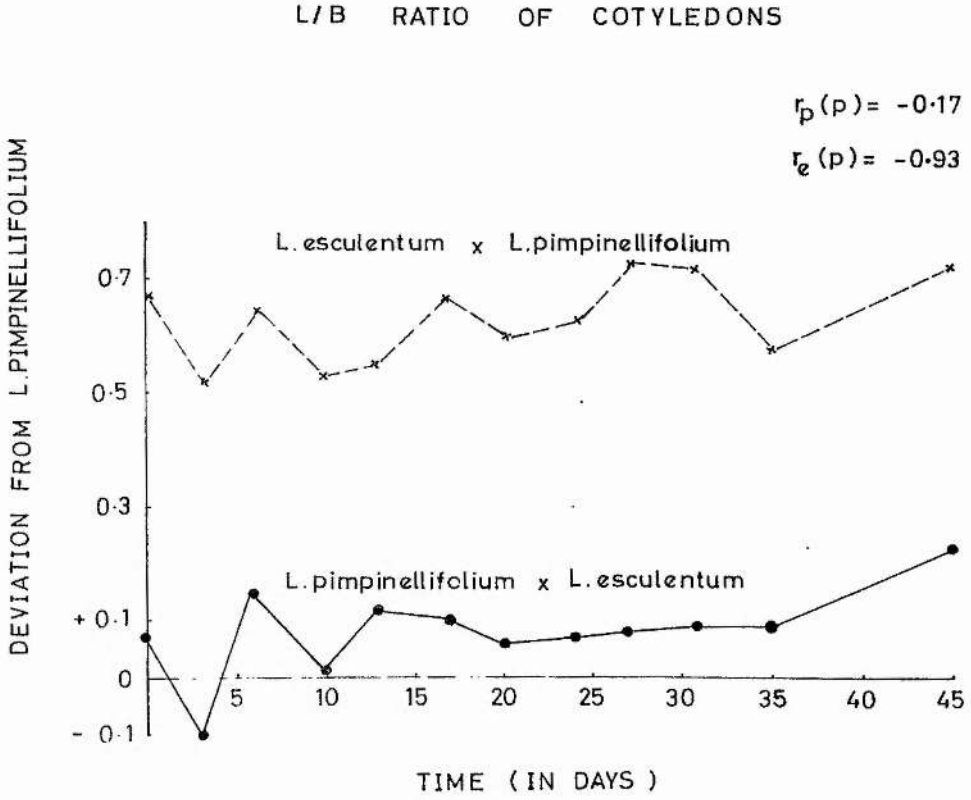
Marked differences in shape and size of the cotyledons existed in the inbred strains of L.esculentum and L.pimpinellifolium. In the former the shape was oblong and was approximately 1½ times as long as in L.pimpinellifolium. The latter had cotyledons which were more or less triangular with a broader base and tapering tip. The width as measured at the widest point, was not much less than that in L.esculentum, so that its length-breadth ratio was less than that in L.esculentum. The hybrids when considered as a whole, were intermediate with respect to length, breadth and length-breadth (L/B) ratio. Plate 1 shows the cotyledon characters in seedlings of the same age.

Between the reciprocal hybrids, differences in cotyledon characters existed, there was a tendency towards inheritance of maternal characteristics as regards cotyledon size. There was a significant correlation between cotyledon characters of each hybrid with those of the respective pistillate parent.

The accompanying graphs (Text fig:1 (a) and (b)) illustrate this correlation effect clearly. In Fig: 1(a) the L/B ratio values of L.esculentum are taken as zero and the L/B ratio deviations of PE and EP hybrids from it are



Text fig: 1(a). A graph showing correlation effect in L/B ratio of cotyledons of EP hybrid with its pistillate parent, L.esculentum.



Text fig:1 (b). A graph showing correlation effect in L/B ratio of cotyledons of PE hybrid with its pistillate parent L.pimpinellifolium.

plotted against time. Text fig: 1(b) is similar graph where L/B ratio values of L.pimpinellifolium are taken as the abscissa.

The first measurements of cotyledons were made when the seedlings were about 5 days old and were continued for one and a half months until the cotyledons were constant in size. Therefore it is worthwile noting here that the cotyledons do not cease growing for about 50 days from the time of germination.

Among the seedlings of L.pimpinellifolium 35 per cent showed a peculiar character in the cotyledons. One cotyledon of a pair was just half the length of the other. There was no evidence to suggest that this was caused by the accidental cutting off of the apical portion when it emerged from the seed coat. Selfed seeds from such plants raised to maturity were sown in the hope of finding out if this unequal cotyledon character was a single gene effect. But among the 2936 seedlings raised only 7 had the same unequal cotyledon character, and the rest were normal. It was more probable that this character was the result of an environmental effect rather than of a gene.

Leaves

Plate 2 shows the range in variation of the leaf shape and size in the hybrids as compared with the parents. No:1 is a picture of a mature leaf from L.esculentum parent; No: 2 from L.pimpinellifolium and No: 3, 4, 5 and 6 are those from several F_1 hybrids between the two.

Variation of leaf forms was found to occur even on the same plant so that it was not possible to say definitely which hybrid resembled which parent. On the whole three types of leaves were observed in the hybrids viz: (a) esculentum-like (b) pimpinellifolium-like and (c) intermediate between the two. Their distribution was as follows:-

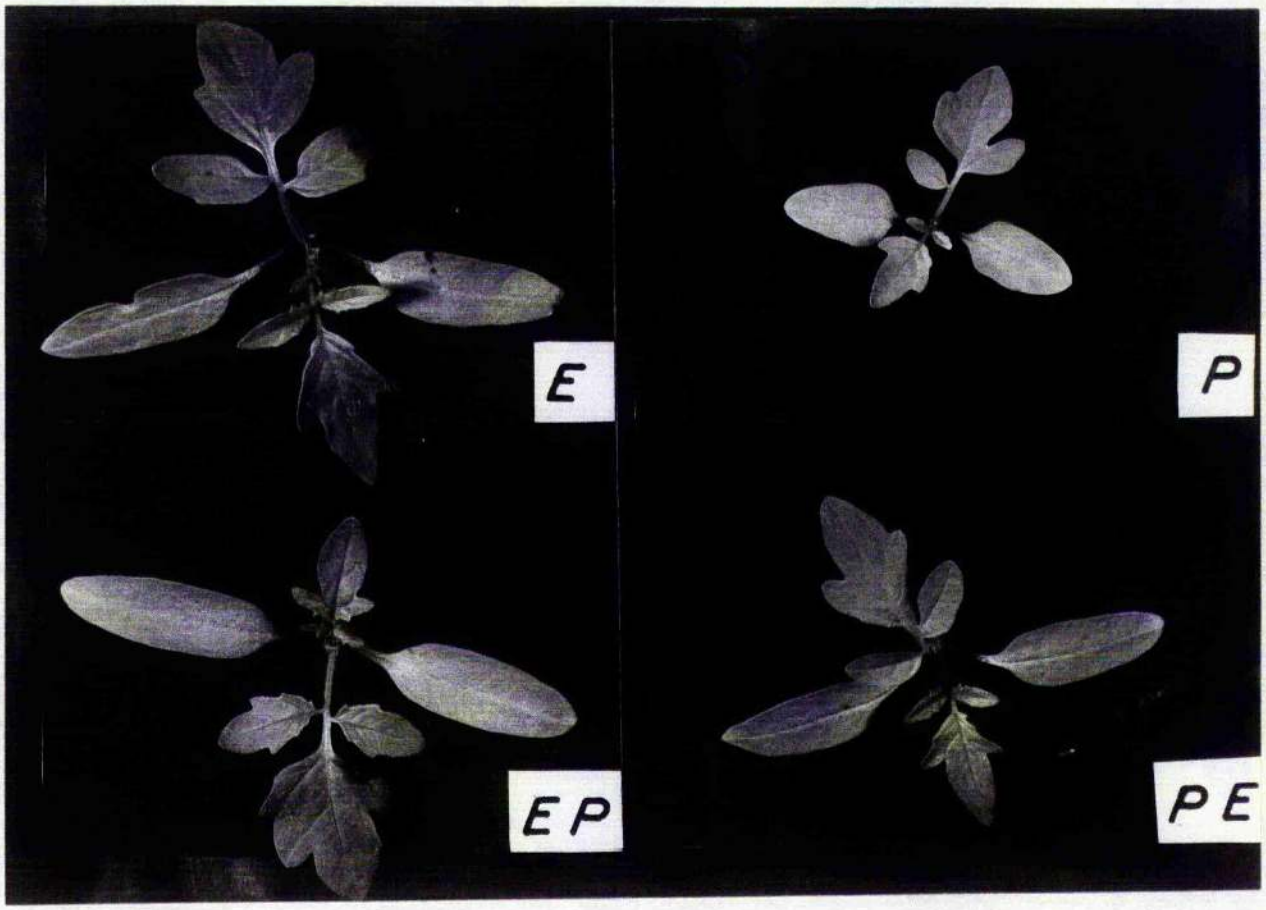
(1) Types (a) and (b) irregularly on the same plant or type (a) on the lower part and type (b) on the upper part of the same p plant.

(11) Type (c) throughout the plant.

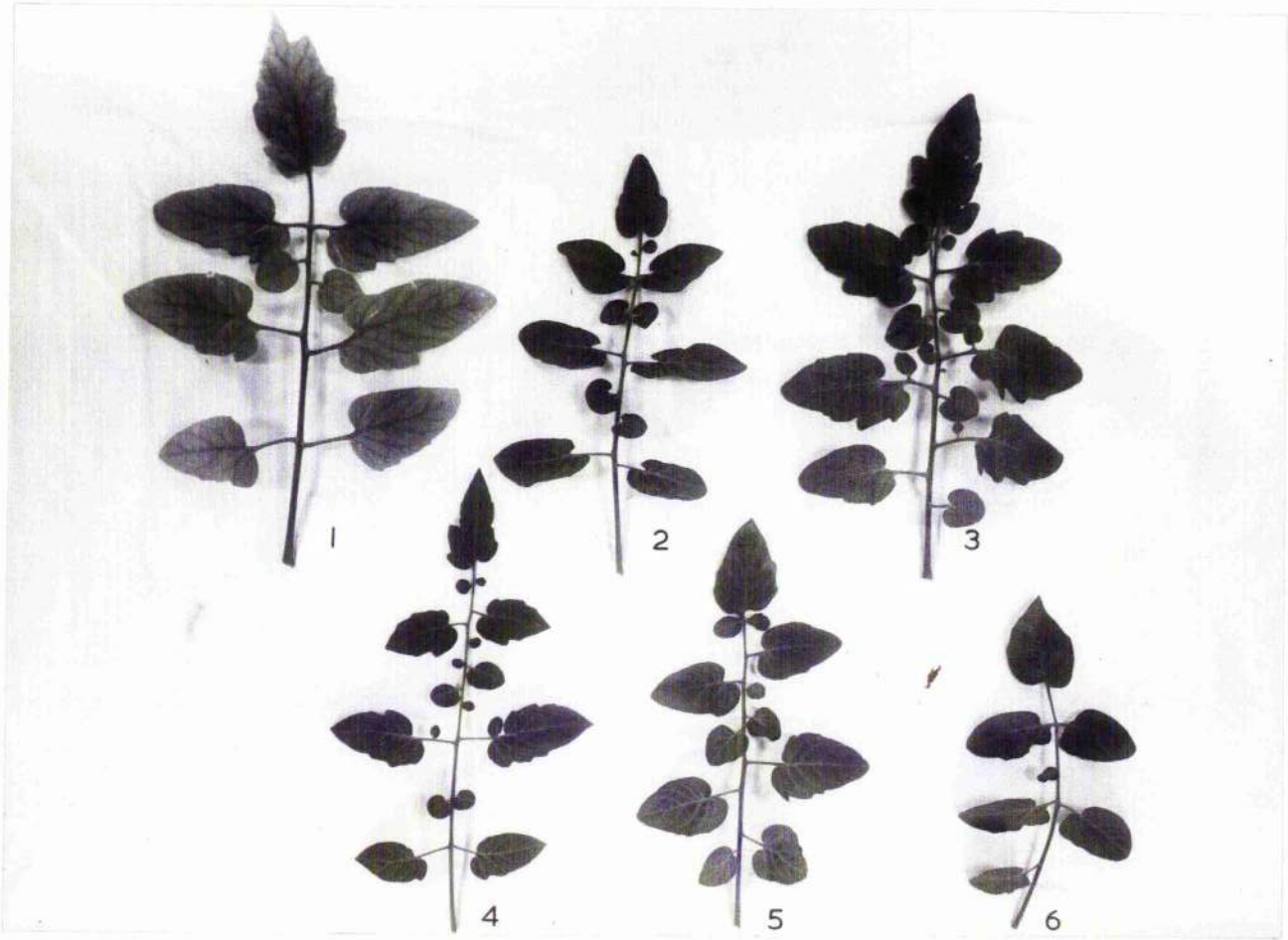
(111) All the three types occurring irregularly on the same plant.

Generally the majority of hybrids had leaves of the esculentum type but there was no apparent relationships between leaf character and the pistillate parent.

PLATE 1.



Seedlings of (E) L.esculentum, (P) L.pimpinellifolium, (EP) L.esculentum x L.pimpinellifolium, and (PE) L.pimpinellifolium x L.esculentum showing cotyledon shapes and sizes.



Leaf shapes and sizes in parental species and F_1 hybrids

1. L.esculentum 2. L.pimpinellifolium 3 – 6. F_1 hybrids

PLATE 2

Epidermal Hairs

Of the seven different types of epidermal hairs found in the genus Lycopersicon (Luckwill 1943), the following four different types are found in L.esculentum. (a) slender trichomes 6 - 10 celled with a bulbous multicellular base of 4 - 5 cells, (b) slender hairs 4 - 8 celled (not as long as type (a)) on a simple basal cell, (c) short patent hairs 1 - 4 celled (sometimes bent into the shape of a shepherd's crook) and (d) glandular hairs with a multicellular base, a stalk of 2 to 3 cells and surmounted by a tetrad of secretory glands. In L.pimpinellifolium, only the latter two types are present. In the present observations only three types were distinguished viz:-

Type 1. Trichomes 7 - 9 celled with multicellular bases (Luckwill's (a))

2. Slender hairs with no compound bases (Luckwill's (b) & (c))

3. Short hairs of 2 - 3 cells surmounted by a tetrad of secretory glands.

This was due to the difficulty of distinguishing two types of hairs viz: slender hairs on simple basal head and

short patent hairs sometimes bent near the tip (types (b) and (c) of Luckwill's) which were therefore combined as one, Type 2, in the table.

The accompanying table (Table 2) shows the percentages of the different types of hairs observed in the seven strains used in the experiment. Thin strips of epidermis were peeled off from the petioles of mature leaves and were put on a slide in a drop of water. The strips of epidermis were so placed that the hairs lay horizontally on the slide and the slide studied under the microscope using a low power objective.The different types of hairs observed were counted and their percentage obtained.

Table 2. Different epidermal hair types found in L.esculentum L.pimpinellifolium and their reciprocal hybrids.

	Types of hairs					
	1		2		3	
	No.	Percent	No.	Percent	No.	Percent
L.esculentum (E 24)	3	2.38	110	87.30	13	10.32
L.pimpinellifolium (P_{52})	2	1.27	81	51.27	75	47.47
L.esculentum x L.pimpinellifolium (EP_{51})	3	1.34	191	85.53	30	13.39
(EP_{122})	2	1.04	168	87.05	23	11.92
L.pimpinellifolium x L.esculentum (PE_{11})	2	0.86	195	84.05	35	15.09
($PE_{1}21$)	5	2.36	172	81.13	35	16.51
($PE_{8}25$)	2	1.87	81	75.70	24	22.43

Type 1 hairs were not totally absent from L.pimpinellifolium but occurred only in a very much reduced proportion. In the different plants examined these hairs were found to vary in sizes; in some cases type 1 hairs were not as long as usual, and type 3 and type 2 hairs were about the same size.

These differences were most probably effected by the age of the plants.

In the F_1 hybrids the distribution of hairs of types 2 & 3 resembled the E parent, but tended towards the P parent for Type 1 hairs.

Stomata

Stomatal size, measured lengthwise along the stomatal openings showed very similar results in the two parental lines, while in the hybrids although they did not differ greatly from the parents they exhibited some variation among themselves.

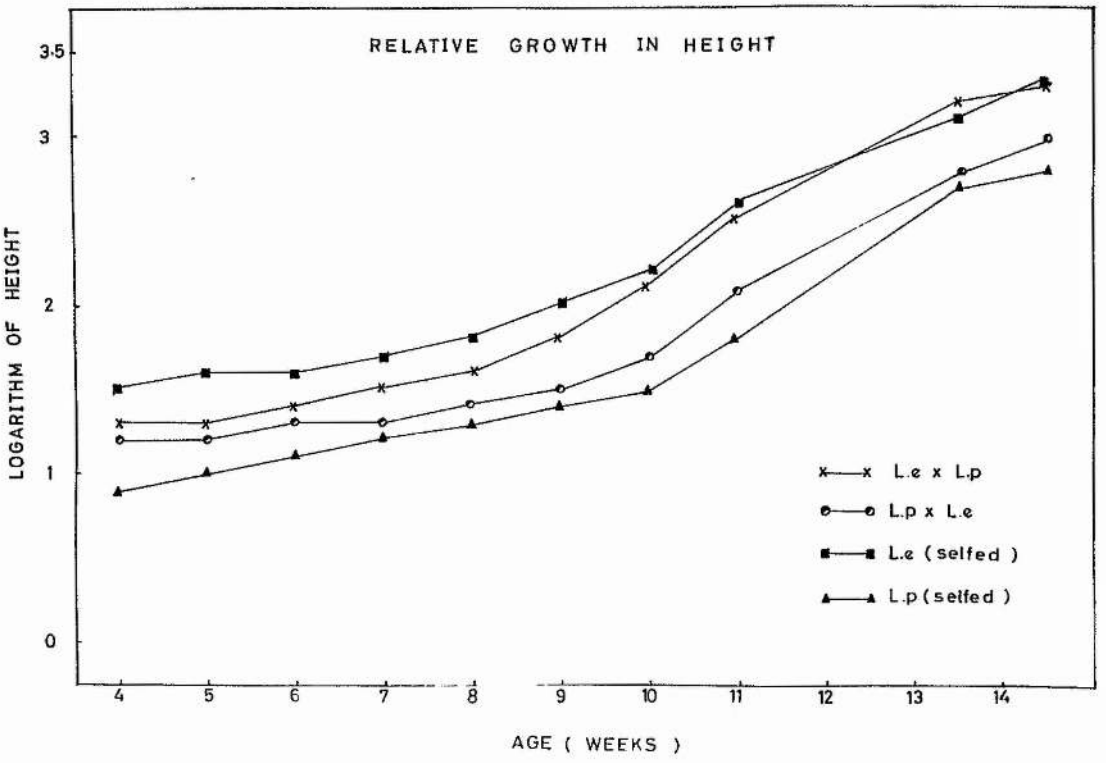
Table 3. Mean stomatal sizes as measured from 20 stomates in each line

	Size (in units)	S.D.
L.esculentum (E_2)	2.8	± 0.39
L.pimpinellifolium (P_5)	2.8	± 0.39
L.esculentum x L.pimpinellifolium		
($EP_1 2$)	2.5	± 0.51
(EP 5)	2.9	± 0.37
L.pimpinellifolium x L.esculentum		
(PE 1)	2.7	± 0.47
($PE_3 2$)	2.4	± 0.49

Growth

Growth of the seedlings of both parents as well as the reciprocal hybrids were studied for the first 14 weeks. Weekly measurements in height were made starting from the fourth week after the date of sowing and a graph was plotted for relative growth as measured in height. Fig: 2 is such a graph. Growth pattern, as will be seen in the graph, was very similar for all the four strains, the hybrids neither exceeding (except at one point) the taller parent nor less than the short parent at any time in height. The hybrid with L.esculentum as the pistillate parent showed a slightly faster rate of growth than either of the parents from after the tenth week of sowing.

There was thus no heterotic effect in respect either of height or relative growth rate in the hybrids. Of the reciprocal hybrids, so far as height was concerned, the hybrids EP approached the taller parent more than its reciprocal hybrid did. However on reaching maturity any significant differences in height that existed between the reciprocal hybrids disappeared. They were nearer to the shorter parent, L.pimpinellifolium. See Table 4 and Plate 3.



Text fig: 2. Relative growth in L.esculentum, L.pimpinellifolium and their reciprocal hybrids.

Seedlings of L.esculentum (L.E), L.esculentum x L. pimpinellifolium (L.E x L.P), L.pimpinellifolium x L.esculentum (L.P x L.E), and L.pimpinellifolium (L.P), all at the age of 13 weeks.

PLATE 3

Table 4. Mean height in inches of L.esculentum, L.pimpinellifolium and their reciprocal hybrids at 21 weeks of age.

		S.D.
E	53.60 inches	± 3.19
EP	48.60 "	± 4.75
PE	44.60 "	± 6.14
P	44.50 "	± 5.26

Fruit

Fruit size was assessed by equatorial measurement in mm. The fruits of all the five F_1 generation lines were intermediate in size with no significant differences within the lines.

Table 5. Means of five F_1 tomato species hybrids and their parents for fruit size.

	Fruit size (Diameter in mm)	S.D.
L.esculentum	30.5	± 3.23
L.pimpinellifolium	22.1	± 3.47
L.esculentum x L.pimpinellifolium		
(EP_5)	29.3	± 1.60
($EP_1 2$)	27.62	± 2.01
L.pimpinellifolium x L.esculentum		
(PE 1)	28.9	± 1.81
($PE_1 2$)	29.9	± 2.94
($PE_8 2$)	29.4	± 2.90

The hybrid fruit size however is definitely larger than the mean of parents (both arithmetical and geometric) as proved by significante tests. This is not in agreement with the results obtained by several other workers where the fruit size of the F_1 was found to be intermediate with a tendency

towards the smaller fruited parent (MacArthur 1941; Larson and Currence 1944; Haskell and Brown 1955). No results indicating maternal or cytoplasmic effect was observed.

No measurements were made of the sugar contents of the fruit but L.pimpinellifolium and the hybrid fruits tasted definitely sweeter than the L.esculentum fruits. This is explicable by the results of Bianchi et.el. (1953).

Seeds

Seed size measured as length and breadth showed that the hybrid seeds were intermediate, but with a tendency toward the larger seeded L.esculentum parent as will be seen in the following table (Table6). No differences in the reciprocal hybrids were observed.

Table 6. Seed measurements of the parents and the F_1 hybrids.

	Mean Length	Mean Breadth	Mean L/B Ratio	S.D.
E	3.45	3.15	1.26	± 0.17
EP	3.24	2.24	1.47	± 0.25
PE	3.11	2.16	1.45	± 0.24
P	2.75	1.98	1.39	± 0.17

(Length and breadth measured in mm.)

IV.

CYTOLOGICAL OBSERVATIONS

DIPLOIDS

Mitosis in species and F_1 hybrids

In both L.esculentum and L.pimpinellifolium mitosis was typical of any somatic division. Prophase was apparently a very prolonged process, in the early stages of which the nucleus assumed an indefinite moshy appearance. Darkly stained bodies were visible scattered in the nucleus. It could not be determined whether these dark staining bodies corresponded in number to the somatic chromosomes. The pycnotic state of the interphase nuclei in vegetative cells is also known to occur in the bryophyte Pellia (Heitz, 1928). Metaphase consisted, unless the root tips were previously treated, of a tangled mass of chromosomes orientated crosswise on the equatorial plate. By this stage the chromosomes were very contracted in length and it was very difficult to make individual identification of each of the 24 chromosomes in the complement. A pair of chromosomes with satellites, which was also the longest, was recognisable. Besides this pair of satellited chromosomes there were two other pairs of chromosomes which were longer than the rest. Of these

latter 4 long chromosomes in the complement one pair was metacentric, and the other pair had a submedian centromere (cf. Afify 1933). The method of staining used here was not very satisfactory for detailed study of chromosome morphology. But no other better method of staining was devised.

At anaphase, disjunction occurred when 12 chromosomes migrated towards each pole, on the arrival at which the individual chromosome lost its definite form in a process comparable to a reversed prophase. Then telophase nuclei were formed and subsequent division of the cell into two, followed with the formation of a cell wall between the two nuclei.

The process of mitosis was identical in all the four lines studied, viz: L.esculentum, L.pimpinellifolium and their reciprocal hybrids. Since it was not possible to distinguish between the mitotic chromosomes of L.esculentum and L.pimpinellifolium morphologically, it was not possible to distinguish the two parental sets in the hybrid. (Plate 5, fig:1)

MEIOSIS IN SPECIES AND F_1 SPECIES HYBRIDS

Parental Species

Pollen mother cell divisions of L.esculentum and L.pimpinellifolium followed the general process of a normal diploid tomato plant and were similar to those described by

previous workers including Jörgensen (1928), Lindstrom and Koos (1931) Humphrey (1934, 1937) and Brown (1949).

In the very early stages, preceding zygotene, the nucleus was made up of a closely packed and densely stained mass of chromatin, in which the individual ~~protonema~~ chromonema threads were indistinguishable. By zygotene the double strand nature of the chromosomes was apparent. Each chromosome appeared as a beaded thread with dark chromatic regions or chromomeres separated by lighter achromatic regions. In a chromosome these chromomeres occurred mainly on either side of the centromere and the distal part of the chromosome was mainly achromatic. Pairing was seen to commence from these distal achromatic regions (cf. Brown 1949) and the chromosomes came to lie side by side, chromomere to chromomere. In pachytene complete synapsis occurred. In this stage the differentiated nature of the chromosomes was more distinct and each chromosome pair could be traced from end to end in good preparations.

The constant morphological features viz: the chromatic and achromatic differentiation of the pachytene chromosomes of the tomato were the results of the unequal staining capacity of the chromatin material which constitute the chromosomes. The dark staining regions were termed "chromatic",

the weak staining ones "achromatic" and the terminal chromomeres "telomeres" by Brown (1949).

In the nucleolar chromosome (chromosome No: 2 of Barton (1950) or chromosome "A" of Lesley and Lesley (1935)) the short arm associated with the nucleolus, and referred to as the satellite, was entirely deep staining and the presence or absence of a terminal chromomere was difficult to ascertain.

Following pachytene, considerable shortening of the chromosomes took place and the closely paired chromosomes of the pachytene stage were separated from each other again. Points of contact between the two chromosomes were observed clearly. Presumably these were chiasmata where crossing over between the two chromosomes took place. On the whole, this was the most difficult stage to study in the meiosis of the tomato. The highest number of points of contact per bivalent was four, but bivalents with three chiasmata assuming a figure of eight configuration were the commonest. But it was not until early diakinesis that chiasmata were more easily recognisable. At this stage the achromatic regions were greatly shortened while in most bivalents the chromatic regions, although somewhat contracted, formed the greater part of the chromosome structure. Chiasma formation was observed to take

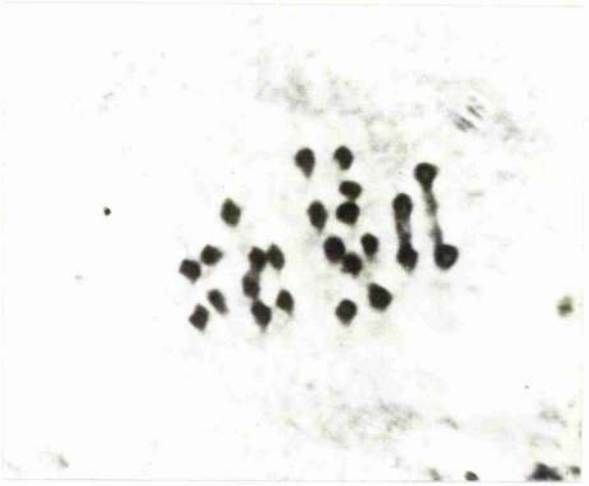
place only in the achromatic regions.

During the early stages of diakinesis the majority of the 12 bivalents appeared as rings, either with terminal chiasmata at both ends or terminal at one end and interstitial at the other. This interstitial chiasma was usually seen when the achromatic regions of the chromosomes at that end had not yet completely contracted. With the further contraction of the achromatic regions the chiasmata seemed to be terminalised. In these ring bivalents, one end of the ring usually opened out first so that rod bivalents were predominant in the late diakinesis stage (Plate 4, fig: 3.

Only 2 bivalents differed markedly from the rest. One was a rod bivalent whose chromatic regions were smaller than any of the rest in the complement. The other was Y shaped, the two chromatic regions forming the arms of the Y while its tail was formed by the paired achromatic region held together by an interstitial chiasma.

The nucleolus remained prominent till late diakinesis though sometimes it was very reduced in size, and the nucleolar chromosome was always associated with it. In prometaphase the nucleolus suddenly disappeared.

