CHROMOSOME BEHAVIOUR AND STRUCTURAL HYBRIDITY IN THE *TRADESCANTIAE*.

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(With Eighty-four Text-figures.)

CONTENTS.

1. Introduction.

The attempt to apply the results of cytological observation in the field of systematics is difficult because it involves two unsettled questions: first, the relation of cytology to genetics; secondly, the relation of genetics to systematics. The former, however, is now a matter of speculation in detail only, for the principle is conceded. In the past scepticism has been justified, for it has been the misfortune of the chromosome theory of heredity that the chromosomes were discovered before the theory. Converse propositions based on the one hand on genetical experiment, and on the other upon cytological observations,

have become entangled with some consequent confusion; for errors are inherent in each method, both in observation and inference. Had genetical theory, based on linkage experiments, advanced to its limits without any knowledge of the chromosomes, the later discovery of these actual organs of transmission would have verified prediction so completely that all objections to the principle would have been at once dissolved.

Later studies have supported the original conclusions with remarkable consistency. We may therefore assume the general truth of the chromosome theory and attack the problem of variation directly from a cytological standpoint, not regarding this method as merely ancillary to the morphological method in genetics. The basis of our method of approach must be some such definition as this: *Variation amongst individuals is the occurrence of differences in their physiological properties correlated with differences in the permanent structure of their cells*[1].

Not only are we on safer ground in applying such a method of enquiry to-day, but we are also provided with the means of attacking the problem more effectively than hitherto. The study of polyploid tulips and hyacinths has shown that the *unit of behaviour* up to metaphase of meiosis is not the whole chromosome but an indefinitely small part of it. If parts of chromosomes are really capable of behaving independently we have a means of studying their genetical structure by observation of the behaviour of their parts. And for the purpose of drawing deductions from this behaviour, principles that have become defined by a study of the polyploid tulips and hyacinths seem again to be applicable. These are, first, that *association takes place at prophase between homologous pairs of elements*, however the nuclear complement may be constituted, and, secondly, that *association takes place at metaphase between pairs of chromatids*, two or more chromosomes being associated merely as the result of exchanges between their constituent chromatids.

Employing these two principles we have a new means of studying the problem of the organisation of the nucleus. *Oenothera* naturally offers itself as the most outstanding example of exceptional organisation, and I have for this reason endeavoured to show (1929) the conclusions to be derived from applying to *Oenothera* the rule of pairing of homologous parts of chromosomes at meiosis. These are, in short, that

[1] The proposition here implied, namely that all variation is determined by physical differences in the nucleus or other permanent bodies, is capable of being tested in only a limited sphere, but this seems no objection to its theoretical validity.

paternal and maternal chromosomes do not correspond as units, but are differently made up in opposite sets. Working on an analogous hypothesis Belling and Blakeslee had arrived at this conclusion in *Datura* on the strongest evidence (1924–7). In order to find indirect support from material cytologically more favourable than *Oenothera* the present study of the *Tradescantiae* was undertaken.

This group, for the most part confined to America, embraces species of which some, like *Tradescantia virginiana*, are exceedingly variable, while others, like *Rhoeo discolor*, are relatively constant. Corresponding with this we find, first, that the relative importance of seed-production and vegetative propagation in these species is very variable; secondly, that the hereditary mechanism plays, as will appear, a very different part in perpetuating the various types; and, thirdly, that the mechanism itself is, in the one case, constant and, in the other, exceedingly variable.

A few notes on related studies in *Fritillaria* and *Commelina* are included.

2. PREVIOUS WORK.

Tradescantia virginiana has been studied cytologically from many points of view, but with results that in the light of our present knowledge seem inconclusive. Farmer and Shove (1905) find "that in this plant the number of chromosomes is not constant during the heterotype division, and it certainly varies between twelve and sixteen," but they do not seek any explanation. Miyake (1905) describes the associations of several chromosomes together in terms which indicate regret at their difficult behaviour rather than interest in their exceptional properties[1]. S. Nawaschin (1911) finds that bodies of chromatin are not included in the interphase nucleus, and concludes that these are lost chromosomes; that there is in fact "chromatin-diminution." These bodies may be either fragments or lost chromosomes. Recently Stow has described and illustrated (1928) the association of as many as ten chromosomes end to end at meiosis in *Tradescantia virginiana*, and finds differences in degree of association subject to changes of temperature.

The accounts of other species invite further enquiry. For example, Hance remarks on variation in chromosome number at meiosis in *Zebrina* without offering any interpretation or convincing illustration. In *Tradescantia fluminensis*, Tischler does not venture (1921) "definitiv zu entscheiden" that the haploid chromosome number is 12. In *Rhoeo*,

[1] "Bei *Tradescantia* war die Untersuchung noch schwieriger weil die Verklebung der Chromosomen länger dauert und zwar bis zur Bildung der Kernplatte."

Suessenguth remarks, first (1920), that the probable haploid number is 6 and, secondly (1921), that gemini are not formed at meiosis. Belling (1927) has recorded and illustrated chain-formation in this species, but his illustration seems rather to represent a perfect ring in which one pair of chromosomes has been separated by pressure. Belling's material was probably identical with that about to be described.

3. Methods.

The root-tips examined were fixed in various modified Flemming solutions of which the one probably giving the most satisfactory results was of the composition 60 c.c. 1 per cent. chromic acid, 20 c.c. 2 per cent. osmic acid, 25 c.c. 5 per cent. acetic acid. Sections of the *Fritillaria* species and the *Tradescantia virginiana* varieties were cut at a thickness of 24–30 μ, the minimum necessary to secure a moderate proportion of uncut plates, since the chromosomes, whose limbs are nearly 20 μ long, may lie almost perpendicularly on the plate. The other species were cut at 16 μ.

The study of pollen mother-cell and pollen-grain divisions was made entirely from smear preparations (cf. Newton and Darlington, 1929). The only notable disadvantage of this method in the present work has been that, while a statistical study of various abnormalities would have been desirable in several instances, this has not been possible in connection with the pollen mother-cell divisions, for they are usually too thickly crowded on a slide to "score" reliably. With the pollen grains, statistical studies have been possible.

Drawings were made at bench level with a Leitz Abbé camera lucida, with a Zeiss 1·5 mm. objective (n.a. 1·3) for *T. virginiana*, a Swift telangic compensating × 15 eyepiece to give a magnification of 3200, and, for the other species, a Leitz periplanetic 25 × B eyepiece to give a magnification of 5700. All drawings, except where specially stated, are reduced to a magnification of 2800. Wherever necessary for clearness unconnected groups of chromosomes in side views of pollen mother-cell divisions have been illustrated separately.

4 Observations on Root-tips.

In several species, inconstant numbers of disproportionately small chromosomes are found. These are referred to, for reasons discussed later, as "fragments," "f." in the tables.

Rhoeo discolor. $2n = 12$ (Fig. 1).

The attachment constrictions are approximately median except in

four chromosomes where the smaller of the two segments is less than half the length of the longer. Two probably dissimilar chromosomes had small trabants on the shorter arm, and one chromosome was frequently seen to have a second constriction very close to the attachment one, separating, as it were, an interstitial trabant (cf. *Tradescantia virginiana* vars. and *Spironema fragrans*). Other subordinate constrictions were less clear and were only occasionally observed.

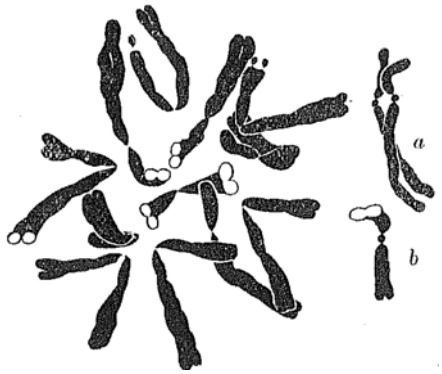


Fig. 1. Somatic metaphase from the root-tip of *Rhoeo discolor*. $2n = 12$. *a* and *b*, figures showing "interstitial trabant."

Fig. 2. Somatic metaphase from the root-tip of *Tradescantia crassifolia* (Kew). $2n = 12 + 2$ ff.

Tradescantia crassifolia. $2n = 12 + 2$ ff. (Fig. 2).

The chromosomes closely resemble those of *Rhoeo*. The form in question (from Kew) has two substantial fragments (cf. the observations at meiosis) with apparently sub-terminal constriction, and it is noteworthy that one of them appears to show an affinity for a chromo-

some which has, relatively to all the rest, a curtailed arm (cf. *T. virginiana*, pollen-grain divisions).

Spironema fragrans. $2n = 12$ (Fig. 3).

The following chromosome types can be distinguished:

two long pairs (almost identical) with sub-median constriction,
one shorter pair with two sub-median constrictions (cf. *Rhoeo* and *Tradescantia*),
one long pair with sub-terminal constrictions,
one shorter pair with sub-terminal constrictions,
one shorter pair with sub-terminal constrictions and trabants.

Fig. 81 *a* on p. 264 shows the trabants lying *behind* the head of the chromosome.

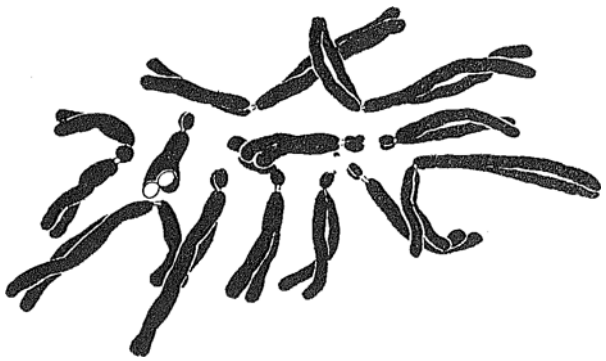


Fig. 3. Somatic metaphase from the root-tip of *Spironema fragrans*. $2n = 12$.

Tradescantia virginiana. $2n = 24$, etc. (Figs. 4, 5, 64–66).

Most of the forms studied have 24 chromosomes varying slightly in size with approximately median constrictions. One or two can frequently be seen with a trabant. This trabant is so small that its behaviour is probably exceptional, and the illustration (Fig. 81) shows the difficulties that are encountered in studying it. This question will be referred to later in discussing the structure of the chromosomes.

The "interstitial trabant" has been observed in a number of forms; it is probably obscured in many plates by special conditions of arrangement. In one form, Medium Blue (No. 4), one chromosome was found with a nearly sub-terminal constriction and shorter than any of the other chromosomes in this or other forms of *T. virginiana* (Fig. 4 *a*, cf. account of meiosis, Figs. 41 *k* and *l*).

Two forms were found with 25 chromosomes, and four forms with

two, three, four and five fragments respectively. These have usually sub-terminal constrictions in which the small segment is of the size of a small trabant. The studies of meiosis and pollen-grain divisions, where correlated, show that these fragments are usually constant in the somatic cells of the plant.

Fig. 4.

Fig. 5.

Fig. 4. Somatic metaphase from the root-tip of *Tradescantia virginiana* var. *montana*. $2n = 24 + 2$ ff. *a*, chromosome with short arm from Medium Blue (No. 4).

Fig. 5. Somatic metaphase from the root-tip of *Tradescantia virginiana*, Chelsea seedling B. $2n = 25$.

Fig. 6. Somatic metaphase from the root-tip of *Tradescantia virginiana* var. *brevicaulis*. $2n = 18$.

A large plant of Medium Blue (No. 1) was divided into eight roots, of which six gave the following different results in respect of number of fragments: (i) 4, (iii) 5, (v) 5, (vi) 5, (vii) 6, (viii) 5 (Fig. 69 *d–h*). Since the number of chromosomes was the same in every sample, and since the fragments varied about a mean of 5, the most likely conclusion is that 5 is the original number, and that 4 and 6 have appeared as a result of somatic loss and gain. This possibility is suggested by the

occasional behaviour of the fragment at mitosis in lying to one side of the plate.

Tradescantia virginiana var. *brevicaulis*. $2n = 18$ (Fig. 6).

The chromosomes of this form resemble those of the tetraploid *T. virginiana* forms in general character but are slightly smaller. It has three chromosomes with trabants. The difference of chromosome number which makes the distinction from the tetraploids possible is also the one which makes its perpetuation by normal seed-production impossible, so that the question of its specific rank may be conveniently passed over.

Fig. 7. Somatic metaphase from the root-tip of *Trelcasia brevifolia*. $2n = 24$.

Trelcasia brevifolia. $2n = 24$ (Fig. 7).

The chromosomes resemble those of some vars. of *Tradescantia virginiana*, but there are three or four with constrictions more nearly terminal than is usual in that species.

Zebrina pendula. $2n = 24$ (Fig. 8).

The number suggests a tetraploid relationship to the first three species, and the numbers of the different chromosome-types broadly agree with this assumption. These consist of

4 with median constrictions (much the longest),
8 with sub-terminal constrictions,
12 with terminal constrictions (trabants).

Those of the second class have different sizes of distal segment.

Tradescantia navicularis. $2n = 32$ (Fig. 9).

This species differs from the foregoing in not falling into the six-

series, and in all its chromosomes having sub-terminal constrictions. In the bulk of its chromosomes it seems to approximate to *T. virginiana* ($2n = 24$). (This species was referred to earlier (1929) as *T. navicola*.)

Fig. 8.

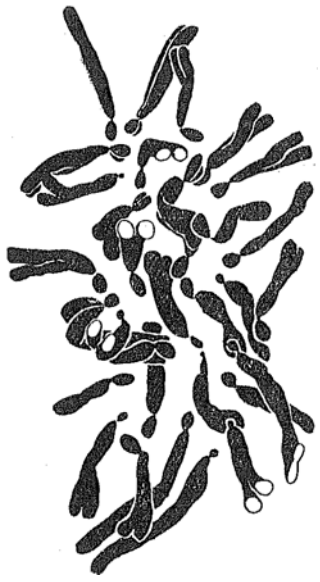


Fig. 9.

Fig. 8. Somatic metaphase from the root-tip of *Zebrina pendula*. $2n = 24$.
Fig. 9. Somatic metaphase from the root-tip of *Tradescantia navicularis*. $2n = 32$.

Fig. 10. Somatic metaphase from the root-tip of *Tradescantia geniculata*. $2n = 32$.

Tradescantia geniculata. $2n = 32$ (Fig. 10).

Although the chromosome number is the same as that of *T. navicularis*, the chromosomes of the two species evidently do not correspond

individually. The chromosomes of *T. geniculata* are markedly smaller, and eight of them have median or sub-median constrictions.

Dichorisandra thyrsiflora. $2n = 38$ (Fig. 11).

The complement resembles that of *T. navicularis*, but numerous chromosomes have sub-median constrictions. Probably as a somatic variation a root-tip with 36 chromosomes was found (cf. pollen-grain counts).

Fig. 11. Somatic metaphase from the root-tip of *Dichorisandra thyrsiflora.* $2n = 38$.

Fig. 12.

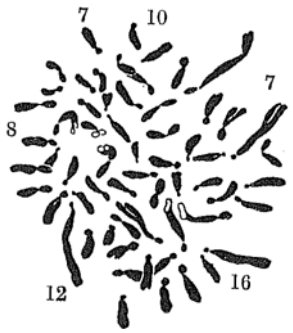


Fig. 13.

Fig. 12. Somatic metaphase from the root-tip of *Coleotrype natalensis.* $2n = 36$.
Fig. 13. Somatic metaphase from the root-tip of *Tradescantia fluminensis.* $2n = 60$.

Coleotrype natalensis. $2n = 36$ (Fig. 12).

The complement resembles that of *T. geniculata*, and eight chromosomes have sub-median constrictions.

Tradescantia fluminensis. $2n = 60$ (Fig. 13).

Green and variegated forms of this species were identical. As Tischler observes (1921) in giving the haploid number as 12, or perhaps more

("weil manche Präparate mit der Interkinese mir eine grössere Zahl als 12 Chromosomen vortäuschten"), the size of the nucleus is no greater than in *Rhoeo*. Fifty-four of the 60 chromosomes are no bigger than the fragments commonly found in other species of *Tradescantia*, and have for the most part the sub-terminal constriction characteristic of these. The remaining six are longer and also have sub-terminal constrictions.

Fig. 14. Somatic metaphase from the root-tip of *Tinantia fugax*. $2n = 68$.

Fig. 15.

Fig. 16.

Fig. 15. Somatic metaphase from the root-tip of *Cyanotis somaliensis*. $2n = 28$.
Fig. 16. Somatic metaphase from the root-tip of *Commelina benghalensis*. $2n =$ probably 68.

Tinantia fugax. $2n = 68$ (Fig. 14).

The chromosomes resemble those of the last species, but the six of the large type are wanting.

A selfed seedling had the same chromosome number as its parent. In the pollen mother-cell divisions 34 bivalents were formed (see Section 7 (iv)). Connections were sometimes observed between one or two pairs (Fig. 82). Evidently 34 is to be regarded as the haploid number of this species.

Cyanotis somaliensis. $2n = 28$ (Fig. 15).

The chromosomes individually and as a whole are smaller than in any other species of the *Tradescantiae* that I have examined. Six have sub-median constrictions; in many of the others the smaller element is a trabant.

TABLE I.

Summary of Chromosome Counts.

Tradescantia virginiana L. (U.S.A.) varieties and sub-species.

	$2n$ (root-tip number)		n (pollen-grain number)	
	Chromosomes	Fragments	Chromosomes	Fragments
Medium Blue (No. 1)	24†	4, 5 and 6†	10–14†	0–5†
,, (No. 4*)	24†	—	10–13	(found once)
Small Red*	24	—	11–12	—
Taplow Crimson	24	4	—	—
*hirsuta**	24	—	11–13	(found once)
alba (Kew)	24	—	11–13	(found once)
Pale Blue	?	—	12	—
alba (Aldenham)	?	—	11–12	(found once)
Chelsea seedling (No. 1)	24	—	—	—
,, (No. 2)	25	—	—	—
*bracteata**	24	—	11–12	—
humilis	24	3	—	—
montana	25	2	11–14	0–2
congesta	24	—	11–13	—
caerulea (double)	24	1	—	—
delicata	24	—	—	—
reflexa	24	—	—	—
lilacina	24	—	—	—
rubra	24	3	—	—
*brevicaulis**	18	—	7–11	(found 7 times)

Species	Chromosome number	Distribution
Rhoeo discolor Hance*, †, ‡	$2n = 12$	Mexico
Tradescantia crassifolia Cav.*, †, ‡	$2n = 12 + 2$ ff.	South America
Spironema fragrans Lindl.†	$2n = 12$	Mexico
Trelcasia brevifolia Rose†	$2n = 24$	,,
Zebrina pendula Schnitzel*, †, ‡	$2n = 24$	,,
Tradescantia navicularis Ortgies*, †	$2n = 32$	Peru
T. geniculata Jacq.†	$2n = 32$	Tropical America
Coleotrype natalensis C. B. Clarke†	$2n = 36$	Natal
Dichorisandra thyrsiflora Mikan†, ‡	$2n = 38$	Brazil
Tradescantia fluminensis Vell.†	$2n = 60$	Brazil to Argentine
,, ,, variegated	$2n = 60$	—
*Pinantia fugax**, †	$2n = 68$	Tropical America
,, selfed seedling	$2n = 68$	—
Cyanotis somaliensis C. B. Clarke†	$2n = 28$	E. Africa
Commelina coelestis Willd.†	$2n = 90$	Mexico
C. benghalensis L.†	$2n = 68$	Tropical Asia and Africa
C. nudiflora L.	$2n = 56$	Tropics

* Pollen mother-cell divisions examined.
† Illustrated in the text.
‡ Pollen-grain divisions examined.

Commelina nudiflora. $2n = 56$.
Commelina benghalensis. $2n =$ probably 68 (Fig. 16).
Commelina coelestis. $2n = 90$ (Fig. 17).

These three species resemble *Tinantia fugax* very closely in the size and shape of their chromosomes. In *C. benghalensis*, with the same number, the chromosomes with median constriction are more numerous.

