THE NATURE OF TETRAPLOIDY IN *PRIMULA KEWENSIS*

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(With Sixteen Text-figures)

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1. Introduction

Primula kewensis is of special interest for genetics as the first hybrid whose property of breeding true was shown to be the result of doubling its chromosome complement. It was first found at Kew in 1900, a single seedling among a batch of *P. floribunda*. Its appearance led to the assumption that it was a chance hybrid with *P. verticillata*, and this was confirmed in the following year by crossing *P. floribunda* × *P. verticillata* (Watson, 1900; *Kew Bull.* 1910, p. 325). The new hybrid was almost completely sterile. It produced no seed either on selfing or on crossing with its parents' pollen. Its pollen on *P. floribunda*, however, gave a few seedlings of intermediate character (Newton & Pellew, 1929).

On three occasions, in 1909 in the nurseries of Messrs Veitch, in 1923 at Kew and in 1926 here, fertile branches have arisen spontaneously on the sterile plant. The seed from these developed into plants of similar type to *P. kewensis* but with broader leaves and larger flowers. These in turn were fertile and bred nearly true. In 1912, Digby first showed the nature of the difference. The original *P. kewensis*, like its parents, has 18 somatic chromosomes. The fertile form has 36 and is therefore tetraploid as the result of somatic doubling.

Crosses with the parental species and the sterile hybrid were then attempted using the fertile form. No offspring was obtained with *P. verticillata* or *P. kewensis* (2*x*) whichever way the cross was made. The pollen of *P. floribunda*, however, on the tetraploid gave rise to a few

plants most of which proved to be tetraploid, not triploid as would be expected. A summary of these results is shown in the following table:

TABLE I

♀	♂ *P. floribunda*	♂ *P. verticillata*	♂ *P. kewensis* 2x	♂ *P. kewensis* 4x
P. floribunda	*ff*	*fv* *ff* (apo)	*fv*^{f} *ff* (apo)	*ffv* *fffv*
P. verticillata	*ffvv** *vv* (apo)	*vv*	*vv* (apo)	*vv* (apo)
P. kewensis 2x	—	—	—	—
P. kewensis 4x	*ffv* and *fffv**	*ffvv* (apo)	—	*ffvv*

Note. *f* and *v* are sets of *floribunda* and *verticillata* chromosomes respectively, v^{f} is a set of *v* and *f* chromosomes mixed by recombination. (apo) signifies purely maternal progeny arising by apomixis of undetermined type.

* Action of unreduced gametes.

We have now two kinds of tetraploid, those derived from the fertile spike, which I shall call *duplex tetraploids* (*ffvv*); and those obtained by back-crossing with *P. floribunda*, which I shall call the *triplex tetraploids* (*fffv*). As might be expected from their constitution, their behaviour has proved to be entirely different.

The sterile hybrid has never been obtained again, although numerous attempts have been made. The reciprocal cross (*P. verticillata* × *P. floribunda*) has, however, on two occasions given a tetraploid hybrid seedling indistinguishable from that obtained by doubling the sterile hybrid. This could have arisen in two ways, by somatic doubling at one of the early divisions of the embryo, or by the fusion of two unreduced gametes. The second method would not be surprising in view of the frequency with which unreduced pollen grains from *P. floribunda* function on the style of the tetraploid hybrid. These cases also make it likely that the "maternal" progeny from various crosses are the result of diploid parthenogenesis.

Two years after Digby had shown the nature of the difference between the sterile and the fertile *P. kewensis*, without however offering any explanation of how it came about, Farmer & Digby (1914) asserted that the chromosomes had split transversely. This unfortunate suggestion confused the interpretation of Digby's discovery for fifteen years. It was not until 1929 that Newton & Pellew showed that this could not possibly be so, and adopted Winge's hypothesis (1917) of the longitudinal duplication of the whole chromosome complement, an explanation which accounted in genetic terms not only for the increased chromosome

number but also for the restored fertility, characteristic of the whole class of true-breeding hybrids.

I now propose to re-examine the chromosome behaviour of *P. kewensis*, for two reasons: first, to clarify the breeding behaviour of allotetraploids in general by making use of the quantitative methods now available for the study of meiosis, and secondly, to discover the basis of certain unexplained anomalies of inheritance which were formerly thought to be peculiar to *P. kewensis*, but now seem to be of widespread occurrence.

Material. The parental species and the diploid hybrid examined are the stocks maintained at this Institution. The duplex tetraploid was obtained from Kew and the triplex tetraploid from Miss Pellew's genetical stock.

For meiosis the anthers were dissected and fixed in 2 BD (La Cour, 1937) to which a little extra saponin had been added to wet them and so to hasten the penetration of the fixative. Sections were cut at 16 μ and stained in Gentian Violet.