PLATE 4



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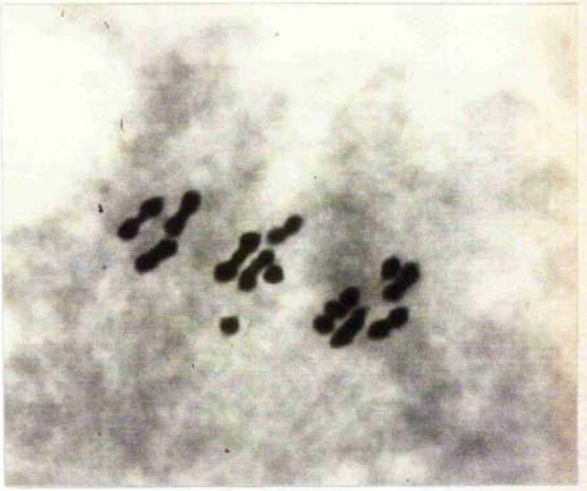

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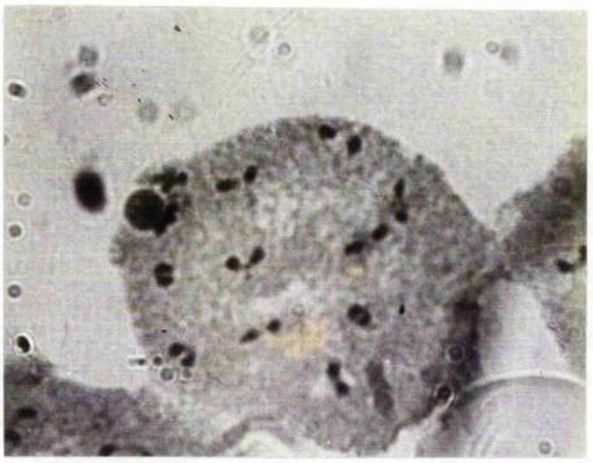

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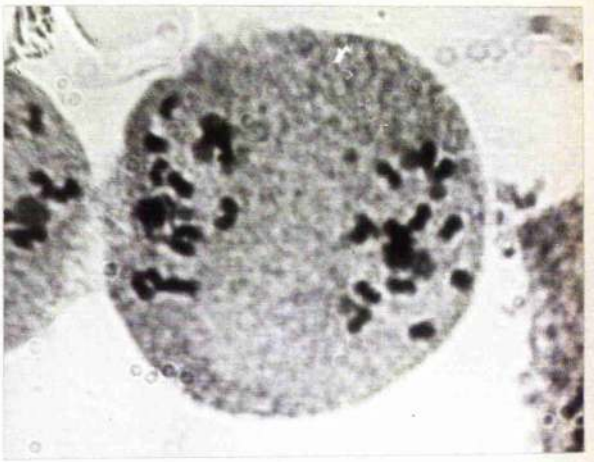

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Meiosis in parental species: -

Metaphase in (fig: 1) L.pimpinellifolium and (fig: 2) L.esculentum.

Fig: 3 Diakinesis and fig: 4 Interphase in L.pimpinellifolium.

Metaphase chromosomes became greatly contracted, thick, darkly stained bodies with no trace of achromatic regions left in them. There was no apparent differences in size among them as they became orientated along the equatorial plate. Normally, in both parents, one pair of chromosomes was precocious in anaphase separation (Plate 4, fig: 2). Disjunction was sharp and no lagging chromosomes were observed.

From late anaphase when counting the number of chromosomes was easiest, the chromosomes quickly passed into second meiotic division. Plate 4, fig: 4 is interphase of second meiotic division following which at prometaphase the chromosomes are orientated at random. By second metaphase the chromosomes came to lie at the equator. At anaphase II spindle formation was either parallel or perpendicular to each other and 12 chromosomes separated towards each pole. At telophase, 4 daughter nuclei were seen, and after cell wall formation a tetrad was formed. Soon after the release of the pollen grains from the pollen mother cell, about 5 to 7 days before anthesis, pollen grain mitosis occurred.

Generally up to the stages of diakinesis all the anthers in a bud were well synchronised in their meiotic divisions but

later on, stages from metaphase 1 to tetrads may be observed in a single anther. This is probably due to the fact that succeeding stages of metaphase 1 proceed rapidly giving little chance of overlap when all the cells are at the same stage of meiosis.

Relation of Mitotic to Meiotic Chromosomes

In the differentiated pachytene chromosome complement of the tomato, the nucleolar chromosome was most easily recognised due to its being attached to the nucleolus. This pair of chromosomes was also recognisable in the somatic complement as it was characterised by the presence of a satellite or trabant.

There were 2 other chromosome pairs in the somatic complement which were different from the rest in virtue of their greater length. These chromosomes could not be related to those in the meiotic complement without considering it from two points of view.

Firstly, according to their length they must be two of the first three chromosomes in the pachytene complement, excluding the nucleolar or chromosome 2. Taking into account Barton's (1950) descriptions of the individual pachytene chromosomes, chromosome 1 being more symmetrical than chromosome 3 would easily correspond to the long somatic chromosome pair

with a median centromere. Chromosome 3, having one arm much longer than the other would probably be the chromosome pair with submedian centromere in the somatic metaphase complement.

An alternative assumption to the above would be that since achromatic regions contributed little to the total length of the contracted chromosomes of metaphase (Brown 1949), the longest chromosome in the somatic plate need not be the longest in the meiotic complement. The fully condensed mitotic chromosomes of metaphase would therefore correspond only to the chromatic regions of the pachytene chromosomes. According to Barton's (1950) measurements, the chromosome pair with the longest and nearly equal chromatic regions on either side of the centromere could be either chromosome 1 or chromosome 5 and the pair with asymmetrical chromatic arms on each side of the centromere could most easily be chromosme 10.

Pachytene Chromosomes

It was possible to identify and measure 4 pachytene chromosomes in each of the complements of L.esculentum and L.pimpinellifolium. Measurements were made from 10 camera lucida drawings of each chromosome and the mean value was taken. Morphologically these 4 chromosomes corresponded very closely

to chromosomes 2, 5, 6 and 12 of the chromosome complement in tomato described by Barton (1950). From the data given in Table 7, the following comparisons between the chromosomes of L.esculentum and L.pimpinellifolium were made.

(1) Chromosome 2. A difference of 10 μ in the total length of this chromosome between the two species is found. But the arm ratio and the ratio of chromatic to achromatic material are very similar. The chromomeric pattern is apparently identical. In both species, this chromosome is the long satellited type. The satellite of this chromosome in L.esculentum is 7.02 μ in length and exceeds that in L.pimpinellifolium by 0.5 μ.

(2) Chromosome 5. This chromosome is very easily identified by its symmetrical pattern with a median centromere flanked on each side by nearly equal lengths of chromatic and achromatic regions. In L.pimpinellifolium this chromosome is found to be shorter by 2 μ in total length than in L.esculentum but the arm ratios in both species are very similar.

(3) Chromosome 6. This is the most easily recognisable chromosome, besides the nucleolar one, in the whole complement because of its particular chromomeric pattern. This chromosome

Table 7. Pachytene Chromosoms measurements in L.esculentum and L.pimpinellifolium

Chromosome No.	Species	Total Length (μ)	Arm Ratio	Chromatic Short arm (μ)	Chromatic Long arm	Achromatic Short arm (μ)	Achromatic Long arm	Chromatic/Achromatic
2	E	42.7	2.4	12.3	7.0	-	22.8	0.84
	P	32.6	2.4	9.7	5.4	-	17.2	0.87
5	E	28.4	1.0	4.6	5.6	8.8	8.8	0.60
	P	26.1	1.3	4.7	6.7	6.2	7.4	0.80
6	E	27.3	4.1	1.2	3.5	4.6	16.4	0.22
	P	22.3	3.1	1.4	2.8	3.8	13.2	0.24
12	E	17.0	1.2	3.9	3.9	3.1	4.8	0.98
	P	15.7	1.5	2.3	3.6	3.2	4.9	0.72

was first identified by Brown (1949). It has 3 large chromatic zones along its whole length, one on the short arm and two on the long arm. Here again this chromosome in L.esculentum exceeds that in L.pimpinellifolium by 5 μ, but the arm ratio and chromatic / achromatic ratio do not differ between the two species.

(4) Chromosome 12. This is the smallest chromosome in the complement, and a slight difference of 1.3 μ in length is seen between the two species.

Thus in the 4 chromosomes compared above, it is seen that the chromosomes in L.esculentum are longer than the corresponding ones in L.pimpinellifolium. But as far as the macrochromomeres are concerned the chromosome morphology between the two species are seen to be very similar.

Considering the chromosomes of L.esculentum alone, the present measurements do not agree very well with those made by Barton (1950). All the chromosomes measured here, except for the nucleolar chromosome, are slightly smaller than the corresponding chromosome lengths given by him. The two measurements of the nucleolar chromosome, however, corresponds very well, but Barton used the variety, "Sutton's Best of All", which has a short satellited type of nucleolar chromosome and

the size of the satellite is only 1.5 μ. The present material, variety "Early Red" has a satellite size of 7.02 μ. The arm ratio in the same chromosome obtained by Barton is 7.1, while it is only 2.4 in the present case, and the ratio of chromatic-achromatic material here is 0.8 to his 0.3. Apart from chromosome 2 the other chromosomes viz: 5, 6 and 12, though smaller in size, have similar arm ratios and chromomeric patterns as described by Barton (1950).

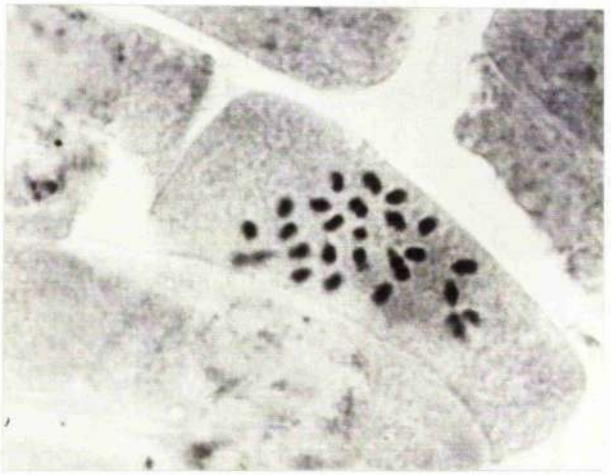
The different varieties of tomato used are to be accounted for the differences between the present measurements of the chromosomes and those of Barton (1950). In fact Gottschalk (1951, 1954) found that the total lengths, proportional lengths of arms and number of macrochromomeres were remarkably variable and this variation (at least in the satellite size of 19 different varieties of tomatoes he studied) was found even in cells of a single anther.

F_1 Species Hybrids

(a) The General Pattern

In the very early stages of pollen mother cell division the process of meiosis in the hybrids was very similar to the parents, a regular zygotene where two beaded chromosome strands come into close association lying side by side and chromomere to chromomere followed by pachytene which was almost normal.

Pairing of chromosomes commenced at zygotene or that was the stage when pairing was first observable. In most cases pairing was observed to start from the distal achromatic regions moving inwards toward the centromere. Plate 6, figs: 1 & 2, show chromosome pairing at zygotene stage. Pairing is still incomplete in the chromatic regions. Note that the chromomere patterns of any two pairing chromosomes are very similar. There were instances, though rare, when pairing was later in the achromatic regions than in the chromatic regions; The centromere, however, was the region where pairing first occurred. Within the chromosomes of a cell, this centromere pairing seemed to occur at different rates. In Plate 6, fig: 3, complete pairing of pachytene chromosomes is shown.



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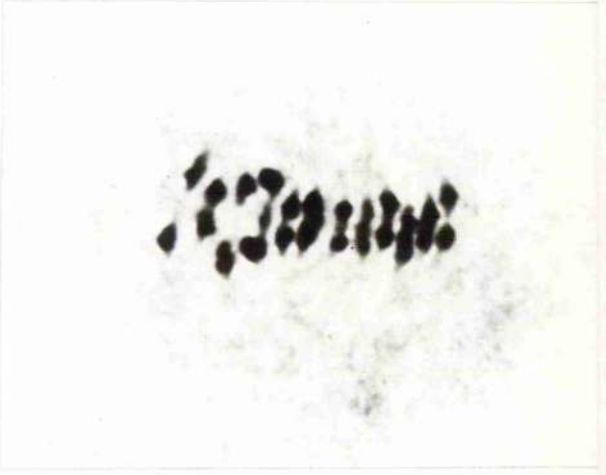

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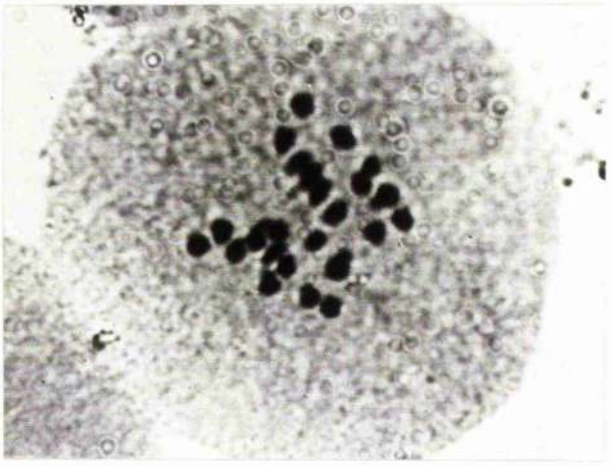

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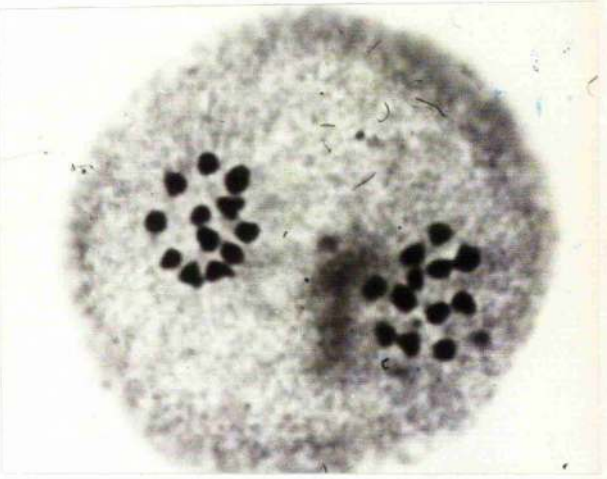

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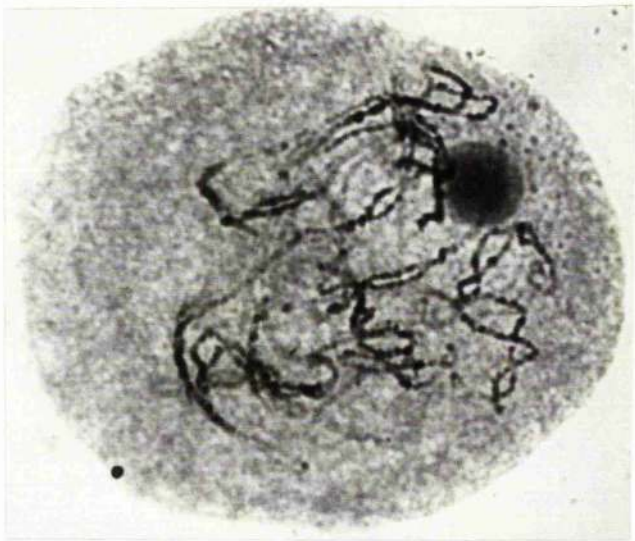
Fig: 1. Somatic metaphase plate

Fig: 2. Metaphase plate in PMC

Fig: 3 & 4. Early and late anaphase stages in reduction division of F_1 species hybrid.



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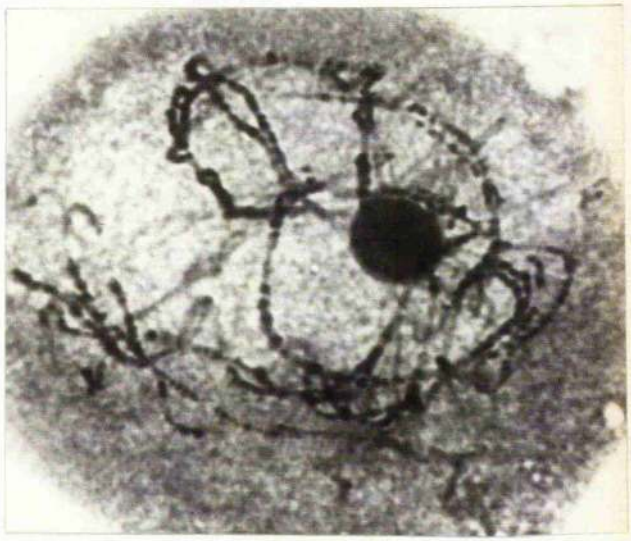

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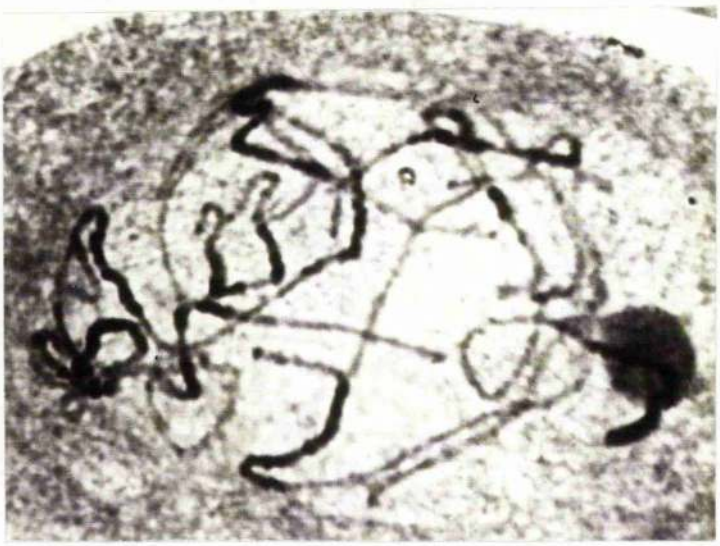

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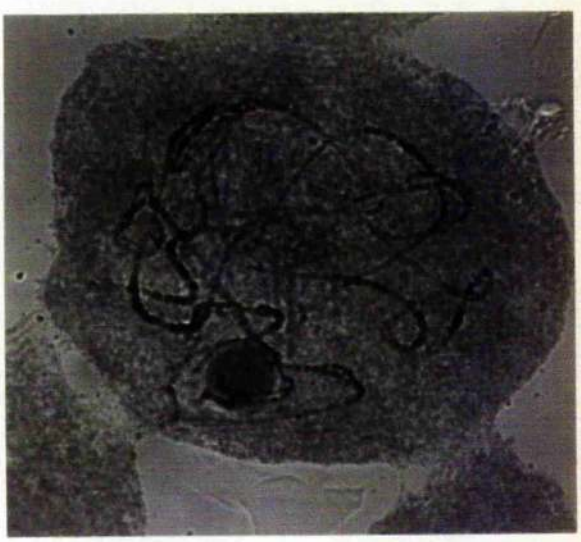

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Prophase in PMC division of F_1 species hybrids

Fig:s 1 & 2. Zygotene

Fig: 3. Pachytene showing normal complete synapsis

Fig: 4. Pachytene showing regions of incomplete pairing

(All fig:s - x 1920)

The only deviation from normal prophase stages as described above, was detected in late zygotene and early pachytene stages. In parental species synapsis of zygotene chromosomes was more or less uniform in the sense that by early pachytene pairing was complete in all the 12 pairs. In the hybrids, there was apparent delay in pairing in some chromosomes so that even by pachytene stage regions of incomplete pairing were still observed (Plate 6, fig: 4). Loosely paired pachytene chromosomes were also observed where the two chromosomes could be easily distinguished, unlike the normal tightly paired ones.

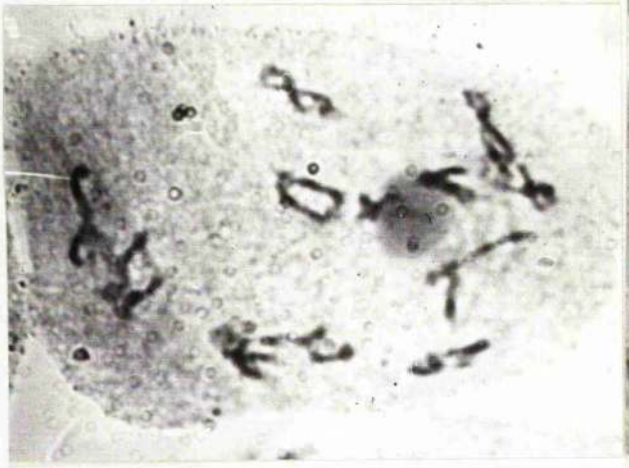
The occurrence of one nucleolus in PMC s was the rule in all the parental species of L.esculentum and L.pimpinellifolium studied, as well as in their reciprocal hybrids. However, samll numbers of pollen mother cells, about 10 - 20 per cent, were often observed containing two nucleoli. These cells with double nucleoli tend to group together in isolated patches presumably resulting from some local physiological condition.

Following pachytene, there was diplotene which was a difficult stage to study chromosome behaviour. In almost

all cells, where the chromosomes were well spread out, 12 bivalents was the rule. As in the parental material, 3 chiasmata per bivalent occurred most frequently and 4 chiasmata per bivalent was the highest number observed.

In several cases, 4 chromosomes (as many as 2 or 3 such associations per cell) were observed close together but it was unlikely that they were true quadrivalents although the fact could not be ascertained. As seen in Plate 7, fig: 1, only achromatic regions were observed to be involved in the formation of chiasmata. Figure of eight configurations are clearly seen in the same figure.

Diakinesis (Plate 7, figs: 2 & 4) was not different from a normal type. The small interstitially paired bivalent was still noticeable (arrow). The nucleolar chromosomes were always found to pair interstitially on the arms other than those attached to the nucleolus. With the exception of one case, the satellite region was never seen to form a chiasma. In one anther however, there were a number of cells in which the satellite regions of the nucleolar chromosomes were in contact (Plate 7, fig: 3 (a) & (b)) but the actual formation of chiasma at the point of contact was not certain.



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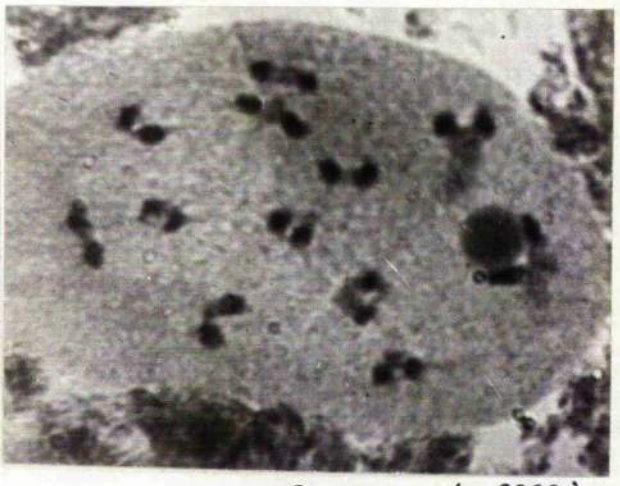

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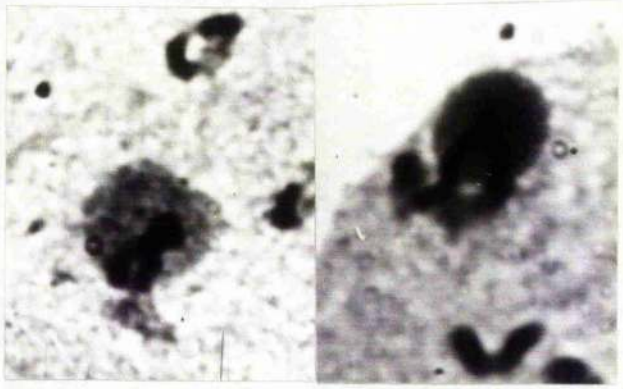

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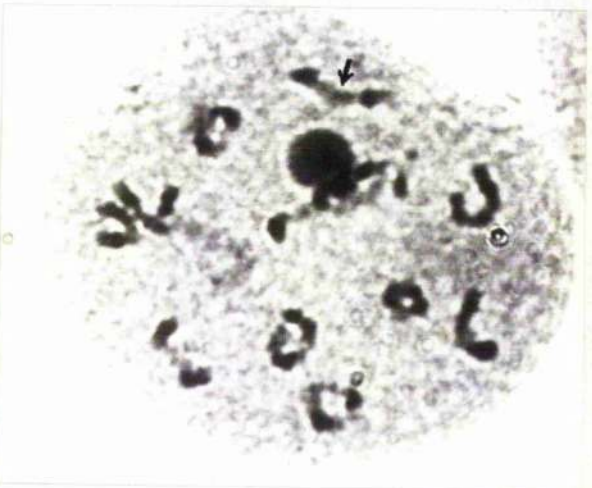

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Different stages in meiosis of diploid F_1 hybrids: Fig: 1, diplotene; fig:s 2 & 4, diakinesis in PE and EP hybrids respectively; fig: 3(a) and (b), points of contact in the short achromatic arm of the nucleolar chromosomes.

Metaphase was typical of any normal diploid metaphase (Plate 5, fig: 2). The chromosomes of each bivalent were equal in size (cf Lesley & Lesley 1935), though Humphrey (1934) claimed to have seen distinct size differences between the meiotic chromosomes of L.esculentum and L.pimpinellifolium. The succeeding stages did not show any deviation from the normal diploids.

(b) Irregularities

Meiosis in the hybrids was therefore normal in general but in some plates irregularities were encountered. These abnormalities may be divided into two groups, one found in EP hybrids and the other found in the reciprocal PE hybrids.

(i) EP Hybrids Abnormalities were first observed at diakinesis and were chiefly concerned with chromosome association. This occurred in only one plant viz: EP52/60. Although 12 bivalents was usual, chromosome associations other than 2 were also observed.

Table (8) gives the various associations encountered.

Table 8. Chromosome Associations at Diakinesis

	Univalents	Bivalents	Trivalents	Quadrivalents	No: of Plates observed
1.	-	12		-	77
2.	-	10		1	14
3.	2	11		-	5
4.	-	9	2	-	2
5.	1	10	1		1
6.	4	10			1

Besides the normal 12 bivalent types, cells with 10 bivalents and 1 quadrivalent occurred with the next highest frequency. Of the latter kind of association, two types were seen. One kind which occurred more frequently than the other was the chain type and the second kind was X - shaped type.

Chromosome distribution however was normal. No inversion bridges indicative of any structural alterations in the chromosomes were observed, although laggards, either a whole bivalent or just a chromosome were quite common. This was expected from the occurrence of univalents at metaphase which normally tended to lag behind the other chromosomes at Anaphase separation (Darlington 1929). In late anaphase no secondary associations were noticed (Plate 5, fig: 4), and in late telophase 4 nuclei were observed in a pollen mother cell with 1 to 3 nucleoli in each microspore nucleus.

(2) PE Hybrids. The irregularities observed in the hybrid PE15/60 were not dissimilar to those found in its reciprocal hybrid, they were as follows.