Fig. 17. Somatic metaphase from the root-tip of *Commelina coelestis*. $2n = 90$.

5. Observations on Pollen Mother-cell Divisions.

The following account deals more particularly with pairing at metaphase of the first pollen mother-cell division in three diploid species, *Tradescantia crassifolia*, *T. bracteata* and *Rhoeo discolor*, and two polyploid species, *Tradescantia virginiana* and *Zebrina pendula*. Other related observations are briefly referred to.

(i) *Tradescantia crassifolia*.

This magenta-flowered form from Kew has 12 chromosomes and two fragments in somatic divisions, and usually forms six recognisable bivalents at meiosis. The fragments, as such, are univalent and may either lie free (Fig. 18), or one (Fig. 19) or both (Fig. 20) can probably be associated with a particular part of one of the bivalents; the one interstitially, the other terminally.

The bivalents take the form of rods, rings or crosses, that is to say, speaking in terms of chiasmata, the chromosomes may be associated by one or two interstitial or more or less "terminal" chiasmata.

The fragments usually pass to the pole undivided, and split at the second division. But three presumably half-fragments have been seen in one second division and one in the sister-cell. They are sometimes

lost in the cytoplasm. Three fragments have been seen at the first metaphase, one presumably a new one resulting from a change at prophase.

Association occurs occasionally between the bivalents (Fig. 19) giving an apparently reduced haploid number.

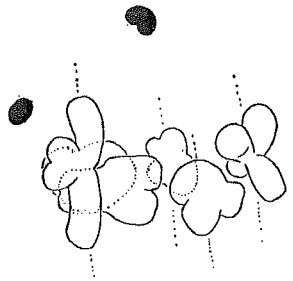


Fig. 18.

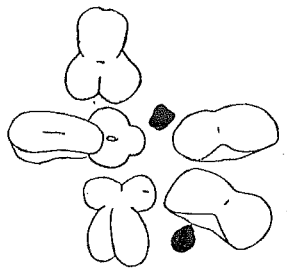


Fig. 19.

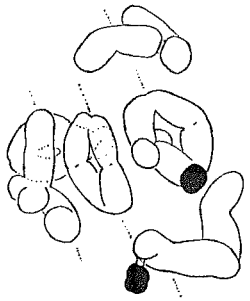


Fig. 20.

Figs. 18–20. Metaphase of the first division in *Tradescantia crassifolia* (Kew).

Fig. 18. Side view: 6 bivalents and 2 univalent fragments.

Fig. 19. Polar view: 2 bivalents associated and one fragment associated with the middle of a bivalent.

Fig. 20. Side view: both fragments paired.

(ii) *Tradescantia bracteata*[1].

This form, for the preparation of which I am indebted to Dr O. Meurman, resembles *T. crassifolia* in its chromosome behaviour at meiosis. Six bivalents are usually found at metaphase, but occasionally pairing may fail in one and we find two univalents (Fig. 21). In the

[1] Dr Randolph kindly tells me that this is a white-flowered form that he has obtained from Nebraska. His description agrees with *T. bracteata* Small, illustrated in Britton and Brown's *Illustrated Flora*, III, p. 510 (1898). I have not examined somatic divisions.

case illustrated one of these is attached to one of the bivalents interstitially.

The pairs may show various kinds of attachments with one another (Fig. 22). These are of two types, genetically considered: the first indicates that the four associated chromosomes contain the same element repeated four times (with probable inversion of a segment), *i.e.* they are tetrasomic (*c* and *e*); the second, that all four chromosomes are differently constituted, so that no element need be assumed to be present more than twice (*a*, *b* and *d*). The certain determination of these novel and variable forms is naturally not possible in every instance, and I cannot therefore judge in what proportion of the cells they occur. The general impression is that between 5 and 15 per cent. of divisions have abnormalities of this kind.

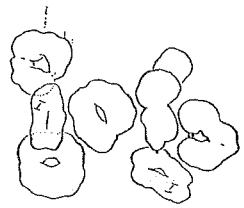


Fig. 21.

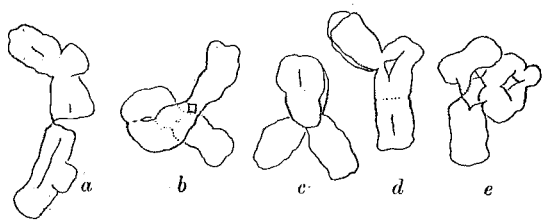


Fig. 22.

Figs. 21 and 22. Metaphase of the first division in *Tradescantia bracteata.*

Fig. 21. Side view showing one chromosome associated laterally with one chromatid of a bivalent, and its fellow unpaired.

Fig. 22. Side views of five types of association of pairs of bivalents (see text).

(iii) *Rhoeo discolor.*

In the simplest case a ring of 12 chromosomes is formed at metaphase (Figs. 23, 24, 32 and 33). But, probably as a result of one or more of the chiasmata between chromosomes breaking down before metaphase (for only complete rings have been seen at diakinesis), a chain (Fig. 25) or chains of chromosomes frequently take the place of the ring at metaphase. Of the proportion of cases in which these several results obtain I can again give only a general impression. It is as follows: ring, ca. 30 per cent.; one chain, ca. 45 per cent.; two chains, ca. 20 per cent.; three chains, ca. 5 per cent. These proportions indicate that the occurrence of one break, or failure of chiasma (as the case may be), diminishes rather than increases the chance of a second occurring.

The 12 chromosomes may be divided into the several chains in any proportions; where two chains are present the combinations 11 + 1

(Fig. 26), 10 + 2 (Figs. 27 and 31), 9 + 38, + 4 (Fig. 28), 7 + 5 (Fig. 29) have been observed; where three chains, 7 + 3 + 2 and 6 + 4 + 2.

At early metaphase the ring or chain of chromosomes begins to arrange itself with reference to the spindle and evidently all the chromosomes do not establish their relationship simultaneously. Rather the influence of the spindle appears to be felt first independently at various parts of the ring. A chain of 12 that is illustrated (Fig. 30) seems to show that in three places the successive chromosomes in the chain are already orientated so that alternate members will pass to opposite poles. It is interesting in this case to notice that the independent behaviour

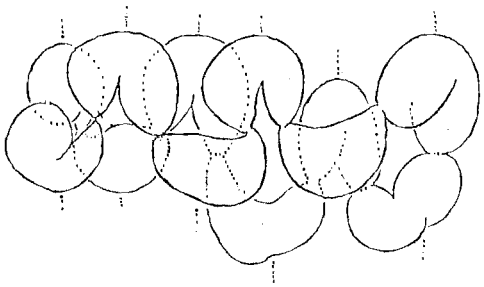


Fig. 23. Complete ring of chromosomes at metaphase of the first division in *Rhoeo discolor*; alternate chromosomes orientated towards opposite poles. × 5700.

of different parts of the chain need not be expected to interfere with the regular orientation and disjunction of alternate members in the intervening parts of the ring. In other words, the three movements *agree*. This is not by any means a rule; indeed only in a bare majority of divisions are all the alternate members found to be turning to opposite poles (as in Fig. 23).

Where we have alternate members turning to the same pole at one point this irregular orientation must also occur at some other, whether on the same side (Figs. 32, 64) or on the opposite side (Figs. 24, 36). This principle applies for genetical purposes whether the ring is closed or open.

Where two chains are formed the non-disjunctional arrangement is less frequent, and where three chains were found the arrangement was always regular, an argument in favour of believing it the result of failure of the spindle relationships to agree in different parts of the long chain. The different chains themselves also usually agree where the numbers are odd; where the numbers are even each chain has its end members pointing to the opposite poles, and it is therefore impossible to tell in the absence of a size differentiation whether the two chains agree or

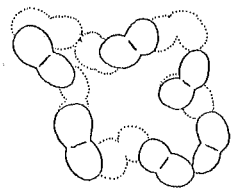


Fig. 24.

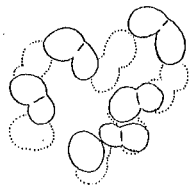


Fig. 25.

Figs. 24 and 25. Polar views of metaphase of the first division in *Rhoeo*.

Fig. 24. Complete ring with two non-disjunctional pairs at opposite sides.

Fig. 25. Broken ring; all pairs disjunctional.

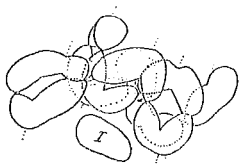


Fig. 26.

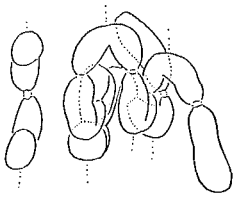


Fig. 27.

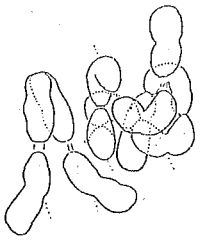


Fig. 28.

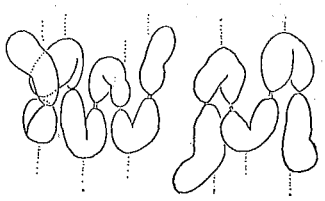


Fig. 29.

Figs. 26–29. Side views of metaphase of the first division in *Rhoeo*, showing different results of the double breaking of the ring after diakinesis.

Fig. 26. Configuration of 11 + 1.

Fig. 27. Configuration of 10 + 2.

Fig. 28. Configuration of 8 + 4 (non-disjunction in the four).

Fig. 29. Configuration of 7 + 5.

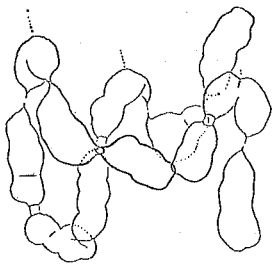


Fig. 30. Early metaphase in *Rhoeo*: three chromosomes in the chain of 12 showing marked relationship with the spindle.

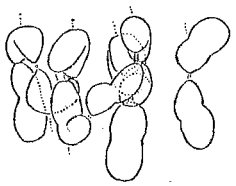


Fig. 31.

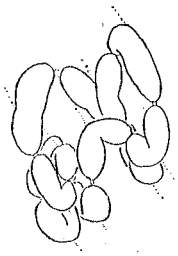


Fig. 32.

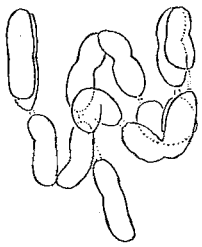


Fig. 33.

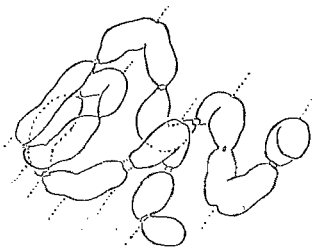


Fig. 34.

Figs. 31–34. Side view of metaphase of the first division in *Rhoeo* showing different non-disjunctional arrangements.

Fig. 31. Configuration with 10 + 2; one chromosome left on the equator (cf. Fig. 35).
Fig. 32. Double non-disjunction on the same side in a ring.
Fig. 33. Quadruple non-disjunction; three cases on one side, one on the other.
Fig. 34. Double non-disjunction on the same side in a chain.

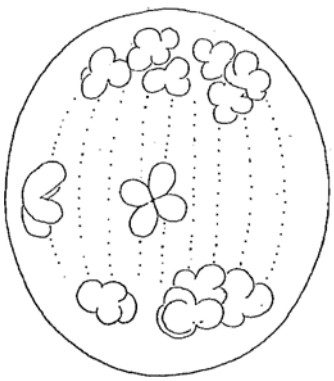


Fig. 35.

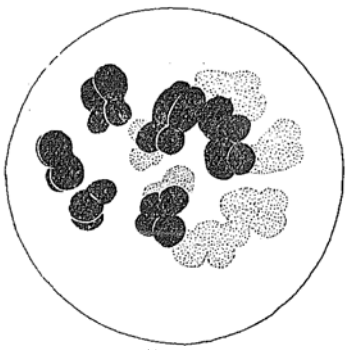


Fig. 36.

Fig. 35. Anaphase of the first division in *Rhoeo*, two chromosomes left lagging on the equator.
Fig. 36. Anaphase of the first division in *Rhoeo* after double non-disjunction on opposite sides.

are really non-disjunctional. The more usual agreement, where the numbers are odd, supports the view that the occurrence of chains is due to breaking of the ring after diakinesis. As many as four non-disjunctional arrangements may occur along a chain of 12 (Fig. 33).

Following non-disjunction the associated chromosomes remain attached at anaphase (Fig. 36) and metaphase of the second division (Fig. 37); even at the second anaphase half-chromosomes may be seen to be attached to different halves passing to the opposite pole after they have separated from their sister half-chromosomes.

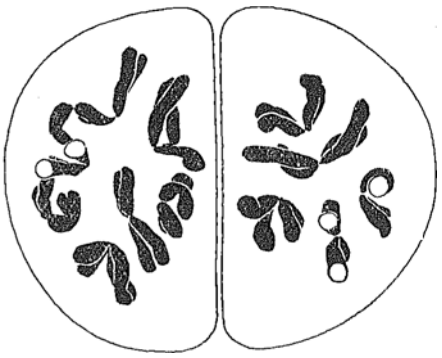


Fig. 37. Metaphase of the second division in *Rhoeo* following double non-disjunction on the same side: indicated by attachments of half-chromosomes; 7 + 5.

In about 40 second divisions in which both plates were examined three had the aberrant numbers 7 and 5 (Fig. 37), none had divided univalents. Where, as frequently happens in the ring or chain (Fig. 31), one chromosome has been lying on the equator between two turned to opposite poles (cf. *Oenothera biennis*, Cleland, 1926) this chromosome may evidently lag at anaphase (Fig. 33), and perhaps occasionally divide (as a "univalent") at the first division.

(iv) *Zebrina pendula.*

The chromosomes are much contracted lengthwise at meiosis so that exceptional associations, apart from those affecting the large chromosomes, are not so easily distinguished as in the other species described.

Apart from the usual formation of bivalents, including two large ring or rod pairs, one exceptional process is worth recording. In a small proportion of divisions these large chromosomes enter into one or two ring associations of the type illustrated (Fig. 38 *a* and *b*). These rings, which are identical, each consist of two chromosomes larger than any

of the others, and two chromosomes of approximately half their size. The ring may consist of chromosomes united end to end, or the larger pair may be associated at an interstitial chiasma. Now, if reference is made to the somatic division illustrated (Fig. 8), it will be seen that the identity of the large chromosomes (which have median attachment at meiosis) is unmistakable: they are the four long chromosomes with median constrictions. They correspond morphologically, and form rings of four as might be expected from their tetraploid number. But they never form a ring by themselves, that is, of four morphologically similar

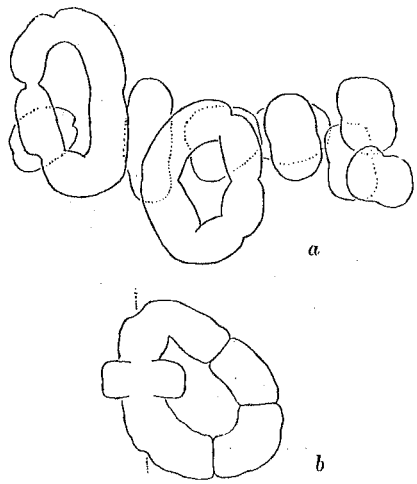


Fig. 38. *a.* Side view of metaphase of the first division in *Zebrina pendula*; 8 pairs and 2 rings of 4; terminal chiasmata only.
b. Single ring of four from another division showing medial chiasma.

chromosomes. Probably therefore the two rings and their constituents have no relationship, and the morphological evidence of tetraploidy in the somatic chromosomes is entirely irrelevant.

The later course of division is generally irregular, as described by Hance. The "restitution nuclei" of Rosenberg have been observed. A stronger growing form, still under investigation, has 24 chromosomes at metaphase of the second pollen mother-cell division, and is therefore relatively tetraploid.

(v) *Tradescantia virginiana* and related forms.

Diakinesis has been less thoroughly examined because at this stage, although connections between chromosomes are more strongly apparent than at metaphase, it is more difficult to know when a connection is

significant. The constant relationship of all the chromosomes with the spindle at metaphase enables one to tell from their attitude whether they are free or attached.

The association of the chromosomes at diakinesis and metaphase is for the most part terminal or approximately so. Occasionally, however, chromosomes are engaged in an obvious interstitial chiasma which may be sub-terminal (Figs. 42 *e* and 44 *f*) or median (Fig. 47). This is perhaps a property of the individual pair of chromosomes, notwithstanding the fact that a string of four chromosomes may be associated at chiasmata at varying distances from the end, for such a string may consist of dissimilar chromosomes (Fig. 41 *l*).

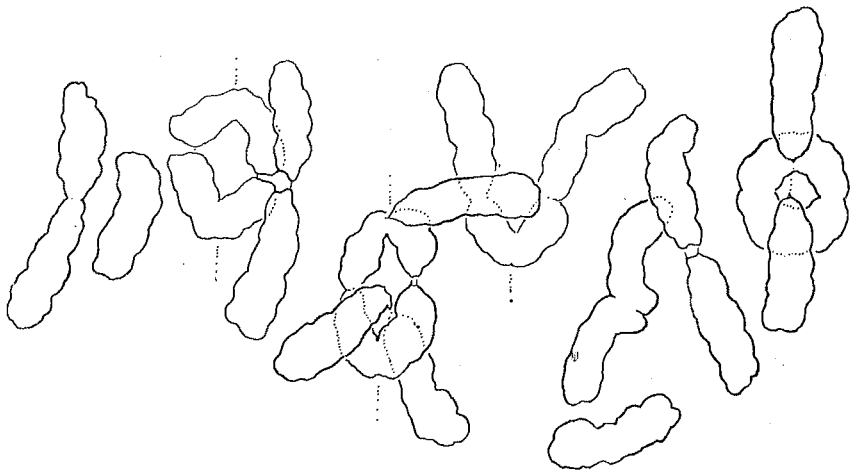


Fig. 39. *Tradescantia virginiana*, Medium Blue (No. 4): 8 bivalents, 2 "trivalents," 2 univalents. One unequal pair at the left-hand side; the second apparently unequal pair is due to the chromosome not lying flat. One rod bivalent passes through a ring bivalent to which a third chromosome is attached laterally.

When the association is actually what would be described as "end-to-end" its nature is shown to be in no way distinct from an ordinary chiasma (cf. Newton and Darlington, 1929), first, by the fact that the separate connections of the two pairs of chromatids are usually distinguishable and, secondly, by the frequent observation of the two bodies which are distal to the chiasma (Fig. 46). In other words gradations occur between the theoretical position of the "terminal chiasma" and that of the interstitial chiasma.

In this way it is usual to see the chromosomes at diakinesis associated in pairs, fours, eights, and even tens, to form simple rings or

strings (cf. Stow, 1928). In most varieties, on an average, three rings of four are formed, the rest of the chromosomes being associated in pairs. The number of fours varies from one to six. One or two univalents are frequently found, usually together with trivalents. It is to be noted that where, in a ring, one of the connections of the ordinary double chiasma at one end has been broken, the chromosomes at this point are usually pulled further apart than at the other chiasma where a double connection is retained (Fig. 40). The first modification of the

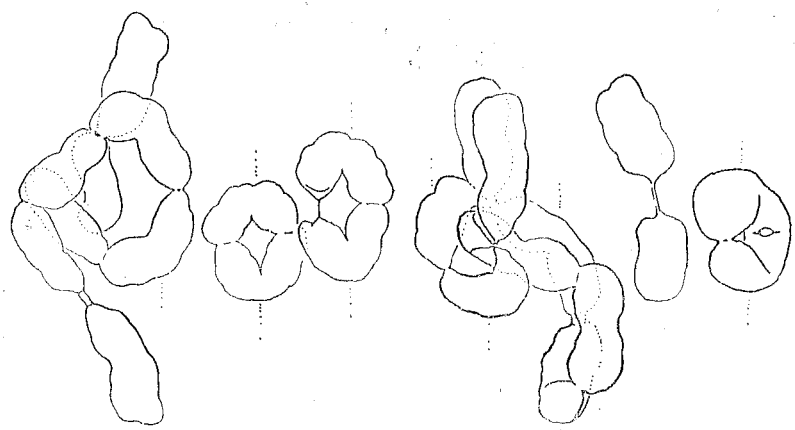


Fig. 40. *T. virginiana*, Medium Blue (No. 4): 2 "octavalents," one a branched chain with a quadruple chiasma, the other a branched string with two triple chiasmata, 4 bivalents, one an unequal rod pair, another a ring with one imperfect chiasma at which the chromosomes are more widely separated than at the perfect one.

ordinary chiasma that deserves mention is the case where three of four chromosomes, instead of two, are associated at a *multiple chiasma* (see Fig. 84, p. 268). In these chiasmata one chromatid of each of the chromosomes involved is associated terminally with a chromatid of another chromosome, and the second chromatid with a chromatid of a third chromosome. These associations are sometimes strikingly clear (Figs. 41 and 42, *b* and *d*); in other cases the relationship can only be inferred, owing to the chromosomes concealing their own connections, one chromatid lying over another (Fig. 40). Probably failure of a connection in double as well as multiple chiasmata is due to another chromosome or chromosomes having broken away from a still larger association. This occurrence corroborates the suggestion that metaphase pairing is due to attraction between chromatids rather than between chromosomes.