For the pollen grain division, the pollen grains were teased out into iron acetocarmine, warmed slightly and examined at once. This method, although temporary, has the great advantage that pollen grains showing side views of metaphase may be rolled over to give polar views by tapping the cover-slip with a needle. Attempts to make the preparations permanent resulted only in the loss of the greater number of the pollen grains.

2. CHROMOSOME ASSOCIATION

Chromosome behaviour at meiosis in the two parental species is what is usually described as "normal". That is to say the 18 chromosomes are regularly associated as 9 bivalents having 1, 2, 3 or 4 chiasmata each (Table II). The chiasmata are distributed at diplotene and by the time metaphase is reached have almost all terminalized (Figs. 1, 2). The chiasma frequency differs very slightly, being 2·00 for *P. floribunda* and 2·13 for *P. verticillata*. In polar view, the bivalents can be seen to be very regularly disposed on the spindle (Fig. 4, *a–f*). In *P. floribunda* a ring of 7 bivalents with 2 in the middle is commonest. In *P. verticillata* an arrangement with 1 bivalent in the middle is common, while a ring of 9 bivalents (Fig. 4, *f*) occurs not infrequently. The bivalents with single chiasmata lie in the ring or in the centre at random. There seems to be no evidence of their being pushed to the edge of the plate as in *Silene* (Fyfe, 1936).

In the diploid hybrid, behaviour is more irregular. In nearly every

cell there is one pair of univalents, and there may be two (Table II and Figs. 3, 9). The chiasma frequency is 1·36, about two-thirds that of the parents. The regular arrangement of the spindle which characterizes the

TABLE II

Chiasma frequency of diploid species and hybrid

Species	No. of nuclei	Number of chiasmata 0	1	2	3	4	*T*	Chiasmata nucleus	Chiasmata bivalent
P. floribunda	10	0	10	70	10	0	180	18·00	2·00
P. verticillata	10	0	11	58	18	3	192	19·20	2·13
P. kewensis	20	17	82	80	1	0	245	12·25	1·36

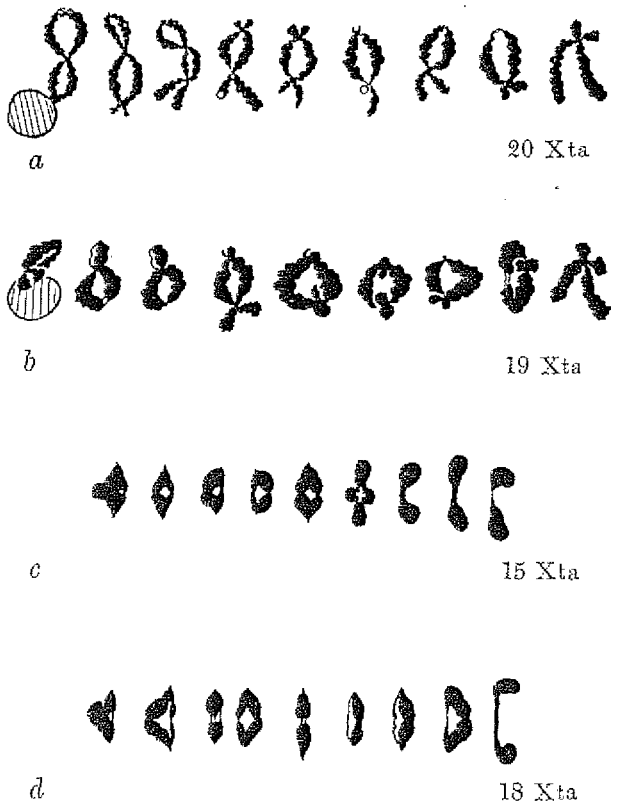


Fig. 1. Diplotene to metaphase in *P. floribunda* (× 4000), the bivalents drawn separately. The total number of chiasmata is given for each nucleus.

parents is entirely lost (Fig. 4, *g–j*). Lagging or non-disjunction of the univalents leads to the production of second metaphase plates with unequal numbers of chromosomes and occasionally to restitution nuclei