(a) In metaphase of the first division, a few chromosomes were left out of the equatorial plate (Plate 8, fig: 1). At anaphase these moved either along the spindle towards each pole or were left stranded in the cytoplasm and thus lost from the normal complement. In one case, a block of chromosomes was seen suspended in the spindle of the two separating groups. It is quite feasible to suggest that this block probably was previously outside the equatorial plate at metaphase 1. Presumably, as a consequence of this abnormality at metaphase

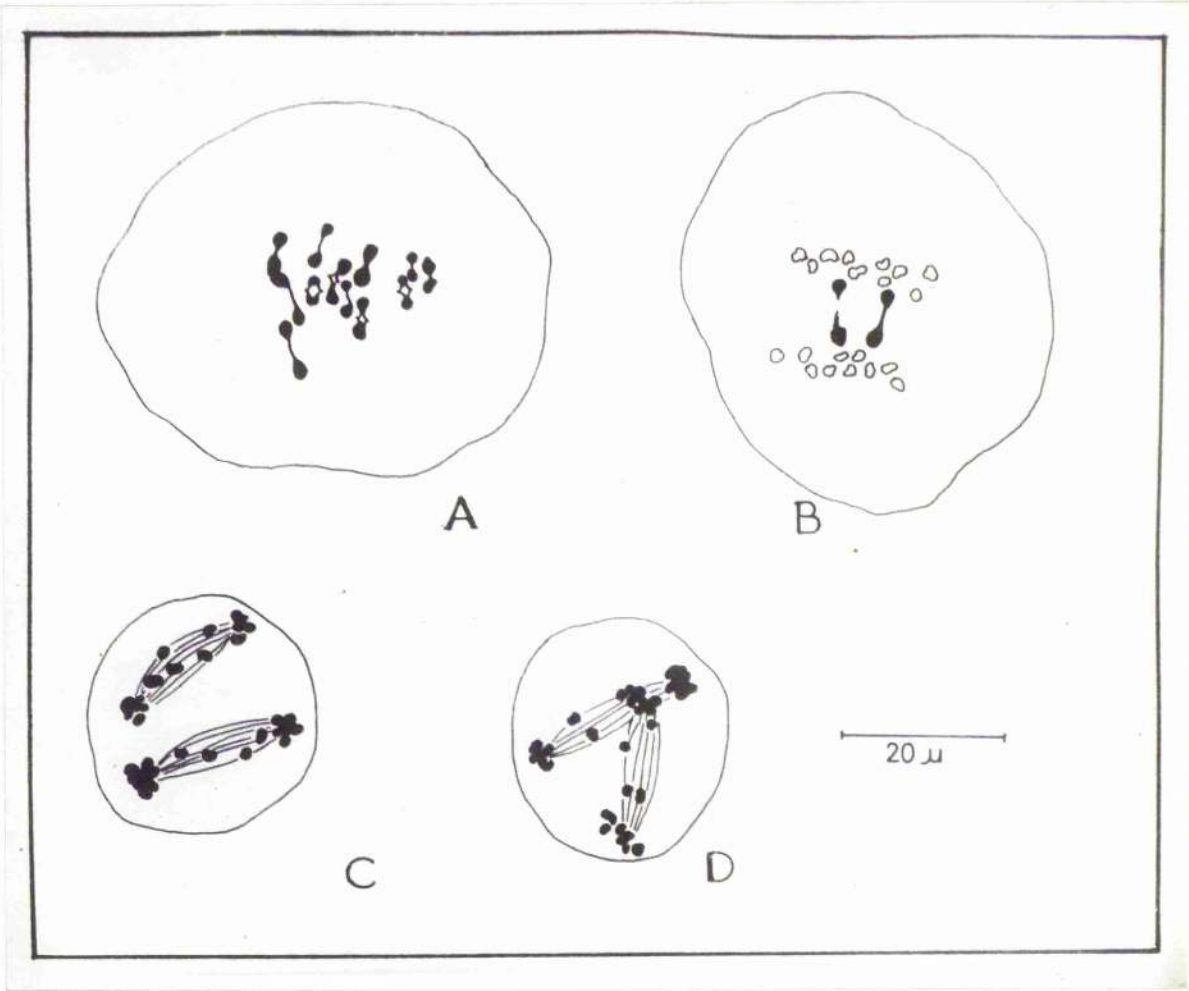
a high percentage, approximately 45 per cent, of the cells in anaphase 1 showed 1 or 2 laggards along the spindle. Normally these finally reached the poles to be included in the interphase nuclei. In a few cases the distribution of chromosomes was somewhat unequal, e.g. there was a case where there were $11\frac{1}{2}$ and $12\frac{1}{2}$ ~~bivalents~~ chromosomes respectively at each pole.

(b) Attenuations at early first anaphase (Plate 5, fig: 3) and bivalents delayed in separating (Text fig: 3, B). One such bivalent per cell was the commonest.

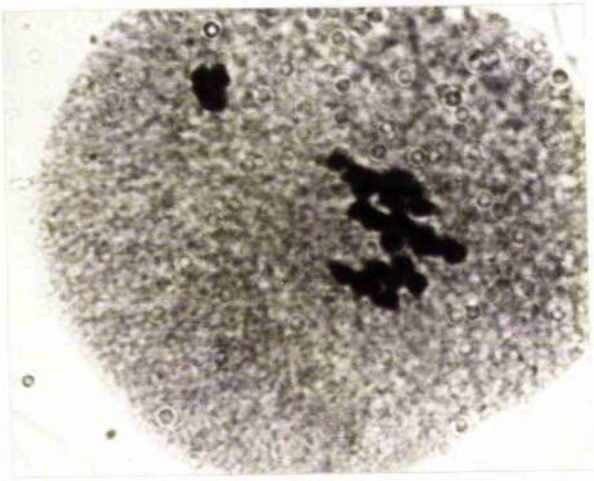
(c) The 12 bivalents at metaphase 1 failed to separate but whole bivalents moved along the spindle towards each pole so that at the poles, although the number of chromosomes was normal, i.e. 6 bivalents or 12 chromosomes, there would be duplication of some chromosomes and reduction of others at either pole. Plate 8, figs: 2 & 3 show movement of whole bivalents towards each pole and fig: 4 of the same plate shows a cell with 6 bivalents at each pole.

(d) At second metaphase the chromatids lay loosely along the equatorial plate.

(e) Laggards were very common in anaphase 11, the highest number per spindle being five (Text fig: 3, C & D). The chromosomes either reached the pole or were left excluded from the telophase nuclei and were finally lost in the



Text fig: 3. Irregularities in PE hybrids:- A. Metaphase plate in which there seemed to be one association of four chromosomes; B. Attenuations of separating chromosomes at anaphase 1; C & D. Laggards on the spindles at anaphase 11.



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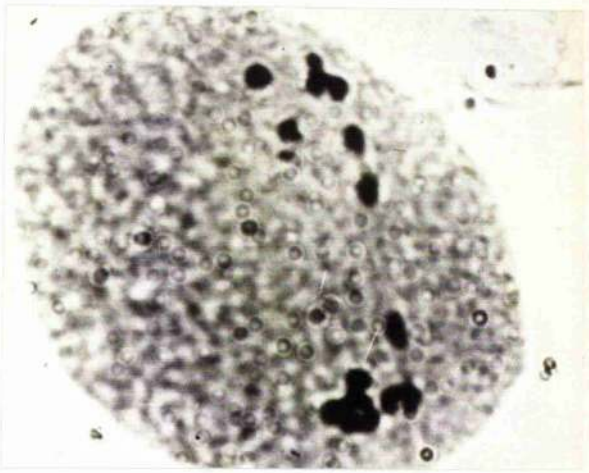

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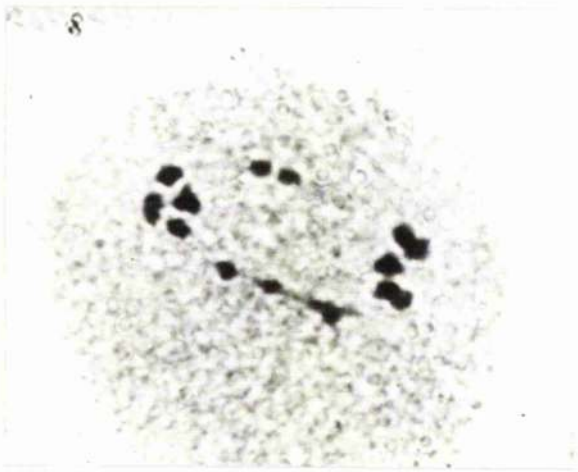

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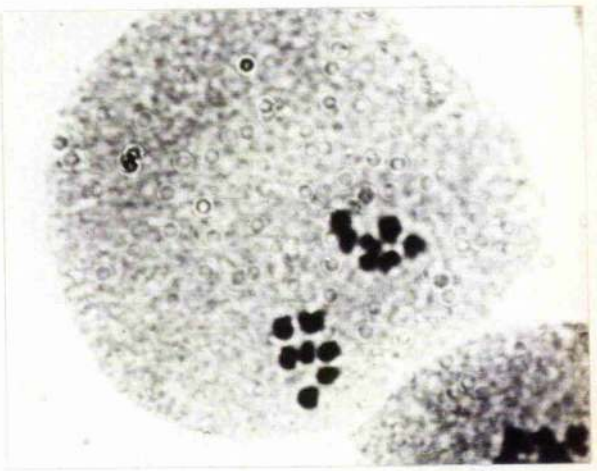

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Irregularities in meiosis in F1 species hybrids:
Fig:1, a few chromosomes left outside the metaphase plate
Fig:2 & 3, movement of whole bivalents towards the poles at anaphase
Fig:4, six bivalents at each pole at late anaphase

cytoplasm. These laggards were either in the spindle or sometimes in the cytoplasm when their chances of reaching the telophase nuclei were remote.

(f) In very few cells, less than 1 per cent, failure of separation of chromatids at anaphase II occurred, and the chromatids lay scattered in the cytoplasm. The final fate of such cells could not be ascertained.

Irrespective of such irregularities as mentioned above, the pollen mother cells at tetrad stage contained 4 microspore nuclei, which however, varied in size. This variation in size of the microspores was probably related to chromosome numbers in them. The considerable differences in size of the nuclei at telophase stage were indicative of the irregular chromosome number contained in each nucleus.

Attenuated Chromosomes

The attenuated chromosomes were observed in almost all the anaphase separations of chromosomes in the F_1 hybrids and occasionally one or two bivalents were delayed in disjunction. According to Upcott (1935) tomato is one of the species in which complete terminalisation of chiasmata at metaphase takes place. In chromosomes with completely terminalised chiasmata, anaphase separation should be sharp and clear (Darlington 1937).

In the present case the failure of sharp segregation of chromosomes at anaphase was presumably because of the presence of interstitial chiasmata still at metaphase stage. In the parental species the terminalisation coefficient was lower than has been recorded by most previous workers (Table 9), and in the F_1 hybrids the terminalisation coefficient was even lower. Afify (1933) and Brown (1949) did not find complete terminalisation of chiasmata in the metaphase chromosomes. They also found that the presence of interstitial chiasmata at metaphase had delayed the separation of certain bivalents. Beasley (1943) claimed that previous workers (names not mentioned) had assumed that the large number of attenuated chromosomes in certain hybrids could be a physiological effect resulting from hybridisation. But he found this to be untrue in Gossypium hybrids for here the chromosomes failed to become attenuated in the polyploids produced from the hybrids. Similarly the induced polyploids of the tomato hybrids in the present experiment did not show any attenuation of chromosomes in early anaphase. These observations suggest therefore, that attenuation is due to an impairment of the process of pairing for this occurs in diploid F_1 hybrids but not in tetraploid derivatives of such hybrids where each chromosome has its homologue present.

Greatly attenuated chromosomes also occurred in meiosis of male cockroaches during the stage known as "pre-metaphase stretch" (John and Lewis 1957). But it differs from the anaphase attenuation of chromosomes in that it occurs immediately following pachytene and it does not result in complete separation of the homologues as recontraction of the chromosomes takes place to form a metaphase plate.

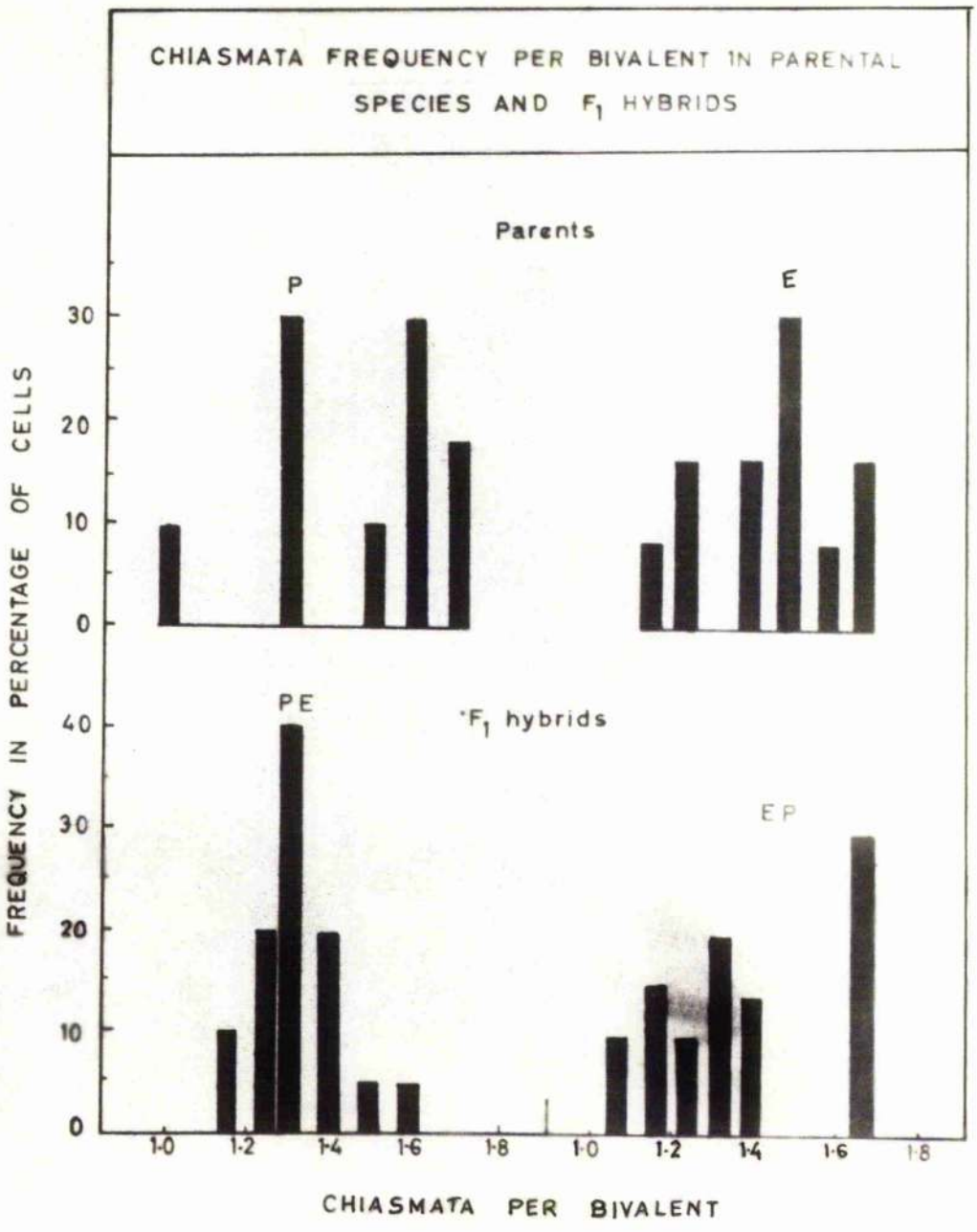
Chiasmata Frequency

The chiasmata frequency values as well as the terminalisation coefficients obtained from L.esculentum, L.pimpinellifolium and their reciprocal hybrids were tabulated and compared with those obtained by other workers (Table 9).

The value of the chiasmata frequency and coefficient of terminalisation in both parental species were very similar. The terminalisation coefficient values were found to be lower than the other workers' values. In spite of the similar degree of pairing in the two parental species studied, the hybrids showed a lower chiasma frequency than the parents. When the two reciprocal hybrids were considered, the values of chiasmata per bivalent and the terminalisation coefficients were remarkably similar. Variation of chiasmata frequency

Table 9. Mean chiasmata Frequency and Terminalisation Coefficient in species and F_1 hybrids

Species	Chiasmata frequency per bivalent	Coefficient of Terminalisation	Reference
L.esculentum	1.66	complete	Upcott 1935
"	1.30	0.87	Afify 1933
"	1.35	0.70	Brown 1949
"	1.47	0.65	Author
L.pimpinellifolium	1.49	0.60	"
EP	1.34	0.55	"
PE	1.33	0.58	"
L.racemigium	1.30	0.86	Afify 1933
L.esculentum x L.racemigium	1.30	0.86	"



Text fig: 4. Distribution of chiasmata frequency per bivalent in L.esculentum (E), L.pimpinellifolium (P) and their reciprocal hybrids EP and PE.

within the F_1 hybrids was less than within the parental species. Although the range of variation in chiasmata frequency in the hybrids was the same, the distribution within hybrids was very different (Text fig. 4). In the hybrid PE the distribution is normal but that in the reciprocal hybrid EP has a negative skewness.

It is known in rye, an inbred species, that chiasmata frequency values for the F_1s of the different inbred lines transcend those of the parental lines (Rees 1955). In the present case, the hybrids did not show any heterosis in the chiasmata frequency values but exceeded the parents in the fact that they showed less variation in the distribution of chiasmata frequency.

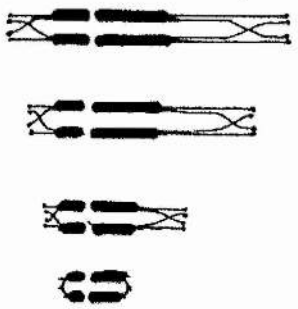
Terminalisation of Chiasmata

According to Darlington (1937), terminalisation of chiasmata in the chromosomes at metaphase is caused by the distal movement of the interstitial chiasmata. This movement is said to be caused by the increasing force of repulsion between the two separating centromeres of the paired chromosomes.

Thus it is usually in the shorter chromosomes that terminalisation is completed first.

In the tomato, chiasmata formation is localised in the achromatic regions of the differentiated prophase chromosomes as observed in the PMC divisions (cf. Brown 1949; Lesley and Lesley 1935; Barton 1951). Up to the stage of diakenesis the interstitial chiasmata could still be seen in the partly contracted achromatic regions of the chromosomes. At metaphase the achromatic material had greatly contracted so that only the deep staining regions of the chromosomes were visible. These achromatic regions contributed little to the total length of the metaphase chromosomes. Considerations of these points make it difficult to assume that in the tomato terminalisation of chiasmata resulted from the outward movement of interstitial chiasmata formed at random along the length of the chromosome. It seemed more plausible that terminalisation was brought about by the pushing in of the chiasmata along the chromosome caused by the contraction of the achromatic distal regions since the chiasmata presumably were stable in their position. Finally at metaphase when achromatic material had contracted more or less completely, the chiasmata pushed along the chromosome would be at the end of the chromatic

regions and would appear terminal. The whole process could be diagrammatically represented as in Text fig: 5.



Text fig: 5. Diagrammatic representation of assumed chiasmata terminalisation in the differentiated chromosomes of the tomato.

It could be argued that terminalisation in the usual way occurred before the complete contraction of the achromatic regions in the chromosomes. In that case there would be a stage somewhere between diakinesis and metaphase when the chromosomes still had chromatic and achromatic regions and the bivalents appeared as rings with the achromatic arms at the point of contact. Such a stage has not been come across. Ring bivalents sometimes had interstitial chiasmata and sometimes terminal but the chromosomes in the bivalents were then completely deep staining.

Afify (1933) and Brown (1949) did not observe complete terminalisation at metaphase. They observed interstitial chiasmata still at metaphase. If terminalisation of chiasmata occurred by their distal movement along the length of the chromosome terminalisation should be complete whether the achromatic regions had completely contracted or not.

There is not enough evidence at present to prove that terminalisation of chiasmata is brought about by the contraction of the achromatic regions rather than by the distal movement of the interstitial chiasmata. It would, however, be very interesting to see if the usual rule of chiasmata terminalisation is applicable to the differentiated chromosomes of the tomato

Pollen Grains

The percentage of good pollen was estimated by counting the number of pollen grains stainable in aceto-carmine.

The L.esculentum parental line had 10 per cent more abortive pollen grains than the L.pimpinellifolium species. The range of pollen viability within plants was also wider in the case of the former. The hybrids also showed a wide range of pollen fertility within themselves, with the highest value

as high as in the L.pimpinellifolium parent.

Regarding the sizes of pollen grains, as measured diameter wise, the parental strains were very much alike as will be seen in Table 10. At least 250 pollen grains were counted in each plant for assessing the mean value of good pollen grains and 20 pollen grains in each plant for the size measurements. Humphrey (1937) described differences in size between the L.esculentum and L.pimpinellifolium pollen grains being larger in the former than in the latter. This may be due to the different varieties of material being used.

Table 10. Mean diameter and percentage stainability (in aceto-carmine) of pollen grains.

	No. of plants from which the mean is taken	Pollen grain diameter (units)	S.D.	Percentage stainability of pollen grains	S.D.
L.esculentum	4	9.65	± 1.55	87.30	± 5.98
L.pimpinellifolium	4	9.40	± 1.59	97.64	± 2.09
EP	12	10.15	± 0.94	89.25	± 5.4
FE	18	10. 3	± 0.84	91.44	± 4.09

Seed set per fruit per truss.

In order to obtain some indication of the fertility of the plants, the number of seed set per fruit was studied instead of the number of fruits set per plant. The reason for doing this was because flower buds were removed from the plants for cytological purposes and therefore the number of fruits set would not be true index of fertility of the plants.

Table 11. Mean number of seed set per fruit per truss for the first three trusses

	1st Truss		2nd Truss		3rd Truss	
	No. of seeds	S.D.	No. of seeds	S.D.	No.of seeds	S.D.
E	63.7	±10.07	74.03	± 12.31	74.2	±7.87
P	42.6	± 9.19	46. 0	± 9.95	53.8	±4.95
EP	53.6	±11.65	66. 1	± 12.68	73.6	±15.96
PE	54.2	±11.65	73. 3	± 12.46	77.1	±10.75

In P and E each mean was based on 4 plants. In PE and EP each mean was based on 16 and 12 plants respectively.

The L.esculentum parent was seen to possess the capacity to produce greater number of seeds per fruit than L.pimpinellifolium and the reciprocal hybrids were almost the same in the production of seeds per fruit. It was noted

that although L.pimpinellifolium had higher good pollen grain percentage than L.esculentum it had a smaller number of seeds per fruit. The fruits of L. pimpinellifolium were much smaller than those of L.esculentum and presumably the size of fruit determined the number of ovules contained in it. Hence the smaller number of seed set per fruit in the smaller fruited L.pimpinellifolium.

Seed set per fruit within trusses inter- and intra-plants however, were not significantly different.

COLCHICINE INDUCED POLYPLOIDS

The effects of the treatment were first observed in the very early stages of germination when the hypocotyl became swollen and stunted. The cotyledons were also affected. Right up to the stage when they dropped off, except in 3 plants to be mentioned later, they never enlarged beyond 1cm in length but remained as thin narrow ribbons. The swollen hypocotyl rotted away when the seedlings were transplanted. Later roots grew from the region immediately below the cotyledons. The first one or two true leaves were simple leaves and were distorted.

Of the 70 per cent of hybrid seedlings which survived the treatment all were affected. Once the seedlings were established in the soil growth was quite normal though not as speedy as the untreated normal diploids. None of the treated seedlings of L.esculentum and L.pimpinellifolium species survived.

The affected seedlings were numbered as follows: -

EP_2/C_1 - EP_2/C_7 (7 plants) for EP hybrids

PE_6/C_1 - PE_6/C_7 (7 plants) for PE hybrids

Of these 14 seedlings raised, three viz: EP_2/C_7, PE_6/C_4 and PE_6/C_6 went blind in growth after the emergence of 1 or 2 true leaves. Only the cotyledons enlarged, became succulent and the axial growth ceased totally. After a few weeks they died. It is now regretted that their somatic chromosome numbers had not been examined. It might have provided information as to whether this blind effect was just a result of colchicine treatment or the effect of too high a ploidy level.

Morphological Characters

In the early stages of growth, leaves were thicker and coarser in texture and darker green in colour than in the diploid hybrids. The cotyledons remained as they first emerged from the seed coat. Internodes were shorter and the stem thicker. The appearance on the whole was much more vigorous than the diploids, though much slower in growth than the latter. Between the two reciprocal hybrids of EP and PE, the latter grew much slower than the former.

There were three plants, EP_2/C_4, PE_6/C_1 and PE_6/C_3 which looked different from the rest, and were suspected to be of a higher ploidy than 4x. Chromosome counts proved them to be octoploids.

Allotetraploids or Amphidiploids

Three grades of modification in morphological characteristics were discernable among the eight induced tetraploids. Five plants, EP_2/C_1, EP_2/C_2, EP_2/C_3, EP_2/C_6 and PE_6/C_7 were typical of all tetraploids, showing vigour in habit, stems, leaves and flowers. All tended to have leaves similar to L.esculentum while occasional pimpinellifolium-like leaves appeared in some plants, especially later in the life of the plants. Stems, petioles and leaves were mostly covered with short glandular hairs, and slender trichomes isolated here and there. The whole plant body, as a result, was sticky to the touch.

Two other plants viz: EP_2/C_5 and PE_6/C_5, had curly rugose leaves which were thickened and irregular and dark green in colour. Slender epidermal hairs were seldom seen. The leaves resembled those of octoploids rather than those of tetraploids.

Finally, there was one plant, EP_2/C_2 (Plate 9,(1)) which had esculentum-like leaves but were larger and more succulent and rugose. The whole plant was much more stunted than the other tetraploids.

Two tetraploid hybrids of different habits.

1. EP_2/C_2

2. $PE6/C_5$

(See text for an explanation)

One peculiar feature noticed in all these tetraploids was the appearance of thin, green, pointed structures on the stem. Judging by their position on the stem, which was half way up the internode and usually appearing first on the 5th or 6th internode, they were believed to be modified trusses. In most plants these structures were observed at least at the position where the first inflorescence ought to be. In some plants however, they occurred more than once. Besides these structures, normal trusses occurred which set normal fruit. In the plant EP_2/C_2 however, most inflorescences did not develop properly, and those few which did develop to maturity merely dropped off from the joint of the pedicel without setting fruit.

Crossability with Diploids

When selfed, the tetraploids readily set fruit containing viable seeds. Artificial crosses between diploids and tetraploids however were very difficult. Thirty three crosses, diploid x tetraploid, were made out of which 24 were successful to the point of setting fruit. Ten reciprocal crosses were also made and all set fruit. With the exception of 5 of the total of 43, none of the fruits contained seeds.

These 5 fruits, 3 from the tetraploid x diploid and 2 from the diploid x tetraploid crosses contained one well formed seed each. It has not been possible to see if these seeds were the result of actual fertilisation or parthenogenic development of the embryo. The diploid plants used for crossing were either parental or F_1 sibs.

The sizes of fruit thus developed were of the average size of fruits borne on the pistillate parents, e.g. tetraploid x diploid fruits were no different in size from the tetraploid fruits and diploid x tetraploid fruits the same as diploid fruits.

Difficulty in effecting fertilisation between diploids and tetraploids in tomatoes had been encountered by earlier workers as well. The degree of difficulty ranged from complete sterility in reciprocal crosses (Lindstrom and Humphrey 1933) to partial sterility (Jørgensen 1928, Lesley and Lesley 1928, 1930). In the latter case, though fruits set readily, only very occasional visble seeds were contained in them. Lesley and Lesley (1930) were able to obtain a triploid from such a cross. Jørgensen observed similar results in Solanum 4x X 2x crosses. Upcott (1939) was able to get a few plants raised from the seeds obtained by crossing tetraploid Primula kewensis with diploid P.floribunda These plants however were all

tetraploids and no triploids occurred. This she attributed to the action of unreduced gametes.

In the presence case it is quite obvious that the pollen, though not effective in fertilising the ovules, at least stimulates the carpellary wall to swell. Kostoff (1939) observed similar phenomenon in his F_1 hybrids of Nicotiana glauca x N.Langsdorffii. An amphidiploid was produced from a backcross of this F_1 species hybrids to N.Langsdorffii which he attributed to parthenogenic development of an egg cell in which reduction division failed to occur, stimulated by the N.Langsdorffii pollen tube. Watkins (1932) after studying crosses between Triticum vulgare (6x) x T.turgidum (4x) discussed the relations between endosperm, embryo and mother plant tissue as well as between pollen tube and stylar tissue which might all affect the sterility and inviability of the hybrids. Brink and Cooper (1947) also extensively reviewed various degrees of abnormalities in embryo and endosperm development following incompatible crosses in several genera.

The present failure of seed development in diploid x tetraploid and reciprocal crosses is likely to be the result of either inhibition of pollen tube growth in the stylar tissue or the failure of fusion of gametes.

MEIOSIS IN TETRAPLOIDS

Meiosis or reduction division was studied in the PMCs of the following plants - EP_2/C_1, EP_2/C_3, EP_2/C_5, $EP_2/C6$ and PE_6/C_5. As the process of division in these plants was more or less the same, the description given below covers all the plants concerned.

From prezygotene stages little could be interpreted about the behaviour of the chromosomes. By zygotene individual strands of chromosome could be made out and at synapsis two by two pairing was observed. Parasynaptic pairing of 4 chromosomes at one time was hardly seen. By pachytene, the parasynaptic pairing of two strands at a time was still maintained. Sometimes the chromosomes were lying side by side but with no association of any kind between them, and very often the two paired chromosomes were well separated. But this did not mean that no associations existed at all between any two paired pachytene chromosomes. / Secondary Chromosome associations of the following kind were encountered in several cells at pachytene.

(a) The two ~~paired~~ pairs of pachytene chromosomes lay side by side with only the telomeres attached. This occurred quite frequently.

(b) Extremities of the chromosome length, i.e. the achromatic regions were attached but the middle portion free.

