CHROMOSOME STUDIES IN THE GENERA LYCOPERSICON AND SOLANUM

A Thesis

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by

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Abstract

This thesis comprises two Parts and concerns the cytology of *Lycopersicon* and *Solanum*.

Part I was a study of pachytene in *Lycopersicon* and began as an attempt to assess the usefulness of this stage as a source of information to aid classification. The principal findings of this thesis were as follows:

1. Chromosomes at pachytene were examined by the 'isolated chromosome technique', the precision of which was augmented by reference to probability theory.

2. In no case was it possible to identify all 12 chromosomes in any species examined but in *L. esculentum* sub sp *humboldtii* 9 chromosome types were elucidated.

3. Detailed interspecific comparisons of two distinctive chromosomes revealed that differences were of two kinds (a) difference in chromomere size and number, (b) differences in chromomere sequence presumably due to inversion.

4. Since such a small proportion of the genome was available for reliable interspecies comparisons pachytene morphology probably has little taxonomic value.

5. The occurrence of distinctive types once rather than twice in each nucleus provided morphological evidence for a basic number of 12 rather than 6.

6. Examination of diakinesis confirmed that at this stage the bulk of the chiasmata were confined to the

achromatic region. This observation was however re-interpreted as evidence of terminalisation.

7. Evidence was obtained that chiasma formation occurred in both chromatic and achromatic regions.

8. The observation that chiasmata occurred at random throughout the tomato chromosome considered jointly with the existence of very small chromosome changes provided instances of 'cryptic structural differentiation' and may account for a proportion at least of the genetic breakdowns observed in F_2 populations of interspecific Lycopersicon and Solanum hybrids.

Part II was a study of meiosis in polyploid species of Solanum and the principal findings were as follows:

1. Solanum juzepczukii may well have originated from the cross S. acaule x S. stenotomum.

2. S. curtilobum probably originated from a cross between S. juzepczukii and S. tuberosum.

3. During this study an attempt was made to define precisely types of chromosome configuration encountered.

OF STUDIES

Crafty men contemn studies, simple
men admire them, and wise men use
them; for they teach not their
own use; but that is a wisdom
without them and above them, won
by observation.

Francis Bacon.

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Studies on the Pachytene and some Subsequent Phases of Meiosis in the Genera Solanum and Lycopersicum.

INTRODUCTION.

In 1925 N.I. Vavilov organised an expedition to collect species of Solanum in South America. Since that time Russian, German, Swedish, American and British collecting expeditions have added greatly to the range of potato material maintained at various herbaria and research stations throughout the world. Because of the availability of material and the need for a classification, the taxonomy of the genus Solanum, particularly among the tuber bearing representatives, has been extensively studied - notably by Bitter (1912, 1913) Juzepczuk (1937) and Bukasov (1933, 1938, 1939, 1941). More recently J.G.Hawkes (1956) published a revised conspectus of all the tuber bearing species more in line with modern cytogenetical concepts.

Within the taxonomic framework cytological and genetic studies have proceeded with increasing momentum over the last fifty years. Recently Swaminathan and Howard, (1952) published a comprehensive review of the literature on the cytology and genetics of the cultivated potato (Solanum tuberosum) and its related species. It is sufficient to summarise only those cytological conclusions of particular relevance to this thesis which are as follows.

(1) A long standing controversy concerns the basic chromosome number of _Solanum_ - whether it is $x = 6$ or $x = 12$. No tuber bearing species is known with less than $2n = 24$*. Reviewing the evidence (derived largely from studies of meiotic metaphase chromosome associations) these authors are of the opinion that the evidence for a basic number of $x = 6$ is inconclusive other than at a remote period in the past.

(2) Meiosis in diploid _Solanum_ species and their hybrids is quite regular and there is no evidence of multivalent formation. Chiasma frequencies are uniformly low and little importance can be attributed to necessarily small variations and thus the facility and regularity with which _Solanum_ chromosomes pair would appear to restrict genome analysis. Consequently, Swaminathan and Howard looked to a possible reduction in the number of species recognised.

(3) Chromosome counts in this genus indicate a polyploid series extending in increments of 12 fron $2n = 24$ to $2n = 72$. Swaminathan and Howard pointed out the complexity of the cytological (and genetical) behaviour and the consequent difficulty of applying cytogenetical concepts to species relationships at the higher levels of ploidy.

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* Two Australasian non-tuberous species _S.aviculare_ and _S.laciniatum_ have $2n = 46$ and $2n = 92$. The haploid number is $x = 23$. (Baylis 1954).

Recently two interesting attempts have been made to elucidate species relationships in the *Solanaceae* by means other than those cytogenetical techniques which have now become traditional. One attempt (Gell Wright and Hawkes 1956) was based upon serological principles. Anti-sera from two species were raised in rabbits and then allowed to react in an agar diffusion system with a tuber extract from one of forty species. Comparison of the precipitation spectra allowed a classification of the species into five well defined groups. Of these five groups, four corresponded with the particular taxonomic series proposed by Hawkes (1956) and one serological group contained species from several of Hawkes' series.

Another attempt at developing, among other things, new taxonomic criteria within the *Solanaceae* has been the work of Gottschalk (1954), who claimed that the pachytene morphology of any chromosome was dependent upon the species in which it occurred. This will subsequently be discussed in relation to other prophase studies.

The first published account of a pachytene analysis was that of Wenrich (1916) who worked with *Phrynottetix magnus* which is favourable for this kind of study since the pachytene chromosome length is only about three times that of the length of a chromosome at somatic metaphase. Belling in 1926 working with *Lilium* and *Aloe* species published drawings of the contents of the whole nucleus at the pachytene

phase of meiosis, and from that time until the present other plants have been similarly described, for example, Bellevalia (Dark 1934), Anemone spp. (Moffett 1932), Trillium erectum (Huskins and Smith 1939), Maize M.M.Rhoades (In Sinnot Dunn & Dobzhansky 1950) and Secale cereale (Lima de Faria 1952) to name but a few. (See also Table II). Fungal chromosomes are generally considered to be difficult cytological material and the field has not been much explored. None the less, Singleton (1953) successfully elucidated the karyotype at pachytene of Neurospora Crassa.

Arising out of studies of pachytene a major contribution to cytogenetics has been the advances of Barbara McClintock and her co-workers. Not least among the contributions from this school was the elegant demonstration of the position on a chromosome of one gene locus to within a fraction of a chromomere, (McClintock 1944). It is with maize that pachytene studies have most helped our understanding of heredity. Pachytene has been little studied in Drosophila since the salivary gland chromosomes have been more suited to the requirements of cytogeneticists.

Prophase in Lycopersicon was studied by Lesley and Lesley (1935,1938). They established the occurrence of different sized satellites with which were correlated differences in nucleolus size. Different satellite lengths in tomato were confirmed by Gottschalk (1954) and also in this thesis.

In 1949 Spencer W. Brown produced the first in a series of detailed studies in tomato cytology which was as regards pachytene investigations immensely superior to anything that had gone before. Brown's conclusions are important and will be summarised in some detail (the terminology used in his paper will be discussed later in this thesis.)

Brown established that pachytene chromosomes of tomato were differentiated into proximal, chromatic and distal achromatic portions and possessed distinct dark staining terminal knobs. The two types of chromosome material during various phases of meiosis showed differential rates of contraction, the achromatic regions contracting more quickly. It was suggested that chiasma formation was confined to the achromatic regions and a similar view was adopted by Barton (1951). Neither worker, however, presented adequate chiasma frequency data for the whole chromosome complement.

Barton (1950) published a pachytene analysis of L.esculentum where he claimed to have identified all 12 chromosome types of the tomato genome. Gottschalk (1951) again described the pachytene morphology of the tomato both in irradiated and non-irradiated material. Detailed comparison of these papers is deferred until later. Further irradiation studies of tomato chromosomes at pachytene were published by Barton (1953,54) where it was claimed that there was a higher proportion of deficiences to translocations following U.V.

treatment than following X-ray treatment. Breakage when it occurred was mostly localized in the chromatic regions.

In 1954 Gottschalk published a long paper in which he claimed that Solanum, Lycopersicon and other genera of the Solanaceae differred in respect of their "heterochromatin" and "euchromatin" content and that these differences were a result of evolution. Altogether he examined nineteen varieties of tomato, twentyeight species of Solanum and such genera as Cestrum, Nicotiana and Withania. Among his other conclusions were that high heterochromatin content was found in highly evolved species and that the basic number of chromosomes in the Solanaceae was six and not twelve. In conversation he suggested to the present writer that chiasma formation was confined to the euchromatic (light staining) portions. Those chiasmata observed occasionally in the heterochromatic regions at diakinesis he explained by supposing them to be located in interstitial euchromatic regions. Finally it may be noted that he did not confirm his conclusions about chromosome evolution by a study of pachytene in diploid interspecific hybrids.

Since the work of Gottschalk appeared to have some significance for the study of the Solanaceae the present critical examination of prophase stages in certain species

of this family was undertaken and forms the substance of Part I of this thesis.

The second Part of this thesis is an account of the results of an investigation on the meiotic metaphase behaviour of several polyploid Solanum species. The aim was to detect and clarify their phylogenetic interrelationships. In the interpretation of the various metaphase configurations the studies described in Part I provided a helpful basis.

MATERIALS AND METHODS.

(1) Solanum Material.

All the tuber-bearing Solanums used in this study were derived from the collection of Dr. J.G.Hawkes, at the University of Birmingham, and I am much indebted to him for this material.

(2) Lycopersicon Material.

Representatives of the genus Lycopersicon were obtained from several sources.

SPECIES	SOURCE.
Lycopersicon pimpinellifolium (W.B.)	The University Botanic Garden, Winterbourne, Birmingham.
L.pyriforme.	Botanic Garden, Cambridge.
L.pimpinellifolium (L.M.8)	Hortus Botanicus Hauniensis
L.esculentum sub sp. typicus (L.M.7)	" " "
L.esculentum sub sp. (pyriforme) (L.M.6)	" " "
L.esculentum sub sp. Humboldtii (L.M.5)	" " "
L.esculentum sub sp. Galenii (L.M.4)	" " "
L.rirstum	C.M.Rick Davis, California.
L.peruvianum	" " "
L.esculentum var, Suttons Best of All.	Messrs. Barrows Stores, Birmingham.

(3) Plant Management.

Seeds were sown in compost of the following composition :

PARTS BY BULK	2 loam 1 peat 1 sand	Plus 1½ ozs/bushel of superphosphate.

Subsequently, seedlings were potted on into a potting compost of the following composition :

PARTS BY BULK	7 loam 3 peat 2 sand	Plus John Innes Base ¼lb/bushel (For Winter work a small amount of lime was added-1 oz/bushel.)

Usually once during the period of growth the plant was top dressed. From October to April plants were grown under artificially extended day length conditions, by means of Phillips "Gearless" fluorescent Tubular Lighting.

(4) Tuber Dormancy.

Usually, freshly harvested potato tubers are dormant. Dormancy was broken by treating cut tubers in the vapour of a 1.2% solution of ethylene chlorohydrin for twenty-four hours at room temperature (Denny F.E., Boyce 1938).

(5) Pollination Technique.

All the tomatoes were grown in a greenhouse insect proofed by fine nylon mesh placed over all vents and lights.

The bagging of flowers used in pollination was shown to be unnecessary since some seventeen flowers were emasculated and deliberately left unpollinated. None of which, together with others emasculated but not used, set seed and it was concluded, therefore, that aerial pollen transference was

negligible under these conditions of cultivation. Tomatoes are normally self fertile and easily self-set seed. For crossing work emasculation was essential and was done with forceps, sterilised in alcohol, on young buds.

An attempt was made to repeat the technique of M.S.Swaminathan (1955) for obtaining wide interspecific *Solanum* hybrids. No success was obtained but the small number of attempts does not justify criticism of that work.

(6) Staining Technique.

i. Aceto-carmine. The modification used of Bellings (1926) Aceto-carmine technique is based on that described by Barton (1950). Anthers were removed from flower buds and fixed in three to one alcohol acetic acid for at least 90 minutes. Before staining the anthers were removed and soaked for about 20 minutes in 4% iron-alum and after washing they were macerated on a slide in a drop of 2% carmine. The cover slip was placed in position and the slide heated on a steam bath for one to two minutes, and then pressed. Slides were made permanent by the method described by Darlington and La Cour (1947), floating off the cover slips in 10% acetic acid and passing through 3.1 alcohol:acetic acid and absolute alcohol. The material was then mounted in "Euparol".

ii. Feulgen Staining. Chromosomes at somatic mitosis were examined after staining with the feulgen technique. Root tips were removed and immersed for two hours in aerated bromo-naphthalene - a 'C' mitotic substance.

By this means division is inhibited and chromosomes accumulate at metaphase. After pre-treatment root tips were fixed in 3.1 alcohol acetic acid overnight and hydrolysed in Normal hydrochloric acid at 60 degrees C. for 7½ minutes. Subsequently, the material was placed in Feulgen stain for 2 to 3 hours and a squash preparation made. Feulgen-stained chromosomes seldom had sufficient contrast for photographing, and, to overcome this difficulty, the Feulgen stained material was heated in 1% aceto-lacmoid for 3 minutes, at 60 degrees C.

(7) The Recording of Chromosome Data.

At pachytene chromosomes were examined in the form of bivalents. The following system was adopted for recording data from bivalents. Bivalents were drawn with the aid of a Zeiss camera lucida on separate pieces of Bristol board approximately 2½" x 4". On the card were recorded the sequence number in the analysis, the number of the nucleus, the slide number and the slide location. Other information included the lengths of the various morphological features measured in arbitrary units. By drawing only one bivalent on each card any one might be compared with any other. Measurement was with an eye-piece micrometer calibrated in by reference to a standard scale.

The optical system of the Zeiss camera lucida consisted of a Prior x 100 apochromatic oil immersion objective lens with a Leitz x 25 eye-piece.

(8) Photography.

Photographs presented in this thesis were taken using either Ilford Rapid Process Panchromatic plates or Kodak P.25. plates. The plates were quarter size and were developed in the following solution :

Solution I.	Solution II.
Hydroquinone 25.grams.	
Potassium metabisulphite 25 grams.	5% caustic soda.
Potassium bromide 25 grams.	
Distilled water to 1,000 mls.	

The developer comprised equal parts of Solutions I and II. The camera was one designed by Dr.Osterstock formerly of the John Innes Horticultural Institution.

I should like to record my indebtedness to Mr. Clark the photographer at this Institution for his help in the preparation of the photographs.

(9) Nomenclature.

i. Chromosome Terminology. The dark and light staining portions of the chromosomes were called "hetero-chromatin" and "eu-chromatin" respectively by Gottschalk but I have departed from this usage for the following reasons. Firstly, these words have acquired meanings or inferences beyond that for which they were first intended. Their origin is due to Heitz (1929), who used them to refer to chromosome portions which maintained their metaphase stainability for relatively longer or shorter

periods of meiosis. Secondly, Brown (1949) proposed two rather more non-commital and purely descriptive terms - "chromatin" and "achromatin". The use of the term "achromatin" is open to criticism since it implies the absence of staining capacity and does not, therefore, accurately describe the weakly staining regions. "Sub-chromatin" has been suggested as a more accurate alternative. In order, however, to avoid confusion through the introduction of a third set of terms, Brown's usage has been adopted in this work.

ii. Systematics. The system of classification used for the tuberous Solanums was that of Hawkes (1956). Material belonging to the genus Lycopersicon was classified accordingly to the system of Luckwill (1943). Various workers in the genus have used a variety of taxonomic names and in particular the distinctiveness of Lycopersicon as a separate genus from Solanum has not always been accepted. Rather than pronounce upon the suitability of previous taxonomic names a Table of equivalents has been compiled (Table I), and in this thesis the names used by various workers for any species have been retained.

FIGURE I.

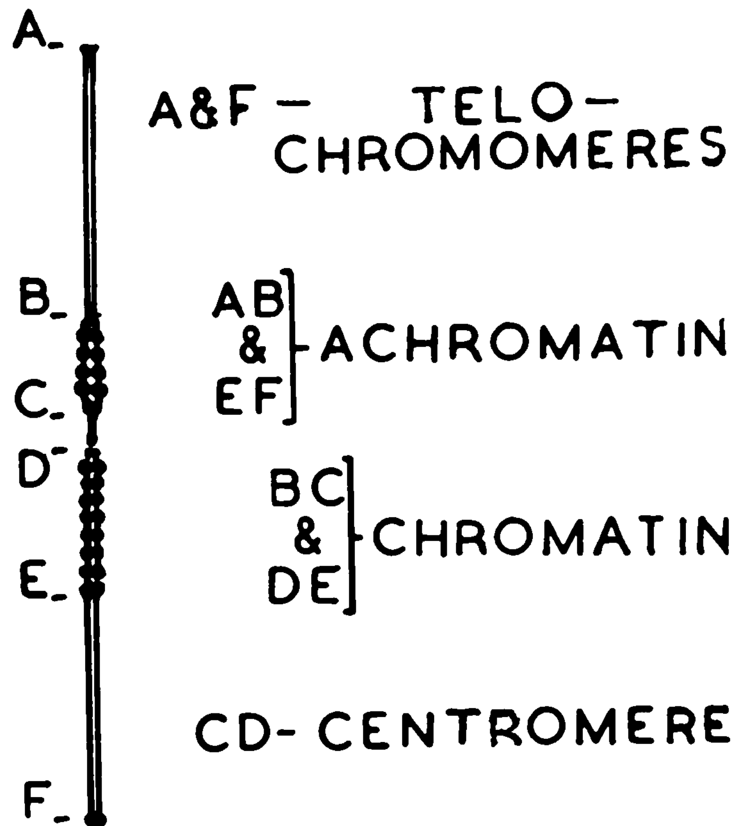


Diagram of a Lycopersicon Bivalent at Pachytene.