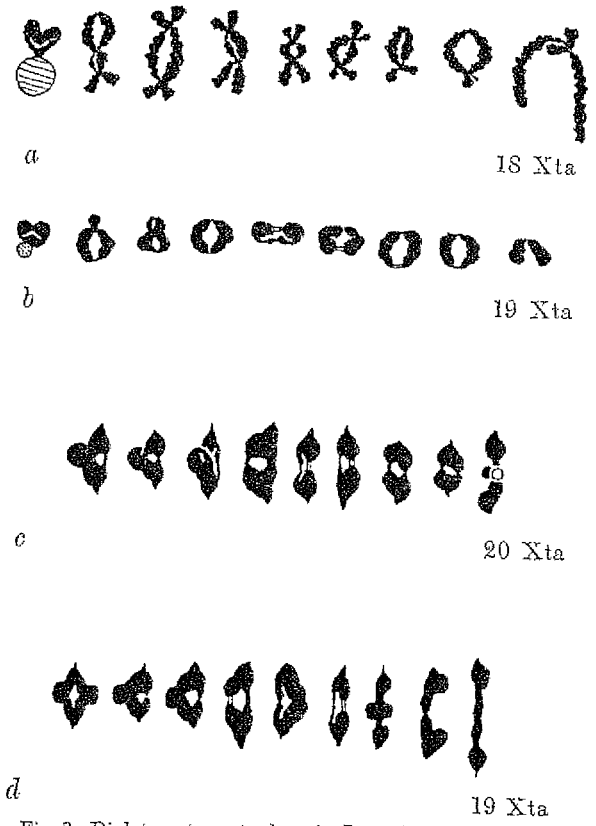


Fig. 2. Diplotene to metaphase in *P. verticillata* (× 4000).

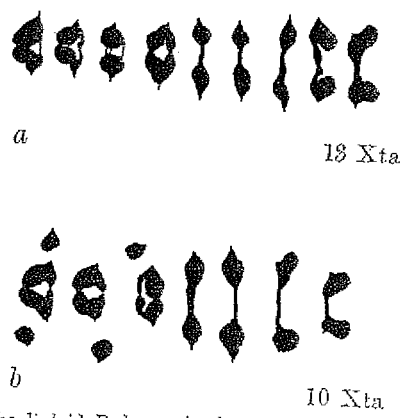


Fig. 3. Metaphase in the diploid *P. kewensis*, showing the reduced chiasma frequency and the occurrence of univalents (× 4000).

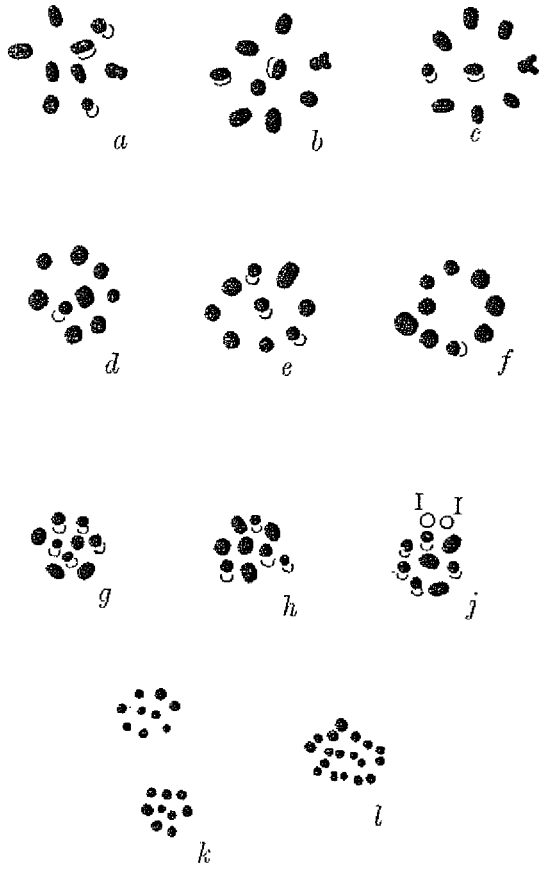


Fig. 4. Polar views of first and second metaphase in the parental species and the hybrid (× 2700). *a–c, P. floribunda; d–f, P. verticillata; g–j, P. kewensis.* The regular orientation and spacing of the parents is lost in the hybrid. *j,* Shows two univalents; *k* and *l,* second metaphase in *P. kewensis,* showing the normal arrangement (9 + 9) and a restitution nucleus.

(Fig. 4, *l*). Table III shows the distribution observed in 20 pollen mother cells. Since most of the pollen grains die before they undergo mitosis,

TABLE III

Distribution of chromosomes at second metaphase in the diploid hybrid

No. of chromosomes	6	7	8	9	10	11	*T* (nuclei)	*T* (chrs.)	*M*
No. of nuclei	—	3	9	24	4	—	40	349	8·725

Loss per nucleus = 0·275.
No. of univalents per nucleus at M_I (from Table II) = 0·85.
Chance of loss of univalents at A_I = 0·32.

this is a useful method of obtaining an estimate of the chance of loss of the unpaired chromosomes (see later).