(c) All the 4 chromosomes ~~strands~~ were attached at the centromere but the arms were free and paired two by two. In about 14 per cent of the cells at least one such association was seen (Plate 10, fig.1; Text fig 6, a). In some cases attachment or pairing occurred in an achromatic region other than the centromere.

(d) Pairing of the chromosomes ~~strands~~ at just one end, the remaining lengths lying free.

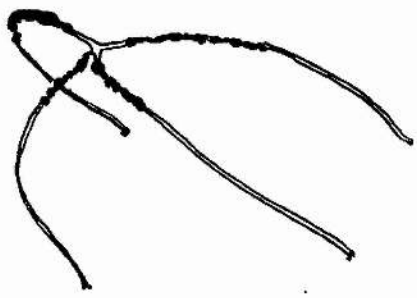
(e) Associations involving all 4 ~~strands of~~ chromosomes (Plate 10, fig: 2,3 & 4; Text fig: 6, b,c & d), but it was noted that pairing was only between two chromosomes at a time.

(f) Four chromosome ~~strand~~ association of an almost complete nature (Plate 11, fig: 1 (arrow)). This kind of association was very rarely seen and the highest number of such an association seen per cell was two.

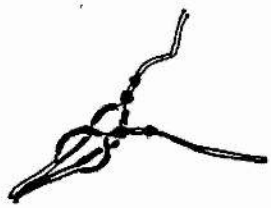
(g) Sometimes the two pachytene chromosomes were intertwined.

Very seldom incomplete pairing was observed between the two chromosomes of a pachytene pair.

Four chromosomes were associated with the tetraploid nucleolus. Pollen mother cells with both one nucleolus and two nucleoli were observed, the former being most common; when



a



b

c

d

Text fig: 6. Photodrawings of the same chromosomes in Plate 10.

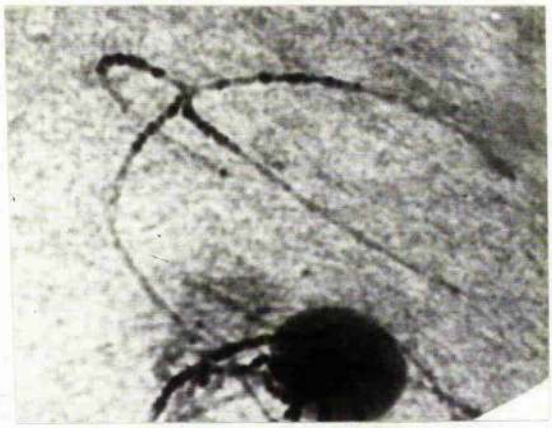
the latter occurred, it did so in isolated groups. In one exceptional case, 3 nucleoli were observed in one cell.

In diplotene, multivalent associations of chromosomes were apparent. The highest number of chromosomes involved in an association was four and the number of quadrivalents per cell ranged from 5 to 12, the mode being 8. In only one cell was a hexavalent seen. These chromosome associations were in chains or rings. (Plate 10, figs: 2,3,& 4). Trivalents and univalents were also seen.

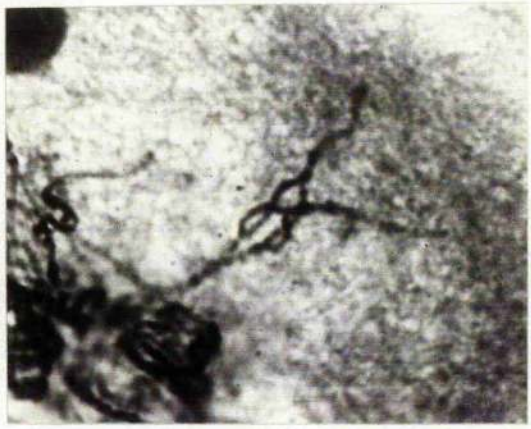
Following diplotene, there was diakinesis where quadrivalents were most distinct. At this stage the highest numbers of such associations observed was 8 and they occurred in about 5 per cent of the cells. The quadrivalents still maintained their ring or chain associations, the latter being more frequent.

At metaphase 1, univalents, bivalents, trivalents and tetravalents were seen in all cells, the number of univalents varying from one to four. It was not possible to make out in all the cells, whether the number of univalents corresponded to the number of trivalents in a cell, due to the overlapping of chromosomes in a plate. But in those cells where the chromosomes were well spread out the number of univalents and trivalents corresponded well.

PLATE 10



1



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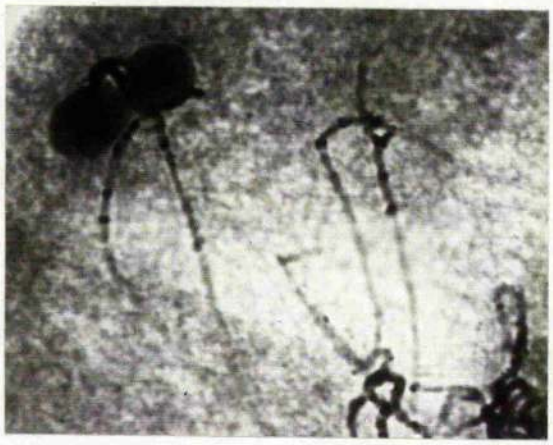

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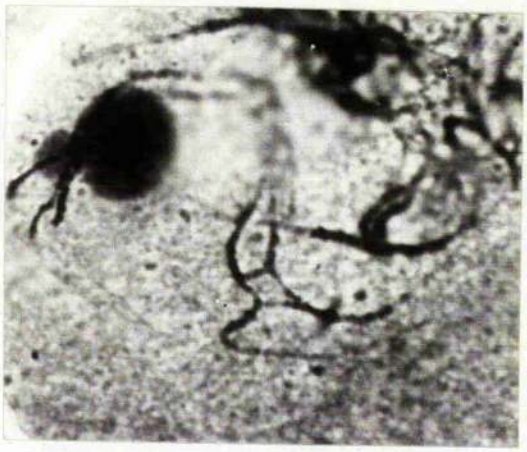
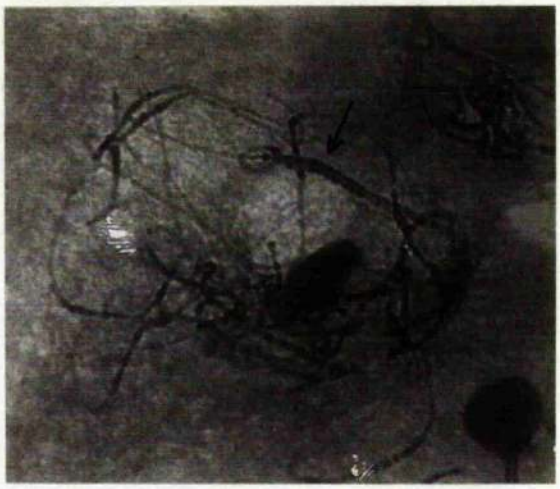


4

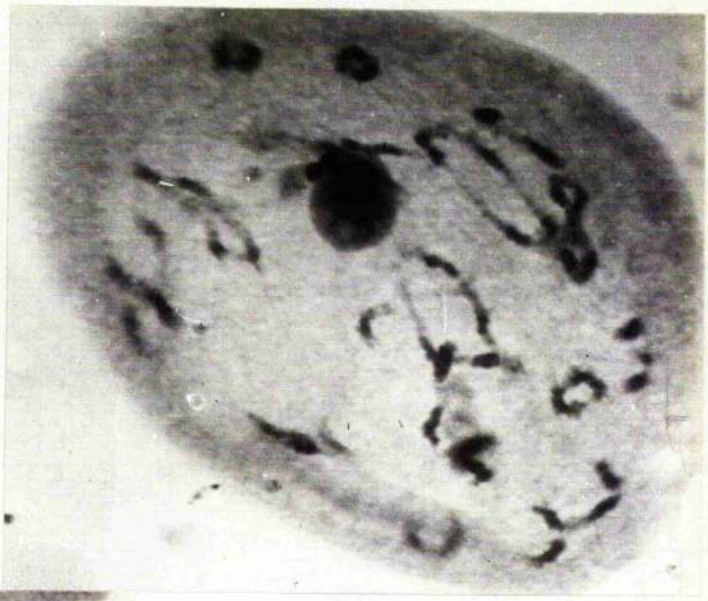
Pachytene chromosomes associations in amphidiploids.

(All fig:s - x1920)

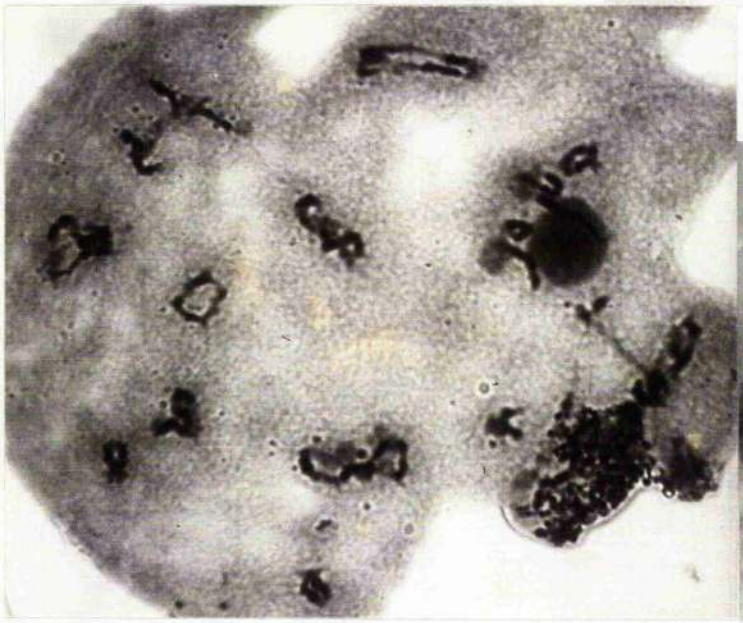
PLATE 11



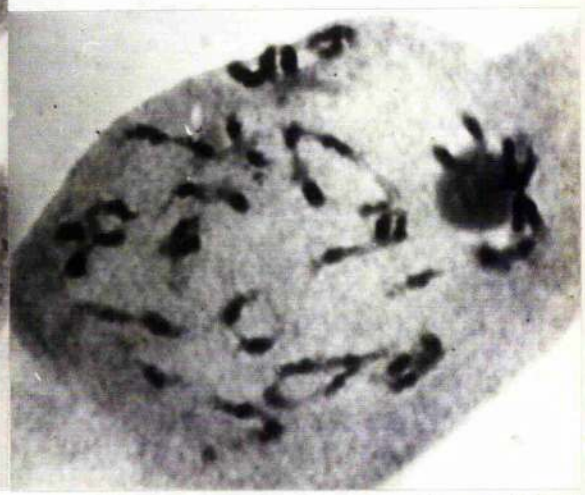
1 (x 1400)



2 (x ca.1200)



3 (x 1200)



4 (x ca.1200)

Meiosis in tetraploid F1 hybrids. Fig: 1, pachytene stage showing one 4 - strand association of chromosomes(arrow); fig:s 2, 3, and 4, early diakinesis stages showing bi -, tri -, and quadrivalent associations of the chromosomes.

At this stage, the highest number of quadrivalents per cell was 3, and the majority of the cells had either a quadrivalent or none, besides bivalents. Text fig: 7, (1) shows a metaphase plate with three quadrivalents. One or two precocious pairs were always away from the equatorial plate at early anaphase.

At anaphase I, the majority of cells showed regular disjunction i.e. uniform distribution of 24 chromosomes towards each pole. Occasional disjunction of 25 and 23 chromosomes was also observed, while in some cells a few chromosomes were left outside the spindle scattered in the cytoplasm. These chromosomes could not possibly reach the poles in time for the formation of interphase nuclei but even if they had the chances of forming interphase nuclei each containing 24 chromosomes were remote. Interphase followed the first anaphase rapidly.

In the second metaphase stage 2 equatorial plates were formed normally although the number of chromosomes at each plate was not always 24 as it should be. Table 12 shows the distribution of chromosomes on each plate at Metaphase II. Over 30 per cent of the cells at this stage showed one or more chromosomes orientated away from one or both of the metaphase

plates. Occasionally only a single chromatid was scattered while its sister chromatid was normally included in the metaphase plate.

Table 12. Chromosome distribution at Metaphase II in amphidiploids.

Chromosome distribution at		No: of cells observed	Percentage of Total
1st equatorial plate	2nd equatorial plate		
24	24	28	58.3
25	23	2	4.17
26	22	3	6.25
24	23 + (1)	7	14.58
24	$22\frac{1}{2}$ + $(\frac{1}{2})$	1	2.08
23 + (1)	23 + (1)	3	6.25
22 + (2)	22 + (2)	1	2.08
22 + (2)	$23\frac{1}{2}$ + $(\frac{1}{2})$	1	2.08
23 + (1)	19 + (5)	2	4.16

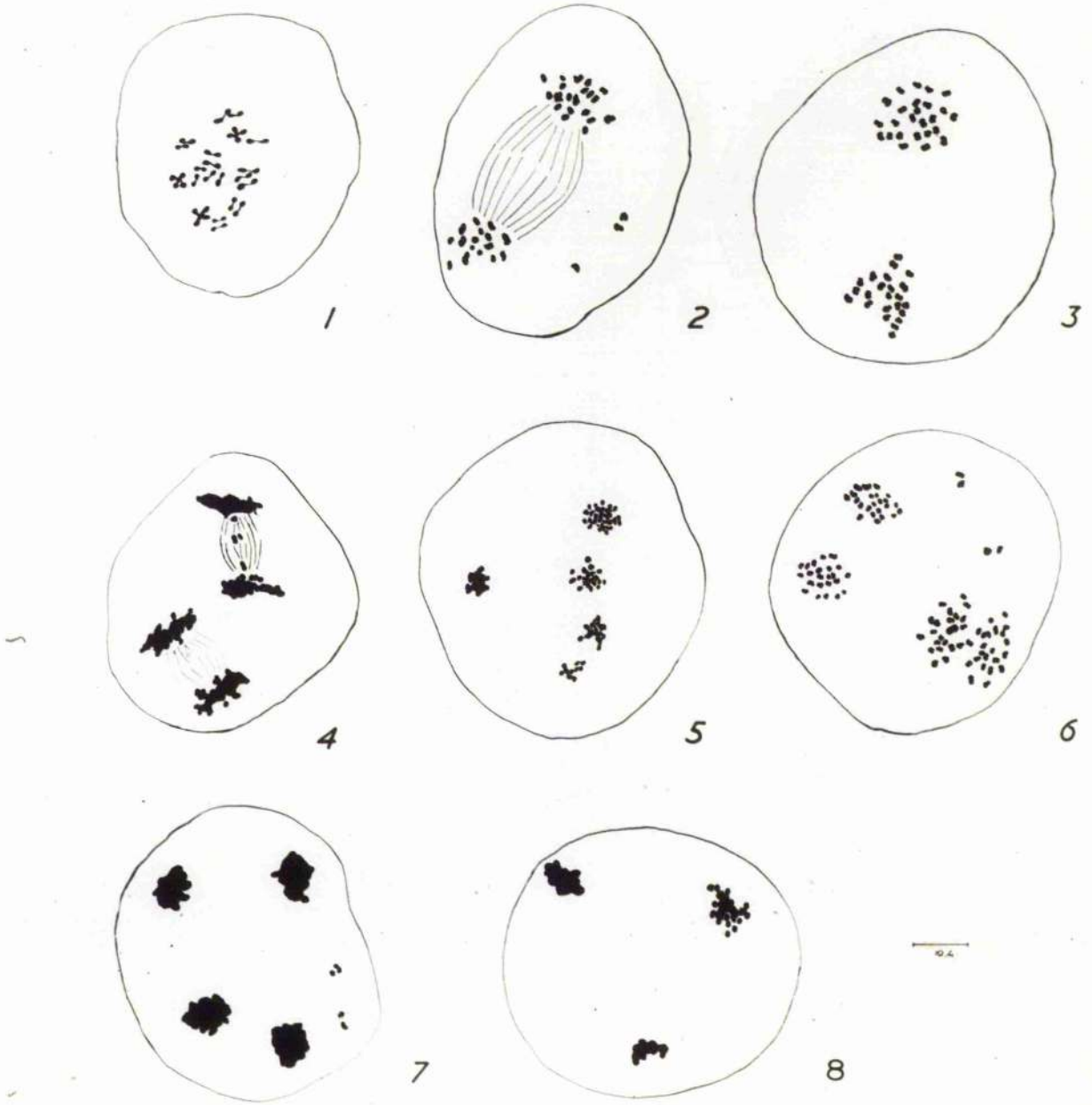
N.B. The numbers in brackets showed the number of chromosomes away from the plate, either scattered or in a group.

Five chromosomes was the largest number of deviations observed from the normal distribution. Text Fig: 7, (2 - 8) showed the various kinds of abnormalities observed from the stages of first anaphase to telophase.

Normally at 2nd metaphase, the scattered chromosomes were on the spindle but outside the equatorial plate, whereas in anaphase I and II they were found lagging on the spindle between the separating groups. Quite often they lay away altogether from the spindle and/were scattered in the cytoplasm.

After the irregular distribution of chromosomes at metaphase II, abnormalities were observed as expected at second anaphase disjunction. 56.9 per cent of the cells showed normal disjunction, which was just 1.4 per cent short of the normal equatorial plates formed at metaphase II. But this difference was not significant in view of the number of cells examined. Table 13 shows the chromosome distributions at second anaphase of the amphidiploids.

Besides cells with normal chromosome distribution, there were cells where chromosomes separated normally as far as the number was concerned but at one pole only. Failure of dusjunction at one plate or both plates were also seen and in the latter case, the chromosomes were greatly scattered



Text fig: 7. Meiosis in tetraploid hybrids: - 1. Normal metaphase plate with 3 quadrivalents; 2. Three chromosomes left outside the spindle at 1st anaphase; 3. Unequal distribution of chromosomes at 1st anaphase with 23 and 25 chromosomes at each pole; 4. Laggards at 2nd anaphase; 6 & 7. Chromosomes scattered in the cytoplasm; 5. five chromosome groups and 8. three chromosome groups at late/2nd anaphase.

in the cytoplasm. Their final fate was not known. Diads, which will be mentioned later, were the results of failure of disjunction at both metaphase plates, and triads resulted from the failure of one spindle formation or the fusion of two spindles at one pole.

Table 13. Chromosome distributions at Anaphase II in tetraploid species hybrids.

Chromosomes distribution on				No: of cells observed	Percentage of Total
1st Spindle		2nd Spindle			
Pole 1	Pole 2	Pole 1	Pole 2		
24	24	24	24	29	56.86
24	24	25	23	1	1.96
24	24	23	23	1	1.96
24	24	22	22	1	1.96
22	22	22	22	1	1.96
24	24	24	22	1	1.96
24	22	22	28	1	1.96
23	23	23	23	1	1.96
24	24	23	23	1	1.96
24	24	46 + (2)		1	1.96
23	25	No spindle formation		1	1.96
48	48	Failure of 2nd disjunction		3	5.88
48	(scattered)	Failure of both disjunctions		4	7.84
24	24	Two laggards in between		1	1.96
Chromosome number in each group not countable:					
1 laggard in 1 spindle, 4 in the other				1	1.96
1 laggard in each spindle				1	1.96
2 laggards in each spindle				1	1.96
4 Telophase nuclei but 2 chromosomes scattered				1	1.96

At late telophase, although 4 microspore nuclei were normally seen, abnormalities were not infrequent. Three and two microspore nuclei, the former of varying sizes, were also seen. Sometimes although 4 nuclei were present at telophase, one of these disintegrated (Text Fig: 8, 4) failing to form a nucleus. The following types of microspores were seen at tetrad stage.

(a) Normal appearing tetrads

(b) Triads - containing three microspores nearly uniform; two normal looking microspores with one dwarf; and one normal looking and two dwarf microspores or two normal looking and one giant microspores.

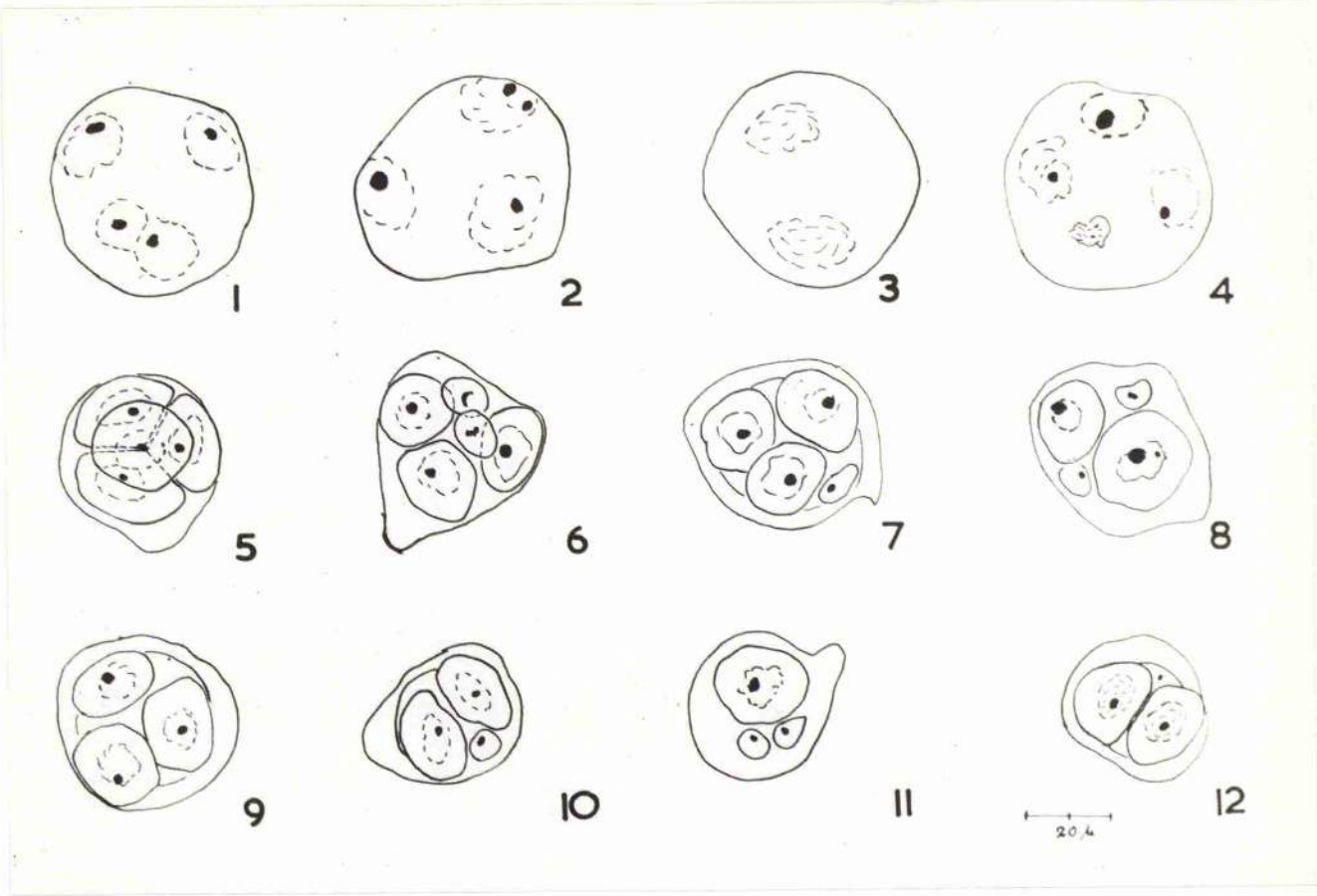
(c) Diads - usually of two equal, larger than normal microspores.

(d) Aberrant tetrads containing 3 normal looking microspores with one dwarf or two normal looking and two dwarf microspores.

(e) Pentads containing 4 normal and one dwarf microspores; 3 normal looking and 3 dwarf microspores.

Text fig: 8 shows all these different kinds of tetrad formations.

In some cells at late second anaphase, one of the 4 groups of cells at each pole divided again and this finally led to the formation of pentads containing 3 normal and 2 dwarf microspores in it.



Text fig: 8. Abnormalities in the formation of microspores in the tetraploid F_1 species hybrid.
1 & 2, fusion of two microspore nuclei which will result in the formation of triads; 3, two telophase nuclei which will result in a diad; 4, disintegration of one of the 4 microspore nuclei; 5, a normal tetrad containing four microspores; 6, 7, 8, 10 and 11, dwarf microspores in pollen mother cells; 9, a triad; 12, a diad.

These undeveloped microspores usually lacked a nucleus, though sometimes a trace of dark material in each was observed.

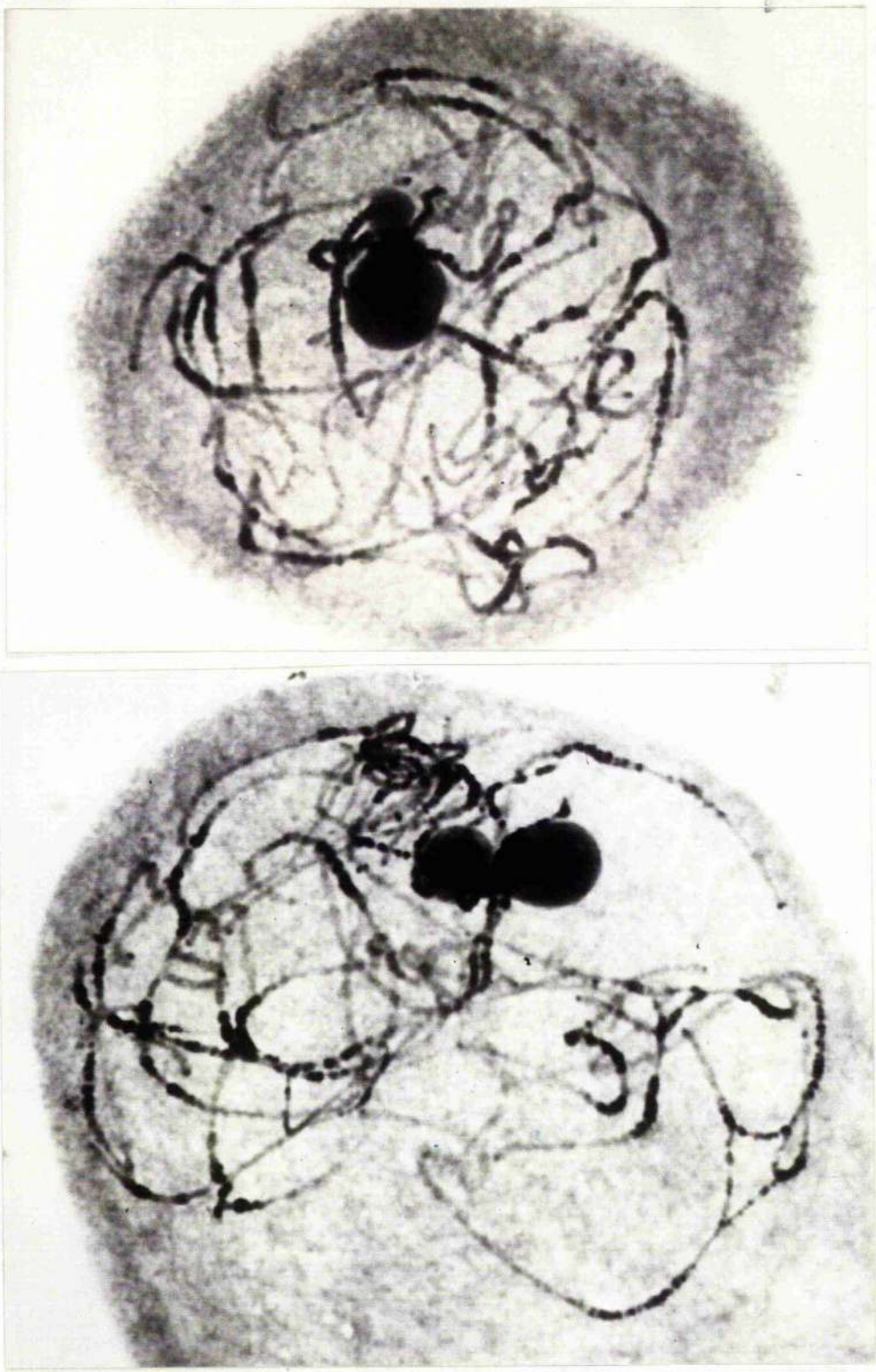
Pollen Grains

Despite the abnormalities at various stages of meiosis mentioned above, the percentage of good pollen was high, the average being 84.4 per cent. There was no doubt that these pollen grains were not all chromosomally balanced.