TABLE I.

TAXONOMIC EQUIVALENTS OF MATERIALS USED IN THIS THESIS.

BARTON	GOTTSCHALK	CHAPMAN.
Lycopersicon esculentum var Suttons Best of All.		Lycopersicon esculentum var Suttons Best of All.
	Solanum lycopersicum	Lycopersicon esculentum
	Solanum humboldtii	Lycopersicon esculentum sub.sp. humboldtii.
	Solanum lycopersicum var rosarigerum.	Lycopersicon esculentum sub.sp. galenii.
	Solanum pimpinellifolium.	Lycopersicon pimpinellifolium.
	Solanum peruvianum	Lycopersicon peruvianum.
	Solanum hirsutum.	Lycopersicon hirsutum.

** In general, throughout this thesis, when these materials are mentioned they will be referred to by the name the various workers used for them. As regards the genus "<u>Lycopersicon</u>" the current spelling recommended by <u>Index Kewensis</u> has been adopted.

A Theory of Pachytene Analysis.

At the pachytene phase of meiosis allele to allele pairing of the chromosomes has already taken place and the simplest behaviour is observed in those diploid species where there is complete chromosome homology. Any departure from this diploid condition induces complexity into the chromosome pairing relationships which is reflected, often, in meiotic aberration perhaps resulting in more or less diminished fertility or skewed ratios of gene assortment and segregation.

Chromosomes at pachytene are relatively uncontracted and appear as long thread-like structures and it is at this phase of meiosis that their chromosome morphology is most detailed. Unfortunately this detail often tends to be obscured by the relative compactness of the nuclear contents. Cytologically there is an added disadvantage which is that in polyploids and polysomics the increased complexity of behaviour is accompanied by a consequent decline of optical resolution. In those cases, therefore, where analyses would be most informative, they are least practicable. The thread-like chromosomes continue to contract throughout pachytene. Mid-pachytene is the most suitable sub-phase for study since it offers a compromise between the nuclear mellay of immediate post-zygotene and the considerable chromosome contraction of incipient diplotene when the finer structures are obscured. Evidently the examination of pachytene potentially could provide valuable

cytological information and several attempts have been made to study this phase of meiosis. A consideration of these gives an idea of the possibilities and limitations of this sort of enquiry. (Table II).

Chromosome numbers vary from two to about two thousand (Darlington 1953) and between these numbers is a more or less continuous series though with a very uneven frequency distribution. (The great bulk of somatic chromosome numbers falls below 2n = 100). Naturally the clarity of a stained nuclear preparation depends partly on the number of chromosomes it contains. At pachytene it depends too, on the complexity of chromosome association, their degree of contraction and, of course, the morphology as revealed by the particular procedure used. Doubtless, the facility with which pachytene threads spread is in some degree under genetic control as Wellwood and Randolf (1957) have shown for maize. (Table II). (See also this thesis).

With increase of chromosome number, there is a point at which it is no longer possible, consistently, to see all the pachytene threads within each cell examined. An important distinction may now be made, namely, between those plants where all chromosomes may be seen and those where only a portion is visible in every cell. Where in any plant not all the chromosomes may be seen together, conclusions drawn about the morphology of the whole genome inevitably contain a greater

TABLE II.

EXAMPLES OF THE DIFFERENTIAL SPREADING OF BIVALENTS AT PACHYTENE.

Category	Basic No.	Species	Reference.
(1) All bivs. consistently distinguishable.	x = 8	Anemone spp.	Moffett (1932).
	x = 4	Bellevalia romana	Dark (1932).
	x = 5	Sorghum Intrans	Garber (1947).
	x = 4	Plantago O.Vata	Hyde (1953).
(2) All bivs. only rarely distinguishable.	x = 12	Lilium pardalinum	Belling (1926).
	x = 8	Salvia horminum	G.Linnert (1955).
	x = 7	S.nemorosa	" "
	x = 12	Lycopersicum esculentum	Barton (1950).
	x = 12	L.pimpinelli-folium	Chapman (this thesis)
	x = 10	Zea mais	Wellwood & Randolf (1957)

Continued.......

TABLE II (Cont)

EXAMPLES OF THE DIFFERENTIAL SPREADING OF BIVALENTS AT PACHYTENE.

Category	Basic No.	Species	Reference.
(3) Only isolated bivs. distinguishable.	x = 10 x = 12 x = 7 x = 12	Zea mais Lycopersicum peruvianum Secale cereale Solanum spp. (both diploid & polyploid)	Wellwood & Randolf (1957). Chapman (this thesis). Lima de Faria (1952). Gottschalk (1954).

This table was compiled from the literature. Those bivalents of species in Category (1) can readily be identified by ordinary observational methods. The bivalent types of species in Categories (2) & (3) would most conveniently be elucidated by the technique of isolated chromosome examination. In these cases some reference to the mathematics of chance would seem to be helpful and necessary.

It must be pointed out that these categories would not be clear cut and that there would be gradations between them. Such distinctions as these are proposed out of convenience.

or smaller element of conjecture.

Those cases where a pachytene cell prepartion reveals a proportion only of the total chromosome content and where all the components of the nucleus stand an equal chance of being included in that proportion from cell to cell provide instances where probability theory may be legitimately applied. This method is not limited by high chromosome numbers. Theoretically any plant however large its chromosome number may be analysed in this way given compliance with probability requirements and indeed the only limiting factor would be the fortitude of the investigator.

This method of pachytene analysis was used for studies of Lycopersicon in this thesis. In diploid species of Solanum and Lycopersicon the twentyfour chromosomes were arranged as twelve bivalents at meiosis. In the majority of instances preparations of pachytene showed bunching of most of the bivalent threads with perhaps one or two chromosomes isolated from the bulk of the nuclear contents. If in such a preparation 120 bivalents were examined and ex-hypothesi each bivalent had in any cell an equal chance of appearing among those isolated from the bulk of the nucleus then each of the 12 types was expected to occur 10 times. Subsequently, the observed and expected frequencies were compared and made the subject of a probability test. Table III shows a range

of hypothetical frequencies compared with those expected, according to the χ^2 test. This treatment was found not wholly adequate but was helpful in appreciating the significance of the various data obtained and provided a useful starting point.

TABLE III

χ^2 ANALYSIS.

The probability of observing numbers (1 - 26) of randomly isolated bivalents based on expected frequencies of 0.16 and 0.32 per observation.

P	OBS. FREQUENCY	0.01 to 0.001	0.02 to 0.01	0.05 to 0.02	0.10 to 0.05	0.20 to 0.10	0.30 to 0.20	0.50 to 0.30	0.70 to 0.50	0.80 to 0.70	0.90 to 0.80	0.90 to
EXP. $\frac{10}{120}$		1,2	-	3,4	5	6	-	7	8	9	-	(10)
		-	-	16	15	14	-	13	12	11	-	(10)
EXP. $\frac{20}{120}$		-	-	-	-	14	15	16,17	18	-	19	(20)
		-	-	-	-	26	25	23,24	22	21	-	(20)

Pachytene Analyses of Lycopersicon Pimpinellifolium Mill.

The work of Gottschalk (1954) established the existence of a qualitative similarity between the chromosome of Solanum and Lycopersicon at the pachytene stage of meiosis. Lycopersicon pimpinellifolium was considered suitable for analysis since an analysis had been published by Gottschalk (1954) and thus a comparison was possible. Further, assuming for the present the basic number of x = 12, the plant was diploid and its 12 bivalents were free of duplicates. Since the material was extremly floriferous finding pachytene was less fortuitous than for Solanum. It was found that Lycopersicon material stained with aceto carmine revealed more detail than did Solanum.

First Analysis.

The individuality of the twelve bivalents at pachytene was explored. 120 bivalents were sampled and the following characters measured (see Figure I), AB and EF the lengths of distal achromatin, BE the length of chromatin and AF the total length. CD could not always be seen. Of the 120 bivalents all but 15 were obtained from one anther since it was important to reduce the variation to a minimum.

The results obtained were plotted as a scatter diagram (see Figure II), The co-ordinates were: on the y axis the length of chromatin and on the x axis the total length of a bivalent.

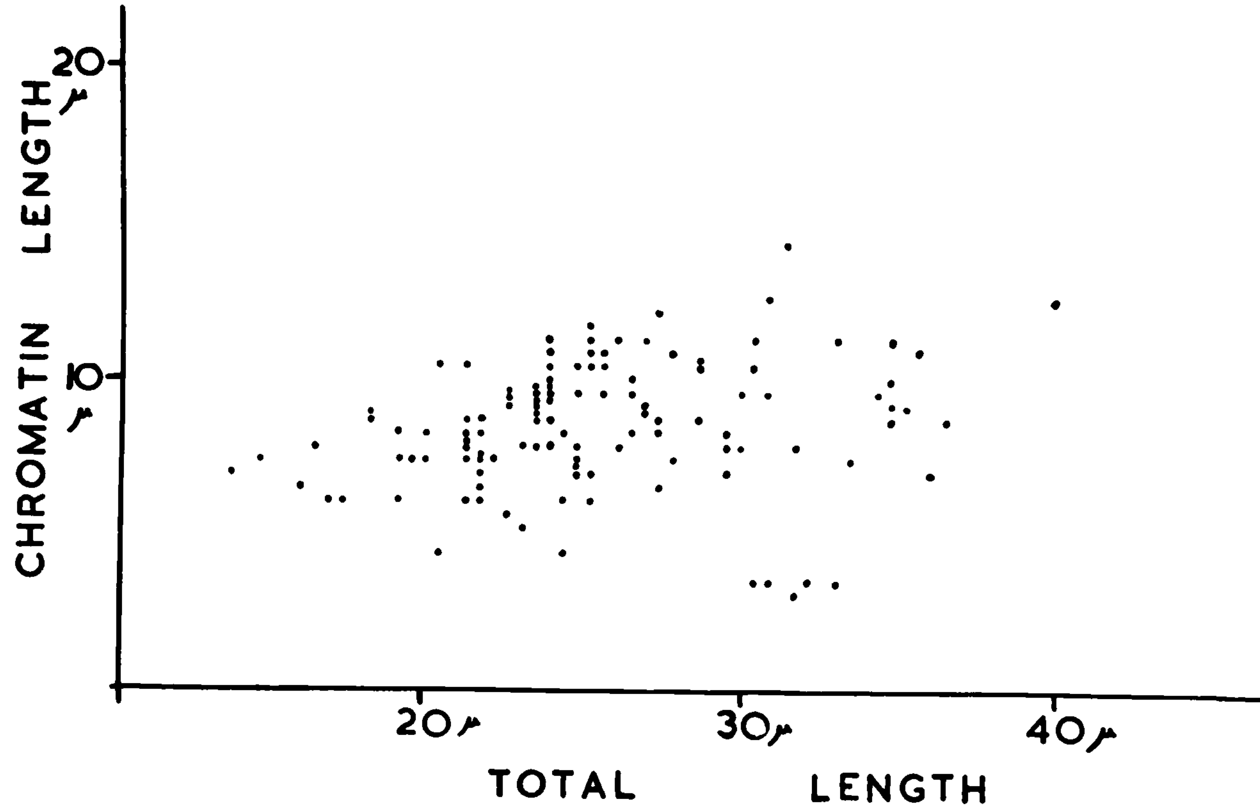
Other co-ordinates suggested themselves but were unsuitable. It was not possible, for instance, to plot the length of the long arm against the short arm since on 20 occasions out of 120 the centromere was not visible. It was not possible to plot achromatin against chromatin since occasionally in the chromatin there were two or more light staining interstices of various sizes and there was no a priori reason why any one should be regarded as the centromere and the others as achromatin. Strictly, therefore, the amount of achromatin was not accurately definable.

From the scatter diagram (Figure II) it was seen that five bivalents were plotted in the region $x = 3.0$ $y = 32.0$. Assuming these bivalents to be all of the same type and expecting a frequency of 10 the probability that these points represented a type was $P = 0.10$ to 0.05. There was too a bunching in the region $x = 24.0$ $y = 8.7$. The nucleolar bivalent was not plotted since it was quite distinct and its identity was not in question.

Conclusion.

From this analysis it was concluded that two bivalents could definitely be distinguished. Namely, the nucleolar bivalent and that having a discreet scatter position around $x = 3.5$ $y = 32.0$. A group of points around $x = 35.0$ and $y = 9.6$ possibly comprised a third chromosome or bivalent type.

FIGURE II.



A Scatter Diagram of Chromatin and Total Lengths at Pachytene.

Second Analysis.

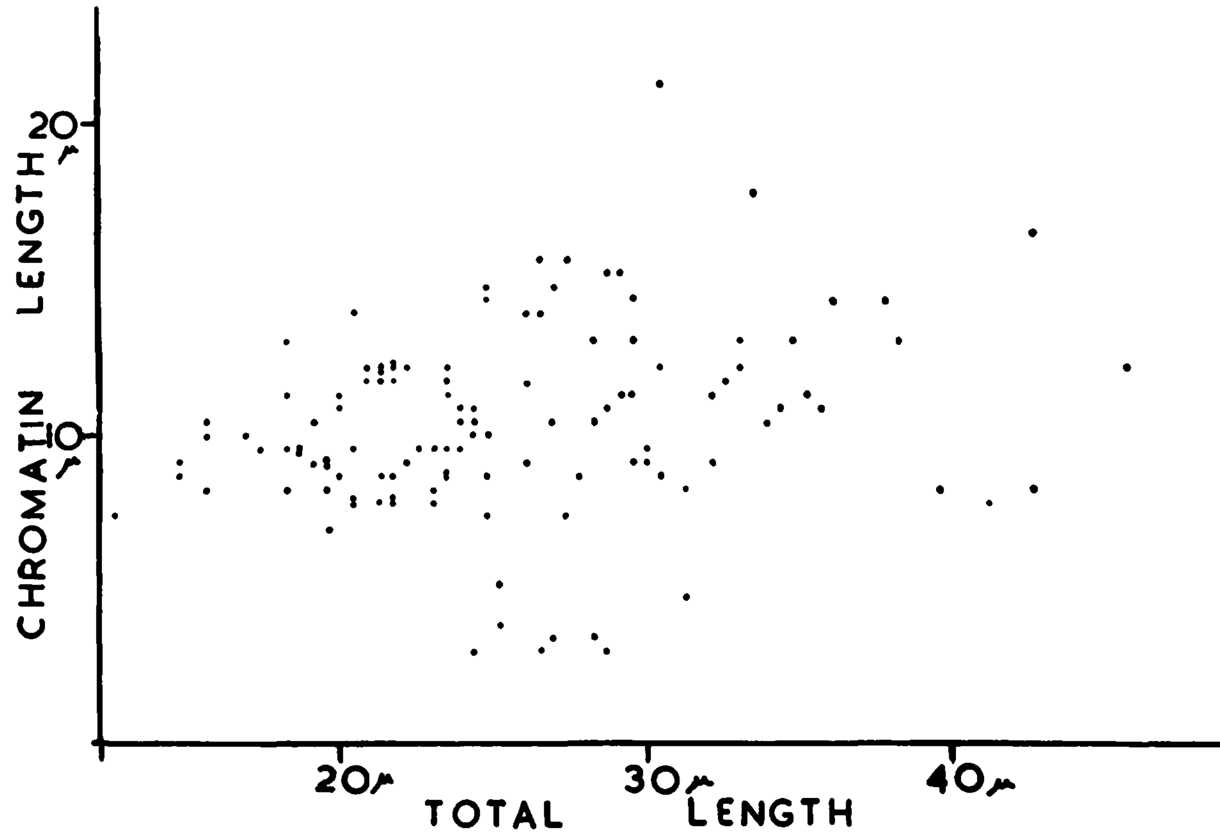
It was thought that the bunched points might be fractionated by adding an estimate of chromomere number when collecting chromosome data. In this analysis chromosomes were sampled from nine anthers. These data were plotted as a scatter diagram (See Figure III).

Although the points were distributed over a wider range as might be expected due to the larger number of anthers used, it had the same general features. (Cf. Figure II) There was a discreet collection of 7 points in the region x = 30.0 y = 4.0 . The data for this and the nucleolar bivalents will be found in Tables VIII and IX. In this analysis the nucleolar bivalent occurred five times. The chromomere data did not significantly assist the further grouping of bivalents. Plate I is of interest in that it shows a cell in which all 12 bivalents could be seen, and in which the two distinctive types mentioned above each occurred once.

Conclusion.