The parental species evidently differ in the linear arrangement of the homologous parts of their chromosomes, for we find inversion bridges and fragments are not uncommon in the hybrid (Fig. 5, *b–d*). In one of the individuals of *P. verticillata* I found an inversion bridge and fragment (Fig. 5, *a*), but only in two cells out of 100. It does not of

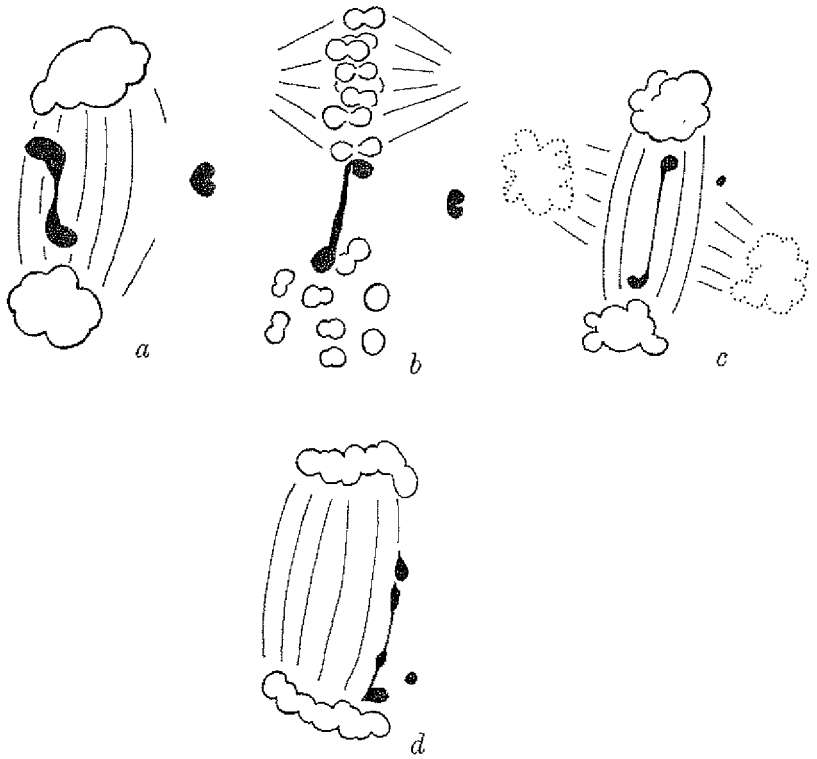


Fig. 5. Inversion bridges and fragments at first and second anaphase. *a–c*, *P. kewensis* (×2700, acetocarmine preparations); *d*, *P. verticillata* (×4000).

course follow that the individual involved in the cross was structurally hybrid, and the greatly increased frequency in *P. kewensis* shows that the structural differences between the species must be much more important than those within either of them.

It is clear that the sterility of this hybrid is essentially due to the incompatibility of combinations of genes from the two species. This distinguishes it from the sterility due to gross structural differences between homologous chromosomes such as hinder pairing in the sterile

diploid *Raphano-Brassica* (Howard, 1938). Sterility is due to the regular segregation of differences and not to irregularities in the segregation itself.

In the duplex tetraploid, my observations agree with Newton's, who stated that there were from 1 to 3 quadrivalents per nucleus (Newton

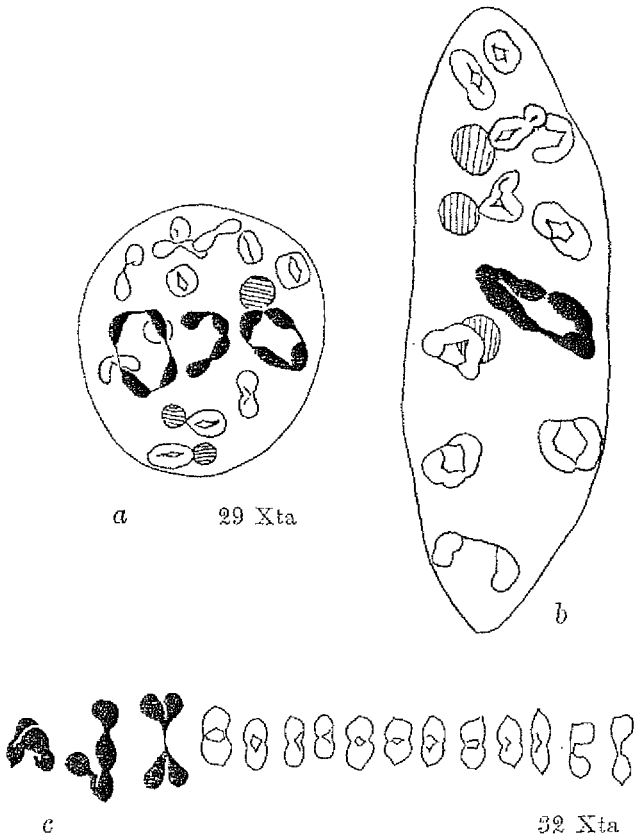


Fig. 6. *a*, Diakinesis in the pollen mother cell and *b*, in the embryo-sac mother cell of the duplex tetraploid *P. kewensis*, showing the formation of quadrivalents: *a* has 29 chiasmata, *b* is incomplete. *c*, Side view of metaphase with 3 quadrivalents (×4000). The apparent difference in the size of the chromosomes is due to different methods of fixation.