AUTOTETRAPLOIDS

It was desired to study meiosis in autotetraploids for comparison with meiosis in amphidiploids. Unfortunately the seeds of the parental L.esculentum and L.pimpinellifolium did not survive the colchicine treatment. By decapitation method, 2 tetraploid plants of L.esculentum variety "Kondine Red" were produced at the Scottish Horticultural Research Institute. The writer is very grateful to Dr. Haskell for letting her use some of the flower buds from these plants for cytological work. It was not possible to study meiotic stages succeeding pachytene due to scarcity of material.

In zygotene, there was two strand pairing of chromosomes and then the paired chromosomes either lay scattered about or came together to lie side by side. Very seldom 4 strand



Pachytene chromosome complements in autotetraploid, showing some associations of four chromosomes.

(Both fig:s - x1920)

pairing was seen and in such cases associations were seen to occur only in achromatic regions.

Behaviour of the pachytene chromosomes was very similar to that of the chromosomes in allotetraploids at the same stage. Twenty four chromosome pairs were seen at this stage and two pairs of homologous chromosomes with their centromeres attached were frequently seen. Four ~~strand~~ chromosome pairing was occasional, in which pairing took place between achromatic regions as well as at the centromere but not including the chromatic regions. The highest number of 4 strand association seen in each cell was 2, but such pairing throughout the length of the chromosomes was never observed.

Plate 12 shows two PMCs of the autotetraploid at pachytene stage of reduction division.

OCTOPLOIDS

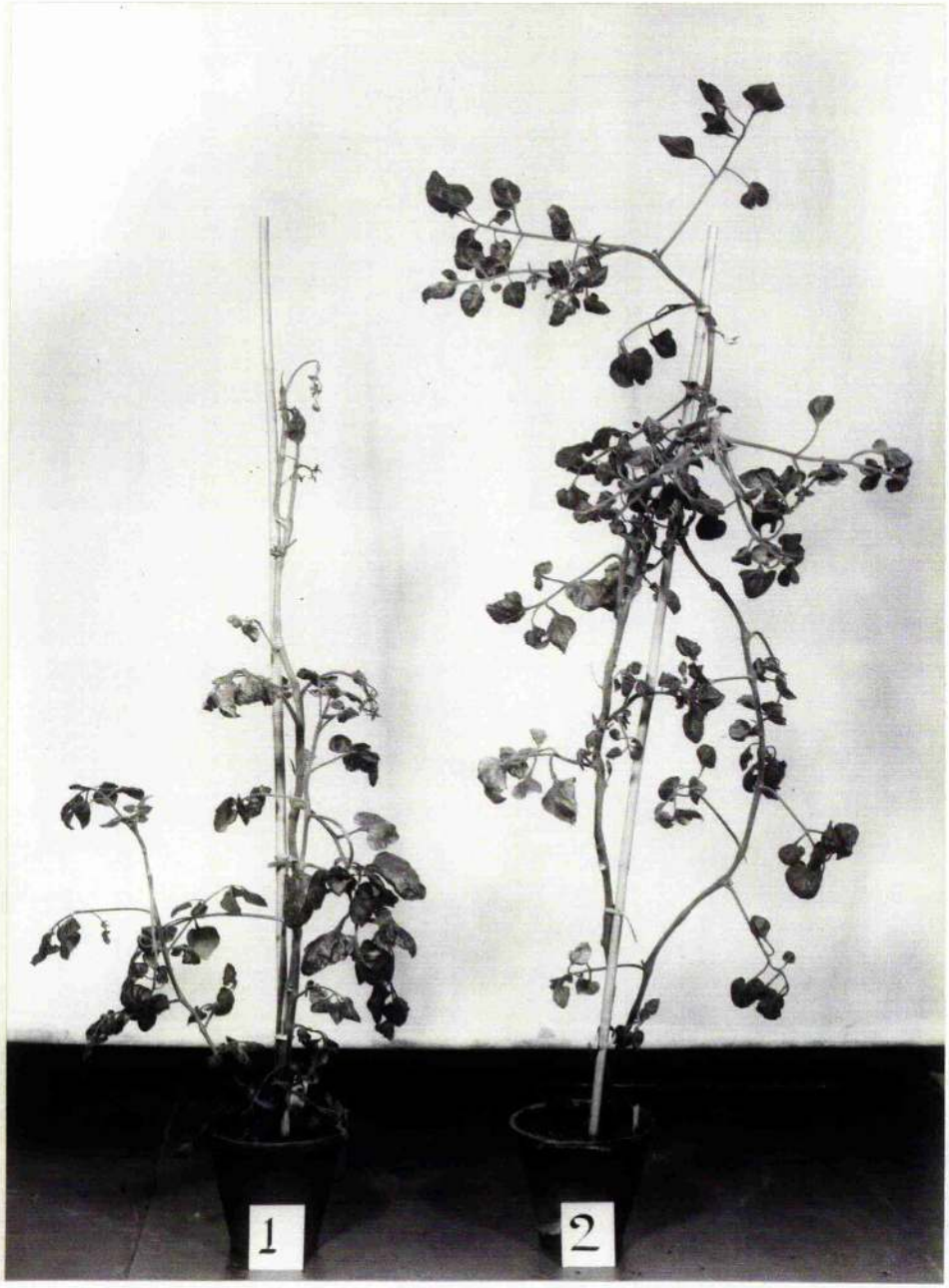
Origin

Among the colchicine induced polyploids of tomato species hybrids, 3 were found to be octoploids. The remaining 8 plants were tetraploids. These 3 octoploids were EP_2/O_4, PE_6/C_1 and PE_6/C_3.

Some morphological changes accompanied the increase in chromosome number of the plants. Leaves were the most affected part of the plant. They were rugose, thickened and brittle and the growth of the whole plant was grotesque. In one plant PE_6/C_3 (Nil in Plate B) the axial growth was terminated by a leaf. As in the tetraploid plants, along the stem where the first truss should be and in several other places above there were green structures. Plate 14 clearly shows such a structure, and on the stem and petioles the short glandular hairs are also noticeable.

EP_2/C_4 was the only plant which bore a comparatively high number of flowers. Even so the number of flowers per truss was very small as compared with the diploid and tetraploid sibs. In the other two plants, PE_6/C_1 and PE_6/C_3 not more than 10 flowers were borne on each. Sometime there was a single flower on a long, stout and brittle peduncle. The sepals were fleshy and some of the flowers on these two plants did not develop to maturity.

Three fruits were set on plants PE_6/C_3 and one on EP_2/C_4 but in both cases the fruits contained no seed at all. There were not even traces of slightly developed ovules. Apparently the carpellary wall swelled to form a fruit by the stimulation of the pollen grains on the stigma. The fruits formed did not



Octoploid Tomato species hybrids.

1. PE_6/C_3 2. PE_6/C_1

Modified trusses on an octoploid Tomato plant.
Note the glandular hairs on the stem and petiole.

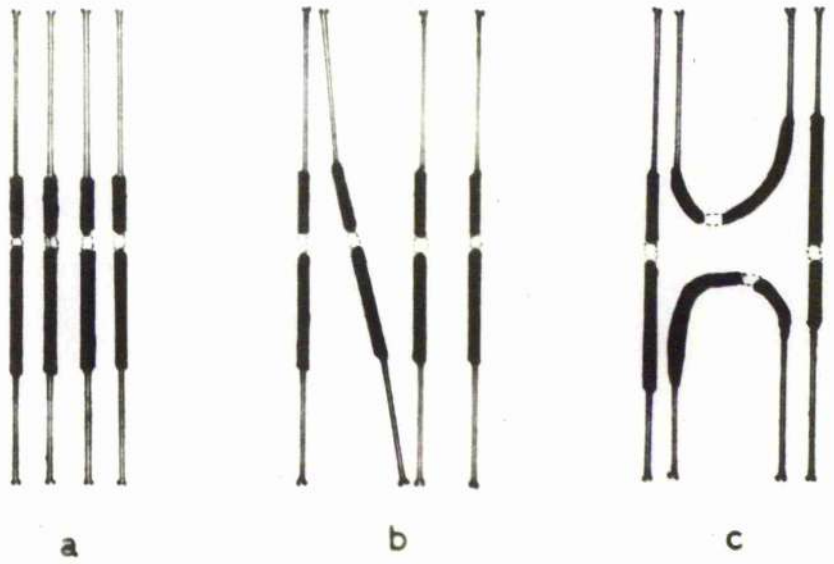
separate easily from the joint, the sepals were stuck on to them. Plant PE_6/C_1 did not bear a single fruit.

Cytology

Due to the scarcity of material, cytological observations in the octoploid plants could not be carried on a large scale. All the post pachytene stages in PMC division were studied in plant EP_2/C_4. The few flowers borne on the other two plants could not be spared as they were required to study the fertility of the plants as well.

In pre-zygotene stages, the high number of chromatin threads in a cell made it difficult to study the exact nature of chromosome behaviour. By zygotene, pairing of the chromosomes was easier to follow. Parasynaptic pairing of two by two was the rule, and no 8 strand association was observed (Plate 15 fig: 1) Two nucleoli, usually apart, were normally seen in the cells. In pachytene, 2 chromosome strand pairing was still prominent, although secondary pairing of the 4 paired pachytene chromosomes was sometime seen. The accompanying figure (Text Fig: 9) is the diagrammatic representation of 3 types of chromosome associations seen.

(a) The 4 paired chromosomes lay side by side or lay scattered with no apparent association between any two.



Text fig: 9. Diagrammatic representation of pachytene chromosome pairing in octoploid F_1 hybrid.

(b) Association of 3 paired chromosomes while the fourth pair remained free.

(c) An association where all the 4 pairs were involved.

At metaphase, bivalent associations were most frequently seen. In one case 48 bivalents were seen in a cell. Unfortunately there was not enough material to make detailed analysis of chromosome associations at metaphase.

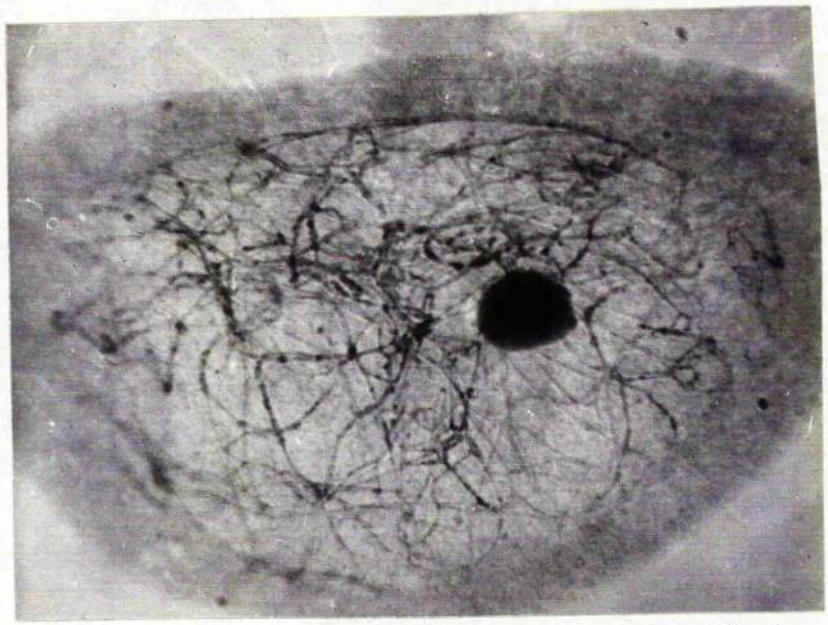
In anaphase of the first division, considerably high percentage of irregularities were seen at disjunction. In 68 per cent of the cells chromosome distribution was normal i.e. 48 chromosomes travelled towards each pole on the spindle. Distribution of chromosomes at each pole is seen in Table 14.

Table 14. Chromosome distribution at Anaphase 1 in Octoploids

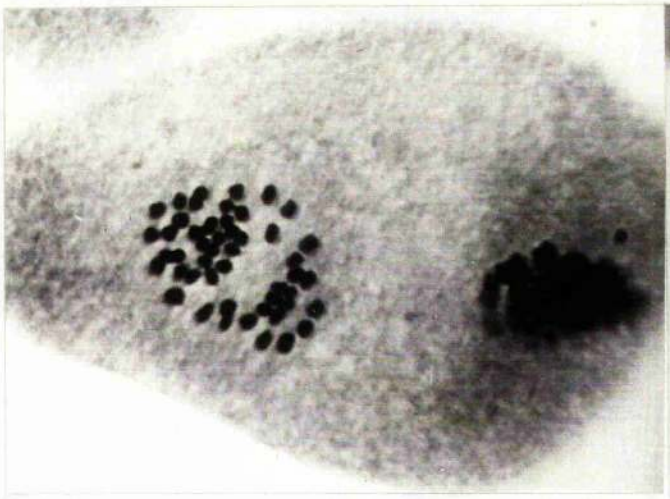
Chromosome distribution at		No. of cells observed	Deviation from normal	
Pole 1	Pole 2		Pole 1	Pole 2
48	48	13	-	-
49	47	2	+1	- 1
50	46	1	+2	- 2
51	45	1	+3	- 3
49½	46½	1	+ 1½	- 1½
54	42	1	+6	- 6

Plate 15, fig: 2 shows 49 chromosomes at 1 pole of the spindle at late anaphase of the first division.

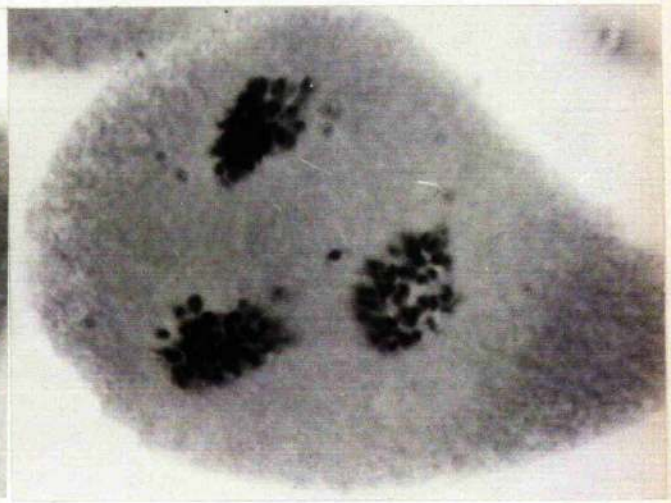
Anaphase of the second division was highly irregular not only in the distribution of chromosomes to each pole but also in the number of spindles formed. As many as 4 spindles per cell were observed. The 4 spindles may or may not be at the same stage of division. Plate 16, fig: 2 is a cell with 4 spindles at the same stage of division; the number of chromosomes at the two poles of one spindle (extreme left in the picture) seemed to be higher than any of the other 6 groups. The beginnings of cytokinesis is also noticeable between the same two groups at this end so that it seems as if the end portion of the cell would be severed. Several cells were seen which were much smaller in size than the other PMCs in the same anther. These small cells contained as little as 12 chromosomes in each and they also occurred in the same anther as where these 4 spindled - cells were seen. It is suggested that these small cells were formed when part of the large cells were cut off by the formation of the cell wall. Plate 16, fig: 1, is another cell with 4 spindles but the chromosomes on each spindle are not at the same stage of division. In one end of the cell, the chromosomes on the 2 spindles are at late



1 (x 1370)

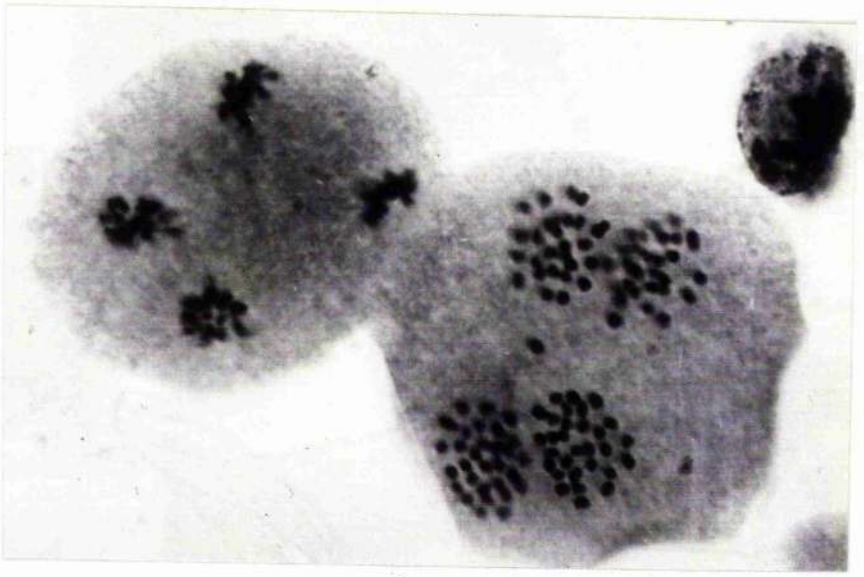


2 (x 1600)



3 (x 1600)

Meiosis in octoploid F_1 hybrids: Fig: 1, zygotene; fig: 2,unequal distribution of chromosomes at anaphase 1 showing 49 chromosomes at one pole; fig: 3, tri-polar spindle with several chromosomes scattered in the cytoplasm.



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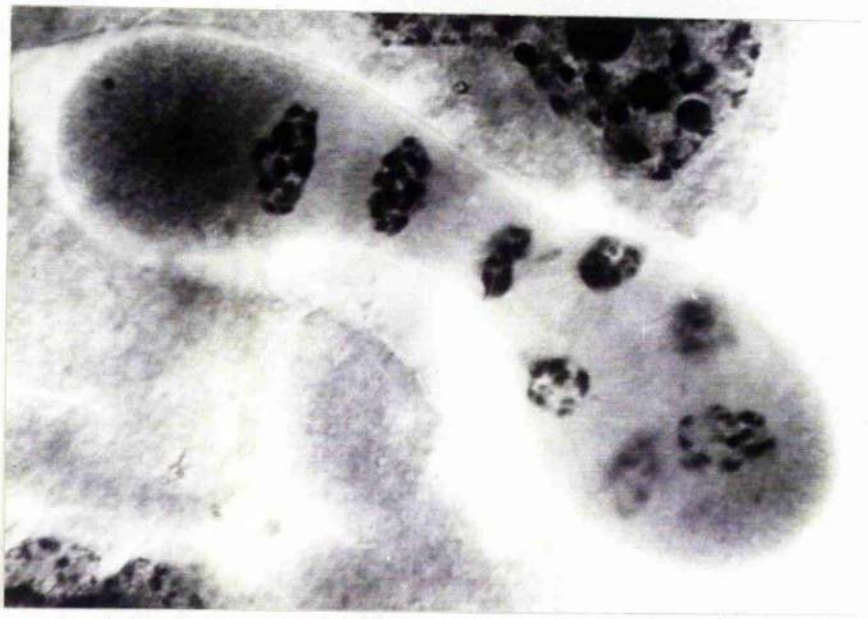
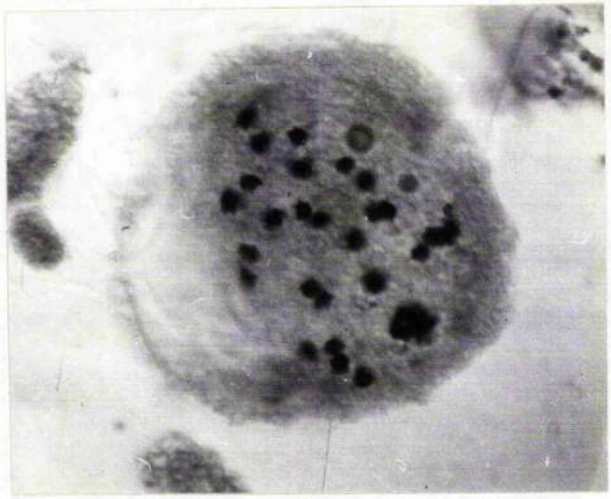


2 (x 1600)

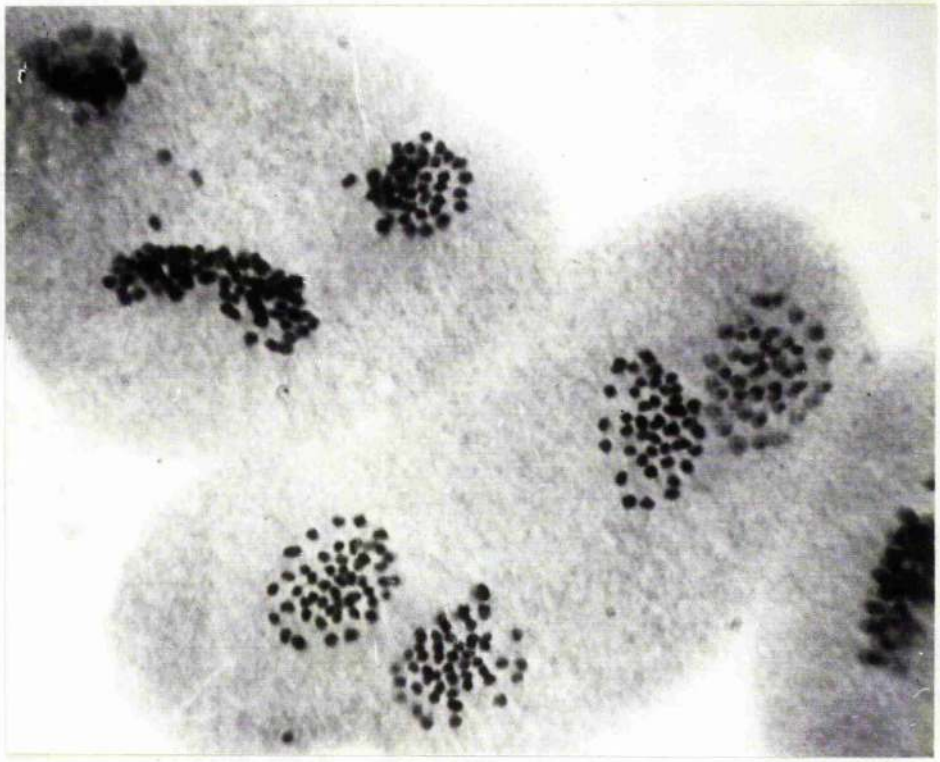
Pollen mother cells with 4 spindles in each.

Fig:1, 2 spindles at anaphase and 2 at telophase

Fig:2, all the nuclei at the same stage of division

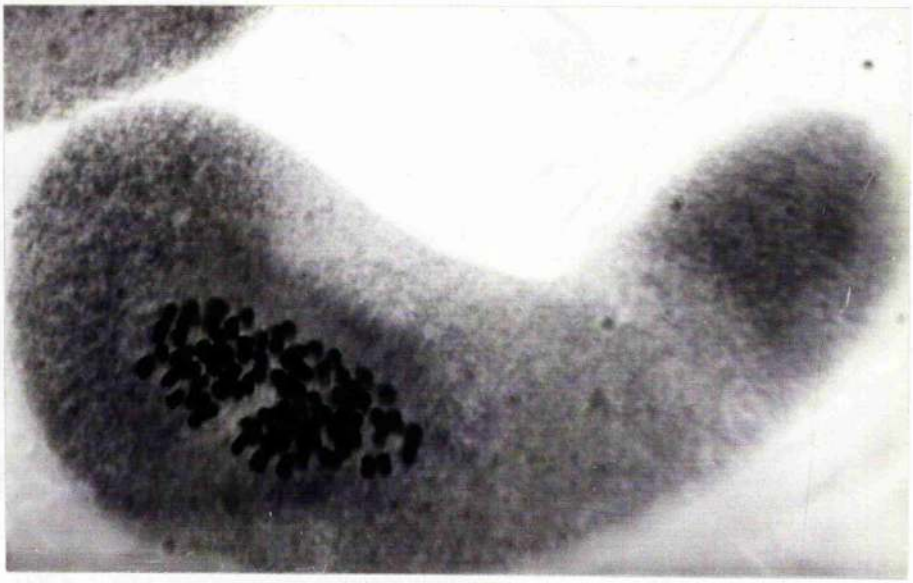


1

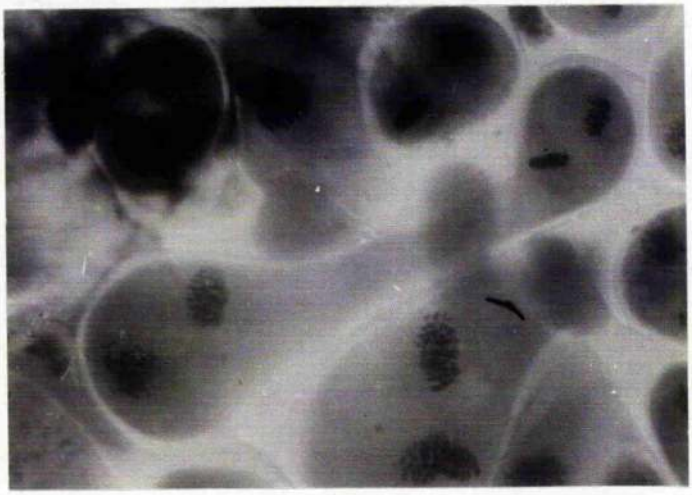


2

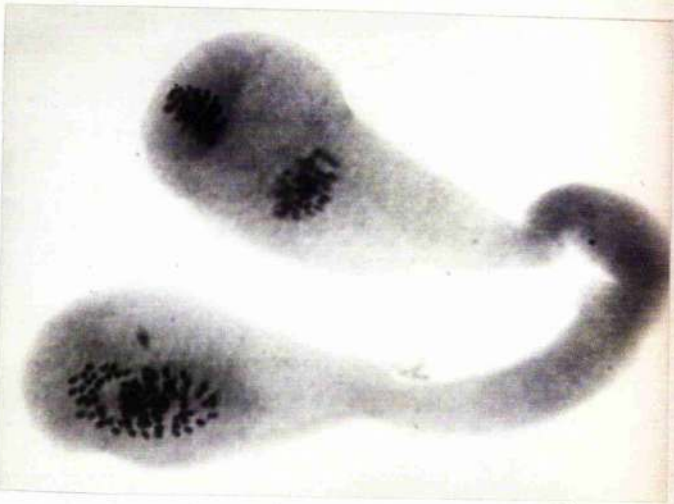
Fig: 1, pollen grain mitosis in octoploid hybrid showing 28 chromosomes (the group at 5 o'clock consists of 3): fig: 2, second anaphase, one plate is normal but there is fusion of the two groups of chromosomes at one end in another plate. (Both figs - x1600).



1 (x1600)



2 (x320)



3 (x 900)

Meiosis in octoploid F_1 species hybrid: 1 - a pollen mother cell in which disjunction has failed at both 1st and 2nd anaphases, 2- a dumb-bell shaped cell with two metaphase plates at each end, 3 - the same as in 2, but there is failure of disjunction at one end of the cell.

anaphase stage and at the other end the chromosomes on the other 2 spindles are at telophase. The number of chromosomes at each spindle is obviously not uniform.

Besides the cells with 4 spindles, cells with 3 spindles were also encountered,)Plate 15, fig: 3) with or without one or two chromosomes still lagging on the spindle. Plate 17, fig: 2, shows a normal second anaphase plate and another cell in which the chromosomes at the two poles of one end of the spindles were fused. Three chromosomes are seen to be still lagging on one spindle. Isolated small groups of chromosomes were often scattered in the cytoplasm at metaphase I and anaphase II.

Occasionally there was failure of disjunction at both anaphases (Plate 18, fig: 1) and a large restitution nucleus was developed in the cell. This was probably the origin of the huge cells seen among other microspores at tetrad formation.

At late telophase, just before the formation of cell walls to form tetrads, the number of nuclei seen per PMC varied from cell to cell. The range of telophase nuclei which would finally form microspores at tetrad stage, can be seen in the following table. (Table 15).

Table 15. Frequency of nuclei per cell at late Telophase stage

No: of nuclei	1	2	3	4	5	6	7	8
No: of cells (per cent)	0.47	11.32	16.5	63.92	6.13	0.47	0.47	0.71

At tetrad stage, the following analysis of microspores per cell was observed (in per cent)

Tetrad	Monad	Diad	Triad	Pentad	Hexad
76.33	0.76	0.76	3.05	15.26	3.82

The microspores in each cell were not often of uniform size. Even among tetrads, the 4 microspores were of unequal size. This was probably the consequence of unequal distribution of chromosomes at second anaphase. Diads were normally equal in size. In pentads, generally 4 microspores were equal in size but one was a dwarf with or without a nucleus in it. Sometimes all the 5 microspores were unequal.

The most striking feature of the PMC divisions in this octoploid plant was the shape of the cells. Most of them were dumb-bell shaped (Plate 18, fig: 2 & 3). It is seen that there are 2 metaphase plates at each end of the dumb-bell. It was evident from this that the cell was originally bi-nucleate, one nucleus at each end of the cell. In fig: 3 of Plate 18,

there was failure of disjunction of chromosomes at one end of the cell. In these dumb-bell shaped cells, it was not possible to trace how cell wall division occurred at tetrad formation.