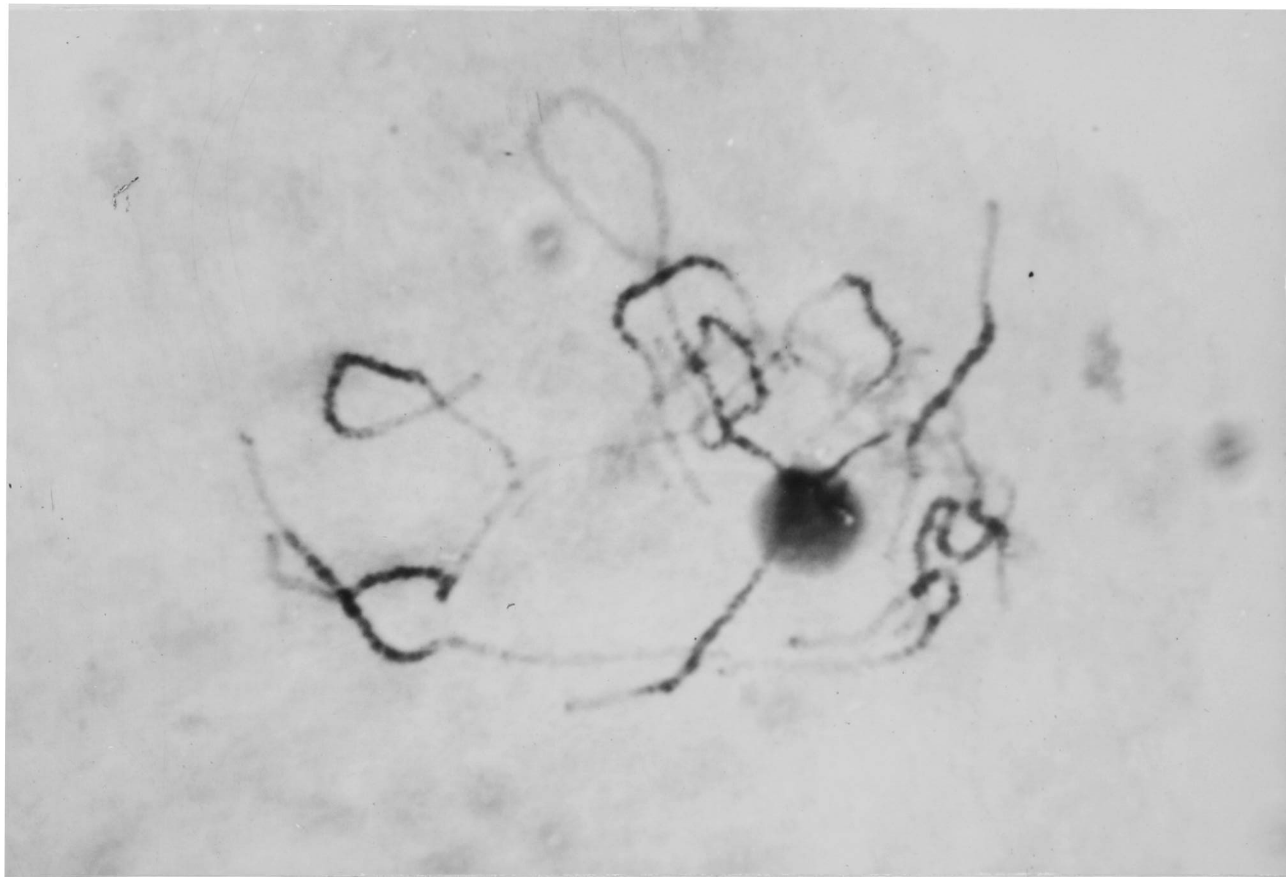
Only two bivalent types were identified satisfactorily and consequently analysis of pachytene in this species was not greatly successful. However, two types were demonstrated and were thus available for comparison with their homologues in other species.

FIGURE III.



A Scatter Diagram of Chromatin and Total Lengths at Pachytene.

PLATE I.



The 12 bivalents of L.pimpinellifolium at Pachytene.
(Resemblences can be seen to most of those subsequently identified in L.esculentum sub-species humboldtii.)

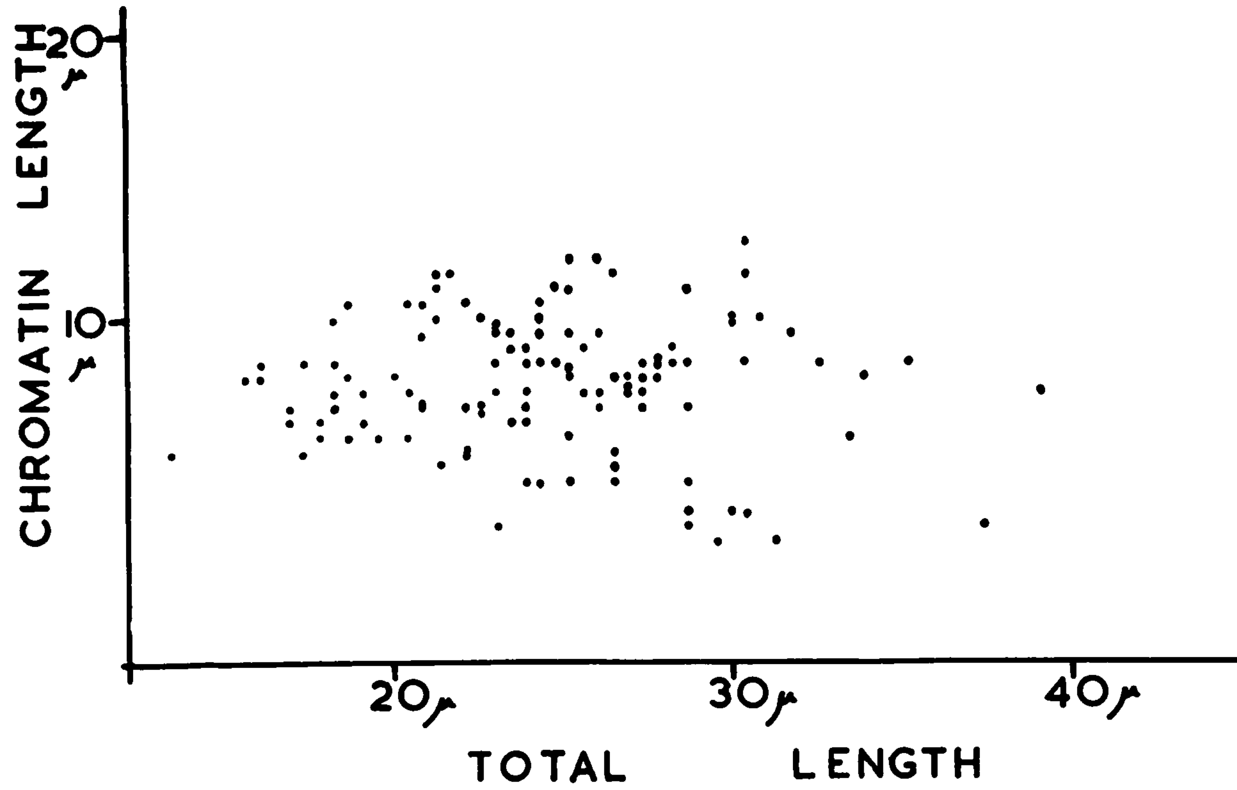
The Elucidation of Nine of the Twelve Bivalent Types of Lycopersicon Esculentum sub.sp Humboldtii.

An examination of pachytene in Lycopersicon esculentum sub species humboldtii showed that its chromomere pattern was relatively more distinct than that of L.pimpinellifolium. Consequently it was of interest for two reasons. Firstly it offered the possibility that some or all of its bivalent types might be elucidated and secondly its two most distinctive bivalents could be compared with the corresponding ones of L.pimpinellifolium.

The elucidation of bivalent types.

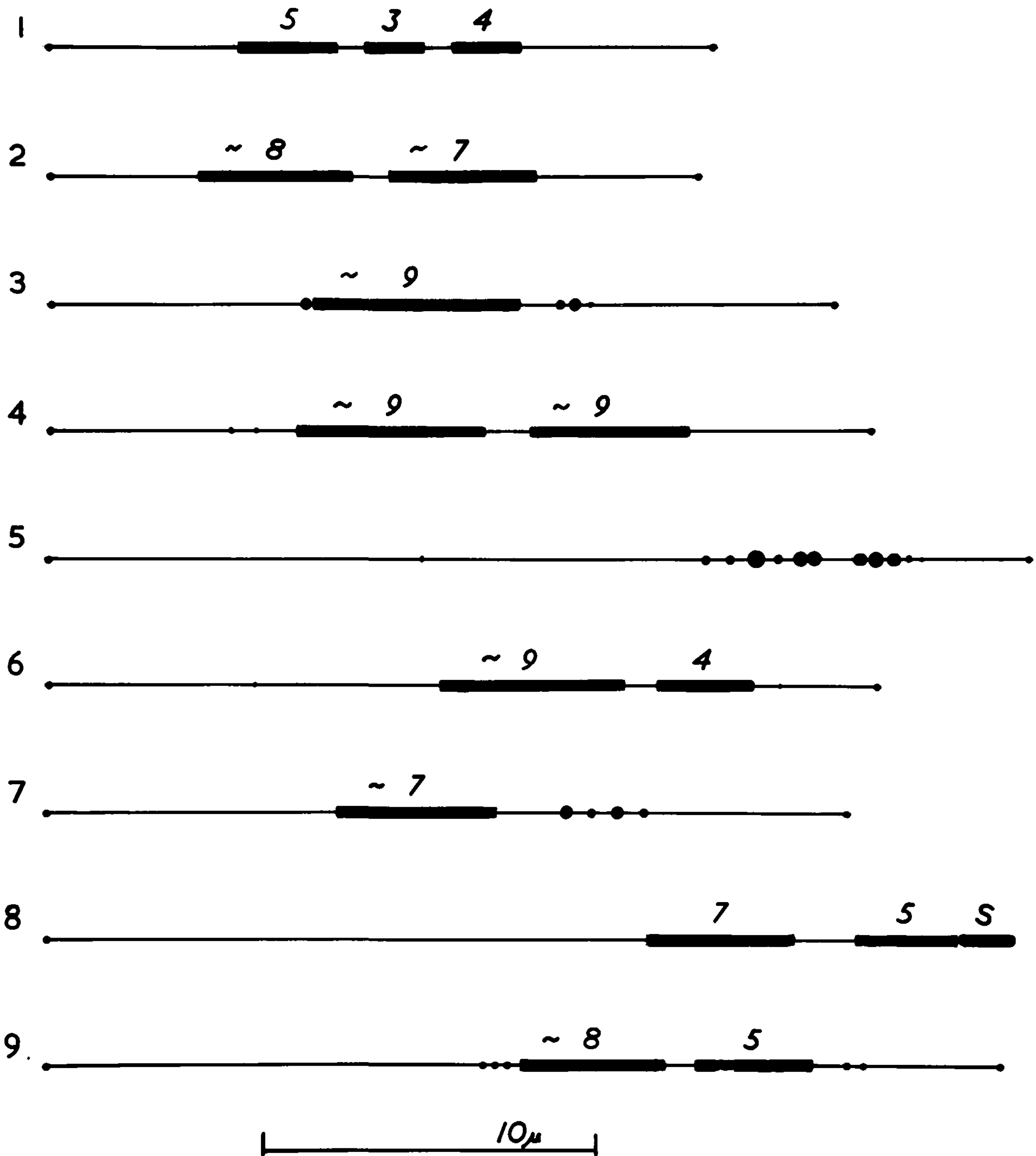
120 bivalents at the pachytene stage of meiosis and isolated from the bulk of the nucleus were scored and the information collected in the manner described in the section "Materials and Methods". The information included length measurements of the various bivalent regions together with estimates of the number of chromomeres present. Subsequently a scatter diagram was plotted (see Figure IV) which included all bivalents except the nucleolar ones, this latter omission avoiding unnecessary crowding on the diagram. A group of points were isolated in the region $x = 29.5$, $y = 4.4$, and in this and other respects the scatter diagram largely resembled that derived from the analysis of L.pimpinellifolium. Such a procedure established a basic similarity between the proportions of bivalents isolating for L.pimpinellifolium

FIGURE IV.



A Scatter Diagram of Chromatin and Total Lengths at Pachytene.

FIGURE V.



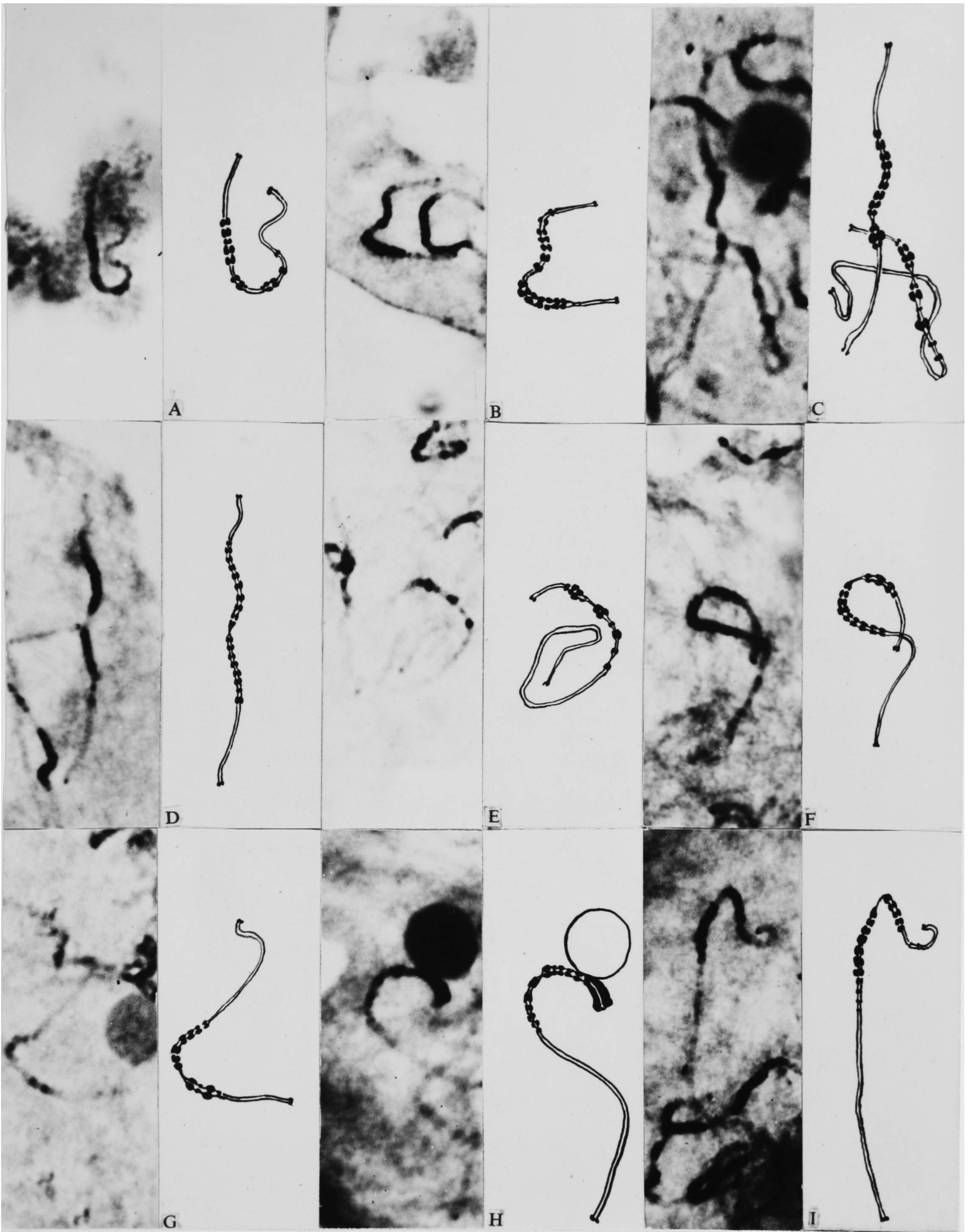
Idiogram of 9 Chromosome Types in L. esculentum

Sub-species Humboldtii.

PLATE II.

The Nine Identifiable Bivalents at Pachytene in L. esculentum sub-species Humboldtii.

II A	Type 1.
II B	Type 2.
II C	Types 3 and 5.
II D	Type 4.
II E	Type 5.
II F	Type 6.
II G	Type 7.
II H	Type 8.
II I	Type 9.


A
B
C
D
E
F
G
H
I

and L.esculentum and also showed that by reference to this procedure alone no more bivalent types could be specified.

An attempt was then made to arrange the sample of 120 bivalents into their 12 types. This was done by putting together those cards whose bivalents were of obviously similar morphology and where necessary subdividing them on the basis of length measurements. During this assorting process reference was made to the original microscope prepartions. By this means it was found possible to elucidate 9 bivalent (and by implication 9 chromosome) types. (See Table IV, Plate II and Figure V).

In Table V the results obtained from this analysis are presented and it will be seen that of the 120 bivalents scored 56 were unclassified. Two reasons may be advanced for this. Firstly, while squashing ensured the essential two dimensional prepartion, the disposition of the nuclear bulk and other cell contents relative to the plane of pressure meant that some isolated bivalents did not undergo uniform pressure along the whole of their length and consequently they were distorted. (By distortion in this connection was meant that a bivalent showed no obvious resemblance to any other. In other words, "distortion" was a relative term and its occurence could only be inferred). Secondly, some bivalents could not be classified as they lacked any consistently distinctive feature.

TABLE IV.

THE CHARACTERISATION OF BIVALENT TYPES AT PACHYTENE IN LYCOPERSICON ESCULENTUM SUB-SPECIES HUMBOLDTII.