Multiple chiasmata are the regular means of association of the

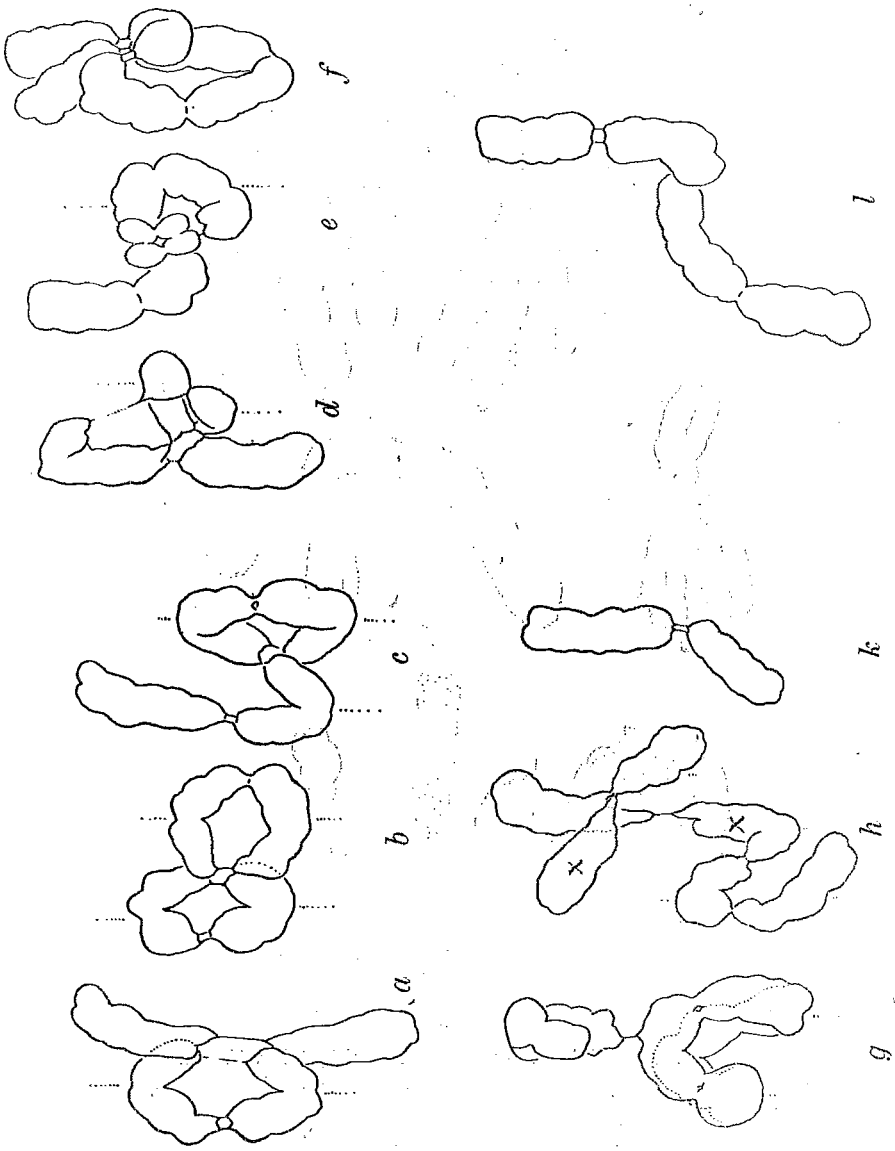


Fig. 41.

Fig. 41. *T. virginiana*: *a–c* and *g–l*, Medium Blue; *d–f*, Small Red.

a–d. Various associations of four by multiple and simple chiasmata.
e. Association of four, simple and interstitial chiasmata.
f. Branched ring of six with one multiple chiasma.
g. Ring of four with one pair attached by a lateral chiasma.
h. The same configuration as *f* but with the ring broken and the multiple chiasma imperfect (a connection between the free chromatids of the two chromosomes marked *X* has been broken).
k. The unequal pair.
l. The unequal pair associated with two others in a chain.

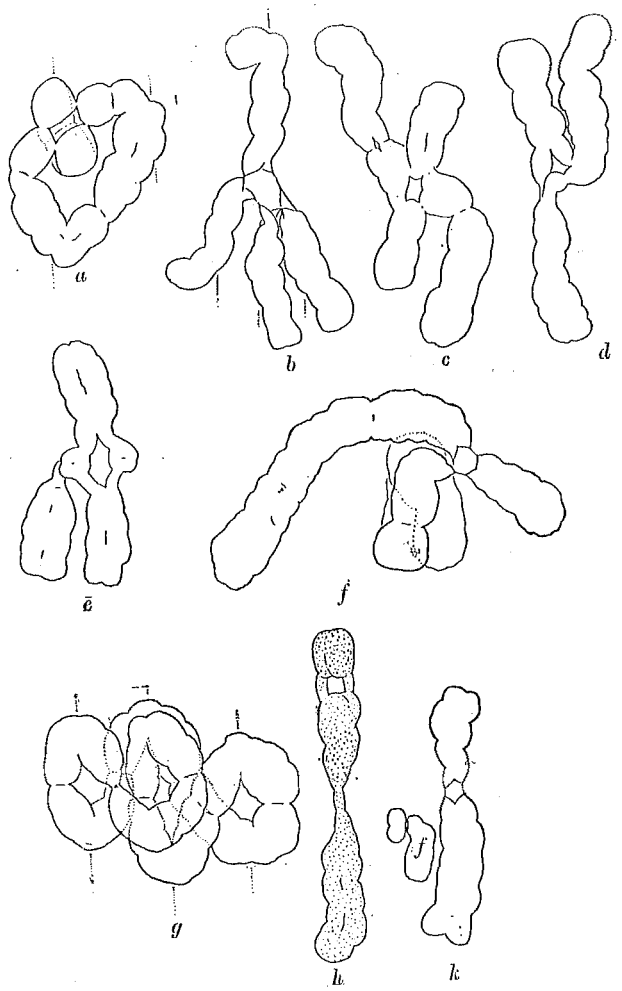


Fig. 42. *T. virginiana*: *a–e*, *g–k*, var. *humilis*, *f*, var. *hirsuta*.

a. A double ring of four, an interstitial chiasma separating the two loops.
b. An association of four at a perfect quadruple chiasma.
c. The same configuration as *a* but with both loops open.
d. Triple chiasma, terminalisation nearly complete.
e. Triple association corresponding to an earlier stage of *d* in which one pair is joined by a still interstitial chiasma; terminalisation incomplete.
f. Association of eight consisting of ring of four, chain of three and single chromosome joined at a quadruple chiasma.
g. Simple ring of eight without non-disjunction.
h. Bivalent in which abnormal lacuna shows in one chromosome—perhaps the result of a structural change at prophase.
k. Fragment with an exceptional unequal pair from the same cell.

branched strings, double rings and other complex configurations (Fig. 41 *f*) so frequently found at metaphase.

Stow (1928) has reported that the formation of compound bodies at meiosis in *Tradescantia* and other plants is affected by the temperature. In order to test this conclusion, separate lots of the form *hirsuta* were kept (i) in a stove house at a temperature of 20–25° C. and (ii) in an ice box at a temperature of 8–10° C. for two days and the pollen mother-cell divisions then examined. In each case, on an average, three quadrivalents were formed with occasional sexivalents[1], trivalents and so on, as in the material growing out of doors (in July). The only difference found was in the occurrence of fragments not ordinarily observed.

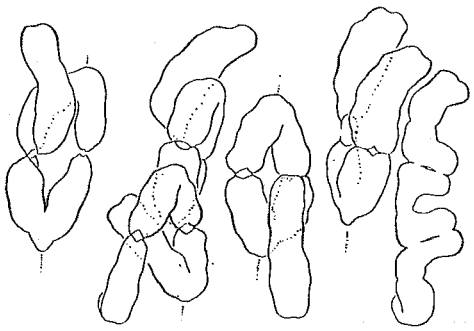


Fig. 43. *T. virginiana* var. *brevicaulis*: 6 trivalents, one with two interstitial chiasmata, and three with triple chiasmata.

In the form Medium Blue (No. 4) in which one chromosome was found shorter than the rest and with a relatively sub-terminal constriction, sometimes an unequal pair (Fig. 41 *k*) and sometimes a string of four (Fig. 41 *l*) containing one shorter than the others can be seen, although the difference is not great enough to be determined with certainty in all cells.

In all the forms examined, while quadrivalents and bivalents are the usual associations found, univalents, trivalents and combinations of higher numbers than four are frequently found. As a result the distribution of the chromosomes at anaphase is irregular. The strings of chromosomes do however, as a rule, so arrange themselves that alternate members pass to opposite poles (as in the diploid *Rhoeo* and *Oenothera*).

[1] The term "sexivalent" clearly can have no genetical significance in a form which is assumed to be a tetraploid. Similarly the other cognate terms lose their genetical meaning in this and comparable plants. I prefer to use them here in a purely descriptive sense rather than invent another set of terms, for the limitations of their proper use must at present remain quite undefined.

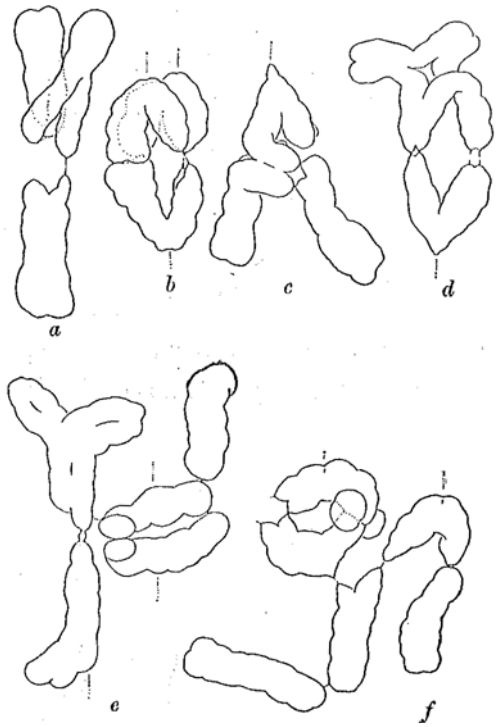


Fig. 44. *T. virginiana* var. *brevicaulis*: trivalents (except *f*).

a. Imperfect triple chiasma.
b. Triple ring with two triple chiasmata.
c. Interstitial and terminal chiasmata (cf. Fig. 42 *e*).
d. The same with a closed ring; the result therefore of triple pairing along at least half of the chromosome.
e. Two trivalents perhaps associated laterally.
f. Association of six involving an imperfect lateral chiasma which is part of a triple chiasma.

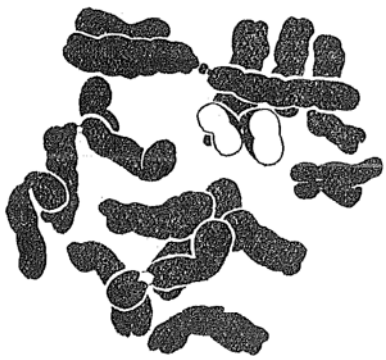


Fig. 45. *T. virginiana* var. *brevicaulis*. Restitution nucleus with 18 chromosomes, one pair still associated at a sub-terminal chiasma. One chromosome shows a new anomalous lacuna or constriction.

In *brevicaulis*, whose chromosome complement is morphologically triploid, the formation of trivalents (Figs. 43 and 44) is much more regular than is the formation of quadrivalents in the tetraploid forms of *T. virginiana*. These, as shown in Fig. 84, p. 268, Nos. 12, 13, are probably formed on the same principles as the compound bodies in the tetraploids, and, as a rule, are associated by means of multiple chiasmata. Associations of more than three can be formed (Fig. 44*f*). As many as four univalent chromosomes have been found at the first metaphase, and divisions are naturally very irregular in regard to numerical segregation. A restitution nucleus has been found (Fig. 45).

The first type of abnormality in all these forms is therefore the association of chromosomes in greater numbers than the chromosome number of the plant would suggest as being homologous on an ordinary polyploid hypothesis. The second type of abnormality is less frequent but equally significant. Certain chromosomes in various forms have the potentiality of pairing with morphologically non-corresponding parts of other chromosomes, that is to say, the end of one chromosome is seen attached, terminally, usually by only one chromatid, with an interstitial part of another (Figs. 39, 41 *d*, and 44*f*). We have thus what must be called a *lateral chiasma*. In Medium Blue (No. 4) and in *brevicaulis*, for example, one chromosome is frequently seen attached to the middle of another chromosome which itself is usually associated in a bivalent or quadrivalent ring.

The third type of abnormality is where the ends of the two chromatids of one chromosome are paired, the first with the end of a chromatid of another, the second with an interstitial part of a chromatid of the second chromosome (Fig. 49, *g* and *h*). This is virtually a triple chiasma which may be perfect or imperfect, and must result from inversion and reduplication of a section of one chromosome. Fig. 84, p. 268, shows how this result can be obtained from triple pairing and terminalisation in *Tradescantia* and in *Aucuba*, where Meurman has also observed it (1929).

Where reduplication of this kind is supposed to have occurred in *Drosophila* the segmentally tetrasomic diploid progeny were non-viable. This is not the case in *Tradescantia*, for the pairing in *T. bracteata* probably indicates segmental tetrasomy. In *T. virginiana* reduplication would not be comparable for, the general condition being tetraploid, it would lead merely to segmental pentasomy.

The behaviour of the three fragments of *T. humilis* is subject to interesting variations in this and in other connections: they may all

appear as univalents at the first metaphase and divide at anaphase (Fig. 48 *b*): two of them may pair together, the third being left as a univalent (Fig. 48 *c*): the members of the pair may divide irregularly

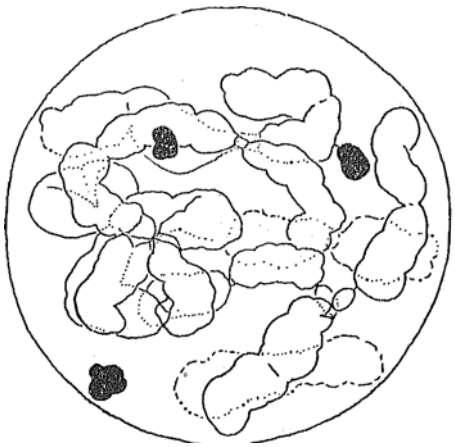


Fig. 46. *T. virginiana* var. *humilis*: diakinesis; one association of ten; one of six, with a very clear sub-terminal chiasma; one of four, with one having a laterally associated fragment; one bivalent; two free fragments.

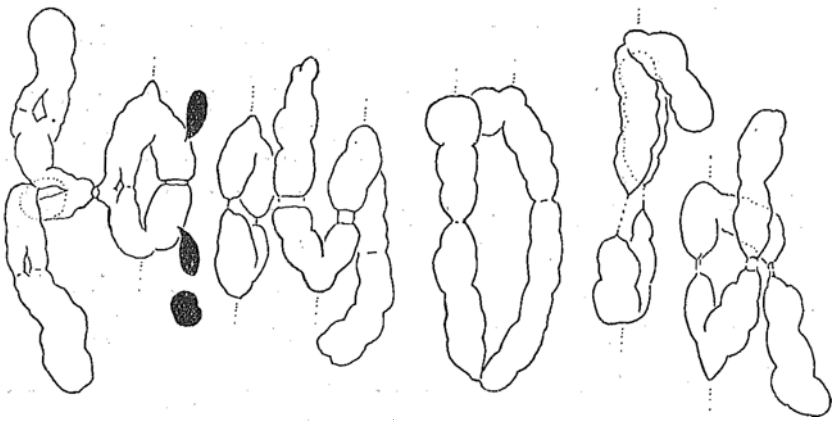


Fig. 47. *T. virginiana* var. *humilis*: an association of six involving one interstitial chiasma; two fragments paired laterally, with corresponding parts of a paired ring; four quadrivalents, one bivalent ring with imperfect chiasmata.

(Fig. 48 *d*), this being possibly associated with the fact that they usually divide earlier than the ordinary chromosomes. The homologous pair may be recognised at somatic divisions (Fig. 48 *a*). The members of the pair may associate severally, or, when paired with one another, with

different parts of probably different, or non-corresponding, whole chromosomes. When one has been associated terminally with a whole chromosome, in spite of the extreme disparity in size of the two bodies, they may be seen separating to opposite poles, as in the case of the ordinary pairing of fragments, rather before the other chromosomes (Fig. 49 *d*). In one case (Fig. 49 *f*) the terminal pairing of the fragment has apparently prevented the ordinary pairing of the whole chromosomes at one point and interrupted the normal formation of a ring of eight. The pair is evidently homologous with an interstitial portion of a pair of whole chromosomes, as well as with the ends of one or more others. Thus they may be seen associated at corresponding points with the members of a bivalent (Fig. 47), or one may be associated at one end terminally with one chromosome and at the other end laterally with a second which is itself paired terminally with a third (Fig. 49 *b*). A fragment terminally

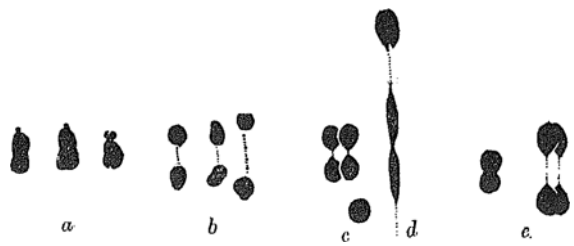


Fig. 48. *T. virginiana* var. *humilis*.

a. Three somatic fragments from the root-tip.
b. Three fragments from a single first division anaphase, dividing as univalents.
c. Bivalent fragment at metaphase of the first division.
d. Irregular division of bivalent fragment at first division anaphase.
e. Bivalent and univalent fragment from a single first division metaphase.

associated may take part in a triple chiasma exactly as a whole chromosome would (Fig. 49 *c*). It is possible that the fragments correspond to the portion of the chromosome with which they pair laterally between the point of lateral attachment and the nearer end.

Fragments have frequently been observed at meiosis which are of irregular occurrence and do not correspond with any present in the somatic tissue (cf. Section 6 (i)). In one case where a large fragment was found, apparently degenerating, a markedly unequal chromosome pair by its side gave a clear indication of its origin by loss (Fig. 42 *k*). A single observation (Fig. 42 *h*) seemed to show that a structural change had occurred interstitially in a chromosome, for the definite lacuna of well-marked boundary seen in the middle of this chromosome is entirely

exceptional. Although ordinary constrictions do not show at meiosis it is possible that this observation may be correlated with that of new constrictions at the pollen-grain divisions, and that these constrictions are therefore the result of structural changes, translocation and the like, at meiosis (cf. Section 7 (iii)).