& Pellew, 1929). In 10 nuclei, I found a mean of 2·4 quadrivalents (Table IV, Figs. 6, 7). A small number of trivalents-and-univalents also occur, and these would explain Digby's unequal counts which Newton could not understand. In some cells she found "18 bivalent chromosomes; the greater number show 17 bivalent chromosomes together with one quadrivalent, and a few show 16 bivalents and one quadrivalent,

and one case was found of 15 bivalents and 2 quadrivalents" (Digby, 1912). With indifferent fixation at diakinesis, it is no easy matter to

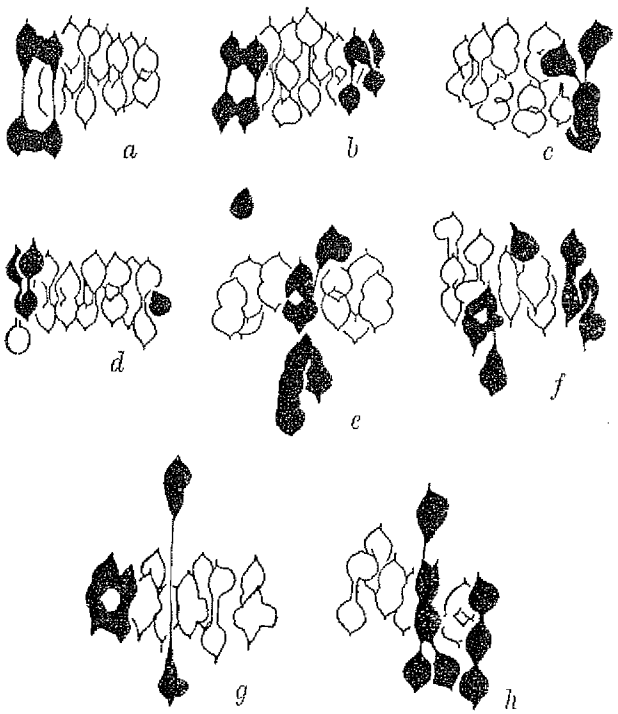


Fig. 7. Types of quadrivalents found in the duplex tetraploid *P. kewensis* (×4000). *a*, *b*, Ring quadrivalents with parallel and convergent orientation; *c*, branched chain; *d*, chain trivalent and univalent; *e*, *f*, trivalents and chain quadrivalents showing different orientations; *g*, bivalent with a single chiasma separating early; *h*, association of five and a trivalent. This is evidence of an interchange.

Fig. 8. Types of configuration found in the triplex tetraploid (×4000) showing the large number of trivalents and univalents.

distinguish bivalents from univalents, especially when the material is cut as thin as 6μ. Further she adds that quadrivalent formation is confined to the pollen mother cells, only bivalents being found in the

embryo-sac mother cells. This would be interesting if true; embryo-sacs would then show a lower chiasma frequency than pollen mother cells of the same plant. I have examined meiosis in the embryo-sac from Miss