Type	Freq.	Achr.	Chro.	Achr.	Chro.	Cent.	Chro.	Achr.	Tl.
1	12	5.8	3.0(5)	0.8	1.7(3)	0.9	2.0(4)	6.0	20.2
Type	Freq.	Achr.	Chro.	Cent.	Chro.	Achr.	Tl.		
2	9	4.6	4.6(8)	1.1	4.5(7)	5.0	19.8		
3	9	7.7	6.6(10)	1.1	1.2(3)	7.3	23.9		
4	7	7.6	5.7(9)	1.4	4.8(9)	5.6	25.5		
5	7	23.5--	-----(6)	0.87	2.3(4)	3.2	29.8		
6	6	11.9	5.6(9)	1.0	2.9(4)	3.8	27.7		
7	6	8.8	4.9(7)	1.9	2.8(4)	5.9	24.3		
8	6	18.2	4.5(7)	1.9	3.1(5)	2.6-(sat)	29.3		
9	2	14.4	4.3(8)	1.0	3.5(5)	5.6	28.9		

Figures in brackets refer to chromomere number. Frequencies refer to the occurrence of each type in a sample of 120. (Theoretically each type would be expected to occur 10 times) 'Sat' equals satelite.
Measurements in μ.

TABLE V.

ANALYSIS OF A SAMPLE OF 120 BIVALENTS OF L.ESCULENTUM SUB-SPECIES HUMBOLDTII.

Bivalent Type.	No. of Bivs.	Obs. Freq.	Significance	Remarks.
1	12	0.10	0.70 to 0.50	Interstitial achromatin.
2	9	0.075	0.80 to 0.70	
3	9	0.075	0.80 to 0.70	
4	7	0.058	0.50 to 0.30	
5	7	0.058	0.50 to 0.30	
6	6	0.05	0.20 to 0.10	
7	6	0.05	0.20 to 0.10	
8	6	0.05	0.20 to 0.10	Nucleolar bivalent.
9	2	0.016	0.01 to 0.001	
-	6	-	- - -	Distorted.) Unclassifiable.
-	50	-	- - -	Non distinctive) Unclassifiable.
TOTAL	120.			

Examination of cells at pachytene where all the bivalents could be seen showed that 3 or 4 were closely similar and this, together with the vagaries of differential contraction and squashing, meant that they could not be decisively characterised. Both Barton (1950) and Gottschalk (1951a, 1954) gave idiograms for all 12 bivalents but agreement between them was found only in about 8 or 9 of the 12 types. (See Table VI).

Each of the 9 types elucidated will now be described together with a consideration of the evidence in favour of its being considered a "type".

Type I.

This bivalent occurred 12 times in a sample of 120 and its most obvious characteristic was the interstitial segment of achromatin in addition to the centromeric region. The bivalent type 11 of Barton (1950) corresponded closely. Gottschalk, although he did not discern this chromosome type in 1951, had nevertheless found it in S.esculentum var rosarigerum and S.humboldtii by 1954.

Type 2.

A bivalent occurred 9 times in the course of the analysis whose chromatin was equally disposed either side of a median centromere with each chromatic section possessing about 8 chromomeres. The type resembled type 12 of Barton

(1950) and it resembled less precisely types 2, 4 and 5 of Gottschalk (1951a). Among the bivalent types described by Gottschalk (1954) for *S.humboldtii* this bivalent most closely resembled his type 4.

Type 3.

The Bivalent referred to as type 3 also occurred nine times in the course of the analysis and was clearly distinguished by a short chromatic segment containing 3 chromomeres all of different sizes. The other arm contained a large chromatic segment comprised of about 10 chromomeres. (The type is illustrated in Figure V). Type 3 showed close resemblance to that designated type 4 by Barton (loc.cit). Among types described by Gottschalk (1951a) type 3 showed greatest similarity to his type 10 and in his later paper (1954) type 3 showed greatest resemblance to his type 6 both for *S.esculentum* var *rosarigerum* and *S.humboldtii.*

Type 4.

Type 4 was discernible by the approximately symmetrical location of its centromere and of two equal sized portions of chromatin each containing about 9 chromomeres. It was distinguishable from type 2 by its greater length. Type 4 closely resembled type 5 of Barton and type 6 of Gottschalk (1951a) for *S.esculentum.* Among those types of bivalent described by Gottschalk (1954) for *S.esculentum* var *rosarigerum* and *S.humboldtii* there were no obvious resemblance to type 4

just described.

Type 5.

Type 5 was, next to the nucleolar bivalent, the most easily distinguished of all those comprising the tomato genome. This bivalent was first described by Brown (1949). In the analysis being presently discussed this bivalent occurred 7 times, and its most obvious feature was the very small amount of chromatin located in an extremely asymmetrical position. Certainly it corresponds to type 6 described by Barton (loc.cit.) and type 11 described by Gottschalk (1951a, 1954) for S.esculentum, S.esculentum var rosarigerum and S.humboldtii. This bivalent is one of the two selected for detailed discussion in a later section since its evolution was of some interest.

Type 6.

This bivalent type was distinguishable by having an asymmetrically placed centromere, a portion of chromatin in its short arm containing 4 or 5 chromomeres and a portion of chromatin in its long arm containing about 9 chromomeres. Its frequency of occurence was 6 in the 120 bivalents sampled. Type 6 showed some similarity to Barton's type 8 and to type 10 of Gottschalk for the species he analysed both in 1954a and 1954.

Type 7.

The 7th bivalent type to be elucidated had a sub-median centromere. The short arm contained a small portion of

chromatin which had a distinctive alternation of 1 large, 1 small, 1 large, 1 or 2 small chromomeres. No other worker observed this type which in the present analysis occurred 6 times. It may be that it is peculiar to this strain of L.esculentum sub species humboldtii. If its chromomeres were more nearly of the same size it would resemble type 7 of S.humboldtii described by Gottschalk (1954).

Type 8.

This was the nucleolar bivalent the identity of which has long been established and which was studied in detail by Brown (1949), Lesley J.W. (1937), Lesley M.M. (1938) and Lesley M.M. and Lesley J.W. (1935). During the present analysis type 8 occurred 6 times. In most of its chromomere detail it corresponded to nucleolar bivalents described by Barton (loc. cit.) and Gottschalk (1951a, 1954). It was, however, note worthy that the satellite was much longer than that found by Brown (1949) and Barton for "Suttons Best Of All" and corresponded more closely to those described by Gottschalk in his analyses. This bivalent type was one of those selected for examination in representatives of the genus Lycopersicon in order that its various evolutionary modifications might be correlated.

Type 9.

This bivalent type was isolated only twice during the

TABLE VI.

CORRESPONDENCES BETWEEN VARIOUS PACHYTENE ANALYSES OF LYCOPERSICON ESCULENTUM.

Reference.	Material.	Type Numbers.								
Chapman (This thesis)	L.esculentum. sub.sp.humboldtii.	1	2	3	4	5	6	7	8	9
Barton (1950)	L.esc.(Suttons Best of All.)	11	12	4	5	6	?	-	2	1
Gottschalk (1951a)	Solanum lycopersicum.	-	2, 4 or 5	10	6	10	11	-	1	12
Gottschalk (1954)	S.lyco. var rosarigerum.	2	4	6	-	11	10	-	1	12
" "	S.humboldtii.	2	4	6	-	11	10	7?	1	12

analysis and as such its frequency of occurence represented a significant departure from expectation. Since however its morphology was distinctive there was little doubt that it ought properly to be considered as a type. The distinguishing marks of this bivalent were its length and the approximately symmetrical distribution of its chromatin about an extremely asymmetrically placed centromere. A reason for the very low frequency of isolation from the nucleus was its length which presented greater opportunities for tangling. This would explain why Barton's estimate of the pachytene length of this bivalent exceeded the present one by some 50%. Very probably, therefore, because of the larger sample Barton used and the low frequency of isolation in the present analysis, his estimate was perhaps the more nearly correct. Type 9 described here corresponded to type 1 of Barton (1950) and type 12 of Gottschalk (1951a, 1954).

Conclusions.

In the analysis, which has just been described, a number of conclusions resulted, some of which related only to L.esculentum and some of which were of more general significance. Firstly, the analysis established that of the 12 bivalent types expected 9 could be identified since they had a characteristic morphology which was frequently observed. Secondly, most of these types which were

identified showed similarities to those described by previous workers.

Between the present work and that done previously by other workers an important difference, namely that of taking a definite limited sample, may be noted and its implication considered. Early workers distinguished "types" but either paid little regard to the concept of frequency as in the case of Gottschalk or used the idea of 'recognition' ambiguously as did Barton. In Barton's investigation types of bivalent were recognised prior to measurement, an approach which leaves the worker perhaps more subject to his pre-dispositions.

One other feature of the present "isolated bivalent technique" was the large number of unclassified bivalents which confronted the worker. Six of these showed resemblances to no others and it was inferred that these were distorted by the squashing process. The remaining 50 were generally similar and sharp differences among them could not be found, possibly because they did not exist. Any attempt to fractionate these bivalents into types would have been hazardous and it is surely not without point that in only 8 or 9 of their types was there agreement between Barton and Gottschalk.

Of more general significance was the qualitative relationship between frequency of isolation and bivalent

length. The longer the bivalent type the less frequently it was isolated. It appeared therefore that by reason of length not every bivalent stood an equal chance of isolation and one of the statistical requirements indicated earlier for identifying karyotypes was not entirely fulfilled. Furthermore since the shorter types tended to be isolated more often, it followed that every type whether long or short tended to have its average length at pachytene underestimated by this sampling procedure. This criticism was to some extent mitigated since the primary criterion of identification was morphology rather than measurement. Lastly, it may be observed that the analysis confirmed those points on which there was agreement between Barton and Gottschalk and provided explanations for some of the discrepancies between them.

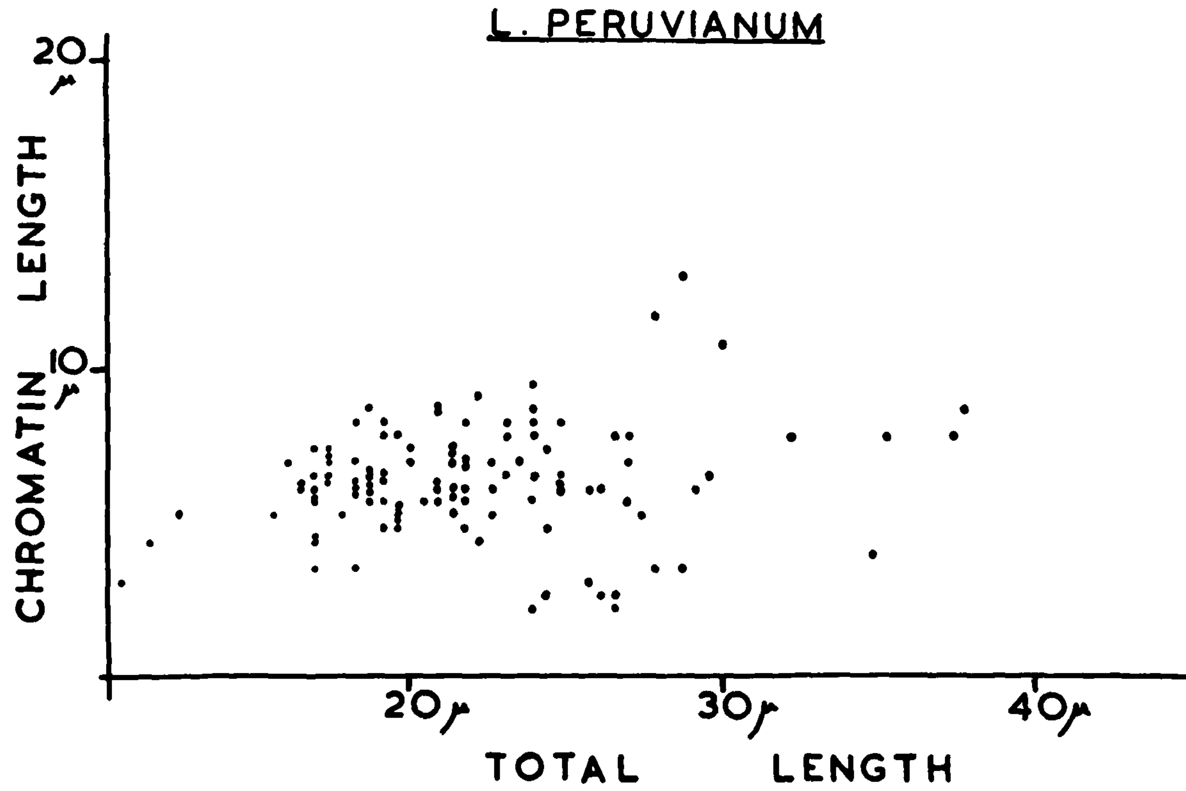
A Pachytene Analysis of Lycopersicon Peruvianum.

Examination of the pachytene structure of bivalents of L.peruvianum showed that the chromomere structure was relatively indistinct. Furthermore, the spreading of bivalents was very poor so that usually no more than 1 was isolated from any nucleus.

An analysis of L.peruvianum at pachytene was made with a view to detecting types similar to 5 and 8 previously described for L.esculentum rather than with characterising the whole genome. That such types probably existed was suggested by the work of Gottschalk (1954) but it was important to establish their frequencies and ascertain how many times each was represented in any nucleus.

120 bivalents were sampled and measurements of the various chromosome features obtained. Where possible an estimate of chromomere frequency was added but was not usually very reliable. A scatter diagram (Figure V) was plotted and this shows the same general shape as those previously presented. Of particular interest was the collection of 7 points situated in the region x = 25.0 , y = 3.0 . The bivalents which occurred here were very similar to type 5 described for L.esculentum except that they had less chromatin in the long arm. Supposing the expected frequency to be 10/120 the

FIGURE VI.



A Scatter Diagram of Chromatin and Total Lengths at Pachytene.

observed frequency of 7/120 did not depart significantly, (P = 0.5 to 0.3).

The nucleolar chromosome occurred 10 times in the analysis and thus exactly agreed with an expectation of 10/120. The morphology of this nucleolar bivalent type closely resembled that of type 8 in L.esculentum and homology was assumed between them. One difference was that the satellite bivalent of L.peruvianum had smaller chromomeres in its long arm. The morphology of types 5 and 8 as they occurred in the genus Lycopersicon will be discussed in detail in the next section. (See Tables VII and VIII, Plates III and IV and Figure VII).

The analysis revealed other bivalent types which showed resemblances to other types described in L.esculentum. They were, however, unsuitable for characterisation or detailed comparison because of their indistinct chromomere pattern.

Conclusion.

This analysis revealed two types presumably homologous to types 5 and 8 described for L.esculentum and to the two outstanding bivalents in L.pimpinellifolium.

TABLE VII.

MEAN MEASUREMENTS OF BIVALENT TYPE 5 IN THREE SPECIES.

Material	Freq.	Long Arm	Cent	Short Arm	Total length
L.pimp.	7/120	24.9 (3S + 2L + 1M)	0.6	5.37 (2M + 2L,+ 1M)	30.87
L.esc. sub sp.humb.	7/120	23.5 (2S + 1L + 1S + 1L + 1M)	0.87	5.7 (1M + 1L + 1M + 1S)	30.07
L.peru.	7/120	19.95 (4S + 1M)	0.7	5.24 (4L + 1S)	25.89

L = Large.)
M = Medium.) Chromomere sizes.
S = Small.)

Figures in brackets indicate chromomere sequence.

TABLE VIII.

MEAN MEASUREMENTS OF BIVALENT TYPE 8 IN THREE SPECIES.

Material	Freq.	Long Arm	Cent	Short Arm	Sat.	Total length
L.pimp.	5/120	22.3 (6L)	-	5.0 (5L)	3.4	30.7
L.esc.sub sp. humb.	6/120	22.7 (1S + 5M + 1L)	1.9	3.1 (1L + 4M)	2.6	30.3
L.peru	10/120	17.8 (3S + 2M)	1.3	3.3 (4L + 1S)	1.4	23.8

L = Large)
M = Medium) Chromomere sizes.
S = Small)

Figures in brackets indicate chromomere sequence.

Chromosome Evolution.

The work of Gottschalk (1954) provided evidence that morphological evolution of chromosomes had occurred, the evidence being differences in chromomere pattern of chromosome homologues in different species. Further data obtained in the course of this work showed the need for modification of some of Gottschalk's conclusions.

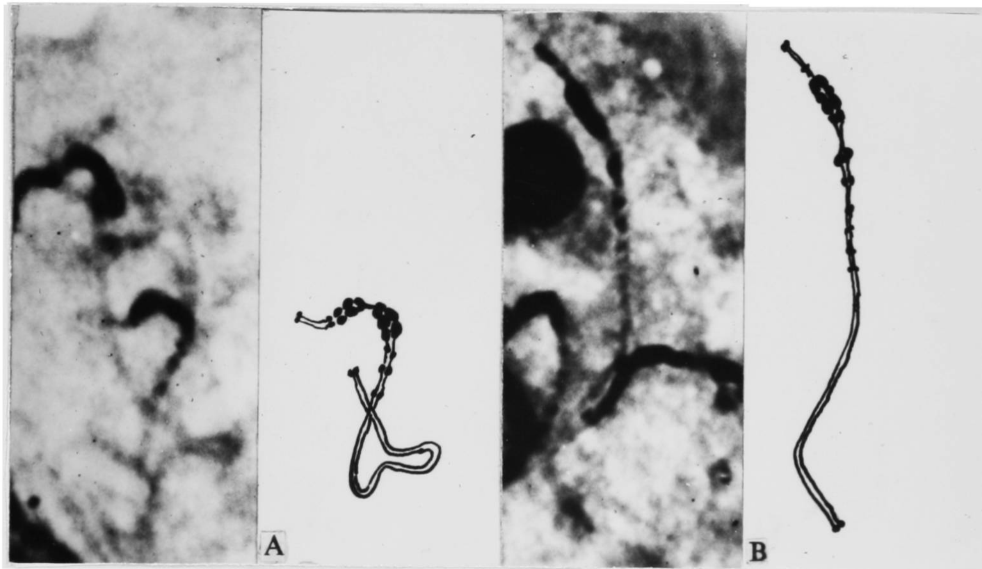
To establish interspecific chromosome differences a number of requirements needed to be met. It was, for instance, necessary to show that the chromosomes being compared were homologous and further that they were compared under as nearly identical conditions as possible. Both these requirements were met by reference to pachytene in Fi hybrids between the species being compared. One required too, that the differences were of sufficient magnitude to be clearly discernible. It will be evident that competing demands were made of those types chosen for examination. On the one hand one required a chromosome type to be reasonably distinct and on the other for it to show sufficient inter-species variation to provide a basis for study. Only types 5 and 8 described in L.esculentum and those similar to them in L.pimpinellifolium and L.peruvianum were considered sufficiently striking.