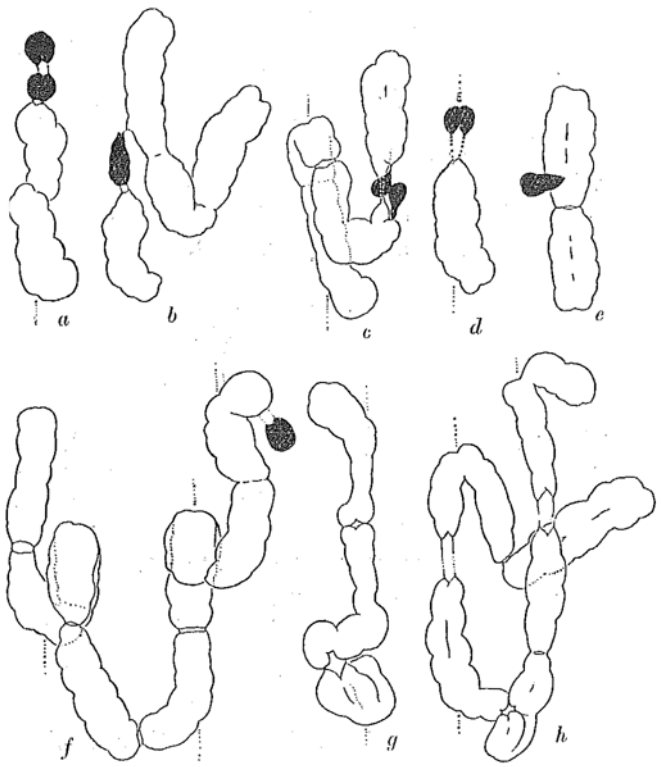


Fig. 49. *T. virginiana* var. *humilis*. Pairing of fragments and exceptional pairing of chromosomes.

a. Bivalent fragment associated terminally with one chromosome of a bivalent.
b. Fragment associated laterally with one member of a "trivalent," terminally with a fourth chromosome.
c. Fragment associated in a triple chiasma with two whole chromosomes forming part of a "quadrivalent."
d. Fragment separating from a whole chromosome, with which it has been paired, at anaphase.
e. Single fragment paired as in Fig. 47.
f. Chain of eight with a fragment paired terminally.
g. "Trivalent" showing an imperfect lateral-terminal chiasma supposed to result from the triple pairing of reduplicated segments of one chromosome with the corresponding segment of a second.
h. Perfect lateral-terminal chiasma in an association of six.

6. OBSERVATIONS OF POLLEN-GRAIN DIVISIONS.

(i) *General.*

The pollen-grain divisions in many of the forms under consideration offer a more favourable field for the study of the somatic chromosomes than do the root-tips, and a more accurate means of studying the results

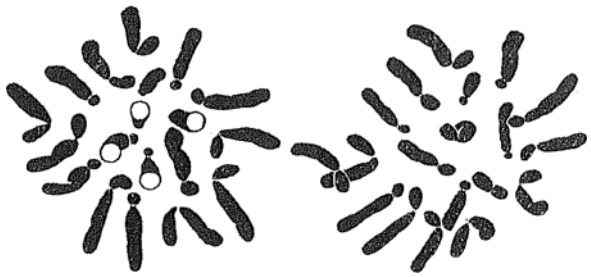


Fig. 50. First pollen-grain division in *Dichorisandra thyrsiflora*, anaphase; $n = 19$.

Fig. 51.

Fig. 52.

Fig. 53.

Figs. 51–53. First pollen-grain division in *Rhoeo discolor*.

Fig. 51. Metaphase; $n = 6$. Fig. 52. Metaphase; $n = 7$. Fig. 53. Anaphase; $n = 7$.

of reduction than the second pollen mother-cell division. It has usually been possible to correlate the observations of pollen-grain divisions with those preceding meiosis (as well as with those of the root-tips), but in the case of Medium Blue (No. 1) this unfortunately was not possible. The root-tips were however examined in all cases, and the results show that

the behaviour of the chromosomes and fragments at the pollen mother-cell divisions in this individual must be in a general way analogous to that observed in *humilis*.

Fixation gave variable results as is shown by the contraction of the chromosomes exhibited in some of the illustrations. These differences are perhaps responsible for the differences in the obviousness of the extra constrictions sometimes found. The second constriction next to the point of attachment (Fig. 58) probably corresponds to a constant condition, for it has been observed in the pollen grains of tetraploid and triploid forms and in the root-tips of *T. virginiana* (Fig. 4), *Rhoeo discolor* (Fig. 1, *a* and *b*) and *Spironema fragrans* (Fig. 3). The form it was observed to take at anaphase in *brevicaulis* is particularly remarkable,

Fig. 54.

Fig. 55.

Figs. 54 and 55. First pollen-grain division in *Tradescantia crassifolia*.

Fig. 54. Prophase; $n = 6 + f$.
a. A new long fragment from another prophase.
b. Chromosome from a prophase, showing large trabant not found in parental cells.

Fig. 55. Metaphase; $n = 6 + 3$ ff. One chromosome has a large trabant or newly attached fragment (cf. Fig. 45).

and almost suggests that it is a side branch of the chromatin thread. The possibility that other anomalous constrictions found in other divisions are the result of particular behaviour of the chromosomes concerned at the preceding meiosis cannot be overlooked, especially where this behaviour is so abnormal as in these forms. Variations in number of the members of the ordinary sporophytic complement, both whole chromosomes and fragments, are shown in the tables. Apart from these, new chromosome forms appear, sometimes corresponding in size to the ordinary fragments, and sometimes (in four recorded cases)[1] corre-

[1] In many more cases the chromosomes may have suffered loss without showing any recognisable alteration.

sponding to the ordinary chromosomes, and recognisably different only in one arm being shorter than any in the sporophytic complement (Fig. 58). These must be considered the product of fragmentation at meiosis.

In certain types extra-nuclear bodies, probably of degenerating chromatin material, were found at metaphase (Fig. 76), though whether these correspond to whole chromosomes or to fragments cannot be said. They are no doubt the accessory bodies referred to by Nawaschin (1911) as occurring notably at the second division.

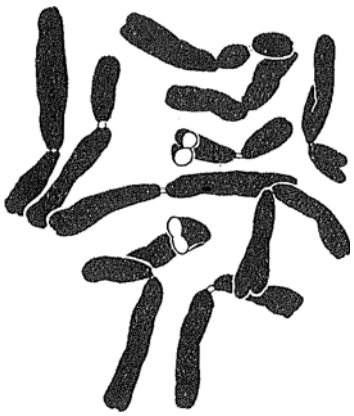


Fig. 56. First pollen-grain division in *T. virginiana* var. *alba*, Aldenham. Metaphase; $n = 11 + f$. Fragment is new and possibly connected with the shortened arm of an adjacent chromosome.

The fragments in the pollen grains of Medium Blue (No. 4), the individual most extensively studied, showed frequently and clearly a peculiarity that I have also observed in *T. crassifolia*, viz. the terminal association of one of the halves of the fragment with one of the halves of an ordinary chromosome (Figs. 60 and 61). This association is so characteristic that it can hardly be without significance. If it is an artifact it is difficult to understand why only one of the halves is attached and not both of them; whereas if it is the actual union of a fragment with one of the chromosomes, this result would be expected, for the two separate halves of the divided chromosome thread might well act independently during the resting stage or at least in early prophase.

(ii) *Chromosome-Number Frequencies.*

Counts have been made of the numbers of chromosomes and fragments in pollen-grain divisions of the various forms of *Tradescantia*

virginiana (including *brevicaulis*), *T. crassifolia* and *Rhoeo discolor*. The frequencies obtained agree with what the observation of the pollen mother-cell divisions would lead one to expect, provided allowance is made for special genetical conditions in the pollen grain. In *Rhoeo*, for example, the proportion of cells with seven chromosomes seems too high to regard as directly determined at meiosis, for, taking into consideration an equal number of non-viable pollen grains with five chromosomes, it corresponds with a numerically unequal segregation (*i.e.* two non-disjunctions on the same side of the plate) in 30 per cent. of cases. This is much too high to agree with observations of either the first or the second division, and would appear to indicate a reduced viability of

Figs. 57–63. First pollen-grain division in *Tradescantia virginiana* Medium Blue. Figs. 57–61, metaphase. Figs. 62 and 63, anaphase.

Fig. 57. $n = 12 + 2$ ff.

the haploid six-chromosome pollen grains relatively to the seven-chromosome grains. This is not surprising, for on the interchange hypothesis (Darlington, 1929), as in *Oenothera*, this type of non-disjunction will always give rise to genetically complete $n + 1$ gametes, while non-disjunction on opposite sides will give rise to genetically defective n gametes[1]. With a further proportion of sixes, therefore, corresponding

[1] Double non-disjunction on opposite sides giving genetically defective "haploid" gametes will be:

$$\frac{AB \quad CD \quad EF.FG \quad HK \text{ (no } L) \quad MN}{BC \quad DE \text{ (no } F) \quad GH \quad KL.LM \quad NA} \quad \begin{matrix}\text{(non-viable)}\\ \text{(non-viable)}\end{matrix}$$

Double non-disjunction on the same side giving one viable $n + 1$ gamete, and one defective $n - 1$ gamete, will be:

$$\frac{AB \quad CD.DE \quad FG \quad HK.KL \quad MN}{BC \text{ (no } D) \quad EF \quad GH \text{ (no } K) \quad LM \quad NA} \quad \begin{matrix}\text{(viable)}\\ \text{(non-viable)}\end{matrix}$$

to the sum of the numbers of five (hypothetical) and seven (observed) types, included to represent genetically defective haploid gametes, the two sets of observations are in agreement.

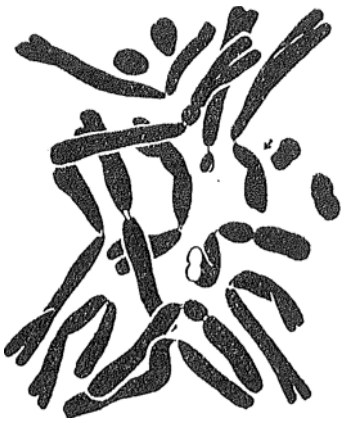


Fig. 58. $n = 12 + 4$ ff. Note one chromosome which has been truncated and its resemblance to part of the chromosome with the second median constriction.

Fig. 59. $n = 12 + 4$ ff.

The observations by Winge on *Vallisneria* (1927) and Belling on *Uvularia* (1925) of the division of pollen-grain nuclei with less than the supposed haploid number of the plant in question is important as indicating a different type of chromosome organisation. Belling is inclined

to think his proportion of $n-1 : n : n+1$ gametes (namely 1 : 96 : 6 or 7) indicates merely that the $n-1$ gametes are slower in development than the others. This argument seems to be ruled out in *Uvularia* by his observation of $n+1$ and $n-1$ grains dividing side by side; nor is it sufficient in *Rhoeo*, for both late and early pollen grains were examined and, although (see Section 6 (ii)) the proportion of $n+1$ pollen grains varied according to the period, none was found with $n-1$. The most reasonable conclusion seems to be that (i) in *Uvularia* the viability of $n-1$ gametes is greatly reduced, and (ii) in *Rhoeo* it is still more reduced, perhaps to zero. It must be remembered that in pollen-grain frequencies at the primary division we have the mildest test of genetic competence that can be applied. It seems none the less significant of either a low degree of differentiation or of a polysomic condition when gametes, short of a considerable proportion of their complement, can live to divide.

The chromosome number frequencies in the pollen grains of tetraploids evidently vary about 12 as a mean. Similar variation in gamete production has been found in the tetraploid cherries (Darlington, 1928). The pentasomic form *montana* has a slightly higher mean, as would be expected. The frequency in Medium Blue (No. 4) B (a single preparation) has a lower mean, although the root-tips had the tetraploid number of chromosomes. This is therefore either the result of the loss of a chromosome in the somatic tissue, or of taking an exceptionally early or late sample of the population. The fragment frequencies, which as far as

TABLE II.

Medium Blue (No. 1). ($2n = 24 + 5$ ff.)

Number of fragments	Number of chromosomes			
	11	12	13	14
0	—	—	1	—
1	1	10	2	—
2	3	19	7	1
3	6	12	—	—
4	—	3	—	—
5	—	2	—	—

Totals for Separate Smears.

		Number of chromosomes				Number of fragments					
	Total	11	12	13	14	0	1	2	3	4	5
Slide 10	11	—	8	2	1	—	—	7	1	2	1
Slide 11	41	7	29	5	—	1	8	16	13	2	1
Slide 14	19	3	13	3	—	—	5	10	4	—	—
Grand total:	71	10	50	10	1	1	13	33	18	4	2

TABLE III.

	Number of chromosomes					
Other Tetraploids:	10	11	12	13	14	Total
Small Red	—	5	17	—	—	22
Pale Blue	—	—	42	—	—	42
bracteata	—	1*	12	—	—	13
congesta	—	4	14	5	—	23
hirsuta	—	3*†	12	1	—	16
alba (Kew)	—	3	9	1†	—	13
alba (Aldenham)	—	1†	6	—	—	7
Medium blue (No. 4):						
Slide A:						
with extra-nuclear bodies	1	8	1	—	—	
without extra-nuclear bodies	—	14	1	—	—	
Slide B:						63
with extra-nuclear bodies	—	3	32†	1	—	
without extra-nuclear bodies	—	—	2	—	—	

* One had an extra-nuclear body. † One had a fragment.

TABLE IV.

montana. ($2n = 25 + 2$ ff.)

	Number of chromosomes				
	11	12	13	14	Total
1 fragment	—	4	2	2	8
2 fragments	2	2	3	—	7
Total:	2	6	5	2	15

TABLE V.

brevicaulis. ($2n = 18$.)

	Number of chromosomes					
	7	8	9	10	11	Total
Smear 181 (*a*)	3	5	24	10	—	42
„ 181 (*a*)	1	1	2	1	—	5
„ 183 (*b*)	—	6	3	3	—	12
„ 184 (*a*)	—	1	1	7	4	13
Total:	4	13	30	21	4	72

(Distributed at random 7 had fragments and 8 had extra-nuclear bodies.)

TABLE VI.

Rhoeo discolor.

Number of chromosomes			
5	6	7	Total
—	37	8*	45

* One with a fragment.

Zebrina pendula: $n = 12$; 3 counts.
Dichorisandra thyrsiflora: $n = 19$ (Fig. 50); 2 counts.

TABLE VII.

Tradescantia crassifolia.

Number of chromosomes	Number of parental fragments					Total
	0	1	2	3	4	
6	5*	20	3	1	1	30
7	—	1	—	—	—	1

* One had a new type of fragment (Fig. 54 *a*).

TABLE VIII.

Summary of Tetraploid Tradescantia.

	Number of chromosomes					
	10	11	12	13	14	Total
Medium Blue (No. 1)	—	10	50	10	1	71
,, (No. 4) B	—	3	34	1	—	38
Small Red	—	5	17	—	—	22
Pale Blue	—	—	42	—	—	42
alba (Aldenham)	—	1	6	—	—	7
alba (Kew)	—	3	9	1	—	13
hirsuta	—	5	12	2	—	19
bracteata	—	1	12	—	—	13
congesta	—	4	14	5	—	23
Total	—	32	196	19	1	248
Medium Blue (No. 4) B	1	22	2	—	—	25

possible are intended to exclude the fragments not already present in the parent plant, show a similar random variation, but in one or two details the results are unexpected. First, in *Tradescantia crassifolia* a number greater than that in the parent was twice found. This would occur as a result of what we may call "double reduction," where both the chromatids of one chromosome pass into the same nucleus at the second telophase[1]. The possibility of such an occurrence in quadrivalents of a tetraploid *Dahlia* has been considered by Lawrence (1929). This might well result from the small size of the univalent fragment in the one case, and from the normal course of quadrivalent pairing and separation in the other.

The second point is that an apparent inverse correlation is noticeable between chromosome number and fragment number, both in Medium Blue (No. 1) and in the variety *montana*. From the observation of meiosis where fragments occur (in *humilis*) I can find no mechanical explanation

[1] Longley (1927) describes the production of $n + 2$ ff. by $2n + 1$ f. parents as the result of "non-disjunction," but the application of this term to the failure of an equational division—a division, that is, of elements that have never been separate—seems a little confusing

of this association, but a genetical explanation is not far to seek. A given fragment may carry material that is already represented four times, as

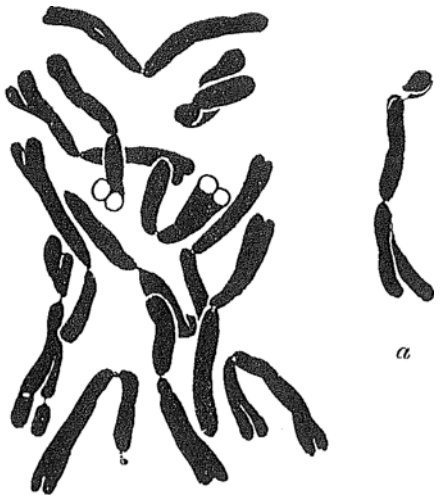


Fig. 60. $n = 13$ (no fragments); note anomalous dissimilarity of two half-chromosomes at 5 o'clock.

a. Chromosome from another metaphase in which one-half of a fragment is apparently joined to one-half of the chromosome.

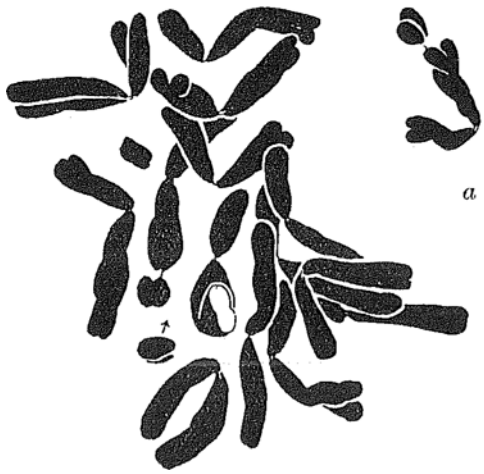


Fig. 61. $n = 14 + 2$ ff. Note chromosome as in Fig. 60 *a*.

a. Third example of unequally attached fragment.

most of the material in the ordinary chromosomes of the tetraploid must be. But it is more likely, especially if any of the fragments are

bivalent (as with the pair in *humilis*), that the same material is only represented again two or three times in the large chromosomes; in other words that the chromosomes which have actually suffered fragmentation are still present, deficient in the material which is carried by the fragments.

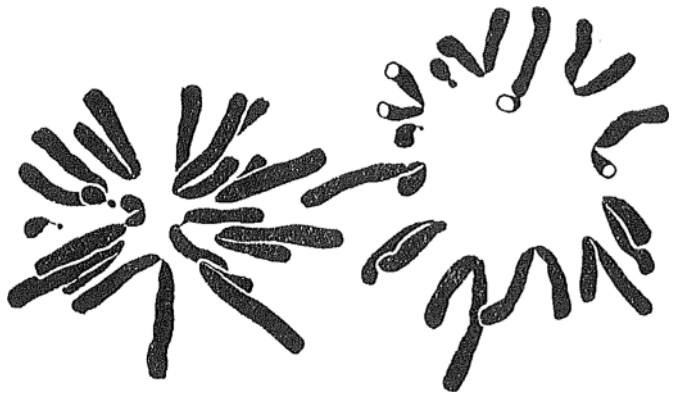


Fig. 62. $n = 11 + 2$ ff. Note the difference in size of trabant in the two fragments.

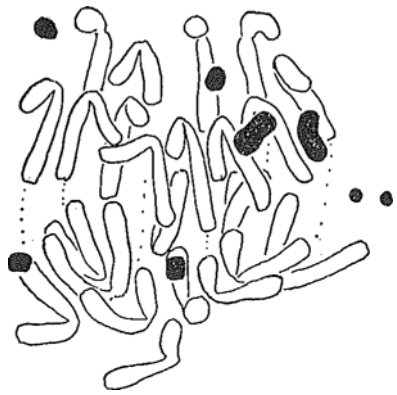


Fig. 63. $n = 12 + 2$ ff. New fragments, two larger than normal, two smaller, are failing to divide.