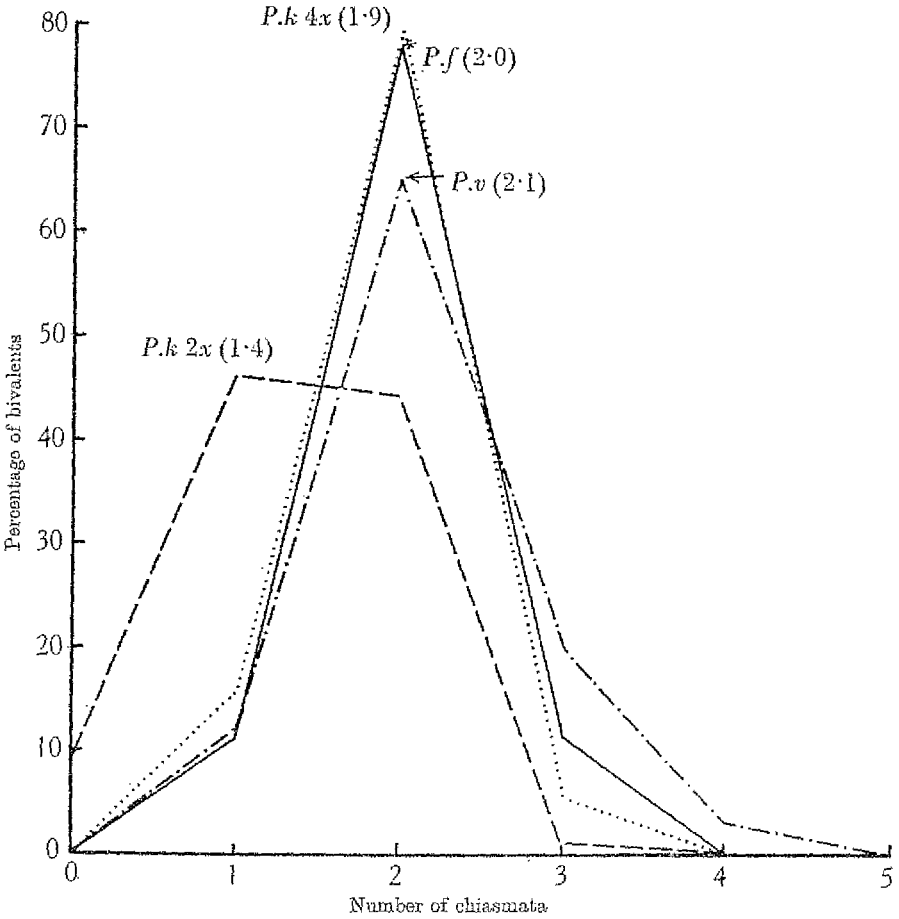


Fig. 9. Graph showing the number of bivalents with different numbers of chiasmata in the parental species, *P. floribunda* and *P. verticillata*, and in the diploid and tetraploid hybrids, *P. kewensis* 2*x* and 4*x*. The figures in brackets show the mean chiasma frequency per bivalent. In the tetraploid the quadrivalents (2·4 per cell) have been divided into bivalents for purposes of comparison.

Digby's own slides,[1] and have been unable to confirm her observation, for quadrivalents do occur (Fig. 6, *b*). The number of nuclei at diakinesis or metaphase is too small to show whether the frequency of quadrivalents is equal to or less than that in the pollen mother cells, but they are of

[1] A privilege I owe to the Botanical Department of the Royal College of Science.

the same types as those most often found in the pollen mother cells. These types are usually of the simplest kind, either rings or chains, owing to the chiasma frequency being rather low (1·90, Fig. 9). Since the arms are nearly equal, this means that the chiasma frequency per arm is less than one.

Two types, however, occurred exceptionally, one the X-shaped quadrivalent where all three chiasmata are in the same arm and on

TABLE IV

Chiasma frequency of the tetraploid hybrids

No. of chiasmata	Configurations: I	II	III+I	IV	*T* (chiasmata)
	Duplex tetraploid (10 cells)				
0	0	0	0	0	0
1	0	19	0	0	19
2	0	95	1	0	192
3	0	10	3	4	51
4	0	0	0	20	80
T (configuration)	0	124	4	24	342
Chiasmata/nucleus					34·2
½ chiasmata/chromosome	0	1·93	1·38	1·92	1·90
	Triplex tetraploid (20 cells)				
0	26	0	0	0	0
1	0	43	0	0	43
2	0	118	35	0	306
3	0	16	9	16	123
4	0	0	0	25	100
T (configuration)	26	177	44	41	572
Chiasmata/nucleus					28·6
½ chiasmata/chromosome		1·84	1·10	1·80	1·59

Note. In this table and in Table VII, I = pairs of univalents, II = bivalents, III + I = trivalents-and-univalents, IV = quadrivalents. Pairs of univalents arise when 2 of the 4 homologous chromosomes form a bivalent and the other 2 fail to pair. Trivalents-and-univalents arise when 3 of the 4 homologous chromosomes pair leaving the fourth unpaired.

terminalization form a quadruple chiasma (Fig. 6, *c*), and the other, the branched chain (Fig. 7, *c*) with a triple chiasma.

In one nucleus I found evidence of interchange between non-homologous chromosomes such as would make possible associations of more than four. The nucleus contained a chain of five and a trivalent. I could not find the corresponding association of four in the diploid, and it is possible that the interchange took place after the sterile hybrid had doubled.

In the triplex tetraploid the frequency of quadrivalents is about the same as in the true *P. kewensis* (2·05 per cell), but the frequency of trivalents and pairs of univalents is very much greater (Table IV, and Fig. 8). In the duplex tetraploid no pairs of univalents occur, but only

those associated with trivalents. In the triplex tetraploid there are more trivalents than quadrivalents, and in addition to these, 0·65 pairs of univalents per cell. A further difference between the two is the lower chiasma frequency of the triplex tetraploid (1·59). Its method of origin and its behaviour at meiosis agree in leading us to regard it as an "auto-allo-tetraploid".

Since we know the chiasma frequency of the parental species and the sterile hybrid, and also the constitution of the two kinds of tetraploids, we can calculate the chiasma frequency to be expected of them.

Let us first consider the duplex tetraploid. Its constitution is (*ffvv*). We might therefore expect the chiasma frequency to be the sum of those of *P. floribunda* and *P. verticillata*. In order to avoid the difficulty of having a variable number of configurations of different size in the tetraploid (bivalents, trivalents and quadrivalents) let us consider the chiasma frequency of the whole nucleus. The expected frequency for the duplex tetraploid is then 18·00 + 19·20 or 37·20 chiasmata per nucleus (from Table II). Actually we find it to be 34·2 chiasmata per nucleus. There is a reduction to 0·92 of the expected value.