These chromosome types are shown in Plates III and IV and data collected from them are presented in Tables VII and VIII. Reference was also made to these types in a second strain of L.pimpinellifolium, L.esculentum sub spp. galenii and typicus and the commercial variety L.esculentum var. Suttons Best Of All. Material of L.hirsutum was grown but did not flower.

Type 5 was characterised in all species by its asymmetry and small portion of chromatin. Within this chromatin there was a distinctive chromomere pattern which was characteristic of each species examined. The chromomere sequence in L.esculentum for this chromosome differed from L.pimpinellifolium only in the order of a large and small chromomere in the long arm, (See Figures VII). In the sub-genus Eulycopersicon it was found that the principal difference between this and the sub-genus Eriopersicon, was that the former contained more chromatin in its long arm, (See Figure VII).

The differences observed in type 5 among the various species examined was explained in the following manner. L.esculentum differed from L.pimpinellifolium in respect of an inversion in the long arm involving two chromomeres. The differences between L.peruvianum and representatives of Eulycopersicon were explained satisfactorily by Gottschalk's theory of chromatinisation. It may be noted in passing that no variation in type 5 was found within the confines of

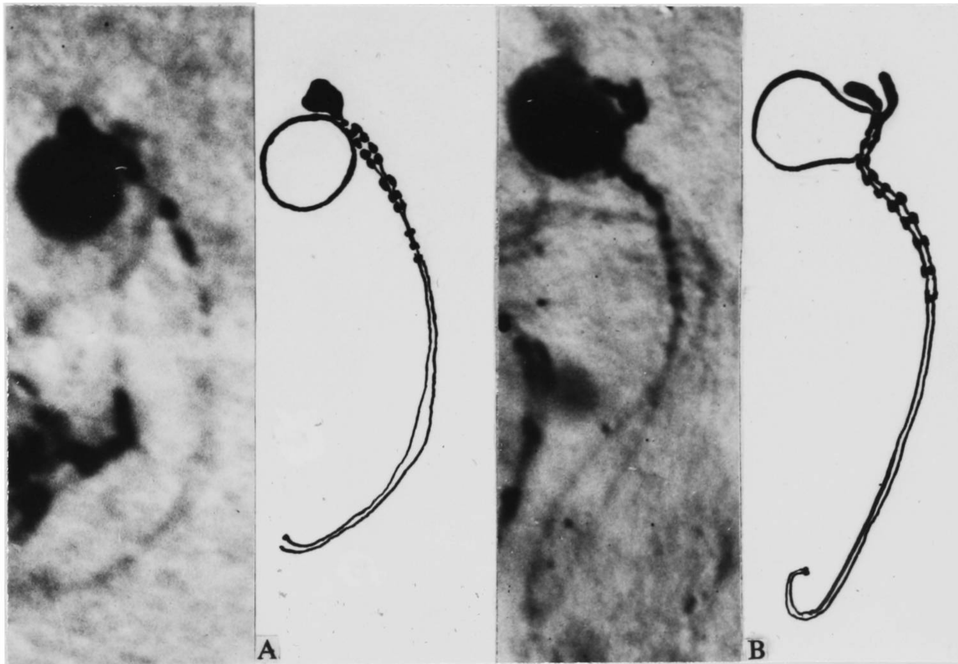
PLATE III.



III A - Type 5 in L.pimpinellifolium.

III B - Type 5 in L.peruvianum.

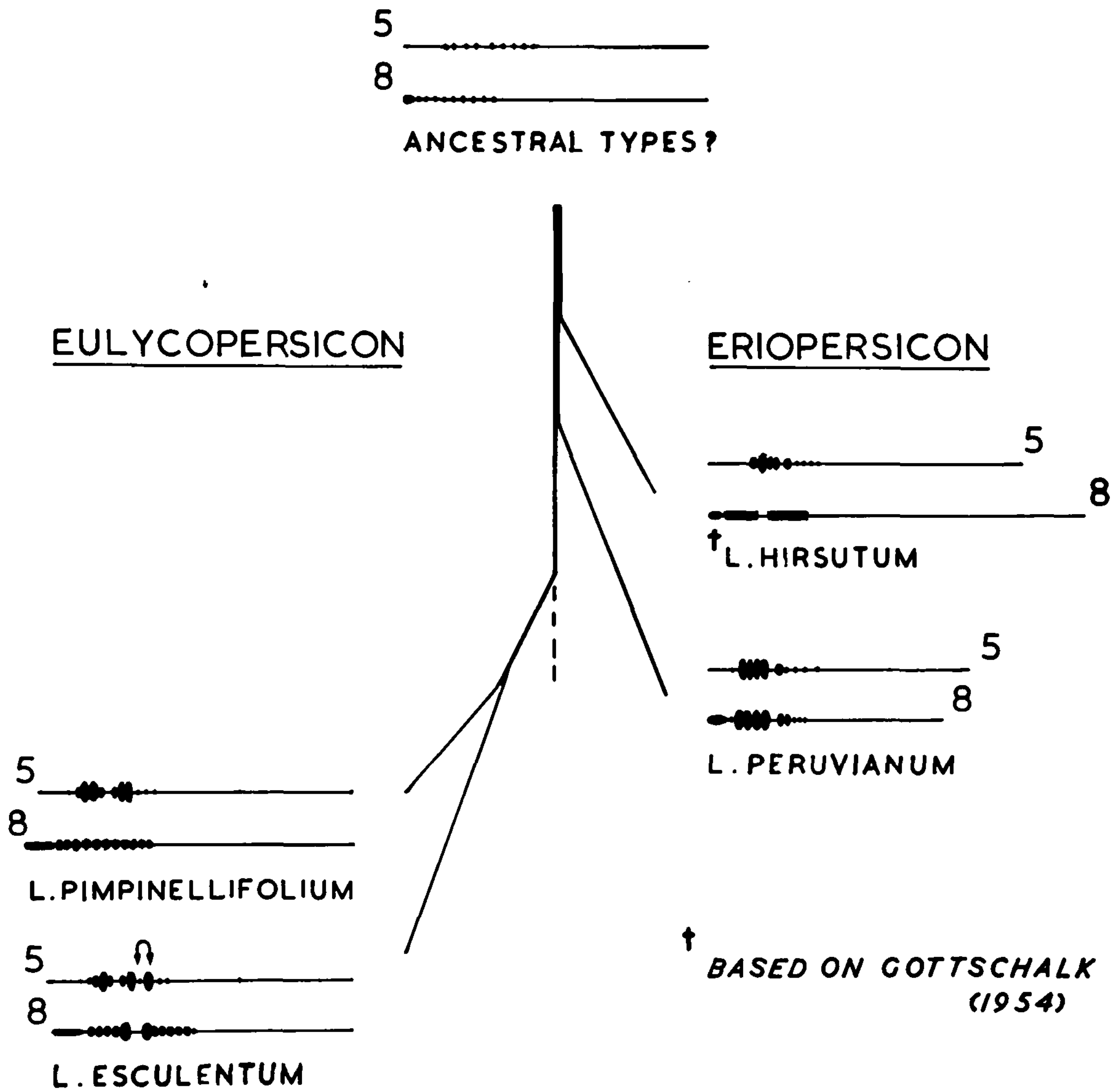
<u>PLATE IV.</u>



IV A - Type 8 L.peruvianum.

IV B - Type 8 L.pimpinellifolium.

FIGURE VII.

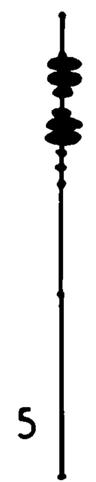


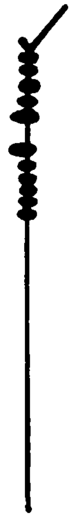
Chromosome Changes for Types 5 and 8 in Four Species of Lycopersicon.

L.esculentum.

Type 8 was much less variable throughout the genus, the most noticable differences occurred in the length of the satellite. These were smaller in *L.peruvianum* and longer in *Eulycopersicon* although Suttons Best Of All was an exception to this generalisation. The long arm of the nucleolar chromosomes possessed smaller chromomeres than those found in corresponding positions in *L.esculentum*, (See Figure VII). For this chromosome type, interspecific differences were adequately explained by Gottschalk's theory of differential chromatinisation.

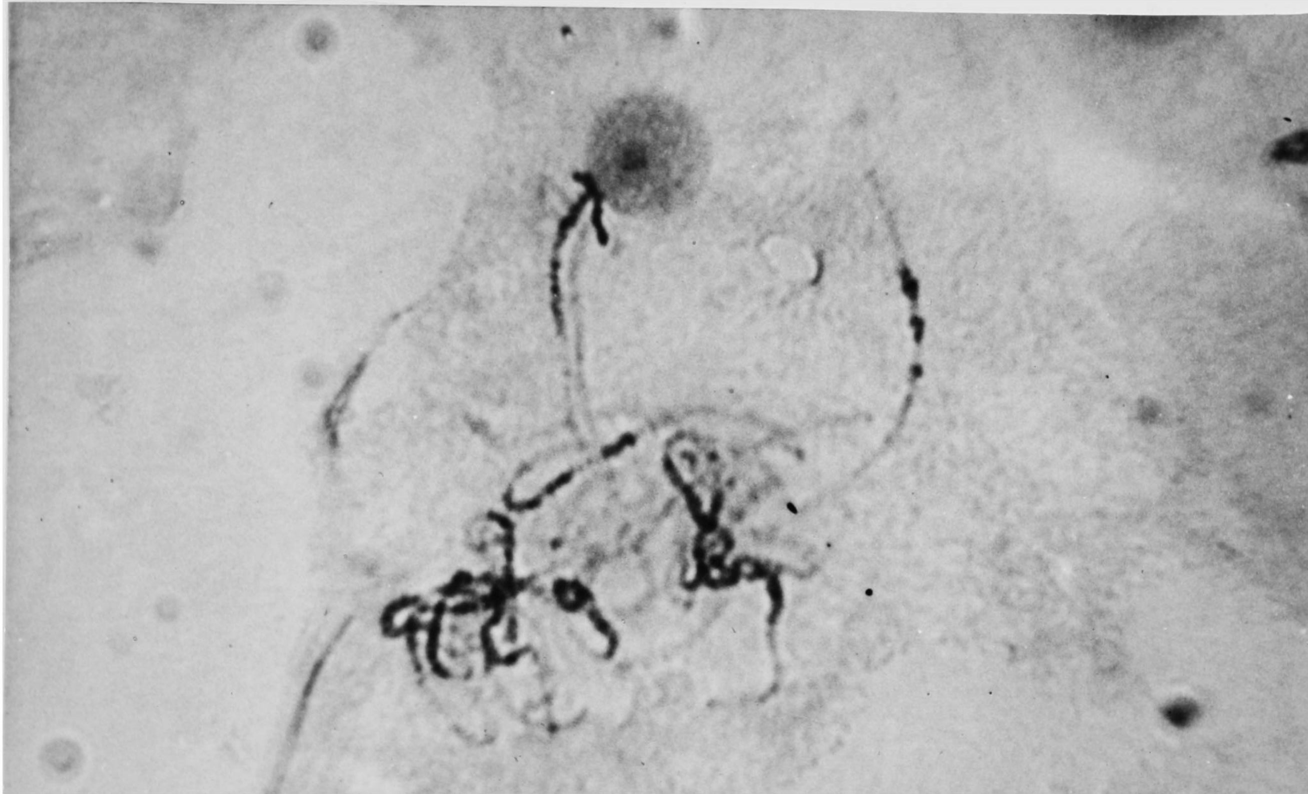
The supposed homology of type 5 representatives in *L.pimpinellifolium* and *L.esculentum* was confirmed as follows. A strain of *L.pimpinellifolium* having a relatively long satellite (about 3.5) was crossed with Suttons Best Of All which had a small knob-like satellite. Fi seed was sown and the plants examined at pachytene. Figure VIII illustrates diagramatically the expected morphology of bivalent types 5 and 8 at this stage in such a hybrid. Plate V shows how far this expectation was realised. The differential satellite morphology provided evidence of *bona fide* hybridity. A similar demonstration of homology was planned for *L.pimpinellifolium* and *L.peruvianum* but unfortunately a seed set was not obtained.





L. PIMPINELLIFOLIUM
8
5
X
8
5
L. ESCULENTUM
VAR. SUTTONS'
BEST OF ALL
F_1 HYBRID
8
5

PLATE V.



Heteromorphic Bivalents in an L.pimpinellifolium X L.esculentum Hybrid.

Conclusions.

Types 5 and 8 showed in the various species some variation of morphology. These differences were explained in one case by postulating an inversion involving two chromomeres and in other cases by Gottschalk's theory of differential chromatinisation. The homology of type 5 as it occurred in two species *L.pimpinellifolium* and *L.esculentum* was confirmed by reference to an Fi hybrid between them.

Evidently, therefore, "chromatinisation" has occurred but probably it is not the only method of chromosome evolution in *Lycopersicon*.

Diakinesis In Solanum and Lycopersicon Chromosomes.

The foregoing work provided evidence of small scale change for two chromosome types as they occurred in several species of Lycopersicon. Could these changes affect the adaptedness of the species possessing them? If these changes do have any effects how are they exercised?

"Each living species may be thought of as occupying one of the available (adaptive*) peaks in the field of gene combinations" (Dobzhansky, 1953). Introduction of foreign genes would tend, generally, to lower the fitness of an organism to its environment. However, the region of the tomato chromosome in which morphological change was observed has been thought by previous workers to be excluded from chiasma formation and consequently genetic recombinations, (Brown, 1949, Barton, 1951, Gottschalk, oral communication). If small scale modification takes place in regions not involved in the precise and orderly processes of meiosis then perhaps they are of little cyto-genetic significance. Suppose, however, that the opposite is true and that these regions are involved in recombination, It is at once evident that for species being crossed similarity of gene sequence is important and the

* In parenthesis added by the present writer.

facility of pairing paramount. The existence of minute differences in their linear orders of genes would give rise to minute deficiencies and duplications and, consequently, to sub-optimal fitness of their hybrid progeny. It was, therefore, important to establish whether or not the chromatic regions were involved in chiasma formation and gene reassortment.

Diplotene was found to be unsuitable in Lycopersicon for studies of chiasma position and frequency since the bivalents were twisted and often super-imposed. Diakinesis was, therefore, studied since it was possible to see in some cells all 12 bivalents and further the distinction between chromatin and achromatin was still evident.

Nine materials were examined at diakinesis including representatives of the genera Lycopersicon and Solanum and two inter-specific hybrids of the former genus. Bivalents were scored for chiasma frequency and location and the results are presented in Table IX. Plate VI shows a pollen mother cell of L.peruvianum where all bivalents were visible. Of particular interest was the chiasma observed in the chromatic segment of the nucleolar chromosomes.

The data presented in Table IX showed that by far the largest proportion of chiasmata were in the achromatic zones at diakinesis. 'Chromatic' chiasma were observed and some of these, possibly, were localised in interstitial achromatic

TABLE IX.

CHIASMA FREQUENCY AND LOCATION IN LYCOPERSICON AND SOLANUM.

Material	No. of cells	Achromatic chiasmata	Chromatic chiasmata	Total chiasmata	Standard error (total).
L.esculentum sub.sp.galenii	15	18.13	0.06	18.20	2.67
L.esc. gal.	15	19.60	0.06	19.66	1.54
Solanum stenotomum	10	17.00	0.50	17.50	1.78
L.esc.Suttons Best of All	10	19.40	0.50	19.90	2.12
L.peruvianum	12	17.66	0.16	17.83	1.92

Continued.

TABLE IX (Cont)

CHIASMA FREQUENCY AND LOCATION IN LYCOPERSICON AND SOLANUM.