In this case a set of six chromosomes in this plant need not be supposed to be a full complement in the absence of particular fragments. Therefore in certain pollen grains where the complement is short of 12 the presence of some of the fragments will be necessary to viability, and from these pollen counts a class will be eliminated with few fragments and less than 12 chromosomes. It follows that there is no simple inverse

correlation, but that we probably have here three separate factors operating: (i) an original higher proportion of pollen grains with chromosome numbers below the mean, owing to loss at meiosis, (ii) an original higher proportion of pollen grains with fragment numbers below the mean for the same reason, (iii) the elimination of pollen grains with certain fragments missing and with less than the diploid number of chromosomes. A deficiency in certain of the haploid combinations from *brevicaulis* would similarly account for the excess of its 10-chromosome pollen grains.

In *T. crassifolia* variations occurred that could only be attributed to changes at meiosis. Large trabants not occurring in the parental cells or in other pollen grains appeared in several divisions (Figs. 54 *b* and 55). In one cell a fragment with a sub-terminal constriction was found (Fig. 54 *a*), longer than those found in the parent. One pollen grain, with seven chromosomes and a normal fragment, was evidently the result of non-disjunction. Occasionally attached to the end of a chromosome at prophase is a small mass of nucleolar material that might be mistaken for a trabant.

7. General Observations.

(i) *Fragmentation in* Tradescantia *and* Fritillaria.

From the present studies of fragmentation in *Tradescantia* the following conclusions can be derived:

(i) Fragments arise at the prophase of meiosis, and not, as in *Uvularia* (Belling, 1925), at anaphase.

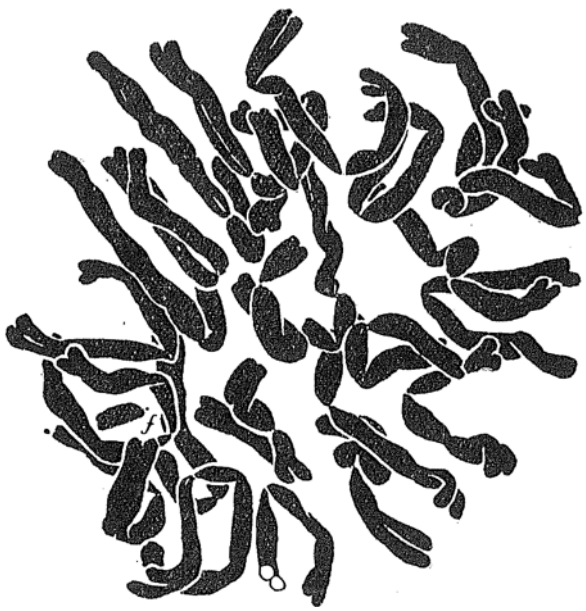
(ii) Fragments arise as a result of the splitting of a chromosome at any point along its length, so that in size the new fragments may bear any proportion to the whole chromosomes (Figs. 63, 71 and 74).

(iii) New fragments sometimes develop attachment constrictions which are evident at the first post-meiotic division. All those which do so bear a certain relationship in size to the whole chromosomes. Those which do not are larger or smaller and can be seen degenerating at meiosis or at the first post-meiotic division.

(iv) All fragments found in the population of varieties and seedlings studied agree in size with those new ones which are found to develop attachment constrictions (Figs. 64–66).

(v) The pairing of fragments at meiosis is irregular but shows that they may be homologous either with terminal or with interstitial portions of whole chromosomes, as well as with one another.

Summarising these conclusions, we find that while fragments (as is the case with polyploids) can arise almost without restriction, they can only be perpetuated subject to two important restrictions, the one mitotic, the other meiotic. Other cases where fragments have been studied extensively, even more extensively than here, yield less decisive results in regard to the homology of the fragments and the conditions of their survival. In *Crepis*, for example, Nawaschin (1926) has found fragments and corresponding losses of parts of chromosomes, but the classification of types of chromosome change based on these discoveries



Figs. 64–66. Somatic metaphases from the root-tip of *Tradescantia virginiana*.

Fig. 64. Var. *caerulea fl. pl.* $2n = 24 + 1$ f.

seems to some extent empirical, for such structural changes as probably occur in *Crepis* express themselves at meiosis (Nawaschin, 1927), not in exceptional types of association but in a failure of pairing altogether[1]. Similarly in *Zea* the material has not been favourable for the genetical identification of the fragments, and the remarkable abnormalities found by Kuwada (1915, 1919) and Randolph (1928) remain to some extent unexplained. For example, Randolph has found four supernumeraries

[1] This is doubtless connected with the fact that even under the most favourable conditions—of pairing between identical chromosomes—only a single chiasma is established.

which may form a quadrivalent or any smaller association. Yet it is scarcely credible that, in addition to the diploid complement, there

Fig. 65. Var. Taplow Crimson, $2n = 24 + 4$ ff.; fragments numbered.

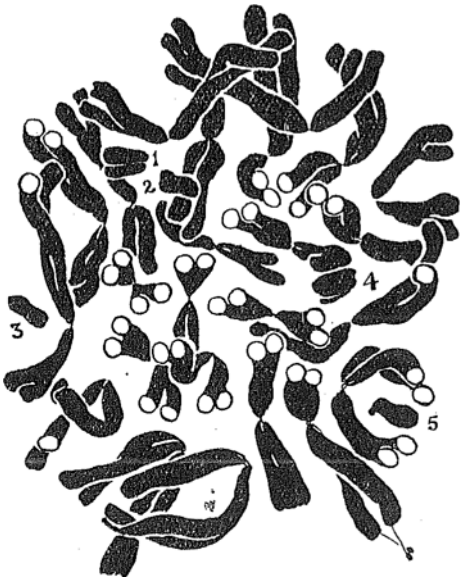


Fig. 66. Var. Medium Blue (No. 1). $2n = 24 + 5$ ff.; fragments numbered.

should be four homologous elements corresponding to one part of the diploid set. It seems more likely that these observations, and those of Kuwada, who found variations in the number of bodies at meiosis, are

comparable with those of *Tradescantia bracteata* and *T. crassifolia*, where five bodies are often formed instead of six. The behaviour of the *Zea* fragments therefore points to the occurrence of segmental interchange (see Section 7 (vii)).

The development of an attachment constriction in a new chromosome, as found by Nawaschin in *Crepis* (1926), is seemingly an essential condition of mitotic life, and we therefore have the new types of chromosome in *Tradescantia* limited to (i) those which have suffered loss but retain their old attachment constriction, (ii) those fragments which develop a new attachment constriction, and these have a certain limited range of size. The effect of this size restriction is seen in the species of *Tradescantia* with higher numbers and smaller chromosomes than *Tradescantia virginiana*, a question I will return to later.

It is interesting to find from a study of the somatic chromosomes in the genus *Fritillaria* conditions rather similar to those suggested in *Tradescantia*. In a variety of *F. imperialis*, the permanent complement of which has already been studied and described by Taylor (1926), six small fragments have been found (Fig. 69, *a–c*). These are of a limited size range and bear the same proportion to the major chromosomes as do the fragments in *Tradescantia*. When we consider the *permanent complement* however we do not find in either genus the extreme disparity of size that exists between the major chromosomes and the supernumeraries or fragments of *Tradescantia* or *Fritillaria*. For example, in *F. imperialis* (Fig. 67), *F. Meleagris* and *F. latifolia* (Fig. 68 *a*) there are 12 pairs of chromosomes, 2 with sub-median constrictions, and 10, about half the length, with sub-terminal constrictions. In the related species, *F. ruthenica*, there are only 9 pairs of chromosomes (Fig. 68 *b*) but the types are represented in different proportions, 5 sub-median, long, 4 sub-terminal, short. Reference to the illustrations will show that the two species correspond closely if we assume that three of the sub-terminal pairs of *F. ruthenica* have broken in the middle to give six short sub-terminals. The analogy between this case and that of the relationship of *Tradescantia virginiana* and *T. navicularis* is fairly close, and shows that similar processes are at work in the two genera.

The second type of restriction, imposed by conditions at meiosis, is probably not applicable to these two genera alone. Work on *Hyacinthus* (Darlington, 1929) showed that the number of chiasmata, on which the pairing at metaphase seemed to depend, was proportional to the size of the chromosomes, so that although the long type of chromosome usually had three or four chiasmata the short ones rarely had more than one.

The same type of variation size and chiasma frequency apparently occurs in *Uvularia* (Belling, 1925). It seemed to follow from this that the

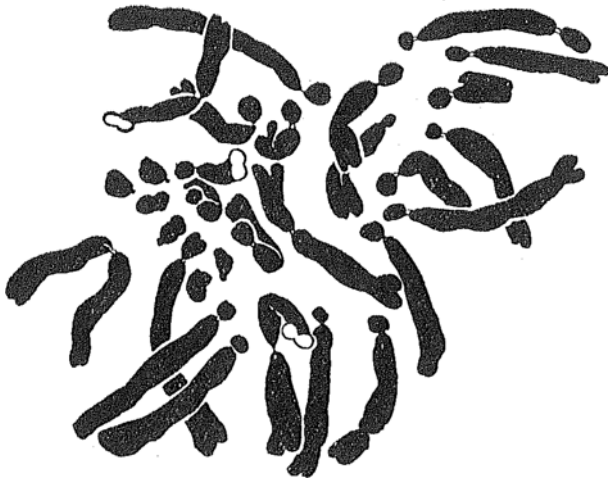


Fig. 67. Somatic metaphase from the root-tip of *Fritillaria imperialis*; $2n = 24 + 6$ ff. Four chromosomes with sub-median, 20 with sub-terminal constrictions.

Fig. 68 *a*.

number of chiasmata was insufficient to permit of the regular formation of trivalents and quadrivalents by the short type. To express this otherwise, chiasma-formation in *Hyacinthus* is fitted to secure regular

Fig. 68. Somatic metaphases from the root-tips, *a*, of *Fritillaria latifolia* ($2n = 24$) and *b*, of *F. ruthenica* ($2n = 18$). Individual chromosomes picked out from a complete side view. Types marked (sub-median, *m*) or numbered: 1–5 with sub-median, 6–8 with sub-terminal constrictions.

pairing in the diploid of the various types of chromosomes between which there is a great discrepancy of size, because the longer ones have numerous chiasmata; but it is not fitted to secure the regular formation of chiasmata, and regular association, between three chromosomes of the short type.

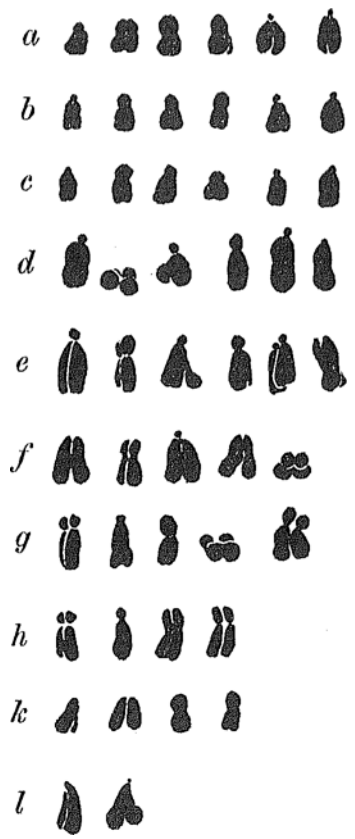


Fig. 69. Somatic fragments from root-tip metaphases drawn separately.
a–c. *Fritillaria imperialis*, 6 fragments.
d–e. *Tradescantia virginiana* var. Medium Blue, No. 1 (vii), 6 fragments.
f. Medium Blue, No. 1 (viii), 5 fragments.
g. Medium Blue, No. 1 (vi), 5 fragments.
h. Medium Blue, No. 1 (i), 4 fragments.
k. Taplow Crimson, 4 fragments.
l. *Montana*, 2 fragments.

This conclusion is in accordance with the observations of Gotoh (1924) and Belling (1925) on *Secale*, and Longley (1927) and Randolph (1928) on *Zea*. In each of these cases small fragmented chromosomes fail to pair

regularly with presumably homologous partners: their chiasma-frequency is too low. The behaviour of the fragments in *Tradescantia* is still stronger evidence in favour of the same conclusion, for the size difference between fragments and whole chromosomes is still more pronounced. Pairing of the homologous fragments is a relative rarity. It is therefore a working corollary of the principle that metaphase pairing is determined by the random formation of chiasmata at diplotene, that gross changes in the size of chromosomes will interfere with the regularity of their pairing at meiosis.

This means that, in so far as sexual reproduction is important in maintaining the species, fragmentation will be restricted so that only such of its products will survive as can pair regularly at meiosis. The fact that we have forms of *Tradescantia virginiana* with fragments that do not answer to the requirements of meiosis merely emphasises the unimportance of sexual reproduction in preserving this species. We may remark, parenthetically, that the same conclusion is to be drawn from the relatively auto-polyploid condition of *T. virginiana*. Its chromosome sets, although of course differentiated (more structurally than genetically perhaps), may be said to be more or less *indifferently differentiated*. It does not affect the question of the conditions under which its relatives have probably varied, and such species as *T. navicularis* and *T. fluminensis* have developed their characteristic chromosome forms. These species are probably derived from ancestors reproducing by seed-production, while *T. virginiana* itself has drifted into an evolutionary backwater in which vegetative propagation has become excessively important. In a seed-producing species, such as *Rhoeo*, chromosome structure could change effectively only as [illegible] changed in *Fritillaria*, by the breaking of chromosomes into two almost equal halves. It could not change by production of the mitotically satisfactory small fragments because these are incapable of regular pairing at meiosis. It is for this reason no doubt that the related species with 24 to 38 chromosomes (*Zebrina pendula*, *Dichorisandra thyrsiflora* and *T. navicularis*) show two types of change relative to *Rhoeo* and *T. virginiana*, namely, loss of small parts, and bisection of the whole chromosome: but they do not show the survival of the fragments found in *T. virginiana*. Such species as *Tradescantia fluminensis* and *Tinantia fugax* (most of whose chromosomes closely resemble the fragments of *T. virginiana*) have arisen probably through such intermediate types as *T. navicularis*, and not directly by the survival of small fragments. For each chromosome type there are two possible types of fragmentation that we must distinguish: effective and ineffective.

We do not know how far fragmentation of chromosomes occurs at random in other groups, but it is evident that the restrictions to which it is subject after the moment of origin must seriously limit its importance. I have earlier remarked, in considering *Ribes* (1929), that fragmentation, when effective, is to a great extent incompatible with freedom of hybridisation. This is indicated by Seiler's results with *Phragmatobia* and Longley's with *Zea*, where irregularities in the segregation of fragments follow their pairing, in the one case, with whole chromosomes, in the other, with fragments like themselves. It is not therefore surprising that in many great plant genera, such as *Rosa*, *Rubus*, *Prunus*, *Ribes*, *Avena* and *Triticum*, where hybridisation and seed-production play an important part, effective fragmentation is absent. Meurman's criticism (1928) of the remark that fragmentation was of little phylogenetic consequence in these groups, on the ground that he had actually found fragments in *Ribes*, does not meet the case. It is not that fragments do not occur. It is that, occurring, they will tend to be eliminated in the course of sexual reproduction by the conditions of meiosis: fragmentation is therefore ineffective.

It is not clear how far gross changes of structure are distinct from genetical changes or "mutations," but it seems evident that, whatever effect mutations may have on limiting hybridisation, the effect of structural changes, fragmentation as well as others, must be at least as drastic. It is only where such changes have developed under strict genetical control, as in *Oenothera* and *Rhoeo*, that they are compatible with moderate regularity of segregation. Where, as in Blakeslee's *Datura* hybrids or in Håkansson's *Godetia amoena-G. Whitneyi* cross, the separated products of structural change are brought together again, the result is irregularity in meiosis or even complete sterility.

(ii) *Chromosome Size and Cell Size.*

Variation in size of corresponding chromosomes in material from the same individual can as a rule be put down to variation in the efficiency of fixation, for the shapes of the chromosomes in these cases themselves indicate such variation. It is, for example, fairly clear that fixation in the pollen grains of *Tradescantia* is not comparable with that obtained in the root-tips. There is contraction in the length of the chromosomes, and the three trabants characteristic of the triploid, and the four of the tetraploid, root-tips (although not always visible on account of the chromosomes lying in the line of vision) are not often found in the pollen grains; although they should always be clear. In *Hyacinthus*

TABLE IX.

Measurements of Longest Chromosomes.

		Root-tips	Pollen grains
Treleasia brevifolia	(4*n*)	10·6 μ	—
Rhoeo discolor	(2*n*)	10·6 μ	7·3 μ
Spironema fragrans	(2*n*)	13·1 μ	—
Zebrina pendula	(4*n*)	14·0 μ	—
Tradescantia crassifolia	(2*n*)	14·4 μ	7·4 μ*
T. virginiana forms	(4*n*)	18·8 μ	15·6 μ
T. brevicaulis	(3*n*)	14·6 μ	(*a*) 13·0 μ†
			(*b*) 7·6 μ†
Hyacinthus orientalis	—	21 μ	21 μ

* Inferior fixation; shape of chromosomes not at all comparable with root-tip material.
† Cf. Table V.

Fig. 70.

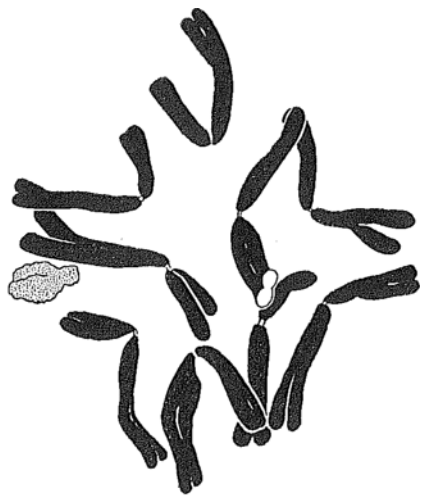


Fig. 71.

Figs. 70–76. Metaphases of the first pollen-grain division in *Tradescantia virginiana* var. *brevicaulis*.

Fig. 70 Smear 184; $n = 10$.
Fig. 71. Smear 181; $n = 9$, large new fragment degenerating.

orientalis, on the other hand, I have found root-tip fixations and chromosome sizes in the pollen grain closely comparable. There seems no reason to doubt that the size of the chromosomes is really the same in root-tips and pollen grains, and that differences between them are incidental to fixation.

In *Tradescantia brevicaulis*, however, one preparation of pollen grains[1]

[1] Smear 183.

proved to be quite distinct from the others both of pollen grains and of root-tips from this individual. In this preparation 12 divisions were

Fig. 72. Smear 181; $n = 9$. New constrictions in several chromosomes.

Fig. 73.

Fig. 74.

Fig. 75.

Fig. 76.

Figs. 73–76. Smear 183; small chromosomes at the same magnification as the other illustrations (× 2800).

Fig. 73. $n = 8$.

Fig. 74. $n = 8 + $ f. (new fragment of normal size, not degenerating).

Fig. 75. $n = 8\frac{1}{2}$, one chromosome has suffered fragmentation at the point of attachment.

Fig. 76. $n = 10 + b$. Extra-nuclear body of degenerating chromatin material. One chromosome shows a second constriction of a type occasionally seen. Two chromosomes have similar trabants not seen elsewhere in pollen grains or root-tips.

examined, four of which are illustrated (Figs. 73–76). They show the same type of segregation as is found in the other smears (see Table I)

and the pollen grains are of the same size, allowing for slight differences between individual grains. Moreover, the same differences in the quality of the fixation occurred in this slide as in the others. Smears 183 and 184 were both fixed in Benda's solution.

The only possible explanation of this difference seems to be a somatic mutation governing the size of the nucleus as a whole. It is the more interesting in this individual which must, it would seem, be regarded as a hybrid between a diploid and a tetraploid (see Section 7 (vi)), for the size of its chromosomes is actually intermediate between that of its diploid and tetraploid relatives.