If we now turn to the triplex tetraploid, we find that there is a similar reduction. The constitution of this tetraploid is (*fffv*) and its chiasma frequency should therefore be the sum of that of *P. floribunda* (*ff*) and that of *P. kewensis* (*fv*). This is 18·00 + 12·25 or 30·25. The observed value of 28·6 (Table IV) gives us a reduction factor of 0·94.

Since we have obtained so close an agreement in the reduction factor of two tetraploids which, although made up of the same chromosome sets, have them in different proportions, and which therefore behave very differently, an examination of the chiasma frequencies of other tetraploids would seem likely to show a similar result. Table V shows the available data for nine other tetraploid species and mutants.

So far as this evidence goes there is a consistently lower chiasma frequency in tetraploids than that which can be deduced from the parental diploids. The reduction factor varies from 0·97 to as low as 0·79.

This reduction might be ascribed to one or more of three possible causes. The simplest assumption would be that, following the precocity theory, the more numerous chromosomes took longer to pair and therefore partially failed to pair in the tetraploid, the frequency of chiasmata being proportional to the amount of pairing. The second possibility is that the chromosomes undo their internal torsion while pairing, and being delayed have less residual stress left for the development of relational coiling and hence for chiasma formation.

The third possibility is that certain changes of partner taking place among the four chromosomes of the tetraploid interfere with their pairing and hinder its completion.

The data at our disposal (Table V) enable us to eliminate some of these possible assumptions. The reduction factor is shown in relation to the size of the mitotic chromosomes, from which the relative sizes of the pachytene nuclei may be inferred, at least in the plants. It is also

TABLE V

The reduction of crossing-over in tetraploids

	Chromosome no. (n)	Total haploid chromosome length μ	Kind of tetraploid	Chiasmata per nucleus in diploid, $\times 2$	Chiasmata per nucleus in tetraploid	Reduction factor
Raphano-Brassica (Howard, 1938)	9	18	Allo	27·40	26·60	0·97
Solanum Lycopersicum (Upcott, 1935)	12	21	Auto	39·84	37·64	0·94
Schistocerca gregaria ♂* (White, 1933, 1934)	11	50	Auto	35·10	33·00	0·94
Primula kewensis (*fffv*) (Present account)	9	17	"Auto-allo"	30·25	28·60	0·94
P. kewensis (*ffvv*) (Present account)	9	17	Allo	37·20	34·20	0·92
*P. sinensis** (Darlington, 1931)	12	28	Auto	45·60	40·80	0·89
Kniphofia Nelsonii (Moffett, 1932 and unpublished)	6	54	Auto	22·10	19·68	0·89
*Allium Schoenoprasum** (Levan, 1936)	8	48	Allo	45·32	40·00	0·88
*Campanula persicifolia** (Gairdner & Darlington, 1931)	8	40	Auto	30·40	25·50	0·83
Allium pulchellum × *carinatum* (Levan, 1937)	8	72	Allo	32·00	25·20	0·79
*A. Schoenoprasum** (Levan, 1936)	8	48	Auto	45·32	35·00	0·77

* One or two cells only of the tetraploid analysed.

shown in relation to the kind of polyploidy. The autotetraploids should have frequent changes of partner, the allotetraploids few.

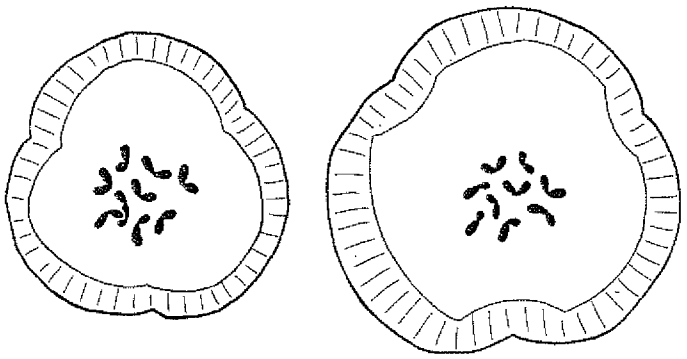
When the results are examined in this light, it is seen (1) that relative auto- and allo-polyploidy has no detectable effect on the reduction factor, and (2) that the size of the chromosomes and the nuclei, on the contrary, has such an influence. This leaves us with the first and second possibilities, namely that the reduction is due to delay in pairing following an increase in the size of the nucleus. The question as to whether the effect is absolute or relative must for the moment be left open.

It should be noted that the only animal from which data could be obtained is the orthopteran *Schistocerca gregaria*. The reduction factor is

0·94, a figure which is considerably higher than that for plants with chromosomes of a similar size. This difference may be attributed to the arrangement and high contraction of the chromosomes at pachytene. In the Orthoptera they are polarized, in plants they lie at random. The regular arrangement naturally assists pairing and offsets the difficulties of the larger nucleus.