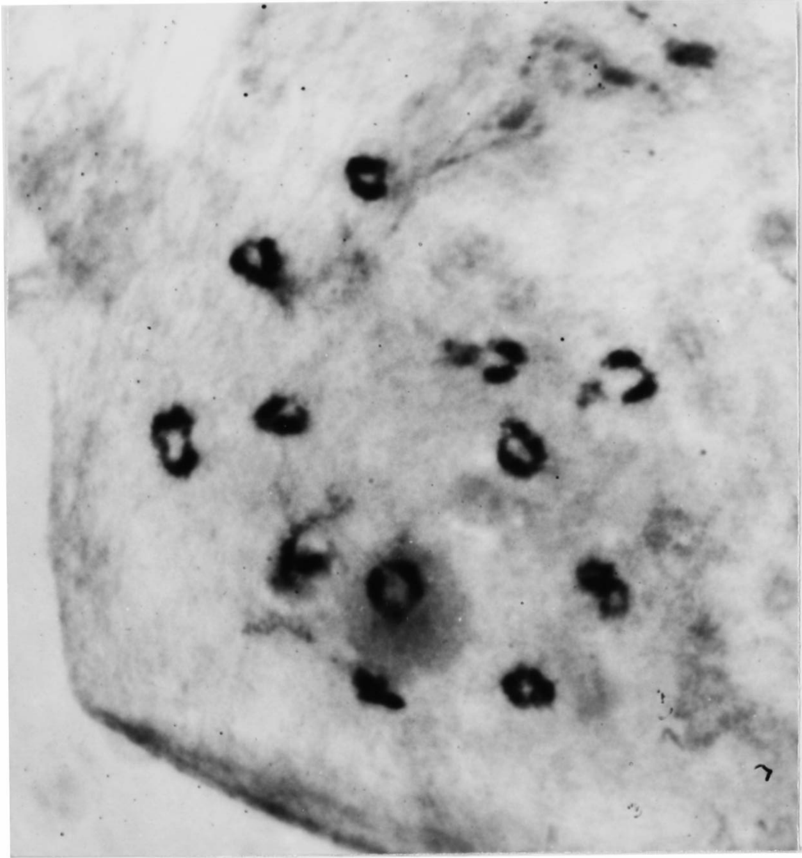
Material	No.of cells	Achromatic chiasmata	Chromatic chiasmata	Total chiasmata	Standard error (total).
L.pimpinelli-folium	10	17.80	0.00	17.80	1.93
L.pimp.	10	19.00	0.10	10.10	1.66
L.pimp. x L.esc. Suttons Best of All.	10	19.00	0.20	19.20	2.02
L.pimp. x L.esc. sub.sp. humboldtii.	10	21.80	0.00	21.80	0.91

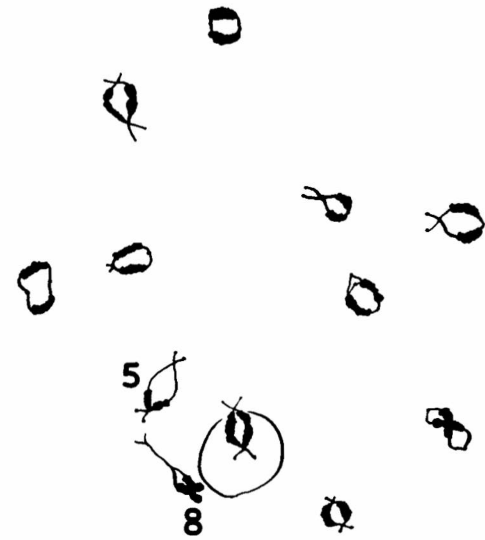
regions. Chromosome type 1 might give rise to this sort of localised chiasmata. More difficult to explain by this hypothesis were the occasional instances of chiasmata in the chromatic sections of the nucleolar bivalent where no interstitial achromatin has been observed.

Conclusions.

Examination of diakinesis showed that the majority of chiasmata were to be found in the achromatic regions. Chiasmata were occasionally seen in the chromatic regions even where no achromatic segments were discernable at pachytene. The distal location of chiasmata at diakinesis could consequently be explained in two ways. Either chiasmata were formed for the most part in the achromatin or else they were formed indifferently throughout the chromosomes with subsequent terminalisation during diplotene. From this evidence it seemed unwise to exclude the possibility of chiasma formation within the chromatin.

PLATE VI.





Diakinesis in L. peruvianum.

Chiasma Frequency in Particular Chromosome Types.

In the preceeding section of this thesis evidence was presented of chiasma formation in the chromatic regions including some instances where no interstitial achromatin was observed at pachytene. Examination has shown that two or three of the bivalent types identified at pachytene may be distinguished at diakinesis. These were types 5, 8 and 9. It seemed, therefore, feasible to explore the relationship between chromosome length and chiasma frequency.

Type 5 in L.peruvianum was of especial interest because of its peculiar morphology. Chromatin formed about 50% of the length of the short arm at pachytene whereas the long arm was almost devoid of chromatin. Consequently, the ratio of chiasma frequency in the two arms was compared to the ratio of arm lengths and other chromosome ratios.

30 pollen mother cells of L.peruvianum and L.pimpinellifolium were examined at diakinesis and in each of the cells it was possible to see all 12 bivalents. The frequencies of chiasmata, for type 5, were scored in the long and short arms. The results obtained, together with various ratios for type 5 in these species, are given in Table X. The various length ratios were based on data collected at pachytene.

The ratio most similar to the chiasma frequency ratio was that for arm length. A statistical analysis confirmed

the apparent similarity between the ratios (long arm/short arm) and (frequency of chiasmata in the long arm/frequency of chiasmata in the short arm) since they were well within the limits of their standard errors. For L. peruvianum this was especially surprising in view of the great difference in the chromatin content of the two arms. Presumably, therefore, chromatin did not hinder the formation of chiasmata.

The ratio of chiasma frequencies was in all cases slightly less than the ratio of arm lengths and this was accounted for in the following way. Brown (1949) established that achromatin contracted to a greater extent than chromatin. Since, therefore, the long arm would contract relatively more than the short arm, then probably at the pachytene-diplotene interphase the arm ratio would more nearly co-incide with the chiasma frequency ratio. It is, of course, that at this point during meiosis chiasmata are formed.

Some chiasma frequency data was obtained for the satellited bivalent at diakinesis and chromatic chiasmata were recorded in the completely chromatic short arm. A ratio of arm lengths was calculated which was noticably larger than the chiasma frequency ratio. Since the bulk of the contraction throughout pachytene would be in the long arm, it seemed reasonable ~~to~~ postulate a closer agreement between the arm length ratio and the chiasma frequency

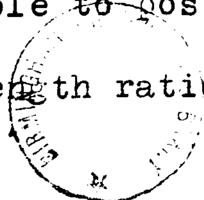


TABLE X.

CHROMOSOME RATIOS AND CHIASMA FREQUENCY RATIOS IN LYCOPERSICON.

Material	(a) L.A. Chrom / S.A.Chrom.	(b) L.A.Achrom / S.A.Achrom	(c) L. arm / S. arm	(d) L.A. chias.	(e) S.A. chias.	(f) (d/e)	Comp.S.E. (c) & (f)
Type 5 L.peru. (late pachytene)	Less than 0.2	6.77	3.98	30	8	3.75	12.65
Type 5 L.pimp. (early pach.)	0.85	7.75	5.12	30	7	4.29	14.76
Type 5 L.pimp. (mid pachytene)	0.81	6.85	4.69	"	"	"	13.68

ratio at the pachytene-diplotene interphase.

Chromosome types 5 and 8 were shown to differ by a small inversion and a different degree of chromatinisation, respectively. This was for L.pimpinellifolium and L.esculentum. Further, the evidence suggested that the regions in which these small changes were detected were subject to chiasma formation and presumably to gene reassortment.

Table XI contains data derived from analyses of diakinesis in two interspecific hybrids of L.pimpinellifolium and L.esculentum. In those arms in which the chromatin variations were detected no depression of chiasma frequency was observed. If, as seemed likely, these chromosomes formed chiasmata at random then the presence of a small inversion in type 5 would lead to the occurrence of duplications and deficiencies in the chromosomes of the hybrid F_2 progeny.

Apposite to this work was some data published by Barton in 1950 and 1951. In the first paper he presented measurements for the 12 chromosome types of L.esculentum at pachytene. His table included data for his type 1 which has been shown synonymous with type 9 described in this thesis. In 1951 he published chiasma frequency data for this bivalent type without apparently noticing any correlation between this and his earlier results.

TABLE XI.

Chiasmata Ratios In 2 Heteromorphic Bivalents.

Material	(a) L. Arm / S. Arm	(b) L.A. Chiasmata	(c) S.A. Chiasmata	b / c
L.pimp. x L.esc. (type 5) i.	L.pimp.4.69 L.esc. 4.12	30	9	3.33
L.pimp. x L.esc. (type 5) ii.	" " " "	60	16	3.75
L.pimp. x L.esc. (type 8) ii.	L.pimp.4.45 L.esc. 7.30	67	12	5.55

The relevant data is set out in Table XII and it will be seen that for this chromosome type too, the chiasma frequency ratio is more similar to the ratio of arm lengths than to any other ratio. The ratio of arm lengths was again slightly higher than the ratio of chiasma frequency. Since the long arm is considerably more achromatic it would contract to a relatively greater extent and at pachytene -diplotene the arm ratio would probably more nearly agree with the chiasma ratio.

Conclusions.

Two conclusions were formed. Firstly, the ratio of arm lengths most closely resembled the ratio of chiasma frequencies and further, chiasmata were found in wholly chromatic regions. This suggested that chiasmata were formed at random in both chromatic and achromatic regions. Secondly, since changes in chromosome morphology were found, which were so small as to be detectable only at pachytene, and which did not influence chromosome pairing, this was considered to be an instance of cryptic structural differentiation in the sense of Stebbins (1950).

TABLE XII.

CHROMOSOME 9, CHIASMA FREQUENCY AND BIVALENT LENGTH.

Modified from Barton, 1950/51.

Plant No.	Achromatic region.			Chromatic region.		Non-class.
	Short Arm 1 xa	Long Arm 1 xa	Long Arm 2 xa	Short arm	Long arm	
824.17	11	27	11	0	0	1
824.30	17	25	7	0	0	-
Total xta	28	52	36	0	0	116

TABLE XII (Cont)

L.A. Chrom. / S.A. Chrom.	L.A. Achrom. / S.A. Achrom.	L. arm. / S. arm.	L.A. Chias. / S.A. Chias.	Plant No.
			4.45	824.17
			2.30	824.30
1.19	7.44	3.9	3.18	Mean.

Discussion.

In many species with small chromosomes the number of chiasmata is low and not much significance can be attached to the necessarily small fluctuations in chiasma frequency which occur among intra and inter species crosses. In an attempt to find other ways of distinguishing between species at the cytological level attention has been directed to the morphology of chromosomes at pachytene.

The approach which was adopted here was essentially statistical. It was suggested that the reliability of any particular chromosome morphology as a'type' depended upon the frequency with which it was observed. Further, it was assumed that each chromosome type would be isolated from the nuclear mass, in a large sample, with a frequency proportional to the number of times it occurred in the nucleus.

The results which were subsequently obtained provide a means of assessing the value of this approach. It was found that it was not possible to identify all the expected 12 chromosome types in any species examined. In the case of L.esculentum, however, 9 chromosome types were defined.

That a karyotype cannot be completely characterised does not proscribe inter-species comparisons provided that attention be confined to the obviously distinctive chromosome types. By this statistical method it was shown that

those chromosome types selected for detailed comparison occurred only once in any nucleus. It was more evident therefore, that genuinely homologous chromosomes were being compared. That they occur only once in any nucleus provides morphological evidence that the basic number among Lycopersicon species is twelve rather than six. If the basic number were in fact six then since that point in time, at which doublings of this number occurred, the'homologous chromosomes' have been modified so as to oompletely obscure their underlying similarity. It may be mentioned that no convincing case of polysomic segregation in tomatoes has been established,(Rick and Butler, 1956.)

The most obvious disadvantage of the present method was that since all the chromosomes were not the same length the longer bivalents at pachytene, having greater opportunities for tangling, were isolated less often. Therefore, the shortest 'types' tended to be isolated more frequently and, further, the shortest representatives of any type were most likely to be isolated. Presumably, therefore, the mean lengths for each type were under-estimated for mid-pachytene, the error being greatest in the case of the longest types. It must be remarked, however, that differences in length assisted the elucidation of the various types.

As regards pachytene analysis, it appears that provided adequate emphasis be layed upon the statistical approach, it is possible to identify a large proportion at least of the chromosome types in a species such as L.esculentum which has a somewhat recondite chromomere pattern. It was moreover found possible to obtain convincing evidence of small scale chromosome modification from species to species, provided examination was restricted to obviously distinctive types.

Against this background, it is possible to appreciate the differences observed in pachytene chromosome morphology in this and other studies of Lycopersicon and Solanum. Gottschalk, (1944), Gottschalk and Peters, (1955) claimed that many species of Lycopersicon and Solanum differ in all their chromosomes. This seems an adventurous conclusion, however, in view of the difficulty of identifying chromosome types and particularly as they attempted in no case to confirm their results by reference to pachytene in F.i hybrids between the diploid species they had studied.

Gottschalk's results were vigorously and cogently challenged by von Wangenheim, (1957) who examined a large number of interspecific hybrids of Solanum at pachytene. He concluded that their karyotypes were very similar since the greatest interspecies chromosome disparity observed was of the order of one or two chromomeres. Differences in the size of paired chromomeres were also reported by

von Wangenheim.

The present work demonstrated the occurrence of chromosome modification on a scale more akin to that claimed by von Wangenheim. It was found that L.esculentum and L.pimpinellifolium differred by an inversion involving two chromomeres. Lima de Faria observed in rye that chromomere size diminishes away from the centromere. This condition was observed to be the usual one in Lycopersicon. Type 5 in L.esculentum differs from normal in this respect whereas L.pimpinellifolium is normal in this respect. It was, therefore, concluded that L.pimpinellifolium Type 5 represents the original condition and its homologue in L.esculentum the derived condition.

Both L.esculentum and L.pimpinellifolium differred from L.peruvianum in possessing more chromatin in their chromomeres, for chromosomes types 5 and 8, a finding Gottschalk would explain by 'chromatinisation'. Satellite material is interesting in that while it has a similar staining reaction to chromatin, its greater or lesser lengths in various species appears to be due to either extension or truncation rather than conversion from achromatin.

The theory of chromatinisation has been put forward by Gottschalk to account for differences in chromomere size and number between the homologues of various species

and genera of the *Solanaceae*. The theory as he stated it implies that greater chromatin content is related to evolutionary advancement. Two considerations militate heavily against this view. It seems for example that the closely related *Petunia* and *Nicotiana* differ markedly in the amount of chromatin they possess - the former having none and the latter having much, Gottschalk, (1954). It seems, therefore, that no phylogenetic significance can be attached to this character. (A similar sort of reasoning might be to suppose that chromosome number *per se* was a measure of evolutionary development. Undue emphasis upon a minority of facts can provide evidence for spurious relationships.) Further, von Wangenheim's careful examination of *Solanum* hybrids shows that for *Solanum* at least the differences the theory seeks to account for are largely non-existent. The theory of chromatinisation therefore, in the *Solanaceae* as a means or as a measure of phylogenetic advance seems ill grounded and is perhaps best discarded.

The assumption that small scale differences do occur, however, among species of *Lycopersicon* and *Solanum* now seems justified. Such small changes have both a cytogenetic and a cytochemical significance. From a cytogenetic viewpoint changes in the chromatic regions are probably of greater or lesser importance depending upon whether they do or do not form chiasmata with

consequent genetic reassortment. The current view among students of Lycopersicon cytology is that chromatin does not form chiasmata. Evidence in support of this view derives partly from Brown, (1949) who claimed that no chiasmata were observed in the wholly chromatic short arm of the nucleolar chromosome. The other data supporting this view was put forward by Barton, (1951) who observed chiasmata at diakinesis only in the achromatic regions of Lycopersicon chromosomes.

Whilst neither Brown nor Barton speculated upon the evolutionary consequences of non-reassortment in sections of Lycopersicon chromosomes, the latter worker did note that large blocks of genes would maintain a similar grouping from generation to generation. Beal B. Hyde (1953) suggested, however, that certain genetic advantages would accrue from non-reassortment notably that a successful genotype would sustain its fitness in an environment to which it was especially well adapted.

There are a number of theoretical objections to this view. A restraint upon reassortment whilst it would prevent the disruption of successful gene combinations equally would preclude the formation of better fitted ones. Further, as Barton pointed out, the only possibility of change within a chromatic region would be mutational.

Genetic theory suggests (Dobzhansky, 1953) that since contemporary species are at or near an adaptive peak, most mutations will be deleterious and, it may be conjectured, such changes within the chromatin would be for the worse. Even a useful mutation would under these conditions have to be immediately and decisively successful since its potential value could not be very conveniently exploited against a variety of gene backgrounds.

These arguments lead one to question the view that chromatin does not form chiasmata and undergo reassortment of its gene material. Furthermore, two results support this. Chiasmata were occasionally observed in the chromatic regions at diakinesis even in the wholly chromatic short arm of the nucleolar chromosome. It was shown too, that provided one allows for the differential contraction of the *Lycopersicon* chromosome, chiasma frequency is proportional to chromosome arm length. The conclusion derived, therefore, was that both the chromatic and achromatic regions form chiasmata and that consequently genetic reassortment takes place throughout the chromosome. The location of chiasmata only in the achromatic zone at diakinesis may be presumed a result of terminalisation.

Since it appears that chromatin is involved in chiasma formation and may be presumed to undergo gene

reassortment the linear order of its genes is important. If, therefore, species differ by small inversions and chiasma formation is at random then whilst an interspecific F.i hybrid might be normal, the genetic material of its progeny would contain small duplications or deletions involving relatively few genes. A probable consequence would be sub-optimal fitness or genetic breakdown in a proportion of the progeny in each of the post F.i generations.

Inversion would still occur in the genus Lycopersicon and M.M.Lesley, (1950) showed that L.peruvianum and L.esculentum differred by an inversion sufficiently large to produce a bridge at meiosis in their F.i hybrid. If, however, inversions arise so small as not to give rise to such bridges and moreover contained chromomeres of similar size, they would be difficult to detect. It is known that genetic breakdown occurs in post F.i generations of interspecific hybrids in both Lycopersicon (Langford, 1948) and Solanum (Hawkes, 1956). Possibly, therefore, a proportion of this breakdown may be explained by the presence of small inversions similar to that detectable only at pachytene between L.esculentum and L.pimpinellifolium. This sort of change comprises an instance of cryptic structural differentiation, (Stebbins, 1950).