The change considered here is essentially one of bulk and has no relation to the change in shape at meiosis described in *Matthiola* (Lesley and Frost, 1927) where change in bulk is not involved. It is never-

Fig. 77. Anaphase group from the first pollen-grain division in the variety *brevicaulis*; $n = 9$. Note "interstitial trabant."

theless possible that the chromosomes of this mutant form might be more than proportionately shorter at meiosis. Some such change in the mass of the chromosomes as a whole must have played a part in the evolution of chromosome form in the *Tradescantiae*, to judge by the comparative size of the chromosomes in species of this group. For example, although chromosome form in *Rhoeo* is strictly comparable with that in *Tradescantia virginiana*, the chromosomes as a whole are definitely smaller, and in *Cyanotis somaliensis* we seem to have an extreme example of the same type of change. Variations of this kind are suggested by Heilborn in *Carex* (1924), but it is not clear from the simple study of the pollen mother-cell divisions what part fragmentation may have played in producing changes of chromosome size in this genus.

In order to test whether this difference of chromosome size was associated with change in cell size, pollen grains of both types in which

metaphase divisions had been counted were drawn in outline, with the results shown (Fig. 78). The size of the grains is of a regularity that could scarcely have been expected. Of the seven pollen grains of smear 183 illustrated, five are not markedly smaller than the pollen grains from the other slides that were comparable in regard to chromosome number, the one with nine chromosomes and a fragment cannot be

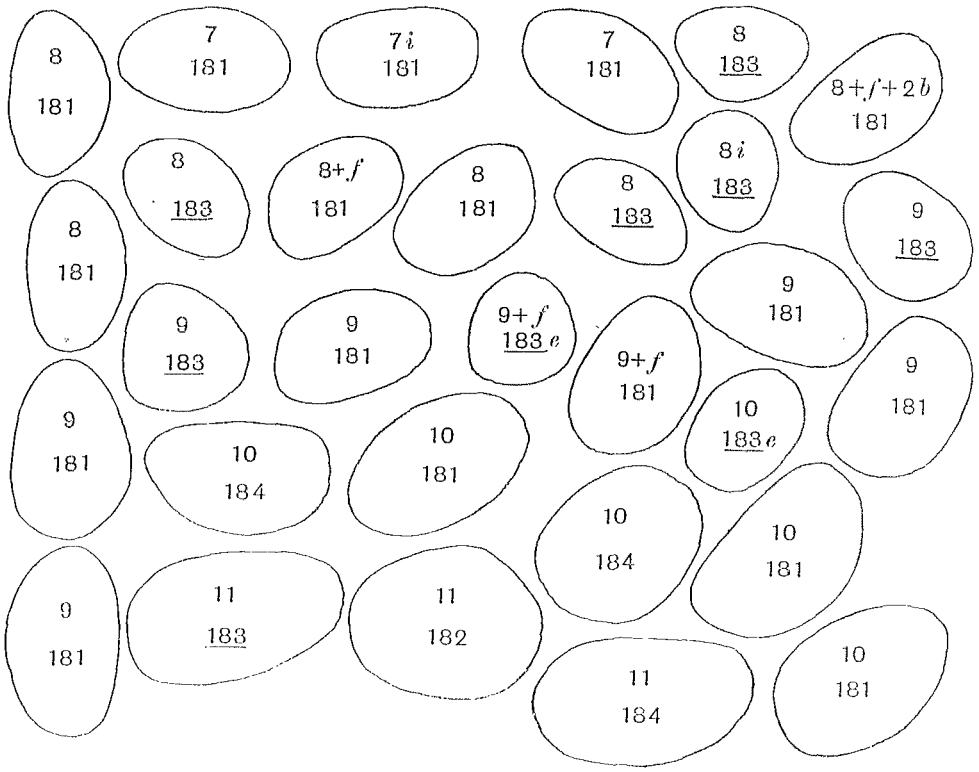


Fig. 78. Plan outlines of pollen grains at the time of first mitosis with the numbers of their chromosome complements.

Tradescantia virginiana var. *brevicaulis*; n = 7–11. No. of slide (181–4) inserted on each pollen grain.

b. An extra-nuclear body present.
f. A fragment. *e*. Size exceptional. *i*. Chromosome complement illustrated.

said to have another comparable with it, and only the seventh, the one with ten chromosomes, shows an apparently reduced size. I conclude that the change in chromosome size is independent of any general genetic change, and the observations support the view that it is due to a single factor mutation.

Taking the observations as a whole, insufficient though they are for any final conclusions, we may consider the variation in pollen-grain size in relation to chromosome number. The chromosomes of *brevicaulis* being of approximately equal size we should expect, other things being equal, increase in pollen-grain size from 7 to 11 chromosomes. Such observations of pollen-grain size as have been carried out in relation to chromosome number indicate a very close connection between the two. Belling found in *Uvularia* (1925) that diploid pollen grains were 1·25 (*i.e.* approximately $\sqrt[3]{2}$) times the diameter of haploid. De Mol's (1928) single illustrations of haploid, diploid and tetraploid tulip pollen grains show a proportion in diameter of 1 : 1·38 : 1·6 respectively. Thus, although the diploid is larger than the expectation, the haploid and tetraploid are approximately in the volume proportion of 1 : 4. This is the case so far as the extreme types are concerned, but all five pollen grains with 8 chromosomes are smaller than any of the three with 7 chromosomes, while those with 9 chromosomes are not significantly larger. Thus, unlike the results of cross-fertilisation in fishes and echinoderms, the variation here shows the immediate effect of a change in the nucleus on the metabolism of the cell (cf. Loeb, *The Organism as a Whole*).

This "sag" in the cell-size curve seems to be in accordance with the view that growth depends on the balance between chromatin elements. We are here dealing with the simplest type of variation in genetic balance, in which different proportions of the chromatin material are merely reduplicated. For the purpose of comparing the degrees of unbalance a satisfactory method in this case seems to be to consider the balance of every possible pair of chromosomes, and to take the relationships of every pair as of equivalent importance (for the chromosomes of *brevicaulis* do not vary in length more than one-fifth). Thus between *A*, *B*, *C*, *D*, *E* and *F* there are 15 different relationships of pairs; in the 7-chromosome individual (pollen grain) of the constitution, for example *AABCDEF*, five of these relationships are abnormal, ten normal; in the 8-chromosome *AABBCDEF* indivdual the one relationship between *A* and *B* is normal and also the six between *C*, *D*, *E* and *F*, while eight are abnormal. In this way the scale of unbalance of the pollen grains varying in number from 6 to 12 will be: 0/15, 5/15, 8/15, 9/15, 5/15, 0/15.

If the degree of unbalance follows any scale of variation such as this the drop in cell size between the 7- and 8-chromosome types is explained.

Further, it will be seen that a fragment may increase unbalance or diminish it. If, in this example, the fragment is a triplication then the unbalance will be sharply increased, especially with the lower chromo-

some numbers. If it is only a duplication it will slightly increase the unbalance with chromosome numbers of 6, 7 and 8 and reduce it with chromosome numbers of 9, 10 and 11. Hence the difference of size between the two "9 + f." pollen grains in opposite directions may mean merely the difference between duplication and triplication.

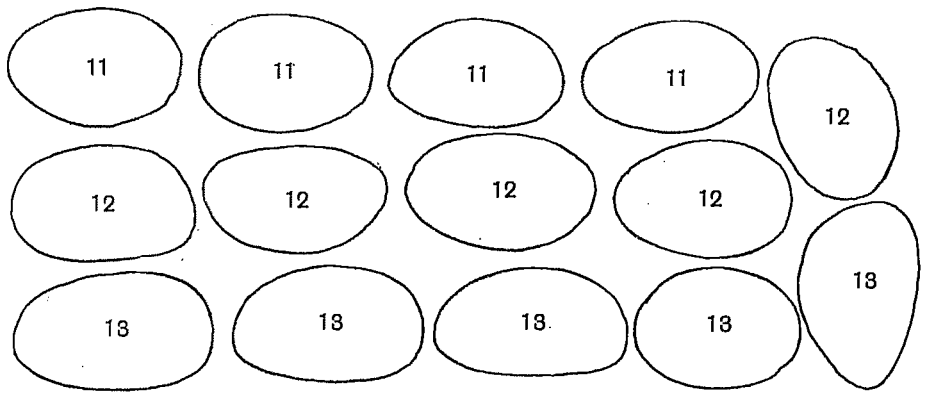


Fig. 79 *a*.

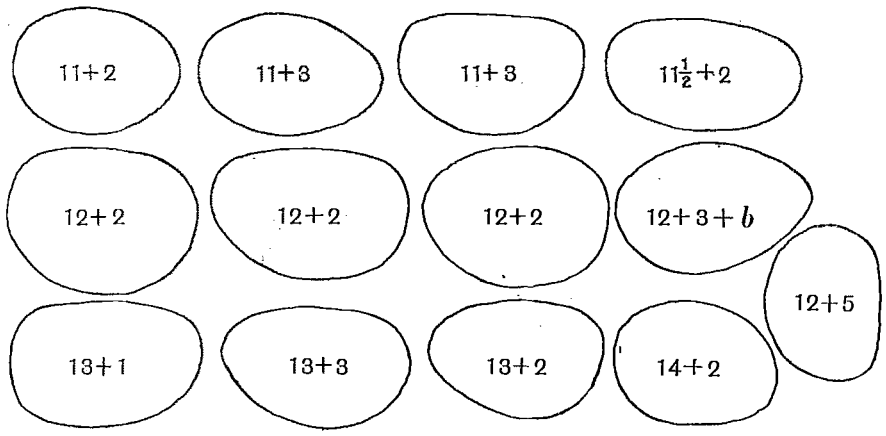


Fig. 79 *b*.

Fig. 79. Plan outlines of pollen grains at the time of first mitosis with the numbers of their chromosome complements.

a. *T. virginiana* var. *congesta*; $n = 11$–13.
b. *T. virginiana*, Medium Blue, No. 1; $n = 11$–14. × 620.

The obvious weakness of the simple scale of unbalance is that it does not allow for the importance of the relationships of the fractions of the chromatin material that have been reduplicated, being probably greater than the relationships of those that have not. The complement

with 11 is shown to be as unbalanced as that with 7, whereas it probably is not. This does not affect the question of the sag between 7 and 9 in the curve of size, but, when we come to consider the pollen grains of the tetraploids, we have this and another complication, for some have non-reduplicated chromosomes (those with 11) and others have triplicated chromosomes (those with 13). Moreover the samples of the pollen grains of the tetraploids examined, from the number-frequencies and correlations discussed already, are evidently very selective. It is not therefore surprising that the pollen grains of *congesta* (Fig. 79 *a*) show no significant

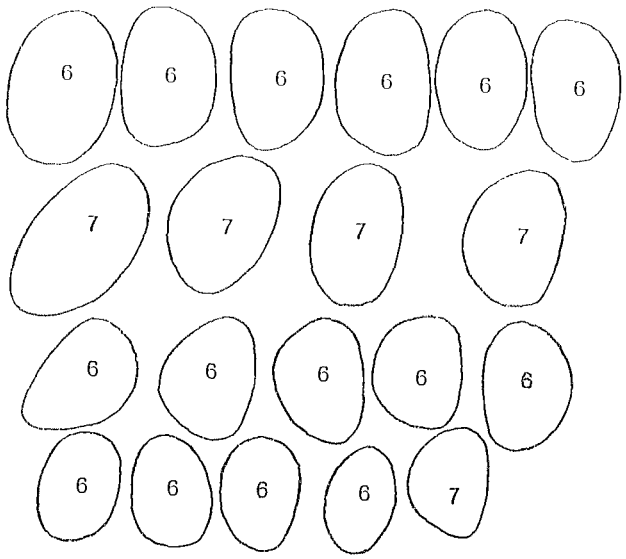


Fig. 80. Plan outline of drawings of pollen grains of *Rhoeo discolor*. The top two rows are from a slide having 6 and 7 chromosome pollen grains in the proportion of 11 : 6. The bottom two rows are from two slides in which the proportion was 12 : 1 and 6 : 1. The number of chromosomes is given in each outline.

difference between the number-types and those of Medium Blue (No. 1) (Fig. 79 *b*) but a slight advantage to the diploid pollen grains (with two fragments).

We may conclude therefore that the evidence from *brevicaulis* shows a sharp differentiation between the members of the chromosome complement in *Tradescantia*, but that the variation in size of the pollen grains with numbers from 8 to 10 shows that the degree of differentiation of the several chromosome types is not equivalent.

Two more sets of observations on pollen-grain size may be noted, although the conclusions to be derived from them are more or less

negative. In *Rhoeo* there was no significant difference between pollen grains with six and those with seven chromosomes, nor were there two distinct types with six, as might have been expected on the analogy of the genetic conditions in some ring-forming *Oenothera* species. Size was fairly constant in particular smears but varied much between different smears (Fig. 80). This can be attributed to the difference in the character of the sample because the slide with large pollen grains had a higher proportion with seven chromosomes. It is plausible that the average period of development should be different in the two classes[1]. Early and late samples would then give different proportions of the two classes: they would also give a different range of sizes.

A large number of drawings of pollen grains of triploid hyacinths (the chromosome constitution of which has been already described, Darlington, 1926) also gave inconclusive results. Particular number-types were extremely variable in size, although a diploid pollen grain was definitely larger than any with intermediate numbers. Considerable differences were noticeable between the varieties studied in general size.

In connection with the occurrence of a physiological differentiation between the properties of the chromosomes it is worth while considering that variations in the degree of this differentiation in different species may be due to variations in the kind of structural change taking place. For example, reversal with reduplication will increase the difference between chromosomes; translocation with reduplication will reduce them.

(iii) *Structure of the Chromosomes and Trabants.*

At the first metaphase of meiosis in *T. virginiana* I found an apparent spiral structure such as has been frequently seen here and elsewhere. It is seen, as Belling has observed, in chromosomes that have been pressed in smearing. A study of the somatic chromosomes suggests that, in accordance with the conception of a spiral structure, they appear to be cylindrical at the ends. This is particularly clear in early metaphase. This interpretation is in agreement with the frequent appearance of division at the ends of the chromosomes at anaphase—the appearance of anaphase duality so often referred to (Darlington, 1926). For if a hollow translucent cylinder is examined in the plane of its axis it appears as though in section, that is, like two parallel rods. The trabant generally found in *T. virginiana* appears to be too small to affect the normal shape of the end, and the illustration (Fig. 81) shows how it may arise from a definite point in the cylinder wall, not corresponding in the two half-

[1] Cf. Section 6 (i).

chromosomes. The explanation of the appearance that seems most natural in this case is that the point to which the trabant is attached is the point at which the spiral thread ends its coil. Where the trabant is larger the connection that it has with the rest of the chromosome becomes physically analogous to an ordinary constriction.

Other illustrations (Fig. 81) show variations in their appearance which may make the determination of small trabants difficult.

The attachment of fragments to chromosomes, or rather half-fragments to half-chromosomes, at the first division of the pollen grain, and the similarity in appearance between this attachment and an ordinary

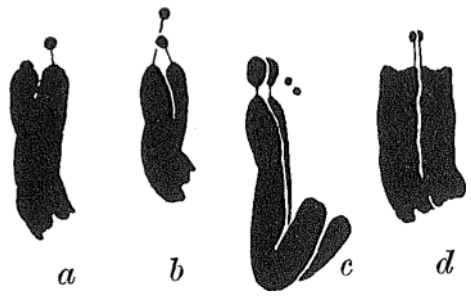


Fig. 81. Forms of trabants and satellites.
a, b, d. In *Tradescantia virginiana*. *c.* In *Spironema fragrans*. × 5700.

constriction, suggests that constrictions may arise from structural changes of this kind. New attachment constrictions appearing after fragmentation cannot be supposed to have such an origin, but the subordinate constrictions might, and the occurrence of these immediately next to the attachment constriction in *Rhoeo*, *Spironema* and the triploid and tetraploid *Tradescantia* varieties is therefore of particular interest in connection with the assumption of segmental interchange made in some of these forms.

(iv) *Chromosome Pairing and Chiasma Formation.*

The material has not proved satisfactory for the study of the stages of prophase between diplotene and diakinesis and the still earlier stages of pairing have not been particularly examined. A characteristic zygotene was however found in *Tradescantia virginiana* and *Colcotrype natalensis*. This interpretation is corroborated by the appearance in the tetraploid of pairs of paired threads, as in tetrasomic *Hyacinthus* (Darlington, 1929), and in the triploid (*T. virginiana* var. *brevicaulis*) of double and single threads lying side by side. Thus the observations by

Miyake (1905) and Hance (in *Zebrina*, 1915), of parasynapsis in this group, are confirmed. Bělař (1928) is also in agreement with this conclusion, for he shows microphotographs of the normal bouquet stage in *T. virginiana*, and this he regards as the clearest sign of parasynapsis.

The behaviour of all species at metaphase is also in agreement with the view that parasynapsis is general in the group. Thus in *Tradescantia crassifolia* (Figs. 18–22), *T. navicularis* (Fig. 83) and *Tinantia fugax* (Fig. 82) the metaphase bivalents are commonly of the cross and ring shapes typically following the maintenance of interstitial chiasmata established at a parasynaptic diplotene. The position in *Tradescantia virginiana* and *Rhoeo discolor* is, from the theoretical point of view, less simple. Association at metaphase is, in the former species, largely, and in

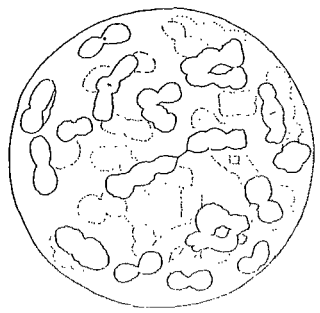


Fig. 82.

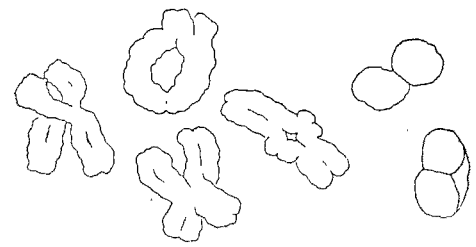


Fig. 83.

Fig. 82. Diakinesis in *Tinantia fugax*. 31 bivalents; one possible association of a pair of bivalents.

Fig. 83. Diakinesis in *Tradescantia navicularis*. Six separate bivalents to indicate formation of interstitial chiasmata.

the latter species entirely terminal, so that the principles of metaphase pairing found in *Hyacinthus*, *Tulipa*, probably *Lilium* (Belling, 1927), and many other genera of plants by the random formation of chiasmata at diplotene cannot directly apply. Terminal association in *Tulipa* and *Hyacinthus* was, however, shown to answer to the same theoretical requirements as interstitial association. In the latter this was by an exchange of partners amongst the chromatids, a chiasma; in the former the segment distal to the chiasma is reduced to the minimum; we have a *terminal chiasma*. Direct observation as well as theoretical arguments are in favour of the same conclusion in *Tradescantia*. In the first place the association of the chromosomes can usually be seen to be double, consisting of one connection between each pair of chromatids, and these connections may be more or less thickened in the middle (Fig. 42 *d*),

as though the distal segments were not, in these cases, reduced to the minimum. Occasionally (always where the attachment is lateral—Fig. 41 *g*) a chiasma can be seen to be single or "imperfect." In these cases, as has been pointed out, the chromosomes are often definitely further pulled apart at this end than at a perfect chiasma at the other end (Figs. 40, 41 *a*). At anaphase the doubleness of the normal attachment is again clearly visible, one pair of chromatids often separating before the other. The same observations apply where more than two chromosomes are associated at one point, a phenomenon which will be discussed in greater detail later.