Pollen Grains

These were very variable in size, and quite a large percentage was abortive. See Table 16.

Table 16. Pollen grain stainability and size.

Plant	Stainability (per cent)	Diameter (units)	S.D.
EP_2/C_4	48.58	15.3	± 1.42
PE_6/C_1	43.88	15.4	± 1.02
PE_6/C_3	49.88	14.8	± 0.95

Most pollen grains had 4 germ pores, while grains with 5 germ pores occurred at about $\frac{1}{4}$ the frequency of the 4 pored ones. The most frequently observed type was pollen grains with 3 germ pores.

In one PG mitosis, 28 chromosomes were seen (Plate 10, fig:1) Most probably many pollen grains were chromosomally unbalanced as this one was.

COMPARATIVE STUDY OF DIPLOIDS, TETRAPLOIDS AND OCTOPLOIDS OF F_1 SPECIES HYBRIDS

Morphological Characters

Typical of all polyploids, the tetraploid and octoploid F_1 species hybrids showed characters which were sturdier and more vigorous than the diploid plants. As compared with the diploids, the tetraploids had stems which were stronger with longer internodes, darker green and coarser leaves with longer petioles and fewer leaflets per leaf. The flowers were larger but fewer in number per truss. Earlier workers on tetraploid tomatoes (Jørgensen 1928; Lesley and Lesley 1930; Lindstrom and Koos 1931; Lindstrom & Humphrey 1933) had observed similar features. In Plate 19, No: 2 shows a diploid and No: 1 a tetraploid F_1 species hybrid plants.

Though the tetraploids showed structural modifications proportionate to the degree of chromosome doubling this correlation did not extend to octoploids in which the normal chromosome set had quadrupled.

The morphological features in octoploids were less vigorous and regular than in the tetraploids. Stems were

Two tomato species hybrids

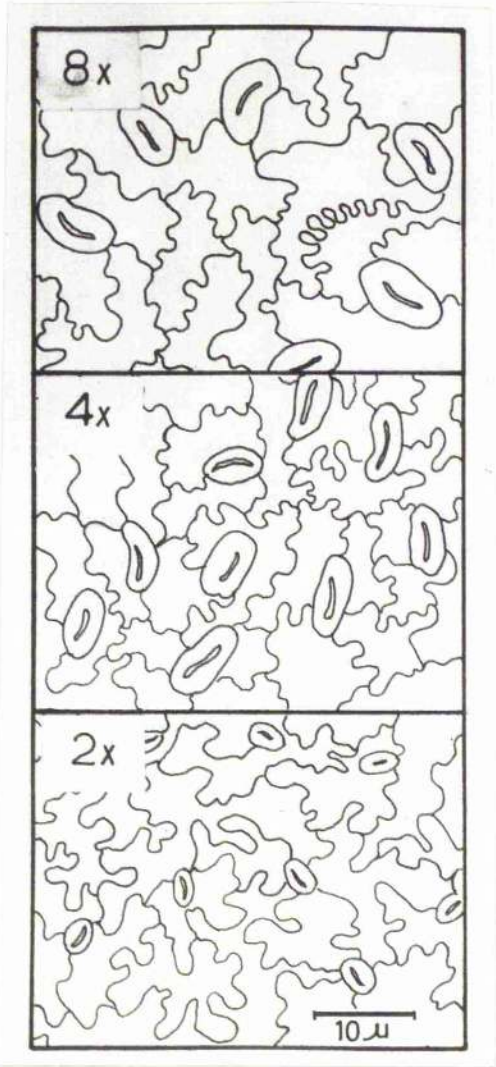
1. A tetraploid (PE_6/C_7)
2. A diploid (PE11/60)

thick and sturdy but internodes were shorter than those of the diploids. Leaves were thick, very dark green in colour and rugose with incurled margins. Petioles were longer than in the tetraploids and the whole plant tended to straggle. Growth of the whole plant was stunted and grotesque. Glandular hairs which covered the whole plant were denser in tetraploids than in diploids, but in octoploids these hairs were most prominent and intense so that the whole plant and even the fruits were sticky to the touch. Of the very few flowers borne by the octoploid plants most dropped off or dried up before fully opening.

Stomata

Though not infallible (Dermein 1940), size of stomata and guard cells has been used as a criterion of chromosome doubling. Grant (1954) and Kostoff (1938) are two of the several workers who observed this correlation between stomatal size and chromosome number in Celosia and Nicotiana respectively.

This characteristic holds true in the case of tomatoes as well. The size of stomata was proportional to the ploidy of the plant. See Table 17, and Text fig:10.



Text fig: 10. Comparative stomatal sizes in 2x, 4x and 8x tomato species hybrids.

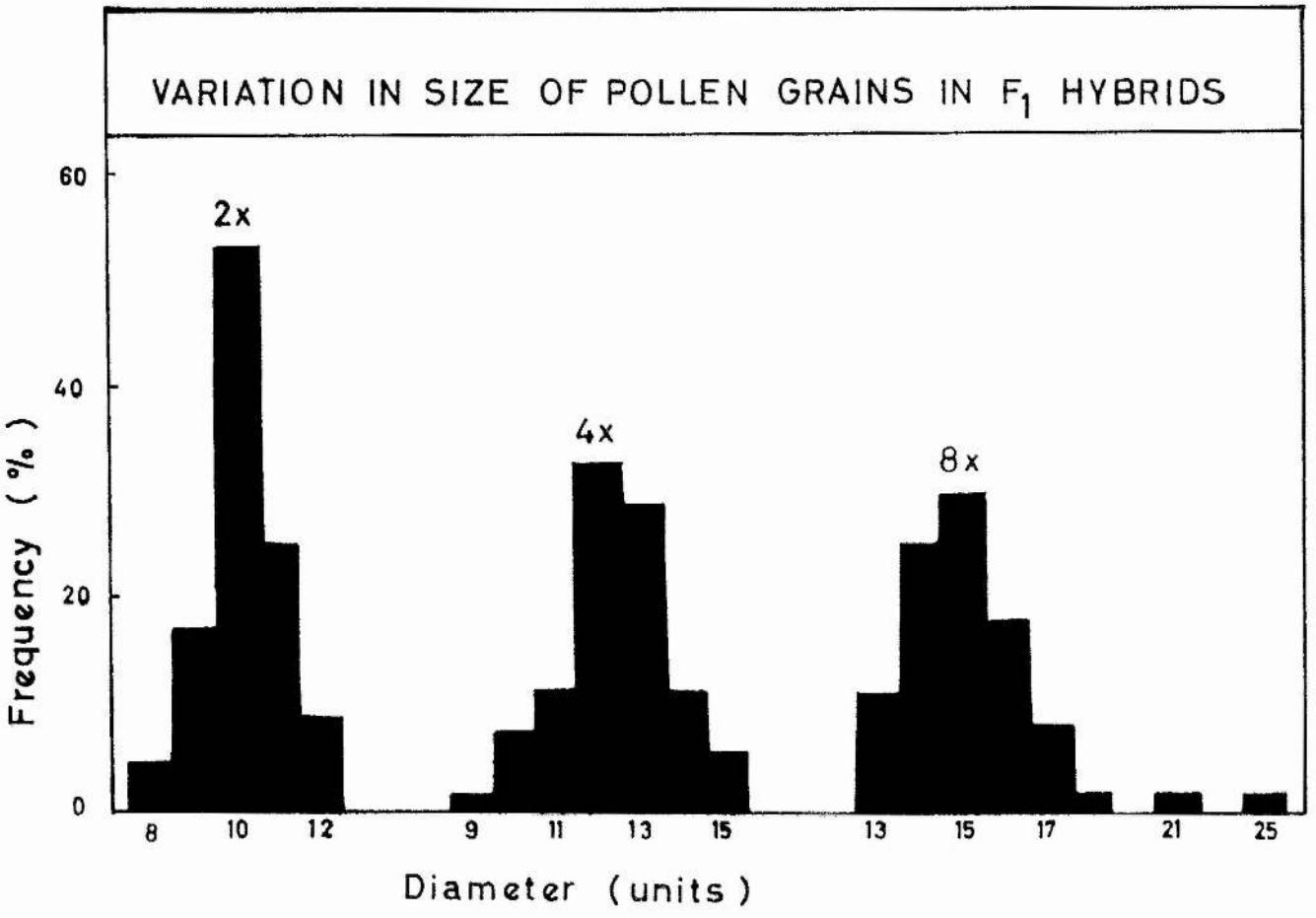
Table 17. Relation between stomatal size and Chromosome number

Ploidy of F_1 species hybrid	Mean size of Stomata (units)	S.D.
Diploid	2.5	± 0.52
Tetraploid	4.7	± 1.18
Octoploid	5.7	± 0.79

Pollen size and Viability

Likewise in stomata, pollen grain size in general was proportionate to chromosome number as well. As a criterion of chromosome doubling this method is much more certain than comparison of stomatal sizes (Goodspeed and Bradley 1942). In accordance with Humphrey (1934) and Lindstrom and Koos (1931) observations, in the present material, pollen grain size increased with the increase in chromosome number.

In the accompanying histograms, Text fig: 11, it is seen that diploids were the least variable in size with a sharp mode. Pollen grain size is most variable in octoploids. It is also seen that the mean size increased with the ploidy of the plant.



Textfig: 11. Frequency histograms of pollen grains in 2x, 4x and 8x tomato F_1 hybrids. Note the range of size at the different ploidy levels.

Another characteristic of the pollen grains which changed with the increase or decrease in chromosome numbers of the plant was the germ pore. Diploid pollen grains normally had 3 germ pores and tetraploid pollens 4 (cf Jørgensen 1928). The octoploids however had pollen grains which were 3, 4 and 5 germ-pored. Pollen grains with 4 germ pores were found most frequently, those with 5 pores about 25 per cent of the ones with 4 germ pores and pollen grains with 3 germ pores least frequently. Warmke and Blakeslee (1939) found similar effects on pollen grains in their colchicine induced Nicotiana tetraploids i.e. 3 germ-pores in haploid and 4 germ pores in diploid pollen grains. In addition they also found that some plants contained admixture of 1n and 2n pollen grains which they attributed to the plant being a chimera with germinal tissue being partly 2n and partly 4n. In the present octoploids three types of pollen grains were observed in the same anther viz: pollen grains with 3, 4 and 5 germ pores. The irregular disjunction of chromosomes at the homotypic division of the pollen mother cell was probably accounted for these different sized pollen grains and not necessarily due to chimera of the plant tissue.

It was also found that abortive pollen grains increased proportionately with the increase in ploidy level. Thus diploids had less than 10 per cent abortive pollen but tetraploids and octoploids had about 16 per cent and 53 per cent of abortive pollen grains respectively. Actually the percentage of good pollen in 2x and 4x hybrids was not much different from each other.

Fruit Size and Seed Set.

In accordance with previous records (Jørgensen 1928; Lindstrom and Koos 1931; Lindstrom and Humphrey 1933) generally the tetraploids fruits did not greatly exceed the diploid fruit size, though Faberge (1936) found that tetraploidy resulted in diminished fruit size. Otherwise there were no other modifications in the form of the fruit. Tetraploids set fruit readily when selfed or when crossed with either parent or with diploid sibs. But the seed set per fruit when selfed was much less than in diploid sibs, and the fruits from crosses with diploids did not contain seeds.

The fruits borne on octoploid plants were smaller in comparison with those of both the diploid and tetraploid. They were sticky being covered with glandular hairs and did not contain even traces of undeveloped ovules. The different

fruit sizes and seed sets in diploid, tetraploid and octoploid plants can be seen in Table 18.

Table 18. Fruit sizes and seed set per fruit in diploid, tetraploid and Octoploid species hybrids

Species hybrids	Mean fruit size (Diameter in mm)	S.D.	No: of seeds per fruit(mean)	S.D.
Diploid	29.6	± 2.98	65.8	± 16.2
Tetraploid	30.5	± 3.25	21.7	± 6.8
Octoploid	26.5	± 4.36	0	-

GENERAL DISCUSSION AND CONCLUSIONS

The fact that a hybrid can be produced, between L.esculentum and L.pimpinellifolium is evidence to show that the parental genomes must be somewhat similar in their genetic make up (Sax 1935). The nature of the similarity between the two species must now be considered.

The F_1 species hybrids between L.esculentum and L.pimpinellifolium studied were, in all outward appearances, very healthy and grew normally throughout their life. Their fertility was as high as, if not higher, than the parents. Several workers had previously studied hybrids in Lycopersicon, and Ashby (1937), Luckwill (1937), Whaley (1939) and Lewis (1955) observed that the hybrids showed marked size heterosis in quantitative characters. According to Ashby, the F_1 hybrids showed significant size heterosis compared with their parents in respect of wet and dry weight, height, number of leaves, leaf area and absolute assimilation rate, but did not exceed their parent in relative growth rate measured either as heightor dry weight. Whaley (1952) found that in a maize cross the hybrid growth was not greater than that of the faster growing parent, and in a tomato cross, an extensive

analysis of both morphological and physiological characteristics showed no significant difference between the inbreds and the hybrids. Luckwill (1937) found that in his hybrids (F_1) of L.esculentum and L.racemigrum (synonym of L.pimpinellifolium) hybrid seeds germinated earlier than the parents, though he pointed out that this was not general, as some other crosses showing heterosis germinated earlier than the parents. Haskell (1955) also pointed out that the relatively more rapid germination of outcrossed seedlings compared with selfed seedlings, from the same mother plant in Rubus, suggested that heterosis in the form of rapid germination rate forms a balance between heterosis and the breeding system of the species.

The reciprocal hybrids of L.esculentum and L.pimpinellifolium in the present experiment showed no hybrid vigour in any of the following characters : germination rate, cotyledon size, growth rate, height, fruit size and seed size. In all instances the hybrids were intermediate in value between the two parents. In fertility, however, the hybrids showed a higher value than that of either parent but the difference was not statistically significant. Again in respect of germination rate, the hybrids did not exceed that of the faster growing parent, although they

were as high as in the latter. One fact always stood out; with the exception of fruit size and height, that the reciprocal hybrids showed a difference between each other with a tendency towards the pistillate parent. In other words a maternal effect was evident. Lewis (1955) observed that his reciprocal hybrids of L.esculentum and L.pimpinellifolium differed in their degree of heterosis; the hybrid with L.esculentum as the female parent being more vigorous and also of higher fruit set than the reciprocal hybrid. But he did not mention of the tendency of the hybrid L.pimpinellifolium x L.esculentum towards the pistillate parent. Previous workers on crosses of L.esculentum and L.pimpinellifolium (Whaley 1939; Luckwill 1937) did not work with reciprocal hybrids. The phenomenon of maternal effect or matrocliny was also noticed in interspecific crosses of Melandrium dioicum and M.album for several characters (Baker 1950). It may be significant that these two species, as those of Lycopersicon studied here, were also clearly very closely related species.

Cytology of the F_1 species hybrids was normal and regular. Chromosome pairing at pachytene in these hybrids showed few signs whereby their hybrid nature could be detected. Pairing was complete and chromomeric pattern between the two species apparently identical. Occasionally, incomplete

synapsis of the chromosomes was observed in certain regions. The distribution of such regions was random but usually related to the chromatic regions of the chromosomes. No such incomplete synapsis was observed in the parental species and even in the hybrids they could easily escape detection. This suggests , therefore, that structural differences are present between the chromosomes of the two parental species but that these differences are too small to have any drastic effect on the pattern of pairing.

Since so far as chromosomes were concerned, the chromosome pattern in both species was apparently identical, the small regions along the chromosomes where there was incomplete synapsis, must represent regions where the structural differences were smaller than one or at most two chromomeres.

The chiasmata frequency per bivalent and the coefficient of terminalisation in both L.esculentum and L.pimpinellifolium were remarkably similar. The reciprocal hybrids were also similar between each other for chiasmata frequency and terminalisation coefficient. The hybrid values, however, were lower than those in the parents. The lower value of terminalisation coefficient was reflected in the occurrence of attenuated chromosomes at anaphase separation in meiosis of the F_1 hybrids.

The succeeding stages after pachytene in the hybrids were very regular and there was high pollen fertility and good seed set. Abnormalities of meiosis, seen in the F_1 hybrids , were not likely to be the result of hybridity since they occurred in only two plants out of thirty studied. Presumably these abnormalities were the result of some genic upset in the plants concerned.

The pachytene pairing in colchicine induced polyploids derived from F_1 hybrids is worth further consideration. From the early stages of zygotene two strand association was apparent. By pachytene, complete synapsis occurred between the two strands so that the complement contained 24 pairs of chromosomes. Secondary associations between pairs of homologous chromosomes did occur from time to time. In such configurations the paired regions were observed to be between 2 strands only in any one region. No complete four strand pairing was observed although Lindstrom & Humphrey (1933) had claimed to observe this in allotetraploid derivatives of L.esculentum x L.pimpinellifolium and Humphrey (1935) in autotetraploids of L.esculentum. Upcott's (1935) observations of autotetraploid L.esculentum were more comparable to the present case. The two strand pairing of the pachytene chromosomes in the tetraploids studied was therefore, like

the chromosome behaviour typical of ~~autopolyploids~~ allopolyploids and dissimilar from that normally found in ~~allopolyploids~~ autopolyploids.(Darlington 1937). The only regions where all the four chromosomes were associated together, in the few cases where such an association occurred at all, were at the centromeres. But it seems unlikely that the chromatids would form chiasmata in such regions (Darlington 1931). A more probable explanation is that this represents non homologous association of heterochromatic regions (White 1954). This two strand pairing still holds true for the tomato octoploids as was seen in the few pachytene complements studied. Associations of two paired chromosomes, but never more than two, were very seldom seen. It was not possible to verify whether the 4 strands in these associations were just lying side by side or whether parasynapsis i.e. parallel pairing, had occurred among the four chromosomes.

In tetraploids quadrivalents were present from diplotene till metaphase and these presumably resulted from the chromosome associations seen in pachytene where all the four strands were involved although only 2 strand pairing was observed. Multivalents, including quadrivalents and trivalents at diplotene persisted through diakenesis to metaphase, and so it was quite evident that these multivalents were held by true chiasmata. The absence of

catenations or chains of more than four chromosomes proved that no structural changes of the chromosomes took place in the hybrids. One apparent hexavalent seen in a cell at diplotene was very likely a false association between a quadrivalent and a bivalent with no actual chiasma formation.

The occurrence of quadrivalents in the tetraploid hybrids ranging in number from 5 to 12 per cell at diakensis was evidence that there was no differential affinity between the two parental sets of chromosomes, as occurs in Primula kewensis(Newton & Pellew 1929); Upcott 1939). This lack of differential affinity in Lycopersicon could account for the lower fertility of the tetraploids observed. Thus the high degree of multivalent formation at metaphase enhanced the unequal distribution of the chromosomes at disjunction and so led to the formation of unbalanced gametic nuclei (Darlington 1937). Though lower in fertility as compared with the diploids, the amphidiploids still gave a fairly high seed set.

Thus it was seen that doubling the chromosome number in tomatoes affected the plants little, apart from the slight reduction in fertility. However, the presence of four homologous sets of chromosomes seemed to produce

unbalance. The octoploids were morphologically abnormal which showed that the effect was initiated in the metabolic mechanism of the plant. Leaves, stems and flower development were abnormal, the flowers barely opened and if they did they just dropped off without setting fruit.

The physiological unbalance within the plant reflecting the genetic unbalance together with the chaotic behaviour at meiosis accounted for the sterility of these octoploid plants, where there was a total failure of seed development.

The perfectly normal development, in both vegetative and reproductive phases of the plants' life, and the high fertility of the diploid F_1 species hybrids of L.esculentum and L.pimpinellifolium indicate that the two species are very closely related. The absence of isolation barrier of any sort between these two species shows that they must be primarily differentiated by genetic factors.

The complete pairing of chromosomes in the diploids and the lack of differential pairing in the amphidiploids which in their behaviour resembled autotetraploids, suggests the homologous nature of the chromosome structure. However, the observation in the hybrids only of small regions of incomplete synapsis at pachytene pairing points to the presence of tiny structural differences between the two

parental chromosomes. Therefore, the chromosomes of L.esculentum and L.pimpinellifolium are seen not to be completely homologous.

The intermediate nature of the reciprocal hybrids with a prevalence of matrocliny suggests that the differences between the two species was genically caused. However, it is not likely to be due to major genes but rather to polygenic complexes.

VIII.

SUMMARY

1. Two species of tomatoes, L.esculentum and L.pimpinellifolium were raised in the first year and crossings between them were made. In the second year, the parental species as well as the reciprocal hybrids were raised and their morphology and cytology were studied.

2. The F_1 species hybrids showed a tendency towards the pistillate parent, at least in the cotyledon characters. In characters like germination, growth, seed size, fruit size and percentage of good pollen grains, the F_1 hybrids did not exceed the higher parent but were intermediate.

3. The pachytene chromosomes of L.pimpinellifolium were found to be smaller than the corresponding ones in L.esculentum. Chromomeric pattern between chromosomes of the two species were found to be very similar. Meiosis in L.esculentum and L.pimpinellifolium were also very similar.

4. The pachytene chromosome associations in the F_1 hybrids were very regular. With the exception of a few instances complete synapsis occurred. In some cells incomplete synapsis was observed and this was attributed to the tiny structural differences between the chromosomes of the two species. The

difference in length between the chromosomes of the two species was not noticeable in the pachytene chromosomes.

5. The F_1 hybrids, with the exception of two out of thirty plants, did not differ significantly from the parents in cytological behaviour. Irregularities observed in the two plants consisted of multivalent associations at diakenesis in one plant, and groups of chromosomes left out of the metaphase plate and failure of separation of bivalents at anaphase in the other plant. In the latter case, whole bivalents moved towards the poles. Attenuated chromosomes at anaphase were observed in most cells of the hybrids. Metaphase chromosomes did not show size differences.

6. Eight tetraploids and three octoploids were produced by colchicine treatment of the F_1 hybrid seeds. The seeds of parental species did not survive the treatment.

7. Tetrploid hybrids showed increase in vigour of plant habit. Cytology of these plants was studied with emphasis on pachytene chromosome pairing. Two strand association was the rule at this stage in both tetraploids and octoploids. Pairing of chromosomes at diplotene to metaphase did not show differential affinity. Lack of differential affinity resulted in the irregularity of chromosome distribution at anaphase and in lower fertility.

8. Pachytene chromosome pairing in amphidiploids was very similar to that in autotetraploids.

9. Morphology and cytology of octoploid F_1 hybrids was greatly affected. The plants were stunted in growth and failed to produce seeded fruits. Meiosis was very irregular, with oddly shaped PMCs, and consequent sterility of the plants.

10. Comparative study of 2x, 4x and 8x hybrids showed that there is a correlation between chromosome number and size of stomata, and size of pollen grains, percentage of abortive pollen and sterility.

11. The affinity between the two species was discussed and the following conclusions were drawn :- (a) that they were very closely related species, and (b) that the chromosomes of the two species were not completely homologous in structure. ~~and (c) that the differences between the two species were caused by polygenic complexes.~~

Part II.

This section is a record of the work done, in addition to the main study on tomatoes, during my stay at the Scottish Horticultural Research Institute, Mylnefield, Invergowrie by Dundee. It includes those hybrids in which reduction division could not be studied for the following reasons :-

(a) In *Ribes* the plants were too young and the duration of study was limited.

(b) In *Rubus* the plant concerned did not and probably would not flower at all. Its physiology was greatly upset by chromosomal unbalance.

Therefore, in both cases only the somatic chromosomes were investigated and the following results were obtained.

FIXITY OF CHROMOSOME NUMBERS IN PLANTS

It has been an accepted fact that the chromosome numbers in animals and plants are most stable at the diploid leveland are believed to be constant. However, a doubling of chromosome number or an increase of the chromosome number to a multiple of the basic number occurs quite frequently. This phenomenon of chromosome doubling is common only in the plant kingdom.

Apart from this systematic increase of the chromosome number there is another type where the chromosome number increase may not be in a euploid series. Thus plants of the same progeny may have different chromosome numbers. This often results from crosses between parents with different 2n numbers:e.g. from a sesquidiploid plant resulting from a cross of 4x L.esculentum (2n=48) x 2x L.peruvianum (2n=24), Soost (1958) obtained aneuploids ranging in chromosome number from 26 to 34. In Rubus, several plants of the progeny of crosses of Boysen x Eldorado and Young x Eldorado showed instability in chromosome number (Britton & Hull 1956). Again these were crosses involving plants with unequal 2n numbers (Young=7x, Boysen=7x and Eldorado is a colchicine

induced tetraploid). These mitotically unstable plants ranged in chromosome number from 13 to 60 in crosses of Boysen and Eldorado, and 18 to 71 in those of Young and Eldorado.

The next kind of irregularity in the increase of chromosome number in plants is where variable chromosome numbers occur even within the same plant. This kind of chromosome instability occurs either in nature or following treatment with colchicine. In Ribes, in the colchicine induced tetraploid, a great range in number of somatic chromosomes (2n=4 to 2n=32) occurred (Varaama 1949). The mode was at 2n=16, i.e. the diploid level. In asexually propagated plants mainly centred around members of the Aroideae, Liliaceae, Amaryllidaceae, and Dioscoreaceae, Sharma & Sharma (1956) observed varying somatic chromosome numbers occurring in different cells, often in cells of the same root tip. The different degrees of chromosome number variation found within stem-tips, root-tips and leaves in Hydrocotyl asiatica, Rauwolfia canescens, Cestrum diurnum, C.nocturnum, C.nocturnum var. foetidissimum and several others led Sharma and Sharma (1957) to postulate a new theory of differentiation in plant cells. They theorised that - "different organs of the body at the time of their origin receive their chromosome set responsible fot their manifestation from the heterogenous

complement of the growing apex. This heterogeneity is represented not only by varying chromosome numbers derived from the original one, but also by varying karyotypes maintained in the normal number. The complement after its initiation into a differentiated tissue behave normally.

Similar variable aneuploid numbers of chromosomes also occurred within root tip cells of Paphiopedilum wardii (Duncan 1945). Löve (1938) recorded PMCs with less than 2n chromosomes in plants derived from pentaploid wheat hybrids; but its occurrence was limited to just one anther. Sachs (1952) found "chromosome mosaics" in amphidiploids of Triticinae and Aegilops. But the mosaics were confined only to the germ line and were not observed in root tips, unlike Snoad (1955) who found varying chromosome numbers both in the pollen mother cells and the root tip cells of Hymenocallis calathium.

In some instances, the variation of chromosome number was accompanied by alteration of the karyotype (Sharma and Sharma 1956, 1957; Duncan 1945). These alterations resulted from duplication of some chromosome pairs and reduction of others. These changes were detectable because the chromosomes were recognisable by the centromere position or the presence of secondary constrictions or the satellites.

The satellite is a part of certain chromosomes to which

it is permanently attached by a tenuous thread, and that each nucleolus takes its origin in telophase from the end of the chromosome at the point where the satellite thread emerges (Gates 1942). Thus the satellite is always associated with the nucleolus. In Spinacia oleracea (n=6) the heteropycnotic satellites in resting nuclei can be used as a method of determining ploidy of the cells (Berger 1941), though Heslop-Harrison (1953) found no correlation between the number of satellites and degree of polyploidy in Rubus.

In Crepis, Navashin (1934) found that one parental species lost its satellited chromosome in the interspecific hybrid's complement. He termed this "differential amphiplasty", as only individual chromosomes of the set were affected, and the term amphiplasty was applied to all the phenomena of hybrid changes. Wilkinson (1941) also found regular amphiplasty in hybrids of Salix alba and S.fragilis viz.(a) differential amphiplasty which resulted in a loss of two satellites and (b) neutral amphiplasty which was revealed in a shortening of some of the chromosomes.

Similar phenomenon, the suppression of a satellite in the hybrid complement was observed in Ribes (Varaama 1949), and in the black currant - gooseberry hybrid (Tun 1959, Keep 1960). But Meurman (1928) saw a pair of satellited chromosomes in

the somatic complement of Ribes nigrum x Grossularia.

Hybrids of blackcurrant and gooseberry were quite frequent either as a chance occurrence or raised for experimental purposes. The common feature in all these hybrids was that they were sterile (Wilson 1900; Meurman 1928; Knight and Keep 1956-1957). Knight and Keep managed, however, to get a fertile hybrid by doubling the chromosome number in the sterile F_1 species hybrids.

MATERIALS AND METHODS

RIBES:

Two varieties of R.nigrum, viz. Baldwin and Amos Black; R.Maximowiczii; Grossularia, the gooseberry and the hybrids of Amos Black x Gooseberry and Baldwin x R.Maximowiczii were used.

RUBUS:

In Rubus, an F_2 progeny of the cross Merton Thornless x Himalaya Giant (R.procerus)was studied. The F_1 material was not available.