The following quotation from Stebbins may serve to describe the phenomenon in greater detail.

"The apparently wide spread existence of chromosomal sterility due to heterozygosity for structural differences so small as not to materially influence chromosome pairing has led the writer to propose a name for this situation: Cryptic structural hybridity ......

"The fact must be emphasized here that much of this cryptic structural hybridity is difficult to discover only because in most plants the best stage for studying gene by gene pairing of the chromosome, the pachytene stage of meiosis, is not clear enough for analysis."

Instances of authentic cryptic change in the chromosomes of natural populations are very few. The tribe maydeae of the Graminae includes the species Tripsacum and Zea. Manglesdorf, (1947), reported that at pachytene chromosomes of Tripsacum differred from those of some strains of Zea in possessing chromatic knobs. Chromosome modification has been studied, in Drosophila in greatest detail and the subject has been comprehensively reviewed by Dobzhansky (1953). From this it appears that a large number of non-visible chromosome changes occur whose positions as gene loci have been accurately mapped. Studies

on the salivary gland chromosomes show a wide range of chromosome changes which occur involving anything from one or two chromatin discs to a whole chromosome arm. But for the existence of the polytene nuclei we should regard many of the chromosome changes in Drosophila as falling in the 'cryptic' range. Many of the changes observed fall within the limits of species and thus it may be concluded that these changes currently, play a part in modifying the adaptive capacity of those individuals that bear them.

Stebbins, (1950) compiled a list of seven inter-species crosses which gave rise in the F.1 to vigorous hybrids and in the F.2 to weak, unthrifty or degenerate individuals and there is little doubt but that the list could be considerably extended. Muntzing (1930a) observed in Galeopsis tetrahit X G.bifida a segregation for fertility among the F. 2 population.

More recently, Stevens, (1950) in a review of genetic behaviour in Gossypium suggested that the theory of 'multiple gene substitution' (Harland, 1936) could be replaced by a theory invoking cryptic chromosome change to explain speciation in this genus. He based this view on the occurence in interspecies crosses of reduced crossing over, skewed backcross ratios and block transference of characters resulting from conscious selection of only one gene.

In none of the cases where F.2 breakdown has been reported has the analysis been pressed to the point whereby minute deletions and translocation have been detected. The present study of pachytene emphasizing the statistical aspect and the conclusions which may be drawn from distinctive chromosomes provides us with a means of studying this problem.

From a cytochemical viewpoint there is very little information available about Lycopersicon chromosomes. Two workers (Gottschalk, 1951a,b Barton, 1954) have reported on the differential breakage rates of achromatin and chromatin but their results are not strictly comparable. The only point of agreement between these workers seems to be that in response to X-radiation more breaks occur in the chromatin than the achromatin. Such information does not provide a basis for a discussion of the biochemical nature of chromatin and achromatin.

It will be evident that the original purpose of this thesis, the study of pachytene to provide a taxonomic criterion was not fulfilled. Since only a small portion of the genome is available for detailed comparison, a comprehensive assessment for each species would not be feasible. None the less, the study of chromosome differentiation in a tomato can contribute to a main stream of contemporary cytogenetic enquiry.

PART II.

A Study of Metaphase of Meiosis in some Polyploid Species of Solanum.

Some Evidence Relating to the Possible Origin of Solanum Juzepczukii and S.Curtilobum.

Introduction.

In 1939 Bukasov sought to account for the origin of two Solanum species, namely S.juzepczukii and S.curtilobum. He supposed that S.juzepczukii which is a triploid (2n = 36) was derived by natural hybridisation from the tetraploid S.acaule (2n = 48) and a diploid species in the series Tuberosa. S.curtilobum was, he thought, derived from a hybrid between S.juzepczukii and S.andegenum. S.curtilobum (2n = 60) is a pentaploid species and presumably an unreduced gamete of S.juzepczukii would have functioned at syngamy. The work of Bukasov was, for the most part, descriptive taxonomy.

A hybrid was described in 1947 by Hawkes, between S.acaule and S.stenotomum (2m = 24) which was triploid and in its morphology resembled S.juzepczukii. The meiotic behaviour was, however, not examined. Both S.juzepczukii and S.curtilobum were mentioned in the papers of Rybin (1933) and Lamm (1945) but neither of these workers presented comprehensive data of meiotic behaviour. Lamm recorded that S.curtilobum spread very poorly and hindered the elucidation of the various chromosome associations. It will be seen, therefore, that taxonomic speculation has proceeded in the absence of a knowledge of the cytology of these two species.

By examination of these species cytologically together with some experimental hybrids of known origin it was proposed to test Bukasov's original suggestion as to their origin. This work formed the object of the present enquiries. During this work it became evident that interpretation of the various configurations previously recorded in the literature of Solanum cytology varied somewhat from author to author, and consequently this subject was considered in some detail.

A frequent source of discrepancy has been the scoring of chiasmata and reference to Swaminathan and Howard (1953) suggested that these were due to differences of opinion among workers and not merely to variations in the material chosen for examination.

Trivalents were observed by Muntzing (1933) who distinguished between "chain" and "Y" types. The latter he referred to as having "triple terminal chiasma". Although three ends may be involved in these configurations the number of chiasmata would be only two. Lamm (1945) considered that although there may be two types of trivalent some uncertainty could arise when scoring since a chain type configuration when drawn into a reflexed position mimicked the Y type trivalent. Swaminathan (1954) observed that trivalents in the pentaploid hybrid (S.demissum X S.tuberosum) were all of the chain type although his diagram showed both chain and Y trivalents. The underlying confusion in all this work seems to have arisen from the varying orientation a

a chain trivalent may take up.

Propach(1938) found mostly chain or ring quadrivalents. It is surprising, therefore, that Lamm (1945) found eight of the various quadrivalent types drawn by Darlington (1933) even though Lamm's study was extensive, embracing representatives from most of the tuber bearing series. Swaminathan (1954) observed in S.andegenum and S.tuberosum quadrivalents of three types, "chain","ring" and "Y". One was led to the conclusion that there was no generally held view as to probable range of multivalent configurations or indeed of the underlying properties of Solanum chromosomes influencing this range. As a prelude, therefore, to the work on S.juzepczukii and S.curtilobum chromosome association in Solanum will be discussed.

MATERIALS AND METHODS.

(1) Plant Material.

The materials used in this study were as follows :-

S.juzepczukii (CPC No.1632).

This clone of S.juzepczukii was derived from the collection of Dr. J.G.Hawkes at Birmingham and was for the purposes of this work propagated clonally.

S.acaule (CPC No.2295/1 X S.stenotomum (CPC No. 878)

This hybrid was made by Dr.J.G.Hawkes and was studied by him prior to this enquiry. Two clones were grown and subsequently propagated by tubers for cytological examination.

S.curtilobum (CPC No. 1943).

This was from the same source as S.juzepczukii and was again propagated clonally.

Complex Hybrids.

One hybrid was obtained thus; the previously described hybrid S.acaule x S.stenotomum was crossed with S.tuberosum sub species andegena (CPC No. 2154). Since the plant was pentaploid the most obvious assumption was that an unreduced gamete of the triploid plant had functioned. The other hybrid

The taxonomic status of S.andegena (Juz and Buk) has been revised. Hawkes (1956) considered that this species ought more properly to be classified as a sub species of S.tuberosum. The spelling was altered to accord with the Index Kewensis. It is, therefore, to as S.tuberosum sub species andegena (Juz and Buk) Hawkes. ^

CPC numbers refer to those originally given by the compilers of the Commonwealth Potato Collection.

was obtained by crossing a hexaploid sibling of the S.acaule X S.Stenotomum cross with S.tuberosum sub species tuberosum var. "Teton". This formed a parallel case with the former pentaploid hybrid in so far as thirty six of its chromosomes were derived from the experimental triploid. All hybridisation was done by Dr. J.G.Hawkes and to him I am indebted for the use of this material.

(2) Cultural Methods.

The cultural procedures for growing the plants were similar to those described in the previous section.

(3) Staining Techniques.

Chromosome counts by means of the Feulgen technique were made and the results are given in Table I.

Chromosomes at metaphase of meiosis were stained by one of two methods involving modifications of Bellings (1926) iron aceto carmine technieque. The first was that due to G.E.Marks (1952). Anthers were fixed for 12 hours or longer at 0-3 degrees centigrade in a solution of ferric acetate in one part glacial acetic acid; three parts absolute alcohol. Subsequently, the material was transferred before staining to a solution containing five parts of ferric acetate in 45% acetic acid: five parts of 45% acetic acid: two parts 1% aqueous formalin for five to fifteen minutes at room temperature. An aceto carmine squash was then made.

The second method, that of P.T. Thomas, (1940) originally developed for staining small chromosomes in general, was modified for use with Solanum. The basic principle of the method is that small chromosomes may be more effectively stained by using a weaker carmine and more iron than is usually the case. Further iron may usefully be added to the prefixative. In the present work a 0.2% solution of carmine in 45% acetic acid was found most satisfactory.

TABLE I.

Chromosome Numbers.

Material	Somatic Chromosome Number.
S.Andegenum CPC 2158.	48
"Tuberosum Material"	60
"Andegena" Material	60 (Hawkes).
S.Juzepczukii CPC 1632	36
S.Stenotomum	24
S.Acaule x S.Stenotomum i.	36
S.Acaule x S.Stenotomum ii.	36

Chromosome Associations in Solanum.

There has been some disparity of interpretation by previous workers of the various chromosome associations seen at metaphase of meiosis in *Solanum* polyploids. The various studies in prophases of plants in the family *Solanaceae* have emphasized the nature of pre-metaphase of meiosis here. Each of the associations seen in the present study will now be discussed.

(1) Univalents.

Univalents were frequently observed at metaphase of meiosis in polyploids. In diploids a pair of univalents was occasionally seen which was attributed to precocious separation of a bivalent. Doubtless, precocious separation occurred at the polyploid level but chromosomes so separated were mostly indistinguishable from unpaired chromosomes (Plate 1A).

(2) Bivalents.

Chiasma frequencies in *Solanum* seldom, if ever, exceed two per bivalent at metaphase and, consequently, they may be classified as 'rods' or 'rings' depending on whether one or both arms have chiasma (Plate IB and C). Swaminathan and Howard (1953) remarked that most chiasmata were terminal. This may be explained by reference to Plate VI and Table IX of the previous Part where it was shown that usually only the distal (achromatic) portions of the chromosome contained

chiasmata, presumably as a result of terminalisation during diplotene and diakinesis.

(3) Trivalents.

Although theoretically possible, no trivalent was observed in which there were more than two chiasmata and consequently only two basic types were possible, namely "chain" and "Y" trivalents, (Plates I.D and E.). A third possibility was the occurence of a pseudo Y type which was really a chain trivalent drawn into a reflexed position (Plate I.F). Because of this, in the opinion of the present writer, it was impossible to estimate satisfactorily the proportions of chain and genuine Y trivalents.

(4) Quadrivalents.

In the present study four main types of configuration were observed, of which three are illustrated, (Plate I.G, H, J.). The four types were Y̶I, I=I, ǂ and possibly X although this latter type was not demonstrated unequivocally. The highest number of chiasmata occurs in the open ring and even here did not exceed one per chromosome.

(5) Pentavalents.

Pentavalents were found only rarely and were considered to be of two types of which one is illustrated, (Plate I.K). Their configuration and orientation were probably decided in a similar manner to that of those multivalents previously discussed. Elucidation of configurations containing this number of chromosomes was not simple.

PLATE I.

Configurations in Solanum

at Metaphase of Meiosis.

I A Two Univalents.

I B Bivalent. One Chiasma.

I C Bivalent. Two Chiasmata.

I D Linear Trivalent. Two Chiasmata.

I E Y Trivalent. Two Chiasmata.

I F Pseudo-Y Trivalent. Two Chiasmata.

I G Ring Quadrivalent. Four Chiasmata.

I H Linear Quadrivalent. Three Chiasmata.

I J Y Quadrivalent. Three Chiasmata.

I K Linear Pentavalent. Four Chiasmata.

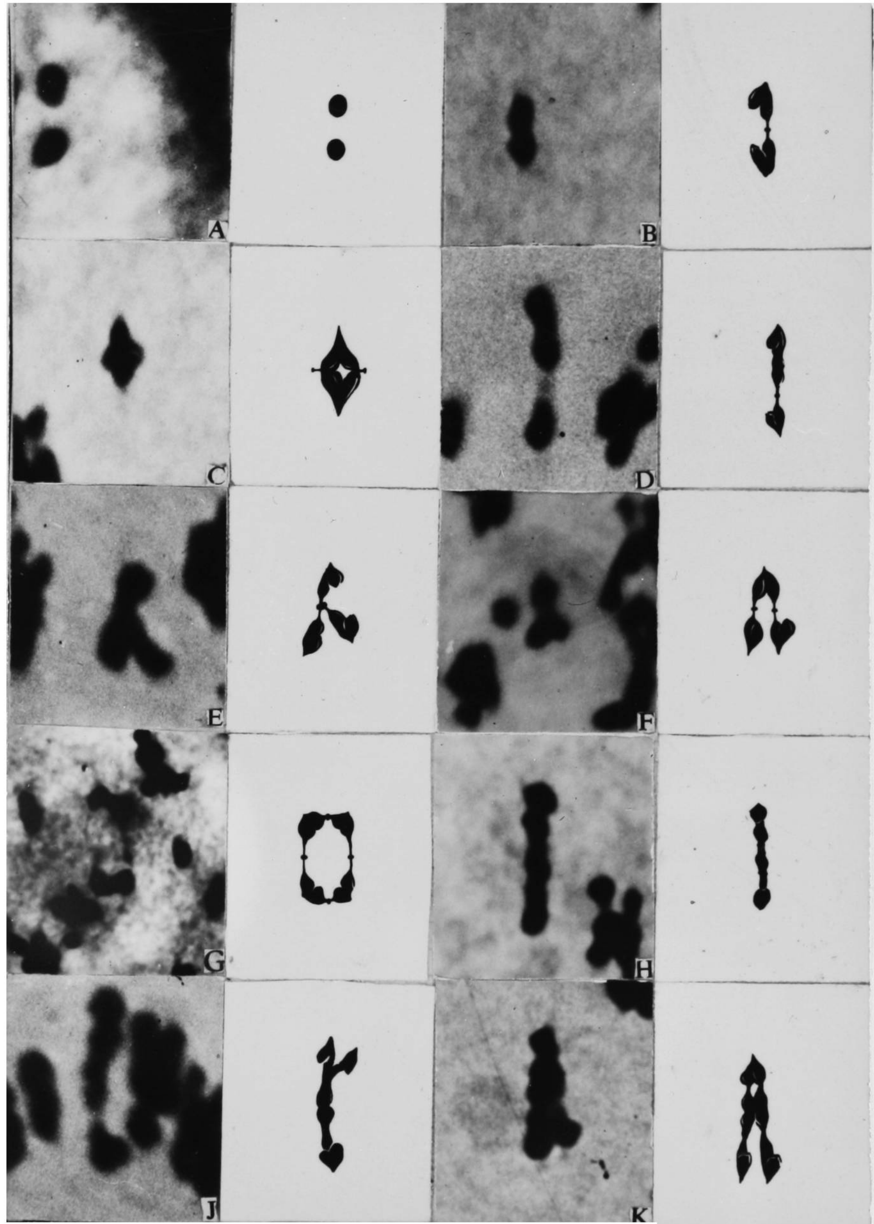
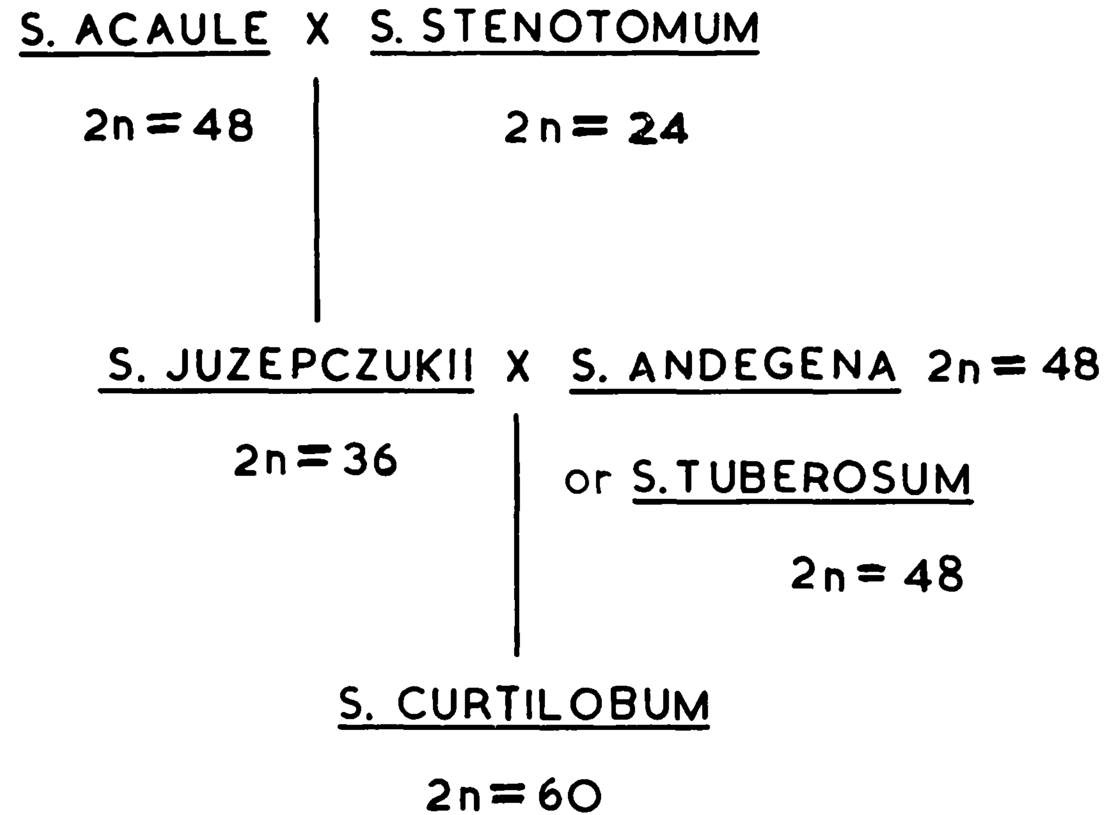

A
B
C
D
E
F
G
H
J
K

FIGURE I.