In *Tradescantia virginiana*, although the terminal chiasma is the most usual form of association, every gradation actually occurs between this and an ordinary interstitial chiasma. The most obvious explanation therefore of the greater frequency of terminal chiasmata is offered by the possibility of a movement of chiasmata after diplotene, such as has been described in *Phrynotettix* (Wenrich, 1916), and is suggested by many observations of meiosis in both plants[1] and animals. This movement in *Tradescantia* must always be *towards* the end of the chromosome or perhaps more properly *away from* the attachment constriction. Observations (unpublished) on slides, of the late W. C. F. Newton, of the very favourable material of *Fritillaria Meleagris* (unpublished) have shown that the post-diplotene stages in this species are characterised by a regular movement of chiasmata *towards* the attachment constriction. The opposite movement, which I will call "terminalisation," affords a sufficient and indeed the only explanation of the exceptional metaphase configurations found in *Tradescantia*.

The movement of terminalisation is analogous to no other process at division except that by which the two halves of a chromosome come together at the metaphase of mitosis, their movement of fusion passing away from the attachment constriction. The analogy is perhaps not without value, for, on the view that chromosomes are associated at metaphase of meiosis merely by the changes in association of their halves, the condition at metaphase of meiosis is essentially the same as that in metaphase of mitosis; the attraction is between half-chromosomes in each case. The difference results from the different conditions at prophase.

The simplest of the exceptional attachments resulting from terminalisation is the *multiple chiasma*. Here, following pairing of three or four chromosomes as observed in *Hyacinthus* and *Tulipa*, chiasmata formed

[1] As, for example, Rosenberg's illustrations of *Drosera* (1905); cf. Bělař (1928).

at random would be terminalised so that the chromosomes are all united at one point (cf. Fig. 84, 11, 13, p. 268), and instead of each chromosome being associated by a single chiasma with another, by a double change of partners, that is, so that n chromosomes are united by $2n - 2$ changes, the number of changes is reduced to n. It has been observed that trivalents are formed much more regularly in triploid *Tradescantia* than quadrivalents in the tetraploid. The reverse is the case in the long chromosomes of *Hyacinthus* (in which there is no effective terminalisation), and the difference is explained by this terminalisation of several chiasmata, for it will be seen (Fig. 84) that terminalisation in one direction of four symmetrical chiasmata between four chromosomes (the most frequent arrangement in *Hyacinthus*) will give two bivalents; only when the chiasmata are formed asymmetrically (as they must always be with three chromosomes) will a quadrivalent or trivalent result. Thus the quadrivalents in *Tradescantia* are usually of a different type from the trivalents. They result from the terminalisation of chiasmata in opposite directions to give a ring of chromosomes which are probably not all corresponding at both ends. It is by the separation of one or two chromosomes from a multiple-chiasma association that the imperfect chiasmata joining bivalents probably arise (cf. Fig. 41 *h* where chromosomes marked *X* have unattached chromatids, 47).

Approximately median interstitial chiasmata, observed occasionally in *T. virginiana*, are presumably the sign of a failure of this terminalisation. A possible explanation of this failure is not far to seek. The middles of some chromosomes are known to correspond with the ends of others (see below), and changes in the correspondence of two chromosomes, segments of which are pairing, may occur: the homology may change (or cease) as a result of translocation at a point between the segments that are associated and the ends of the chromosomes. Terminalisation from chiasmata formed in such segments would therefore be impossible, for it would require association of non-homologous chromatids. But, it may be remarked parenthetically, if the movement of terminalisation has normally to overcome any resistance, it may in these cases be strong enough to break the crossing chromatids and cause crossing over between them. This explanation of approximately median chiasmata is supported by the observation of the group of six (Fig. 47) in which an end on one side of the chiasma is homologous with the end of a second pair, while the ends on the other side are homologous with the ends of a third pair. Unless the plant is segmentally hexasomic there is probably a change of homology in the course of the middle pair of chromosomes.

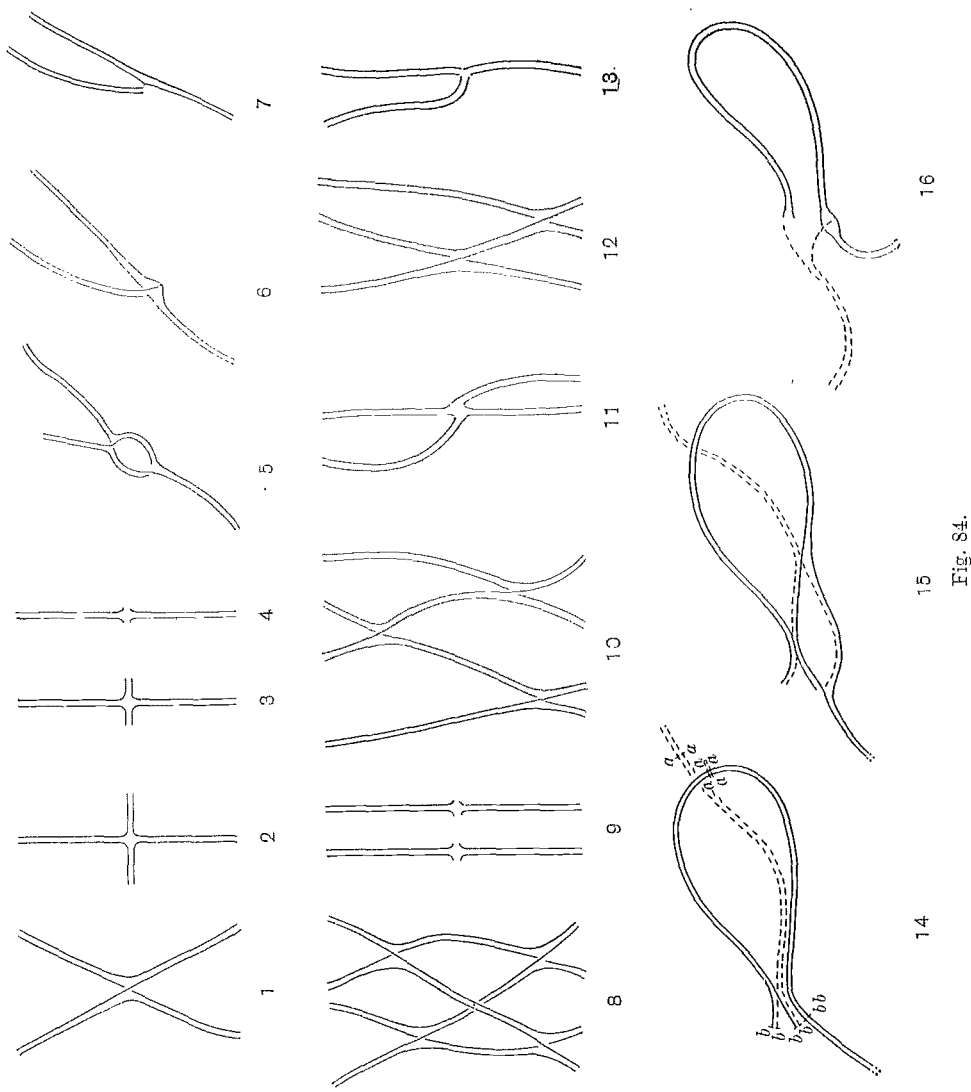


Fig. 84.

Fig. 84. Diagram illustrating probable chiasma behaviour in *Tradescantia* resulting from terminalisation downwards.

1–4. Pairing of two similar chromosomes.

1. Diplotene: interstitial chiasma.
2. Metaphase: metaphase without terminalisation.
3. Metaphase: sub-terminal chiasma.
4. Metaphase: terminalisation complete.

5–7. Pairing of a fragment or non-chromosome with the middle of a whole chromosome.

5. Diplotene: interstitial chiasma.
6. Metaphase: lateral chiasma, perfect.
7. Metaphase: lateral chiasma, imperfect (one attachment broken).

Probable multiple chiasmata have been illustrated in *Datura* by Belling (without comment), but the second abnormal form of association is apparently new. The end of one chromosome (or fragment) is associated with the middle of another by a *lateral chiasma*. Obviously terminalisation is here only possible in so far as one partner is concerned.

Thirdly, we have the mixed lateral-terminal chiasma found in *Aucuba* by Meurman (1929) as well as here in *Tradescantia*. This very exceptional configuration is also intelligible without any additional assumption other than that already advanced with evidence in *Drosophila* by Morgan, Sturtevant and Bridges (1927). They have indicated that crossing-over at their coincident median loci between normal and inverted portions of a chromosome leads to reduplication. Now if such a reduplicated segment is long enough it will follow from our hypothesis that triple pairing will take place and give rise to chiasmata such as those found in the tulips and hyacinths, where chromatids of one chromosome, or in this case segments of a chromosome, exchange partners with chromatids of both the others. Terminalisation in such a case will result in association of the chromatids of one chromosome with different points in the chromatids of the other (Fig. 49, *g* and *h*).

It will be observed that in these cases, as in ordinary lateral chiasmata, the association is usually "imperfect," that is only one of the two pairs of chromatids remains in union (Fig. 49 *g*). This is no doubt due to the fact that the spindle force will in these cases exert an unequal strain on the two sides of the chiasma, owing to the two chromosomes not lying parallel. In these cases the chromatids of the interstitially associated

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Fig. 84 (*continued*).

8–11. Pairing of four similar chromosomes.

8. Diplotene: symmetrical formation of four chiasmata.
9. Metaphase: terminalisation complete, two bivalents formed.
   N.B. If terminalisation of (8) takes place in both directions a ring results.
10. Diplotene: formation of three chiasmata between the four chromosomes.
11. Metaphase: terminalisation complete; formation of a quadruple chiasma.

12–13. Pairing of three similar chromosomes.

12. Diplotene: three chiasmata.
13. Metaphase: terminalisation complete: formation of triple chiasmata.
    N.B. If terminalisation of 10 or 12 takes place in both directions a string of four chromosomes results.

14–15. Pairing of one chromosome with a segment of another reduplicated as a result of inversion.

14. Pachytene: chromatids of two paired segments have exchanged partners and one pair of chromatids is pairing with the third segment.
15. Diplotene: formation of two chiasmata between the three segments.
16. Metaphase: terminalisation complete; formation of an imperfect lateral-terminal chiasma.

chromosome are usually seen to be pulled apart by the strain of the connection of one of them with a chromatid of the other chromosome.

Terminalisation, where one of the pairing chromosomes is shorter than the other (*i.e.* where the end of one corresponds with the middle of the other), must lead to the formation of the lateral chiasmata observed in *Tradescantia*. Where this occurs we may properly conclude (see Introduction) that the portions of the two chromosomes, or of the chromosome and fragment concerned, are homologous, and that in these cases there has been a loss or reversal of a portion of one of the chromosomes leading sometimes to other changes of structure.

The observed rarity of this exceptional lateral pairing may be supposed to depend on two circumstances. First, each chromosome in *T. virginiana*, on a tetraploid hypothesis, is represented four times[1]. Where four corresponding segments are present the association of a particular pair can be expected only in one case in three, and the other associations may be normal in that corresponding parts of chromosomes will associate. Secondly, portions of chromosomes which thus correspond anomalously as a result of reversal and interchange may be very short, and, as was shown in *Hyacinthus*, the frequency of chiasma-formation, and hence, in short trivalents or very short bivalents, of metaphase pairing, is proportional to the length paired at pachytene. Thirdly, a small fragment has a disproportionately small chance of finding its partner at zygotene, for pairing at one end of a chromosome leads to pairing throughout its length. We may therefore conclude that the principles adduced from the behaviour of the tulips and hyacinths are by no means incompatible with different behaviour in *Tradescantia*.

(v) *The Hypothesis of Structural Hybridity.*

Belling has concluded from his important work on *Datura* (1924–7) that, on the assumption that the two ends of a chromosome have specific attractions, interchange of segments between non-homologous chromosomes must have taken place. The premises on which Belling has advanced this hypothesis are considerably strengthened by the observations on polyploid *Tulipa* and *Hyacinthus*, for here it is evident that the attraction of *every particle* of a chromosome is specific. I have therefore felt justified in extending this hypothesis to *Oenothera* where it seems to afford an adequate explanation, directly of the cytological behaviour in this group, and indirectly of the genetical behaviour. It was in order

[1] Actually this simple assumption can be only approximately true; some segments are probably represented only twice or three times, others perhaps more than four times.

to test the possibility of the further application of this method to *Tradescantia* that the present study was undertaken. But Belling himself has in another connection expressed what seems a rather different view from that on which he has based his conclusions in *Datura*. For example: "In *T. virginiana*," he says, "the 24 univalents are in one plant examined united more or less into rings of four but the presence of a continuous chain is not excluded. Such junctions of non-homologous chromosomes postulate different attractions for one end of each of the two homologues, which attract certain non-homologous chromosomes at this end."

Since pairing at metaphase is the final criterion of the homology of chromosomes this seems to involve a contradiction in terms. If on the other hand we assume, as Belling has assumed elsewhere (and there is nothing in these observations to interfere with the assumption), that the pairing of chromatin material at meiosis is the sole criterion of its homology, so that in fact all elements pairing at meiosis are regarded as homologous in so far as they pair, and, conversely, all bodies that are homologous are regarded as capable of pairing, in so far as they are homologous, then the anomalous forms of association must be taken to show an exceptional organisation of the chromosomes relative to one another.

Summarising, we find the following types of association that are not intelligible directly in terms of diploid or polyploid pairing of homologous whole chromosomes:

(i) The end-to-end association in one ring of all the members of the chromosome complement (ring-formation in *Rhoeo*) or association of chromosomes in smaller groups but still involving a greater number than can be supposed to be homologous on a polyploid hypothesis (*Tradescantia virginiana*).

(ii) The association of morphologically dissimilar pairs of chromosomes (ring-formation in *Zebrina*).

(iii) The association of the end of one chromosome or fragment with the middle of another (pairing by lateral chiasmata in *T. virginiana* and *T. crassifolia*).

(iv) The association of a fragment with different parts, interstitial or terminal, of different whole chromosomes (*T. virginiana* var. *humilis*).

(v) The pairing of the same end of one chromosome simultaneously with two different points on another chromosome (lateral-terminal chiasmata in *T. virginiana*).

(vi) The associations of four chromosomes at one point in a diploid (*T. bracteata*).

These observations show, first, that the different parts of a chromosome behave independently in pairing at prophase, thus corroborating the conclusion derived from the polyploid tulips and hyacinths. They show, further, that the chromosomes in these species do not correspond as wholes, but that the separate elements that go to make up their chromosome complements are differently arranged in the opposite sets of the same individual. These plants are, in fact, *structural hybrids*.

The differences that constitute their hybridity can be classified according to their probable mode of origin. The simplest case (from the observational although not from the evolutionary point of view) is that of *Rhoeo* in which the condition is evidently the same as in *Oenothera*, already described in detail as the result of segmental interchange between non-homologous chromosomes (Darlington, 1929).

The analogy in chromosome behaviour between *Rhoeo* and *Oenothera* is remarkably close. In *Rhoeo* irregularities such as non-disjunction are parallel in every way with those described by Cleland in *Oenothera* (1926). They are however more frequent, and this is perhaps due to the position of the attachment constriction in some chromosomes being more nearly terminal in *Rhoeo* than in *Oenothera*. In *Oe. Lamarckiana* and *Oe. muricata*[1] I found that all the chromosomes were constricted approximately medianly. Apart from the attachment constriction, subordinate ones and one pair with large trabants were found in each species.

The only clear difference in behaviour of the two species is in the breakage of the ring after diakinesis in *Rhoeo*, and this is probably due to the enormously greater bulk of the chromosomes in *Rhoeo*, for they must nevertheless be supposed to be joined by connections, chiasmata, of the same strength as in *Oenothera*.

The general behaviour of *Tradescantia virginiana* can clearly be put under the same head, the differences being due to two circumstances: first, self-fertilisation has very probably reduced the degree of structural hybridity; secondly, being a polyploid, without sharp genetical differentiation of its sets of chromosomes, no association of particular pairs can be constant. Under these conditions it is not possible to distinguish between simple translocation and reciprocal translocation or interchange. The occurrence of tetrasomic pairing in the "diploid" *T. bracteata* is however evidence of simple translocation leading to reduplication. The formation of a lateral-terminal chiasma has already been referred to as the natural result of reduplication following inversion. The formation

[1] Raised from seed kindly provided by Professor Renner.

of lateral chiasmata in general indicates simple inversion of the segment between the chiasma and the end of the chromosome.

The behaviour of *Zebrina pendula* could be understood as that of a tetraploid segmentally interchanged type, but a simpler explanation is that the four long chromosomes consist of two non-homologous pairs *A–A* and *B–B*, and that the four short chromosomes with which these are capable of forming the rings are the products of loss, thus: (*A–x*) (*A–x*) and (*B–y*) (*B–y*).

Aucuba japonica, according to Meurman's detailed account of pairing (1929), appears to be closely analogous to *Tradescantia virginiana*. Both are tetraploids showing evidence of the pairing of chromosomes that are not homologous as wholes. But *Aucuba* shows this phenomenon more strikingly because the pairing chromosome types as in *Zebrina* are distinct in form. Thus chromosomes structurally dissimilar are, in part, genetically identical, and it is possible to show that, although the plant is tetraploid, rings of four do not always consist of identical chromosomes. In *Tradescantia* there is no direct morphological evidence. But where a ring of four chromosomes can occur in a diploid no two of these can be wholly identical. In the same way, where a ring of eight chromosomes can occur in a tetraploid, it follows that no four of these can be wholly identical. Interchange must have occurred. There is a further analogy between the two species in the evidence of another type of chromosome change. The pairing of one chromosome with the middle of another by a lateral chiasma in *Tradescantia* indicates inversion. Inversion followed by crossing over should lead to reduplication, and the observation of the terminal pairing of the two chromatids of one chromosome with *different* points in a second (in both *Aucuba* and *Tradescantia*) indicates inversion followed by reduplication.

Pairing in the tetraploid *Aucuba* and *Tradescantia virginiana* affords a different type of test of the interchange hypothesis from that afforded by the diploids, *Rhoeo* and *Oenothera*. For here we are dealing with chromosomes every element of which has three possible mates, while in the diploids there is only one. Pairing is subject to a limited degree of freedom instead of being absolutely determined. But of these three possible associations only one is capable of being maintained throughout the chromosome. We cannot, and do not, have trivalents and quadrivalents like those described by Belling in normal triploid and tetraploid *Datura* where both ends of all three or four chromosomes are associated in what are presumably triple or quadruple terminal chiasmata.

If in *Rhoeo* we have the extreme type of a structural hybrid in a

fertile diploid, strictly comparable with *Oenothera*, at the other end of the scale we have structurally homozygous types like *Tinantia*, and possibly some forms of *Tradescantia virginiana*, resulting from continued self-fertilisation (such as, perhaps, Pale Blue which produced only diploid pollen grains). Between the two, every possible type of structural variation seems to occur. These observations therefore provide us with a bridge between *Oenothera* and the rest of observed material. They enable us to see the accepted normal and abnormal in truer proportion, so that the one appears more extraordinary, the other perhaps less so, than is commonly imagined.

Three possible methods are indicated by which interchange, the most complicated modification of structure suggested, could take place in this group. The first is by the loss of fragments, such as those observed, from non-homologous chromosomes, their preservation at cell division, and their ultimate reunion with the remainders of different chromosomes. This seems to be taking place in the pollen grains of *Tradescantia*. The second is by translocation of an interstitial element from one chromosome to a corresponding part of one not homologous with it. This, leading to reduplication in the next generation, would render possible the pairing of homologous elements intercalated in non-homologous chromosomes. Crossing-over would then give segmental interchange. The third is by double translation such as that referred to by Muller in *Drosophila* (1928). Here, 70 cases of translocation were detected in the progeny of rayed individuals. In two instances, which Muller considers a chance frequency, double translocation occurred simultaneously. If this happened to be reciprocal, and the chance occurrence of these phenomena would lead one to expect this in a small proportion of cases, we should have segmental interchange.

The possibility that the first (observed cytologically) is the actual method of the third (inferred genetically) cannot be ignored, since Muller has evidence that the change is not always effected, or completely effected, at the prophase of meiosis. From sporadic somatic reunions such as those described sectorials would result if the free segments were incapable of regular division at mitosis. This is perhaps preferable to considering the change as affecting only a fraction of a chromiole.