In both Rubus and Ribes, the following method of staining had been found to be very favourable for the study of somatic chromosomes in root tips. Vigorously growing root tips were the best materials for squashing. Root tips were pre-treated in 0.002M solution (aqueous) of oxyquinoline (cf. Tjio & Levan 1950) for 3-4 hours at 0°- 10°C; fixed in 1:3 acetic-alcohol for 12-24 hours and stored in 70% alcohol till required. In Ribes para-dichlorobenzene (saturated aqueous solution) was found to be preferable to oxyquinoline

for pre-treatment. Fixed root tips were hydrolysed in N.HCl at 60°C for 30 minutes and stained in Feulgen for at least 2 hours (Darlington & La Cour 1950). Squashing was done in carmine, with addition of a small drop of acetic acid saturated withferric acetate to the edge of the cover slip; the slide is then heated gently over a low flame as this helps to intensify the staining of the chromosomes. The cytoplasm darkens if the heating is not done immediately after introducing the iron. When conditions are optimal, very darkly stained chromosomes stand out against the almost unstained cytoplasm.

After keeping in the refrigerator for one or two days, the temporarily sealed slide preparations were made permanent in the same way as Celarier's (1956) already described in the first part of this thesis.

Photomicrographs and camera lucida drawings were made from temporary preparations.

XI.

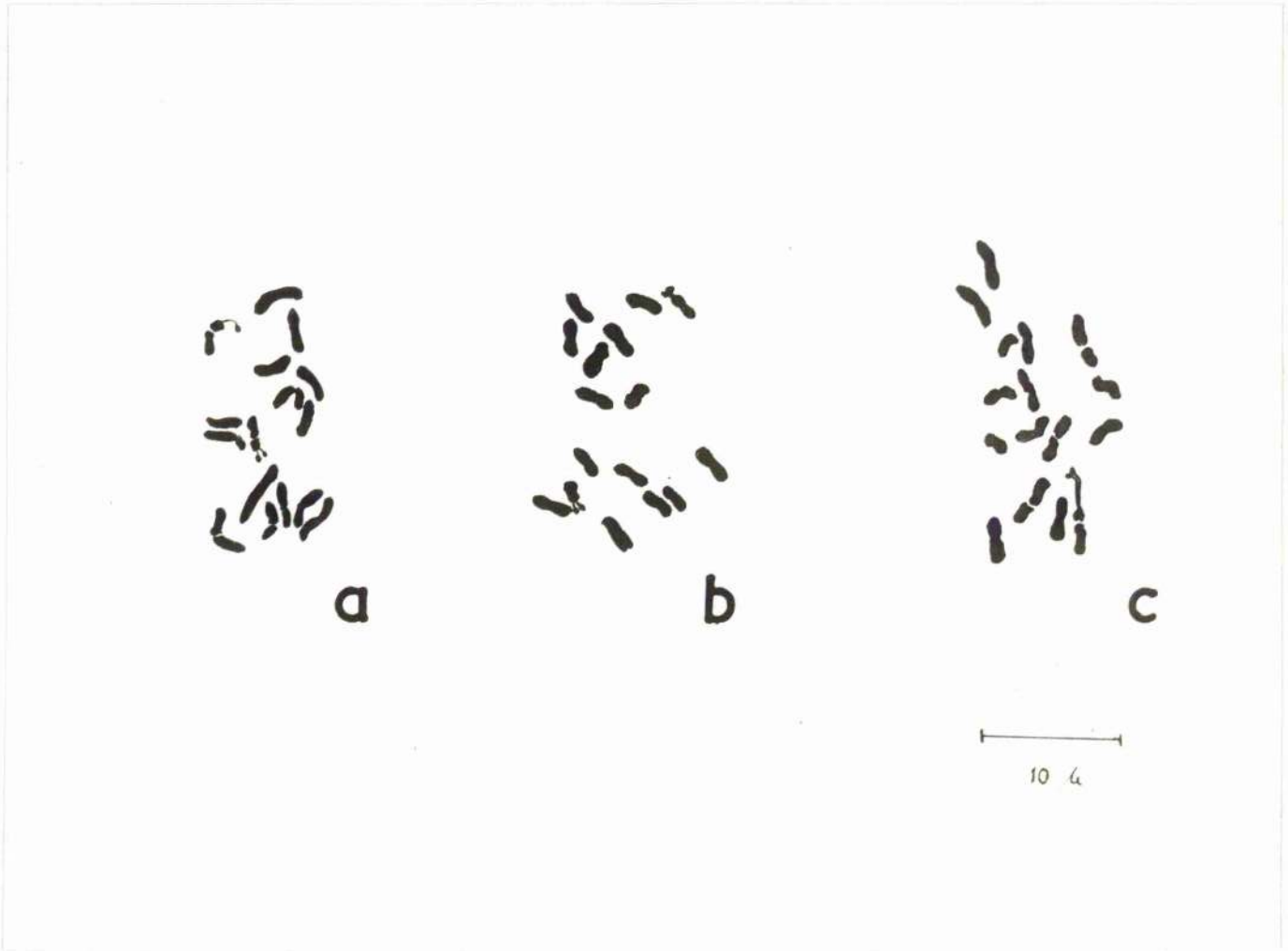
RIBES: BLACK CURRANT - GOOSEBERRY HYBRIDS

According to Berger (1924), currants and gooseberries belong to two different genera viz. Ribes and Grossularia of the family Grossulariaceae, though Janczewski (1907) treated each as a sub-genus of Ribes. Black currants are all descendants of Ribes nigrum. All cultivated varieties of gooseberries, as grown in Europe and chiefly in England are descendants of Grossularia reclinata under the sub-genus Eugrossularia.

The present cross of Amos Black (R.nigrum)and gooseberry (variety unknown) could therefore be considered as an intergeneric cross. Of the six hybrids studied, leaves of the hybrids were intermediate in shape and size. They were of finer texture than the black currant parent, and lacked the glands of the same parent which give the characteristic black currant odour, The spines of gooseberry were absent in the hybrid, and on the whole vigour of the hybrid was less than that of either parent as regards growth rate. Wilson (1900) found the same characteristics in the hybrid of R.nigrum and Grossularia.

Both parents were diploids of 2n=16 and the hybrid between the two was also a diploid of 2n=16. Apart from

the fact that the chromosome complements in both parents were symmetrical, using Stebbins(1950) terminology, the small size of the chromosomes offered little chance for the study of the karyotype. No conspicuous size differences between the two monoploid chromosome complements of the parents were observed. This is in accordance with the results of Meurman (1928), Darlington (1927) and Zeilinski (1952) who found no karyotypic differences among the somatic chromosome sets of several species of black currants and gooseberries. The 2n complement of 16 chromosomes in Amos Black comprises 14 metacentric chromosomes (i.e.chromosomes with median centromere) and a pair of satellited chromosomes with submedian centromeres (Plate 20, fig.2). As reported earlier (Tun 1959), in the hybrids, only one satellited chromosome was observed. It was assumed then that the gooseberry genome lacked the satellited chromosome. But later examination of root tips squashes of the gooseberry showed the presence of a pair of satellites in the complement. This finding of the presence of only one satellited chromosome in the hybrids complement of black currant and gooseberry has/been recently confirmed by the report of Keep (1960). Text fig: 12 shows the camera lucida drawings of the chromosome complements from Amos Black, Gooseberry and their hybrid. See also Plate 20, fig.1, 2 and 3.



Text fig: 12. Somatic chromosomes from root tips of (a) Amos Black selfed; (b) Gooseberry selfed and (c) F_1 hybrid between them. (2n = 16 in all).

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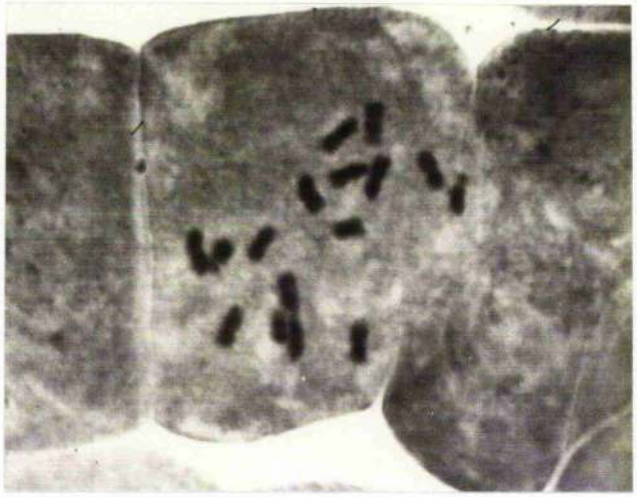
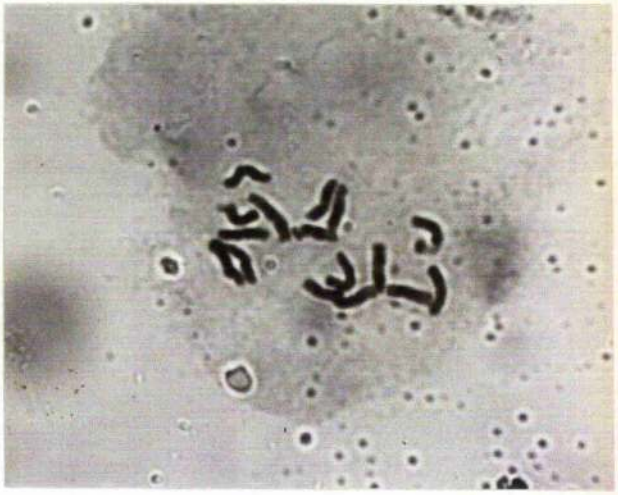


1



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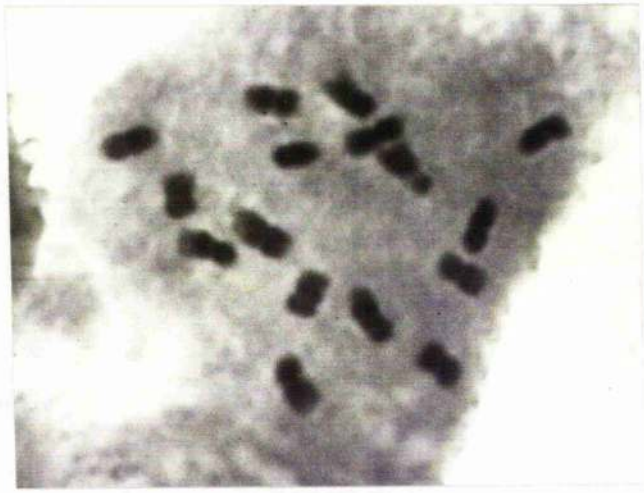

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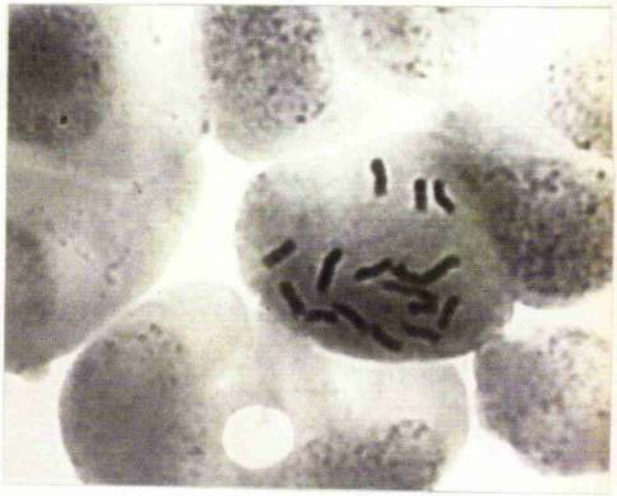


4

Somatic chromosomes in (1). Gooseberry. x1920.
(2). Amos Black selfed. x1920.
(3). Amos Black x Gooseberry. x3200.
(4). Baldwin x R.maximowiczii. x1920.

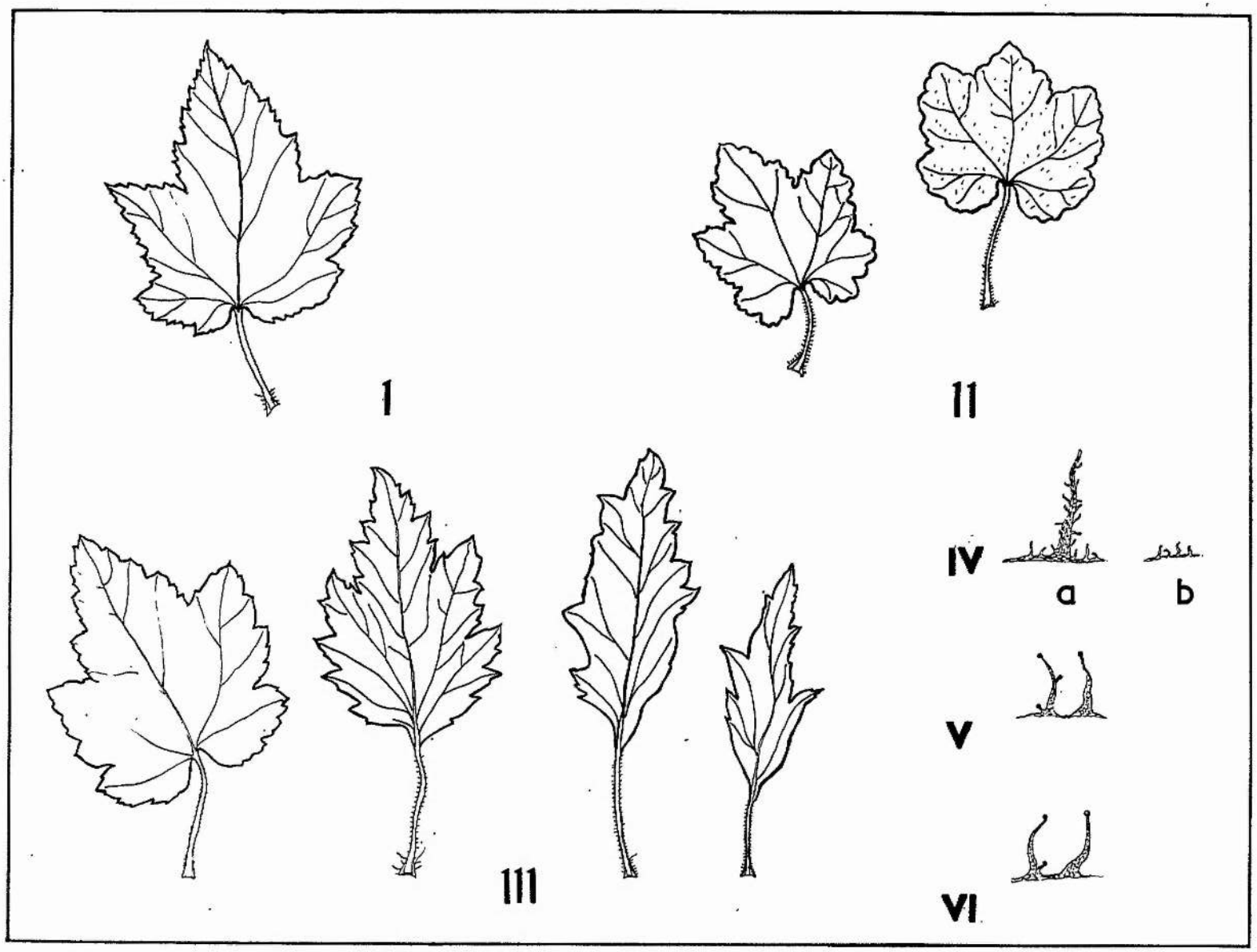
XII.

BLACK CURRANT x RED-FRUITED CURRANT HYBRIDS

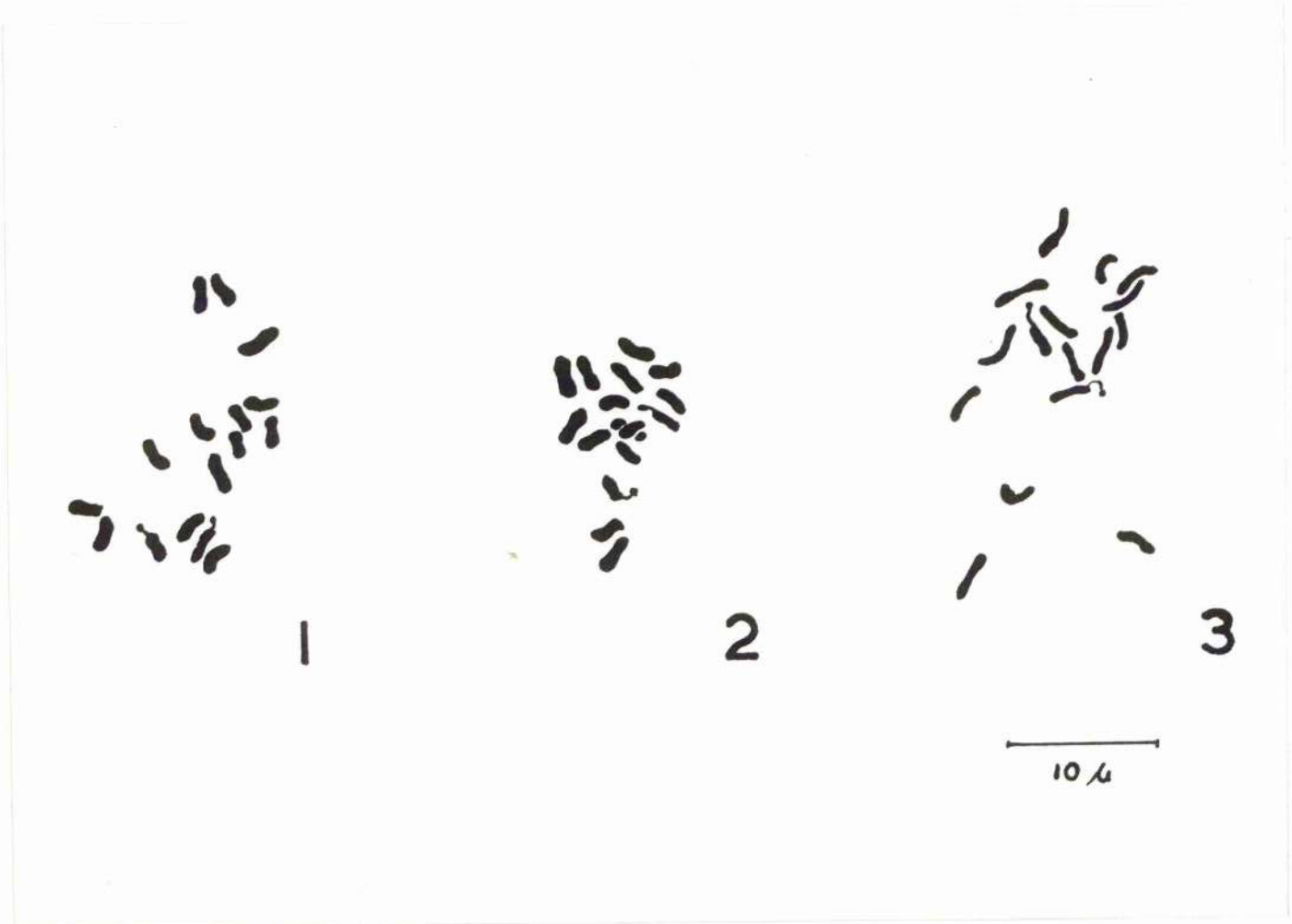
The hybrid studied was a cross of Baldwin (R.nigrum) and R.Maximowiczii, a red fruited currant, native of W.China. R.Maximowiczii belongs to the sub-genus Berisia of the family Grossulariaceae (Janczewski 1907).

Six hybrids were available for study. Leaf shape within these plants was variable. Two plants had leaves very similar to Baldwin, while on one plant they were intermediate between the two parents. Leaves on the remaining three plants were generally long and slender but ranged from moderately broad to thin narrow strips. These latter three plants were dwarfs. Text fig. 13 shows leaves from Baldwin, R.Maximowiczii and their hybrid.

Like the R.Maximowiczii parent no glandular hairs were present on either surface of leaves of the hybrid, except for those present on the petioles and for a few on the abaxial surface veins. No glands were visible at leaf tips and along the serrations of the leaf margin. The yellow glands present on the abaxial surface of the leaves and on the veins of Baldwin, and which were absent in R.Maximowiczii, were also lacking in the hybrid. The type of hairs found



Text fig: 13. The leaves from - I. Baldwin selfed. II. R.maximowiczii selfed. III. Baldwin x R maximowiczii. Epidermal hairs from - IV. Baldwin selfed, (a) bristles from the petiole, (b) hairs from veins and petioles. V. R.maximowiczii selfed. VI. Baldwin x R.maximowiczii.



Text fig: 14. Camera lucida drawings of somatic metaphase chromosomes in (1) Baldwin (2) Baldwin x R. Maximowiczii and (3) R. Maximowiczii.

in the two parents and the hybrids are shown in Text fig: 13. Hairs of the hybrid were very like those in the red fruited currant.

Sixteen symmetrical chromosomes comprise each chromosome complement in Baldwin and R.Maximowiczii, and the genome of either parent could not be differentiated in the hybrid. See Text fig: 14.

XIII.

DISCUSSION

The characteristic odour issuing glands of the black currant are suppressed in its hybrids with both red-fruited currant and the gooseberry, which suggests that it must be a recessive trait if genically caused.

Though R.nigrum displayed amphiplasty in its somatic chromosomes (Varaama 1949), both in diploids as well as tetraploids Meurman (1928), Darlington (1927) and Zeilinski (1952) found a pair of satellites in the normal complements of several species of black currants and gooseberries. In the present observations, in the varieties of black currants, ared currants and gooseberries so far examined, only a constant pair of satellites was observed.

Navashin (1934) found karyotypical changes in the species hybrids of Crepis, involving the "disappearance" of a satellite from one of the parent species. It was also accompanied by either increase or decrease in length and width of the chromosomes of a set. He attributed this to hybridity, though McClintock (1934) was of the opinion that differential capacity of the nucleolar organising element in the two parents was responsible for the disappearance of the satellite.

The present observation of only one satellited chromosome in the hybrid between black currant and gooseberry is comparable to the above in the lack of a satellite, though it could not be certain to which parent this genome belonged. No other karyotypic alterations were observed. Unfortunately it was not possible to study the nature of satellites in the PMCs of the same plants.

If the absence of a satellite from the complement of the hybrid between black currant and gooseberry is a constant feature, it could be a useful marker from the plant breeding aspect.

XIV.

SUMMARY

1. Hybrids of black currant x gooseberry, and black currant x a red-fruited currant species were studied. Both the hybrids lacked the characteristic black currant odour.

2. Only one satellited chromosome was seen in the somatic chromosome complement of the black currant x gooseberry hybrid, although both parents had a pair of satellites in their chromosome complements. Otherwise no conspicuous karyotypical characters were observed which would distinguish one parental genome from another.

3. The two parental chromosome sets could not be differentiated in the hybrid of black currant x red-fruited currant.

4. These findings are discussed in relation to previous and similar observations.

RUBUS

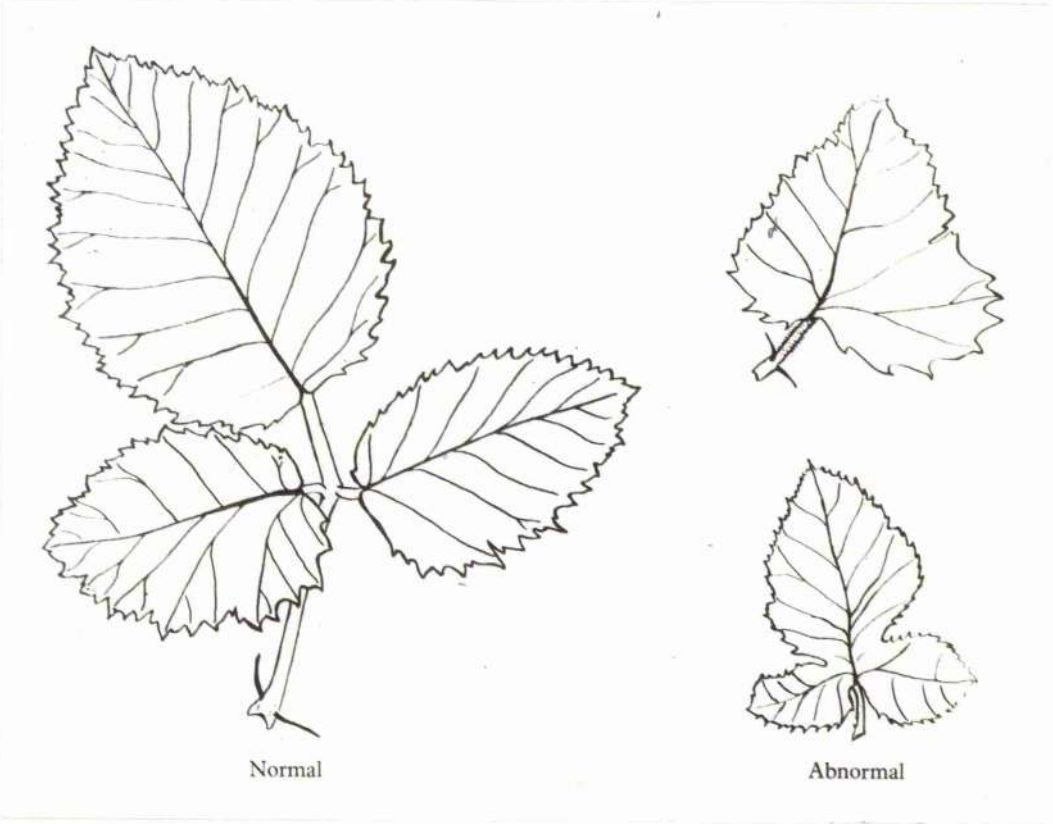
Origin

Merton Thornless (2n=28) is a cultivated species resulting from the multiple cross of apomictic Rubus thyrsiger (2n=28) and a thornless mutant of sexual R.rusticanus (2n=14). The crosses were made by Mr.M.B.Crane at the John Innes Horticultural Research Institute. Himalaya Giant (R.procerus) is a tetraploid apomictic European blackberry.

Among the F_2 thornless segregants of the cross Merton Thornless and Himalaya Giant made by Dr.G.M.Haskell (to whom the writer is indebted for letting her make use of the material) one abnormal seedling was observed, and subsequently studied.

Morphological Appearance

This seedling was very stunted in growth with shortened internodes, rugose and thick leaves which were miniature in size. Instead of the normal tri-pinnate type of leaves, they were simple, entire or pinnatifid (See Text fig: 15). The whole plant was brittle, densely pubescent and very dark green in colour. At 12 months, when its normal sibs had grown to an average height of 266 cm, this seedling was



Text fig: 15. The leaves from (left) a normal stable tetraploid (2n=28) thornless seedling, and (right) from the abnormal, cytologically unstable seedling of the same F_2, taken from similar positions of the respective plants.

Two sibs from the F_2 of Merton Thornless (2n=28) x Himalaya Giant (2n=28). Photographed at 7 months. Right. abnormal plant with varying chromosome numbers. Left. normal plant with 28 chromosomes.

approximately 12 cm high (Plate 21). Both the root and shoot systems were very poorly developed in comparison with the vigorous growth of the normal sibs.

Chromosome Number Variation

Both the parents, i.e.Merton Thornless and Himalaya Giant were constant tetraploids (2n=28). Cytological observations of this abnormal seedling showed a wide range in somatic chromosome number. Consequently chromosome counts were made at intervals although the number of root tips examined at each time and the duration of the intervals were not constant, due to availability of the very poorly developed root system. Table 19 shows the chromosome number variation of the plant observed in sequential chromosome counts. The range of chromosome numbers on the whole was very wide, 9 to 48. The mode, however, in each instance was at 2n=35. Text fig: 16 shows the camera lucida drawings of the different somatic plates within root tips of the abnormal plant. In the fig., M has 2n=28, which is a somatic metaphase plate from a normal control sib.

A. 2n=40	D. 2n=35	G. 2n=29	J. 2n=20
B. 2n=38	E. 2n=34	H. 2n=25	K. 2n=19
C. 2n=37	F. 2n=32	I. 2n=24	L. 2n=9

Table 19. Chromosome number variation in the cytologically unstable rubus plant.