The Supposed Origin of S.juzepczukii and S.curtilobum According to Bukasov (1939).

Some Cytological Evidence Relating to the Possible Origin of Solanum Juzepczukii and Solanum Curtilobum.

In order to test the suggestion of Bukasov to account for the origin of S.juzepczukii and S.curtilobum these species together with some experimental hybrids described in a previous section were examined cytologically.

S.juzepczukii.

Table III showed 3 analyses of this species. Gross variations between the three sets of data were not detected. The average number of univalents was about 6 and in one instance all but two of the chromosomes were paired, suggesting that complete pairing was possible. Presumably this was seldom attained because of mechanical difficulties. Evidently there was little or no lack of homology between the three sets of 12 chromosomes.

Since this species is triploid and presumably reproduces mostly by tubers, the meiotic irregularities would be protected from selection in the direction of fertility. It was possible, therefore, that the material examined was an F.i of a natural hybrid between S.acaule and S.stenotomum, if these were in fact its parent species. A triploid hybrid F.i between the supposed parents may, therefore, be legitimately compared to S.juzepczukii.

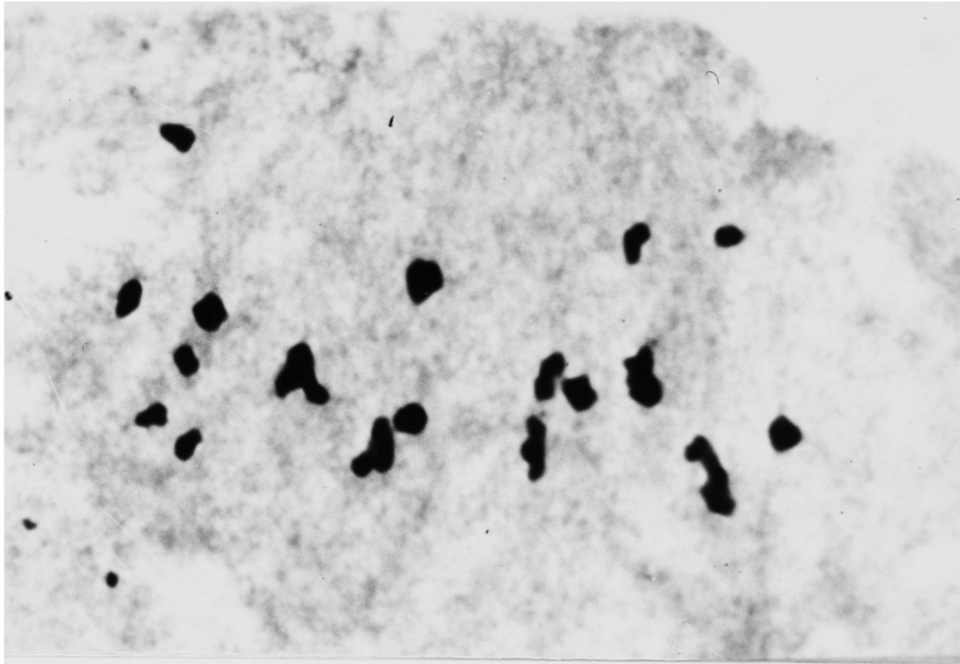
When the hybrid between S.acaule and S.stenotomum was made it was thought that the naturally self fertile

S.acaule would not have been the male parent of the triploid species since, having a short style, its pollen might not be well adapted to growing in the style of other Solanum species. In fact, it may not have been the male parent, though not for this reason. The work of von Wangemheim (1953) suggested that S.acaule was a poor pollen parent since it promoted on some occasions aberrant behaviour of the endosperm.

2 clones of the hybrid S.acaule X S.stenotomum were examined cytologically at metaphase of meiosis and the results are presented in Table III. Agreement between the two experimental hybrids and the naturally occuring species was good as regards all configurations. A useful indication of chromosome homology is the number of chiasma per chromosome. As in cotton (Stevens 1950) pairing in Solanum is in the nature of an "all or nothing " process, and because of this small variations of the order of 0.1 or less may be ignored. Between the natural and experimental material agreement for this figure was again good.

The data so far presented does not establish beyond doubt the origin of S.juzepczukii. The most that can be claimed is that there is no known cytological reason why S.acaule should not have combined to give rise to S.juzepczukii.

PLATE II.



S.juzepczukii 2n = 36
(5 Univalents, 8 Bivalents, 5 Trivalents.)

TABLE II.

Meiosis In Some Species of Solanum.

Species	No.of cells	Univs.	Bivs.	Trivs.	Quads.	Somatic No.	Ref.
S.ACAULE	20	1.0	23.95	0	0	48	Lamm. 1945.
S.STENOTOMUM	10	0	12	0	0	24	Chapman (diak:)*
S.JUZEPCZUKII	28	6.39	9.11	3.75	-	36	Chapman
S.ANDEGENA	20	1.25	19.55	0.75	1.35	48	Lamm. 1945.
S.TUBEROSUM a	a 20	1.05	20.30	0.45	1.25	48	Lamm.45
b	b 41	5.71	17.93	0.68	1.09		Bains.51
c	c 15	4.00	18.07	0.40	1.66		"
d	d 15	3.53	17.87	0.60	1.73		"
S.CURTILOBUM	10	5.6	14.7	6.7	1.1	60	Chapman

* The uniform occurrence of 12 bivalents at diakinesis suggests that occasional pairs of univalents seen at metaphase may reasonably be attributed to precocious separation.

TABLE III.

Meiotic Metaphase Analysis of S.Juzepczukii and an Experimental Hybrid.

Material	No. of cells	Univs.	(se)	Bivs	(se)	Trivs	(se)	Quads	(se)	Chiasma/ Chrom.	Som. No.
S.JUZ:1632	9	7.64	(3.04)	8.33	(2.178)	3.88	(2.11)	-			36
" "	10	5.5	(1.78)	8.80	(1.46)	4.30	(1.42)	-			36
" "	9	6.11	( )	10.22	( )	3.00	( )	-			36
S.ACAULE x S.STEN: 1	15	6.00	(1.78)	10.33	(2.39)	2.73	(1.69)	0.27	(0.63)	0.45	36.07
S.ACAULE x S.STEN: 11	15	6.00	(2.45)	8.85	(2.57)	4.0	(2.09)	-		0.54	36.0
S.ACAULE x S.STEN: 11	10	7.4	(3.10)	8.3	(2.36)	4.0	(2.26)	-			36

S.curtilobum.

This pentaploid species was examined by Rybin (1933) and by Lamm (1945). Neither worker however presented comprehensive data for its meiotic metaphase analysis. Like Lamm, (Loc.cit.) the present worker found that S.curtilobum spread very poorly and in this respect it showed a marked contrast to S.demissum, (Compare Plates III and III), which was remarkable since the latter species has 12 more chromosomes. Table IV shows the analysis of S.curtilobum.

Meiosis was examined in the species hybrids described under "Materials and Methods" and for convenience they will be referred to here as the 'andegena' and "tuberosum" materials respectively. Details of their meiotic metaphase analyses are given in Table IV and these will now be considered together with that for S.curtilobum.

The andegena material did not flower very abundantly and further, well spread metaphases were not very easily obtained. Configurations interpreted as pentavalents were found and in this respect it contrasted with the naturally occurring pentaploids. As regards all other configurations the natural and artificial pentaploids were very similar.

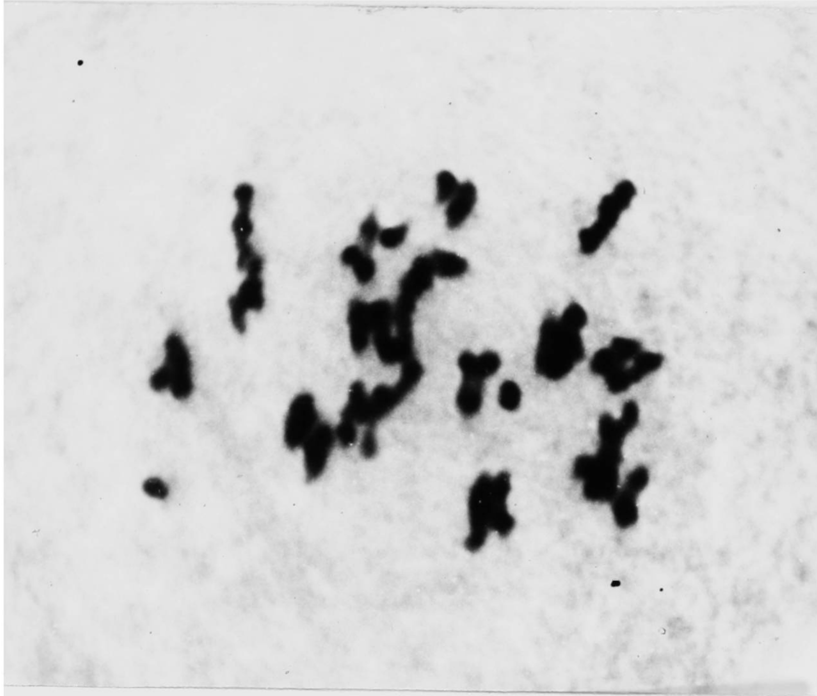
The tuberosum material did not show pentavalents and in this and all other respects showed considerable similarity to S.curtilobum. The figures for chiasma per

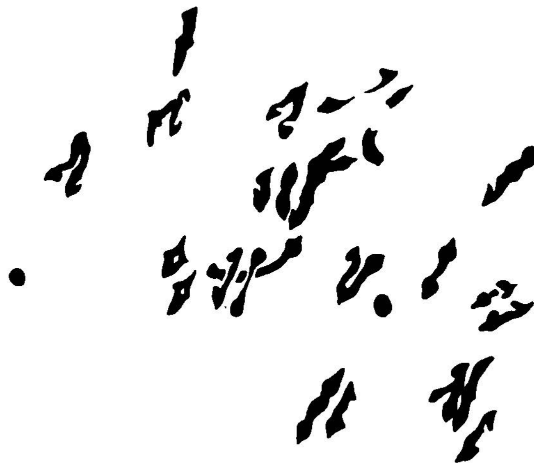
TABLE IV.

Meiotic Metaphase Analysis of S.Curtilobum & two Experimental Hybrids.

Material	No.of cells	Univs.(se)	Bivs.(se)	Trivs.(se)	Quads(se)	Pents.(se)	Chia/ Chrom	Som. No.
S.CUR:1943	10	5.60(2.46)	14.70(1.56)	6.7(1.89)	1.1(0.90)	-	0.59	59.5
S.AND: type	10	3.60(1.77)	16.00(3.16)	5.2(3.16)	1.1(1.10)	0.6(0.54)	0.62	58.6
S.TUB: type	9	5.55(2.56)	17.85(2.06)	5.2(3.8)	0.77(0.66)	-	0.56	59.5
S.TUB: type	11	5.0(2.0)	17.18(3.57)	5.63(1.63)	0.81(0.75)	-	0.57	59.5

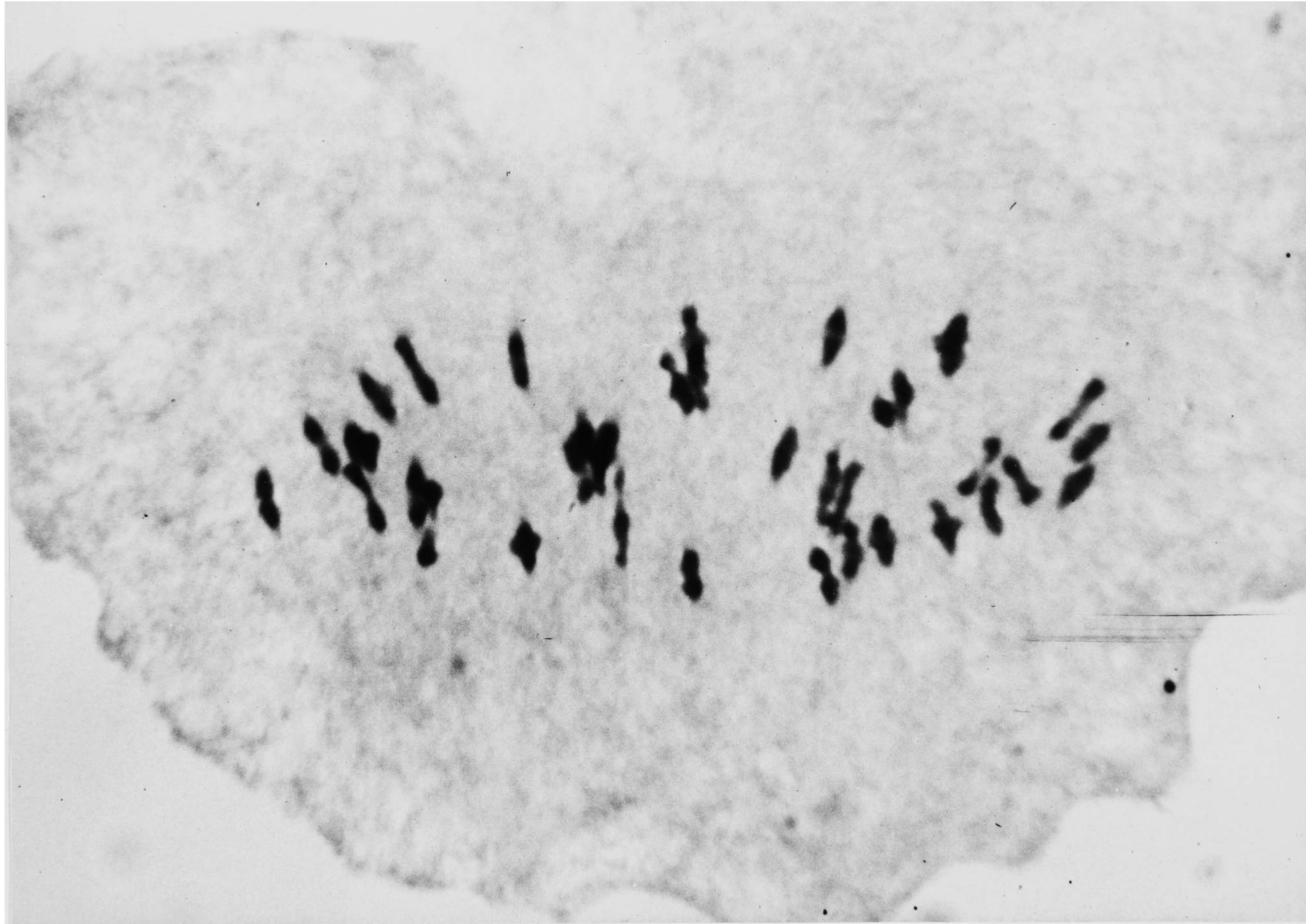
PLATE III.





S.curtilobum 2n = 60
(2 Univalents, 18 Bivalents,
6 Trivalents, 1 Quadrivalent.)

PLATE IV.



S.domissum 2n = 72, (36 Bivalents).

chromosome showed that of the two experimental pentaploids the tuberosum material most closely resembled the wild species.

This cytological data lent support to the view that S.curtilobum originated from a cross between S.juzepczukii and a representative of that rather comprehensive taxonomic entity S.tuberosum. In view, however, of the very real possibility that S.curtilobum has arisen more than once it would on the one hand have a wide range of variability and on the other be difficult for a cytologist definitely to ascertain its probable parental species .

Discussion.

The data presented in Part II provided cytological support for the view that *S.juzepczukii* was derived from *S.acaule* and *S.Stenotomum* and that, furthermore, *S.curtilobum* was derived from *S.juzepczukii* and some representatives of the *S.tuberosum* complex.

Two separate aspects of this work merit emphasis both in regard to the nature of the evidence. The first point is that the evidence shows no reason why the above phylogenetic scheme should not be the correct one. It must be borne in mind that notwithstanding the close agreement between data from natural and experimental materials such evidence as this is inferential.

The other feature of this evidence is illuminated by results obtained in Part I. Here, it will be remembered, that evidence was given for terminalisation of chiasmata during the premetaphase stages of meiosis. Terminalisation has the effect of obscuring all but gross differences in chiasma frequency. This together with the initially low number of chiasmata formed in *Solanum* means that metaphase analysis is a somewhat crude way of clarifying chromosome and consequently species relationships. It was this sort of limitation which prompted the search for alternative methods such as that described in Part I.

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