The hypothesis of structural hybridity that I have put forward in regard to *Oenothera* was based on the principle, adduced particularly from work on the polyploid hyacinths and tulips, that parasynapsis of homologous parts of chromosomes followed by chiasma-formation is in

general a condition of meiosis[1]. The hypothesis was supported by observations on *Datura*, *Drosophila* and probably *Rumex*. It is now supported by the following new observations:

(i) As in the *Onagraceae*, parasynapsis, on which the hypothesis depends, is general in this group, and the resulting chiasmata are the means of metaphase pairing and ring-formation.

(ii) As in the *Onagraceae*, irregular types of ring-formation occur in some forms (cf. Håkansson's *Godetia* hybrids) and perfect ring-formation in others.

(iii) The occurrence of fragments, their method of pairing and that of the whole chromosomes indicate every conceivable rearrangement of chromosome structure in these species. The only one of these rearrangements which is compatible with high fertility in a diploid is evidently segmental interchange. For the resulting ring-formation is the only

[1] Acceptance of the view that the general phenomenon of pairing of chromosomes at the metaphase of meiosis is conditioned by a physical relationship of the pairs—the formation of a chiasma—must necessarily depend on what one regards as evidence of a physical relationship. In this regard the exceptions to the rule of pairing by chiasmata are perhaps not so many as certain more or less accepted interpretations of meiotic phenomena directly suggest.

I have already attempted to show that end-to-end association can always be interpreted in this way. The occurrence of "imperfect" and "multiple" "terminal chiasmata" in *Tradescantia* strengthens this interpretation, for it is incompatible with any alternative hypothesis. Similarly "secondary pairing" (Darlington, 1928), although not usually (at least, in polar views) established by a *visible* physical connection, can be seen in the most favourable observations to be merely an extension of the method to more than two chromosomes. Its continuance in the second division is only marked where the interphase is short (cf. Meurman, 1929; Lawrence, 1929). Bělař has suggested (1928, footnote, p. 198) that the wide separation at the prophase of meiosis in certain Diptera is perhaps comparable with the separation of the halves of chromosomes at interkinesis. In each case we may suppose that a permanent physical connection is maintained, as in the case of the terminal chiasma, although the conditions are unfavourable to its demonstration. Kenneke's observations on *Tephrites* (1924) are perhaps comparable to my own on *Prunus*, where secondary pairing appeared stronger at metaphase than at diakinesis owing to the physical connection being usually obliterated at the latter stage.

Finally it will be seen that the behaviour of the fragments in *humilis* (which agrees both in the method and frequency of pairing with the chiasma hypothesis) is strikingly like that worked out by Wilson (1905) in some Hemiptera for microchromosomes and idiochromosomes. In regard to these different workers have had different results (Wilson, 1907) in the same species (as might well occur in *Tradescantia*). The bulk of the phenomena observed by Wilson would seem to agree as well with the assumption that the pairing of the *m*-chromosomes was infrequent and continuous, as with the assumption that it was regular but momentary; it is then possible to suppose it the result of chiasma formation. Admittedly such anomalous pairing of *particular chromosomes* is well authenticated in many species of animals as leading to more or less regular segregation, but this abnormality does not extend to the whole complement.

alternative to ordinary pairing as a means of regular segregation of alternative elements. Where other irregularities occur—as in *Tradescantia crassifolia* and *T. bracteata*—the consequence must be an impaired fertility. Consequently if they have occurred in *Rhoeo* the results have not been preserved.

(iv) Translocation, of which there is evidence, and fragmentation, the occurrence and results of which have been observed, are shown to provide alternative means by which segmental interchange could take place. Moreover Muller's observations have shown a correlation between the various types of chromosome change. Where all the observable types have been seen we may therefore expect to find evidence of those such as interchange which cannot, perhaps, be directly observed.

(v) The principle on which the application of the theory of segmental interchange to ring-formation most directly depends, namely that of the independent pairing of chromosome-parts, is abundantly confirmed by the behaviour of fragments as well as of whole chromosomes in the *Tradescantiae*.

(vi) Observations on *Aucuba*, *Campanula* and *Pisum* (see later) comparable with these on the *Tradescantiae* show that structural hybridity is not an isolated phenomenon, and that therefore observations of behaviour in widely different groups such as have been brought under consideration are relevant to the *Oenothera* problem.

(vi) *Parasynapsis and Telosynapsis.*

The practical difficulties in the way of regarding ring-formation as the result of structural hybridity in a parasynatpic plant are not therefore very considerable. The theoretical difficulties have already been discussed in some detail (Darlington, 1928) but the alternative hypothesis of telosynapsis (a hypothesis, for its statement involves many assumptions) has not been examined closely enough.

The theory of telosynapsis, that is, of the pairing of chromosomes end-to-end to form, according to some writers, a "continuous spireme" of alternately maternal and paternal chromosomes, rests on two kinds of observation: first, of the supposed formation of such a spireme at prophase; secondly, of the formation of rings at metaphase derived from this spireme. The first of these is founded on untrustworthy evidence, as admitted by many of those who earlier upheld the telosynaptic interpretation. It has never been supported by observations of favourable material, and the observations themselves in some cases contradict the interpretation that is ostensibly derived from them (Darlington, 1929).

The second is, in the light of the hypothesis of structural hybridity, compatible with parasynapsis and terminalisation.

It would seem that the weakness of telosynapsis, from the theoretical as well as the observational point of view, is partly realised by those who still maintain its strength. For example, Gates (1928) has declared that: "The distinctions between these two methods of chromatid-formation and separation (telosynapsis and parasynapsis) are not so fundamental as is generally supposed." Let us examine what these distinctions are:

Parasynapsis (universal)	*Telosynapsis* (occasional)
Pairing at metaphase results from the specific attraction and association of the homologous elements of the chromosomes at prophase.	*Pairing at metaphase* results from an undefined attraction which affects sometimes homologous pairs and sometimes non-homologous pairs of chromosomes, according to rules invented *ad hoc*, which require the assumption, amongst others, of two kinds of non-homologous chromosomes, those which will pair and those which will not.
Formation of bivalents is the result of the association of chromatids in different pairs at different points along their length, *i.e.* of the development of chiasmata *observed* to be formed at diplotene and maintained until metaphase.	*Formation of bivalents* is (sometimes) the result of segmentation of a continuous spireme, which takes place sometimes completely, sometimes incompletely, according to no understood rules.
Ring-formation is the result of the pairing of homologous elements in a particular kind of structural hybrid that has arisen by segmental interchange between non-homologous chromosomes. The processes by which segmental interchange can be effected have been determined by both genetical and cytological observation. Its results in both diploids and polyploids verify genetical and cytological expectations.	*Ring-formation* is *perhaps* the result of "lack of attraction between homologues resulting from hybridity" (Gates, 1927), *or* "failure of the spireme to complete its segmentation" (Gates, 1927), *or* it is determined by "a genetic character inherited according to Mendelian laws" (Cleland, 1927), *or* it is a sign that "the chromosomes in the rings are relatively much more heterozygous than those which are paired" (Cleland, 1927).

I submit that these distinctions, in so far as telosynapsis can be said to have any *form*, are fundamental cytologically and genetically. They are also immediately important, for the attempt to apply this self-regulating hypothesis of telosynapsis to genetics has resulted in serious confusion. For example, Shull (1928) has justly pointed out the weakness of the Gates-Cleland assumption "that the chromosomes are not only arranged in pairs but have a definite position within the pairs," or, as Shull says, "that chromosomes of maternal and paternal origin alternate with each other in the circle in a definitely fixed order." Shull shows that this assumption, embracing as it does the current view

of the homology of whole chromosomes, satisfies certain genetical requirements but does not satisfy others of equal or greater importance. This would seem to show that the assumption of telosynapsis is unwarrantable. But Shull, seeing no alternative assumption, jumps to the conclusion that not merely is the *assumption* bad but the *observations* themselves "have no very fundamental significance." Shull has thus involuntarily destroyed by *reductio ad absurdum* either telosynapsis or the chromosome theory of heredity. The former, in the presence of a satisfactory alternative, will be more readily sacrificed.

Regarding this alternative of parasynapsis as the universal mode of conjugation, we can now apply the same rules to the observation of meiosis in all animals and plants. It is similarly possible to seek the same correlations between cytological and genetical behaviour universally.

(vii) *Structural Change and Species-Formation.*

The relationship of the various kinds of structural change of the nucleus in the different species studied can be usefully considered in relation to their method of reproduction. For it is clear that these processes must affect segregation and seed-fertility profoundly. The four different species studied represent four different types in this respect. The diploid *Rhoeo* is perfectly adapted, like the ring-forming *Oenothera* species, to producing a high proportion of seed of its own hybrid constitution. In both species hybridity is probably maintained by a kind of balanced lethal system. In *Tradescantia crassifolia* and *T. bracteata*, however, the various abnormalities must reduce seed-production to negligible proportions if they reproduce themselves normally. *Zebrina* though apparently tetraploid is completely sterile (Hance, 1915), and is reproduced vegetatively. In a polyploid, however, much grosser irregularities may occur than in a diploid without ruining fertility. To take a crude example, in *T. virginiana* (triploid and tetraploid) pollen grains have been found, viable enough to divide, with chromosome numbers of from 7 to 14. In *Rhoeo* on the other hand only those aberrants with an excess of chromatin material are viable. A single deficiency is, so far as the pollen-grain studies show, immediately fatal; variation is restricted. It is partly to its lack of this control and partly to its generally vegetative method of reproduction that I would attribute the occurrence in *T. virginiana* of every kind of structural variation in the nucleus, such as fragmentation, reversal and translocation, associated with varying fertility. In the diploid *Rhoeo* selection in seed-production has

imposed a rule. The plant under investigation proved to be fertile and self-compatible. Six selfed seedlings were raised from it.

The nuclear instability of *T. virginiana* is associated with the greatest morphological diversity. Early systematists have conveniently included markedly distinct forms under this name. More recently Bush (1904), for example, has distinguished 18 different species from Texas alone; these would probably all resemble the types described cytologically and would be interfertile in so far as they were fertile at all. It need hardly be said that none of them would be consistently true-breeding, and some of them, such as *T. brevicaulis* Raf., if the same as my triploid form, would be entirely incapable of reproducing themselves as such.

Thus seed-production fills an entirely different rôle in *Rhoeo* and in *Tradescantia virginiana*. Both are hybrids, but in one seed-formation is a means of exact perpetuation of the hybrid type; in the other it is merely an instrument for securing variation in that type, which is exactly reproduced by vegetative means alone.

These studies do not lead to any definite conclusion with regard to the systematic relations of the tetraploid *Tradescantia virginiana* and its diploid (*T. bracteata*) and triploid relatives. Doubling of chromosome number in a form very much like *T. bracteata* (which I have unfortunately not been able to examine as to its somatic chromosomes) would yield a form resembling *T. virginiana* except in having smaller chromosomes. In this connection the supposed somatic mutation in the triploid *brevicaulis* is important, for this triploid is approximately intermediate in chromosome size between its probable diploid and tetraploid parents. The sport is in the direction of the diploid parent and its occurrence shows that we need not attach too much importance to the size difference between the chromosomes of two parental types. The tetraploid could actually be supposed to be derived from a ring-forming type like *Rhoeo*, although the reduction in ring-formation and the appearance of new kinds of behaviour indicate that the structural changes we now see going on have been taking place freely for some time since any such doubling took place.

It is not very difficult to see (cf. Section 7 (i)) how the continuance of fragmentation in a tetraploid like *T. virginiana* might give rise, by a process very much akin to "unpacking," to a new form no longer having any of the characteristics of the tetraploid, either in the number or the behaviour of the chromosomes. The direction and method of the change must depend largely on the mechanical conditions of fragmentation and fragment survival. Such a process can take effect without the aid of

hybridisation in any special sense, for the change will depend on irregularities at meiosis whose results can be perpetuated owing to the plant being polyploid, and do not depend on any original hybridisation.

We have already referred to the mutually exclusive effect of hybridisation and structural change. It is only where, either naturally or artificially, the two come to overlap that we can expect to find evidence of structural hybridity. This evidence can only be obtained, where changes of bulk are not involved, by observation of behaviour at meiosis. Present knowledge can therefore only very vaguely indicate the importance of structural changes in species-formation. Recent work however has produced striking advances in this direction.

Consider first the examples already known or suggested. They occur in eight orders of Angiosperms, as follows:

(i) Onagraceae: *Oenothera* and *Godetia*, Gates (1908), Schwemmle (1924), Cleland (1927) and Håkansson (1925).
(ii) Solanaceae: *Datura Stramonium*, Belling and Blakeslee (1924–8).
(iii) Commelinaceae: *Tradescantia*, *Rhoeo* and *Zebrina*, Miyake (1905), Belling (1927) and in the present study.
(iv) Polygonaceae: *Rumex acetosella*, Kihara (1927).
(v) Cornaceae: *Aucuba japonica*, Meurman (1929).
(vi) Hydrocharitaceae: *Vallisneria spiralis*, Winge (1927).
(vii) Leguminosae: *Pisum sativum*, Håkansson (1929).
(viii) Campanulaceae: *Campanula persicifolia*, A. E. Gairdner (unpublished).

Miss Gairdner tells me that in a double white variety of *Campanula persicifolia*, a diploid, she found a ring of four chromosomes which usually resembles the ring illustrated as the first stage of ring-formation in *Oenothera* on the interchange hypothesis (Darlington, 1929).

Winge's observation of the occasional occurrence of a double chromosome in a presumably diploid species of *Vallisneria* points to the formation of a diploid association of four chromosomes.

Kihara's observations on *Rumex acetosella* on the other hand admit of and even compel a hexaploid interpretation in so far as pairing is between similar chromosomes associated to the number of six only. It is only the occasional occurrence of larger associations that requires an interchange interpretation.

To this list should perhaps be added: *Zea* (see Section 7 (i)) where Kuwada (1915, 1919) and Randolph (1928) have found variations in pairing analogous to those resulting from segmental interchange in a polyploid; *Lebistes* where Demerec (1928) has suggested an analogy be-

tween Winge's results and the behaviour of *Oenothera*; *Drosophila* where genetical observations supporting the assumption have been made, particularly as a result of X-ray treatment (Morgan, Sturtevant, and Bridges, 1925, and Muller, 1928).

These observations are not likely to represent the occurrence of structural change in true proportion. First, on account of such change not favouring hybridisation. Secondly, the observations here referred to are the result of prolonged and detailed study, or of the study of particularly favourable material. For example, abnormalities of the kind observed in *Tradescantia* would for the most part pass unnoticed were the chromosomes of the size most frequently found in the Dicotyledons, and the occasional observation of such associations as those found in *T. crassifolia* under less favoured conditions could scarcely be understood unless anticipated. Fragments of a size proportional to those found in *Tradescantia* would be hardly visible in *Prunus* where the chromosomes are no more than one-hundredth as large. Thirdly, observation is simplified in *Tradescantia* by the fairly regular process of the terminalisation of the chiasmata. And finally, most plants studied cytologically are the product of the union of germ-cells of closely related individuals or even of the same individual. Observation of such plants can throw little light on the differences that distinguish incipient species except in cases where structural hybridity is maintained by a genetical mechanism of elimination. When, however, systematic cytological study of interracial hybrids has been undertaken, as in the remarkable work of Blakeslee and Belling (Blakeslee, 1927), the result has been to show a close correlation between racial differentiation and structural change in the chromosomes. Particular races are identifiable by their structure relative to that of other races. We may therefore take it that interchange and other forms of structural variation are much more widespread than cytological observations at present indicate. These variations will usually be associated with, and directly or indirectly the means of, genetical variation.

The frequency with which structural change seems to occur in *Tradescantia virginiana* and the important part it seems to have played in the development in certain forms—as fragmentation in *Tinantia* and segmental interchange in *Rhoeo*—do not enable us to decide whether structural change is responsible for hybridity or hybridity for structural change. But the distinction between evolution by hybridisation and evolution by deficiency, reduplication, and other apparently spontaneous changes in chromosome structure, although perhaps possible on genetical

grounds, has no physiological significance. For both mean change by a rearrangement of materials, by an alteration of balance; both are opposed in principle to the qualitative change suggested by the word "mutation."

8. Summary.

The primary object has been the study of structure and variation in the chromosome complement, particularly by observation of chromosome behaviour at meiosis and its results in the gametophyte. As in *Oenothera*, the principles of behaviour worked out in polyploid *Tulipa* and *Hyacinthus* are made to provide the basis of a structural hypothesis. These principles, viz. that pairing of chromosomes at meiosis is determined by the formation of chiasmata between them at diplotene and that this follows the side by side association in pairs of their homologous parts, are corroborated by the observations of pairing at metaphase.

From a study of the somatic chromosomes in a number of species of the *Tradescantiae* (see Summary, p. 218) it appears that two types of change are taking place independently in this group: reduplication of the whole chromosome set (polyploidy) on the one hand, and breaking up of a whole or part of it (fragmentation) on the other.

Pollen mother-cell divisions were studied in three diploid species, *Rhoeo discolor*, *Tradescantia crassifolia* and *T. bracteata*, two tetraploid species, *T. virginiana* and *Zebrina pendula*, and one triploid *T. virginiana* var. *brevicaulis*. In these, various anomalous types of association occur, such as (i) the association of two ends of one chromosome with ends of two chromosomes which themselves will not associate (ring-formation in *Rhoeo*); (ii) the association of the end of one chromosome with the middle of another (lateral pairing in *Tradescantia virginiana*); (iii) the association of a fragment with different parts, interstitial or terminal, of different chromosomes (*T. virginiana* var. *humilis*); (iv) the association of morphologically dissimilar chromosomes (ring-formation in *Zebrina* and pairing in *T. virginiana*); (v) the pairing of the same end of one chromosome simultaneously with two different points on another chromosome (*T. virginiana* var. *humilis*).

These various associations are shown to be compatible with the method of pairing found in polyploid *Tulipa* and *Hyacinthus* if we assume, apart from polyploidy and fragmentation, (i) translocation of segments from one chromosome to another, (ii) segmental interchange between non-homologous chromosomes, as first suggested by Belling; (iii) inversion of segments of chromosomes followed by reduplication of

parts of them; (iv) a process of terminalisation of chiasmata between diplotene and diakinesis which gives rise to the multiple and lateral chiasmata described and explained in detail (diagram, Fig. 84).

The various types of abnormal chromosome behaviour observed at meiosis occur as a result of a condition of structural hybridity which may be conveniently distinguished by its symptoms (cytological and genetical) from other phenomena seen in zygotes produced by the union of dissimilar gametes.

The possibilities of segregation were examined at meiosis in some forms (particularly in *Rhoeo*, which is analogous in this as in every other respect to the ring-forming *Oenothera* species); but a fuller study of this problem was possible from the first division in the pollen grain, of which chromosome counts were tabulated from several species and varieties (p. 242). These show random frequencies about the half-number as a mean. The genetical conditions in the life of the gametophyte affecting these results are discussed, especially in regard to the behaviour of fragments. The mechanical conditions affecting the origin, survival and behaviour of fragments at mitosis and meiosis are also examined in the light of the observations (*Tradescantia* and *Fritillaria*). Although fragmentation may occur very generally, mechanical as well as physiological conditions are shown to limit its importance as an agent of variation.

The somatic reunion of fragments with chromosomes is described as a possible means of translocation and interchange. Other changes of chromosome structure observed at, and following meiosis, may have a similar significance. Reason is shown for supposing that interchange is but one special form (answering to certain genetical requirements) of structural changes in chromosomes, and that such changes are of widespread occurrence in association with genetic variation and, in a certain sense, the means of it.

Other observations are included bearing on the origin of constrictions, changes in chromosome size, the relation of chromosome balance to cell size in the pollen grain, and the structure of the chromosome.

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A fuller list of references will be found in earlier papers.