Serial No.	No. of RT examined	Number of cells with chromosome number																											No. of plates	Mean chromosome No. per cell
		9	12	14	17	19	20	21	22	23	24	25	26	28	29	30	31	32	33	34	35	36	37	38	39	40	41	46	48		
1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	2	2	3	-	6	1	-	-	-	-	-	-	-	17	33.0
2	3	-	-	-	-	-	-	1	-	-	-	-	-	-	-	2	1	1	3	1	5	1	-	-	-	1	-	-	-	16	33.0
3	2	-	-	-	-	1	-	-	-	-	1	-	-	1	1	1	2	3	2	3	9	1	-	-	-	-	-	-	-	25	32.28
4	2	1	-	-	-	-	-	-	-	1	-	-	-	1	-	-	1	1	1	4	8	1	-	-	-	-	-	-	-	19	32.0
5	3	-	-	-	-	-	-	-	-	-	-	4	-	-	1	-	-	4	6	11	29	2	-	1	-	-	-	-	-	58	33.69
6	5	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	3	1	43	-	-	1	-	-	1	-	52	34.5
7	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	3	-	-	-	-	-	-	-	-	4	33.75
8	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42	1	-	-	-	-	-	-	-	43	35.02
9	4	-	1	1	1	-	1	5	1	-	-	3	1	5	-	-	-	1	2	2	31	3	18	11	1	2	1	-	-	91	33.34
10	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	2	2	16	3	-	-	-	-	-	-	-	24	34.60
11	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	4	25	8	-	-	-	-	-	-	1	40	35.83

Total number of cells with chromosome no. < 35 = 113

35 = 259

> 35 = 44

Text fig: 16. Range of chromosome numbers within root-tips of the abnormal Rubus hybrid seedling.

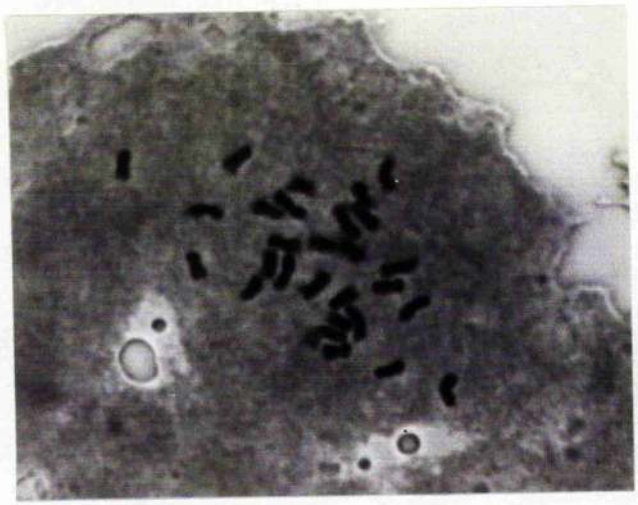
Chromosome counts were also made from stipule squashes inorder to determine whether the variation in chromosome number was confined to the roots or was prevalent in the shoot system as well. But owing to the density of hairs good plates were difficult to obtain. One good plate showed 39 somatic chromosomes, which was an aneuploid number. It was not possible to say whether this somatic chromosome number was constant in the stipules or whether it occurred in only one cell.

The chromosomes in stipules were broader than those in the root tips (Plate 22, fig; 4) which may be accounted for by the difference in pre-treatment, 0.25% colchicine solution (aqueous) being used. But Navashin (1934) had observed in his Crepis species hybrids that chromosomes from adult root tips, petals and sepals were larger than those from embryonic root tips. This suggests that there is a possible relationship between position and chromosome size.

Plates 22 and 23 show photomicrographs of root-tip chromosomes in the chromosomally unstable plant.

Effect of Age on Variation in Chromosome Number

The first chromosome counts were made when the plant was seven months old. Several normal sibs of the plant were examined for their somatic chromosome number as well. This



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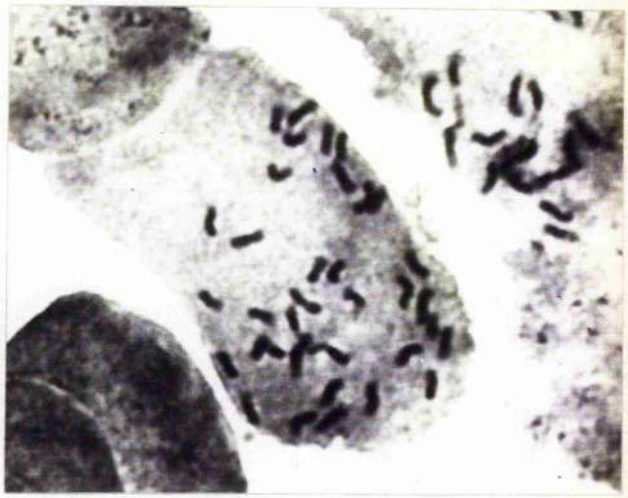

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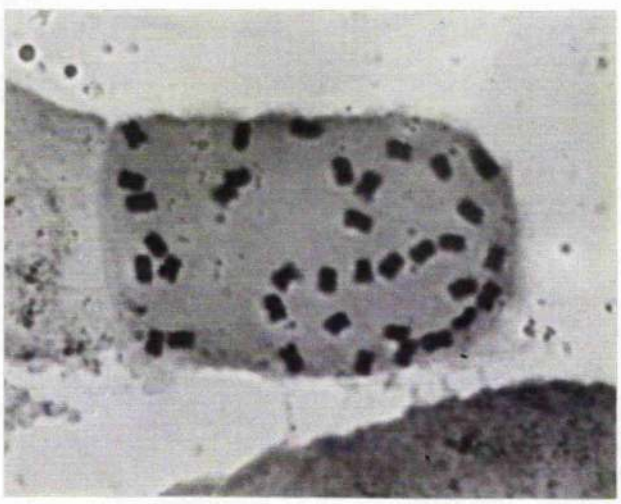

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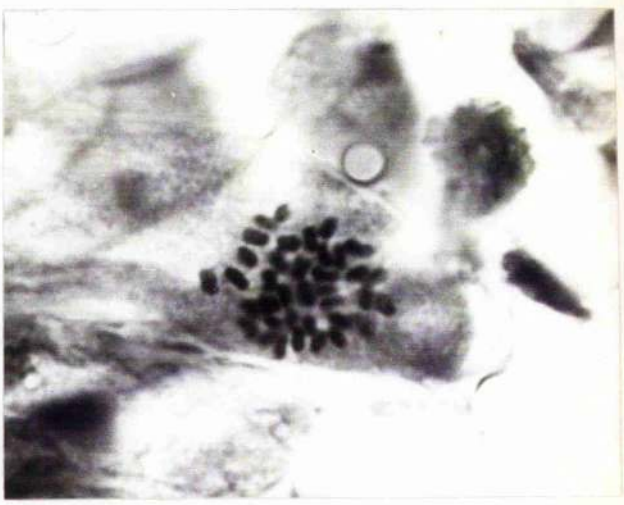
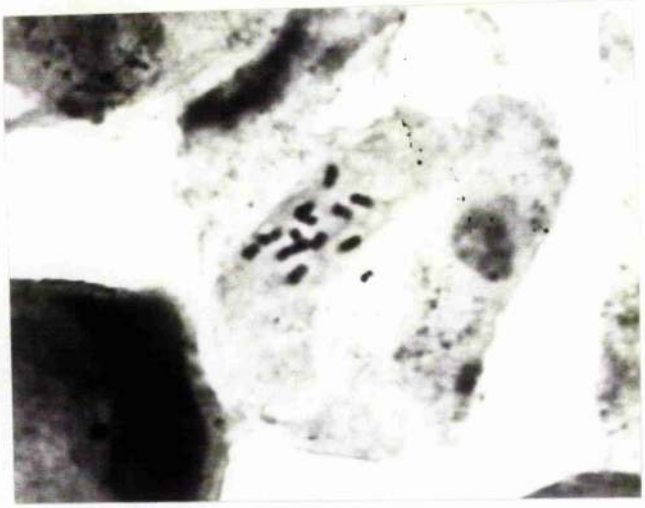


4

(1) Somatic chromosomes (2n=28) in a normal sib of the F_2 progeny of Merton Thornless x Himalaya Giant.
(2) Metaphase plate with 2n=35 chromosomes in the abnormal, cytologically unstable seedling of the same progeny as above.
(3) Metaphase plate with 2n=36 chromosomes in the same plant as in (2).
(4) Somatic chromosomes (2n=39) in the stipule of the same plant as in (2). Two chromosomes are out of focus. (All figs x1920).

Root tips in 1,2 and 3 were pre-treated with oxy-quinoline, and the stipule in 4 was pre-treated with colchicine.



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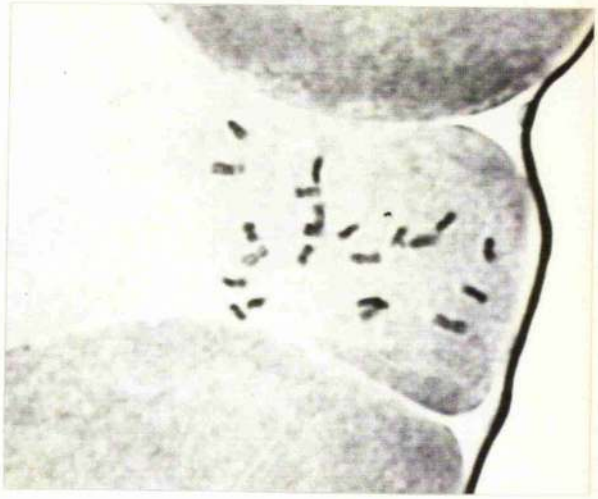

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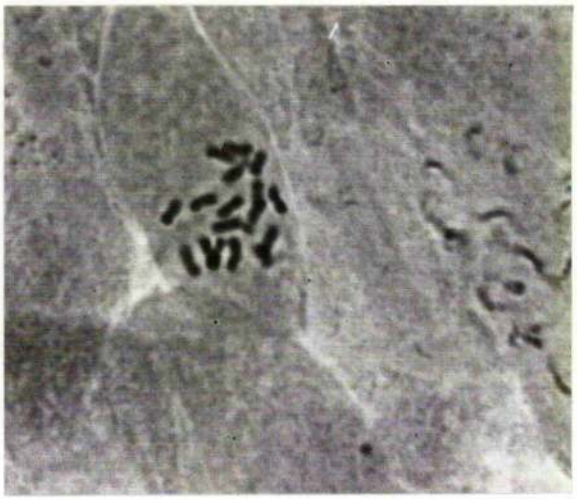

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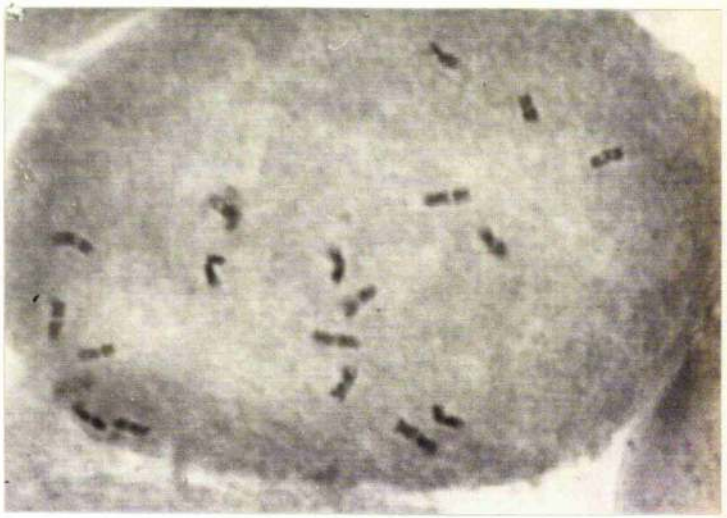


4

Varying somatic chromosome numbers within root tips of the abnormal, cytologically unstable seedling.

(1) 2n=12 (2) 2n=22 (3) 2n=17 (4) 2n=20

(All fig:s are x1920).

was done for once or twice, but later on only one normal plant was checked occasionally for its chromosome number; it was always found to be constant at 2n=28.

As seen from Table 19, as time progressed, the range in chromosome number decreased, while the actual number of cells with 35 chromosomes increased until at one point the plant was almost stable at 2n=35 level. The next count, however, revealed that the pentaploid level did not last for long: variation was seen again. In one later count, cells with chromosome numbers of a multiple of the basic number i.e.7, were observed with greater frequency e.g.cells with 14, 21 and 28 chromosomes. It was also found that cells containing the same number of chromosomes were observed to be in groups so that within an area only cells with 21 chromosomes were observed, while in another area cells with 37 chromosomes were seen.

Altogether 113 cells occurred with chromosomes less than 35 in number and 44 cells with greater than 35, while 259 cells had 35 chromosomes.

Satellites

In diploid species of Rubus the presence of two satellited chromosomes in the complement is a common feature. Heslop-Harrison

(1953) observed 2 satellites in 2x, 3x as well as 5x and 6x plants, the highest number of satellites being three in Rubus vulgaris var. Mollis (2n=21) and Corylifolian R.sublustris (2n=35). In the present study, the normal sibs (2n=28) usually have 2 satellites in the complement while in the abnormal seedling the number of satellites varied from cell to cell. See Table 20.

Table 20. Satellite Range in the Abnormal Seedling

No. of chromosome in the cell	No. of satellites	Frequency
14	4	1
17	2	1
20	1	1
22	1	1
24	1	1
28	3	2
33	2	1
35	1	3
	2	14
	3	7
37	1	1
	3	2
	4	1
38	3	1
	4	1

Although the number of satellites increased with the increase in chromosome number the relation is not precisely correlated with the ploidy level. In cells with chromosome number above the tetraploid level i.e.28, variation in satellite number was greater. In cells of 2n=35, two satellites occurred with the highest frequency, while 1 and 3 satellites were also observed. As many as 4 satellites were observed in cells with 37 and 38 chromosomes. Although the normal tetraploid plants were usually observed to contain 2 satellites, two cells with 28 chromosomes in this plant were seen to contain 3. Another cell of 17 chromosomes contained a pair of satellited chromosomes. The most unusual instance was a cell with the diploid number of chromosomes but containing 4 satellited ones. Possibly different individual chromosomes of the set were involved in the distribution of chromosomes from cell to cell during mitosis.

Correlation of Chromosome Number and Cell Area.

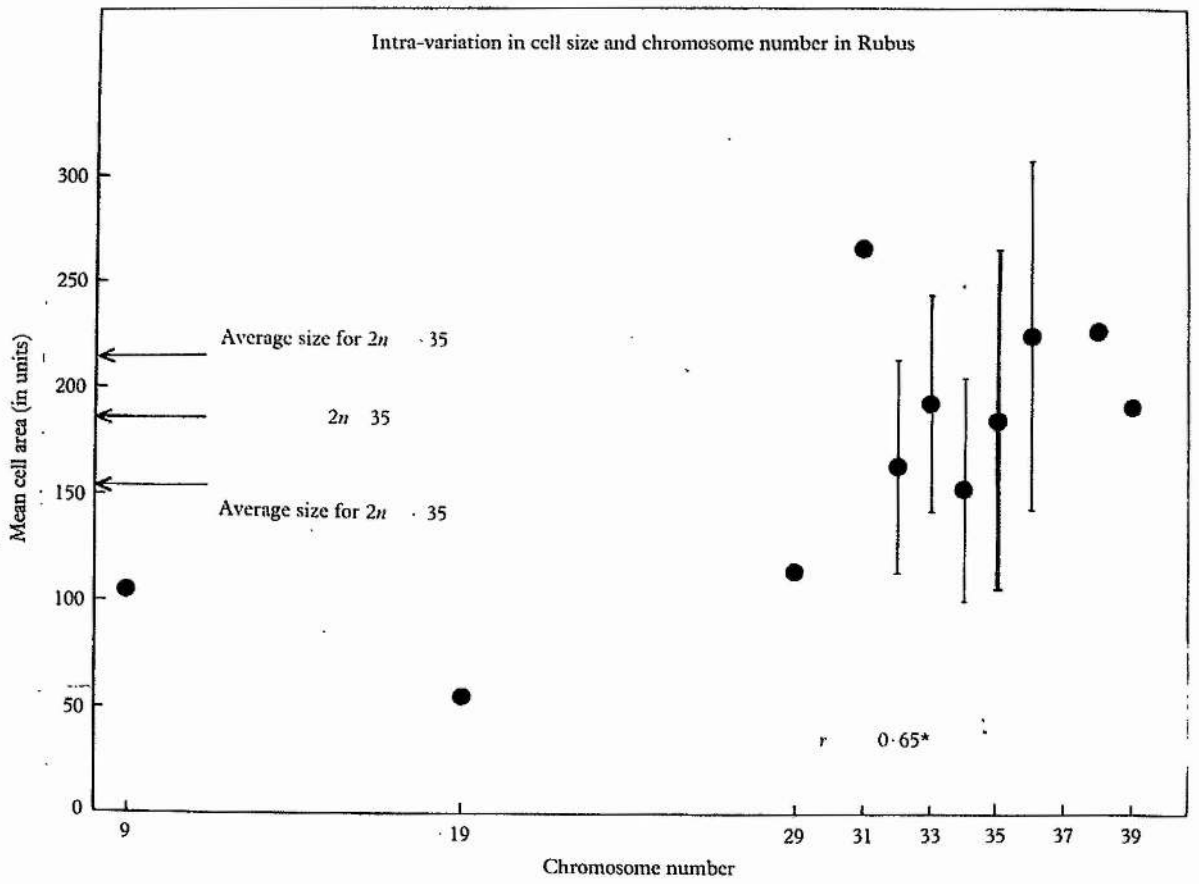
Cell area was assessed as follows. The outlines of the cells were drawn on grid paper by camera lucida at a magnification of x1200 and the number of squares (1 unit=0.01 sq.inch) occupied by the cell was counted.

Correlation was found to exist between cell area and actual

number of chromosomes with $r = + 0.65$. Text fig: 17 shows a graph drawn for mean area of cells plotted against the number of chromosomes contained in those cells. Twice the standard deviation is shown in larger samples. There is a general regression for cell area and chromosome number into which the mean of 2n=35 fits; and the euploid cells, with strict multiple of the basic chromosome number, are not larger than those with aneuploid number of chromosomes.

It could be suggested that differences in pressure of squashing might influence cell area. This could be ruled out when the relation between cell area and chromosome number in the cell was considered. Thus in cells with chromosome number greater than 35, the average cell area was 214 units, in those with 35 , 186 units and for cells containing less than 35 chromosomes the average cell area was 154 units.

Taking cells with 2n=35 only, the variation in size was considerable, being greatest as compared with the other cells (Table 21) with a decrease in the coefficient of variation (c) with the addition or subtraction of 1, 2 and 3 chromosomes.



Text fig: 17. A graph showing mean cell area plotted against chromosome number. Twice the standard deviation is shown in the larger samples.

Table 21. Relation between cell area and number of chromosomes contained in the cell.

Chromosome number	Departure from 2n=35	Mean Area (in units)	Coefficient of variation
36	+1	226.1 ± 31.8	36.18
35	0	186.5 ± 78.4	42.04
34	-1	152.6 ± 52.2	34.21
33	-2	193.1 ± 50.8	26.31
32	-3	163.8 ± 49.8	30.22

Causes of Chromosome Number Variation

The perculiar appearance of the seedling, coupled with the fact that its chromosome number was found to be varying suggested that it might be virus infected, for Caldwell (1952) had mentioned that viruses might have wider effects on nuclear division than have been hitherto supposed. But the tests for virus infection, carried out by Dr. C.H.Cadman,(of the Virus department) gave negative results for the presence of any of the 5 common transmissable viruses of raspberries.

Root tip cells were examined without pre-treatment, as the latter could possibly hinder the observation of actual mechanism of division, like spindle fibre formation, etc.

Unequal division of chromosomes at late anaphase was observed in several cases. While in most such examples the chromosome number in each unequal mass was uncountable, there

were two definite cases where in one cell 9 and 16 chromosomes were observed in each group, and in another cell 8 and 16. Unequal groups range from occasions where there was little difference in the sizes of the two groups to those where they large. Inversion bridges were very rarely seen at anaphase stage. In a few instances, dumb-bell shaped nuclei were seen which may be comparable to the lobed nuclei observed by Britton and Hull (1957) in their mitotically unstable Rubus plants. These kinds of abnormalities were also observed by Hegwood and Hough (1958) in the cell divisions of the White Winter Pearmain Apple showing mosaic chromosome pattern. They observed occasional groupsof chromosomes excluded from the spindle, but no such condition was found here.

The nucleoli observed in somatic cells varied. Most cells had two nucleoli as in normal diploids. However, there was a second type, where although the number of nucleoli was two, their sizes were not the same; one being larger than the other. Occasionally cells with 3 nucleoli were also observed, two usually of equal size while the third was a little larger. Seldom four nucleoli were seen, three equal but one smaller. The significance of these varying numbers of nucleoli will be discussed later in conjunction with the observations of variation in satellited chromosome number from cell to cell.

XVI.

DISCUSSION

Variation of chromosome number in plants is quite a frequent if not a common phenomenon. Instability of chromosome number may occur either within plants of the same progeny or within the same plant. Some of these plants showing variation in chromosome number were the results of crosses involving plants with unequal 2n chromosome number (Soost 1958; Britton & Hull 1956). Chromosome instability may or may not be accompanied by phenotypic effects. Thus in Rubus, in the progeny of crosses of Boysen x Eldorado and Young x Eldorado, several plants which showed instability in chromosome number were not morphologically distinguishable from the other vigorous normal plants. However, in another population of unstable Rubus plants the only symptom found which indicated the presence of mitotic instability was a characteristic "checking" or "mosaicism", in the leaves (Britton & Hull, 1957). The parents were both constant tetraploids of 2n=28 , in the present seedling, and there was no possibility of occurrence of varying chromosome number which might have resulted from the union of gametes with unequal chromosome number. The wide range of chromosome number occurring in this plant had greatly

affected its morphology, which deviated greatly from its normal sibs.

There is a tendency towards retardation of the beginning of flowering, in addition to lower growth rate during the active growth period of the plants in the aneuploids from the progeny of an autoterapioid Ribes nigrum (Varaama 1953). But it is very doubtful whether the plant studied here will flower at all.

Leaf bud cuttings of unstable Rubus plants still maintained mitotic instability (Britton & Hull, 1957). Chromosome number determinations from root tips made when these plants were only a few weeks old revealed many high as well as low chromosome numbers, while later in the season only low chromosome numbers occurred.(e.g. 2n=20 to 35). In the plant studied here, throughout the period of chromosome number determinations, besides the cells with 35 chromosomes which occurred with the highest frequency, there was a greater number of cells with chromosome number lower than 35.

Aneusomaty (variable aneuploid numbers of chromosomes) occurring within root tip cells of the orchard Paphiopedilum wardii was due to recurring duplication of certain chromosomes out of 20 of the complement, giving rise to the differences in chromosome number from cell to cell (Duncan 1945). This

worker was able to recognise the chromosomes in the complement, and of the 7 pairs of atelocentric chromosomes (i.e.chromosomes with median centromeres) in the set he observed that none occurred twice in any of the plates. This is of interest because in diploid Rubus tomentosus (where individual identification of the chromosomes in the complement was very difficult due to their small size) it was still possible to see that apart from the satellited pair which had a submedian centromere, the others were atelocentric or symmetrical. Stebbins (1950) holds the view that symmetrical chromosomes are primary or primitive types from which the more specialised karyotypes are evolved.

Heitz (1931) working with Vicia introduced the word SAT-chromosome, and he pointed out that the number of nucleoli formed at telophase depended upon the number of satellited chromosomes present in the complement. By the interchange of chromosomes involving the satellited chromosome (chromosome 6) and another chromosome (chromosome 9) of the complement in Zea mays, McClintock (1943) was able to show that the nucleolus originated from a nucleolar organising body adjacent to the stalk of the satellite. Hence, in a complement, there is a correlation between nucleoli number and the number of satellited chromosomes. Thus generally somatic tissues of haploids

show one nucleolus, diploids two and triploids three nucleoli. The present findings regarding the relation of nucleoli and satellited chromosome number in a complement paralleled those of Heitz and McClintock. Irrespective of their chromosome number, plates with two satellited chromosomes were observed with the highest frequency and those with three, one and four in descending order. Cells with two nucleoli, equal or unequal in size, were also observed with the highest frequency. Occasionally three nucleoli were observed, two equal in size and the other larger, and only rarely did four or five nucleoli occur. These varying numbers of satellited chromosomes indicate that, so far as the satellites were concerned, their distribution is irregular at mitosis. This might apply to the other chromosomes in the complement as well, but could not be proved as they could not be identified.

Another fact which supports this view that different chromosomes were involved in the different complements was that there was a definite relation between cell area and chromosome number. Moreover, within cells of 2n=35 alone, the variation was great. Therefore this variation in area within cells of the same chromosome number, as well as those of different chromosome number was probably due to differences in the genomic constitution of the individual cells, which

were changing during the processes of unstable mitosis.

Regarding cell size and chromosome number relationship, Heslop-Harrison (1953) also observed differences in cell size between the two regions of octoploid (peripheral) and tetraploid (central) tissues of a periclinal "chromochimera" encountered in a plant of Rubus polygenthemos from Northumberland. Periclinal and sectorial cytochimeras occur quite frequently in plants, as can be seen from the work of many investigators. This is no such behaviour in the present seedling. There was no definite localisation of cells of the same chromosome number; they occurred scattered within the tissue, as was also found by Varaama (1949) in Ribes nigrum. However, the last few counts made showed the occurrence of euploid series of cells(i.e.cells with chromosome numbers which were multiples of the basic "n" number, e.g. 14, 21, 28 etc.) which were isolated in groups. Normal distribution of chromosomes so far as the number was concerned, was evident in those areas.

Müntzing (1943) assumed that the frequent formation of daughter plants with chromosome numbers ranging from 39 to 43 in apomictic Potentilla, originated from reduced embryo sacs which developed parthenogenically. Although the polyploid species of Rubus, comprising most of the European blackberry population are apomictic (Gustafsson 1943), this mechanism

as a possible cause of variation of chromosome number here could be ruled out. The F_1 plant which produced this unstable seedling was vigorous and thorned. It was heterozygous for the gene thorned (S) and thornless (s). The parental R.thyrsiger is a tetraploid apomictic and it could easily reproduce apomictically. But this unstable plant was thornless, which proved that it must have arisen sexually to be homozygous recessive. It is likely, therefore, that instability of chromosome number in this seedling is the result of interaction of genes carried by the parental gametes.

XVII.

SUMMARY

1. Serial cytological observations were made on one abnormal seedling from the thornless segregants of the F_2 progeny from crossing two tetraploid (2n=28) Rubus species. The number of root tip chromosomes was found to vary from 9 to 48 per cell, with the mode always at 35. The wide variation in chromosome number decreased with time, until at one point there was almost stability at 2n=35.

2. The number of satellites per cell was proportional to the number of nucleoli per cell. Evidence from the number of satellites per cell suggests that different chromosomes were being involved in the different complements.

3. There was a correlation between the chromosome number and corresponding cell sizes within the aneuploid series.

4. Virus infection was not responsible for the variation of somatic chromosome numbers from cell to cell. The cause of the instability was not definitely located but it is suggested that its initiation arose from the egg cell, as Rubus pollen is sensitive to chromosome unbalance.

5. Chromosome number instability of this plant is discussed in relation to other examples from the same and different genera.

APPENDICES

1. NUTRIENT SOLUTION.

Pfeffer's Solution (MacLean & Cook, pg 92)

Calcium nitrate	4 gm
Potassium nitrate	1 gm
Magnesium sulphate	1 gm
Potassium phosphate ($KH_2 PO_4$)	1 gm
Potassium chloride	5 gm
Ferric chloride	trace
Water	3 - 7 litres

2. STAINS FOR CHROMOSOMES (Darlington & La Cour, 1950).

(a) Aceto-carmine

45 cc glacial acetic acid

55 cc distilled water

Add 0.5 gm of carmine, when the above mixture is boiling, stir occasionally and simmer on a low flame until the liquid has evaporated down to half. Cool and filter.

(b) Aceto-orcein

Dissolve 2.2 gm orcein in 100 cc glacial acetic acid by gentle boiling. Cool and filter. This is stock solution of 2.2 per cent. Standard solution is 1 per cent in 45 per cent

acetic acid. Dilute the stock solution to 45 per cent as required.

(c) Leuco-basic fuchsin

Dissolve 1 gm basic fuchsin by pouring over it 200 cc of boiling distilled water.

Shake well and cool to 50 C.

Filter. Add 30 cc N.Hcl to filtrate.

Add 3 gm $K_2 S_2 O_5$.

Allow solution to bleach for 24 hours in tight-stoppered bottle in the dark; add 0.5 gm decolorizing carbon. Shake well for about a minute and filter rapidly through coarse filter paper.

Store in tightly-stoppered bottle in the dark.

3. CELLULOSE ACETATE (North, 1956).

Dissolve 2 gm cellulose acetate and 0.7 gm night blue or crystal violet in 100 ml. water.

4. BLEACHING SOLUTION (Manton, 1950).

Add sufficient amount of a stock solution of 10 per cent potassium ferricyanide to the normal stock solution of 20 per cent hypo to produce a deep yellow colour. The exact strength is not critical. The bleaching solution should be prepared immediately before use.

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