3. Chromosome distribution and super-reduction

The sterile hybrid has been known to produce functional haploid pollen grains, but I have never succeeded in finding one undergoing mitosis. Presumably, however, the mitoses would not differ in appearance from those of the parental species (Fig. 10) and would all have 9 chro-



(*a*) *P. floribunda* (*b*) *P. verticillata*

Fig. 10. Metaphase of the first pollen grain division in the diploid parental species (× 3000). *P. verticillata* has larger pollen grains than *P. floribunda*, as Digby (1912) pointed out.

mosomes, since pollen grains with $x \pm 1$ chromosomes will be less viable than those with x. In the tetraploid hybrids, however, dividing pollen grains are numerous and easy to find. The distribution in the duplex tetraploid corresponds very closely with what is generally found in tetraploids, auto or allo, with a few quadrivalents—over half the pollen grains have 18 chromosomes, the greatest deviations being ± 2 (Table VI, Fig. 11, *b*, *c*). In the triplex tetraploid on the other hand, less than 20 % of the pollen grains have 18 chromosomes, the greatest deviation being ± 5 (Fig. 11, *a*, *d*). The variance of this distribution is much greater than that of the duplex tetraploid and resembles that expected in a triploid (Fig. 16). Also although the two means (17·84 and 17·42) do not show a great numerical difference, they imply a considerable difference in the amount of loss at meiosis, namely 0·16 and 0·58 chromosomes per nucleus. This would be expected from the differences in frequency in the two types of plant of odd chromosomes in trivalents and of univalents.

The results indicate in fact a similar chance of loss of such chromosomes, neglecting irregularly co-orientated quadrivalents, of about one-third. This agrees very closely with the value of 32 % found from direct

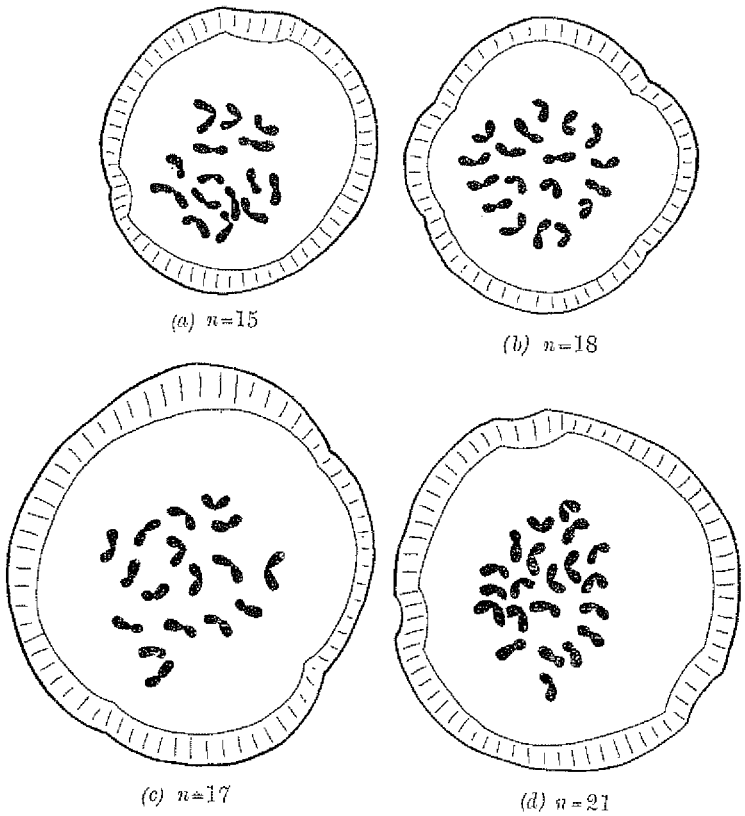


Fig. 11. Metaphases in pollen grains *b* and *c* of the duplex tetraploid *P. kewensis ffvv* and *a* and *d* of the triplex tetraploid *fffv* (×3000).

TABLE VI

Distribution of chromosomes in pollen grains of tetraploids

	T	13	14	15	16	17	18	19	20	21	22	*M*
Duplex tetraploid	150	0	0	0	2	44	81	22	1	0	0	17·84
Triplex tetraploid	150	1	5	12	26	38	30	20	11	5	2	17·42

observation of second metaphase plates in the diploid hybrid (Table III). The value for larger chromosomes varies from zero to 10 %, and the higher value here found agrees with the rule that the chance of loss is a function of the relative sizes of cells and chromosomes (Upcott & Philp, 1939).

In the two tetraploids, differences in variance as well as mean are highly significant and may be compared likewise with the observations of meiosis in the two forms. This I have attempted in Table VII, which shows that the spatial relationships observed on the first metaphase plate agree with and must therefore be supposed to determine directly the inequalities of distribution of the chromosomes in the pollen grains and the observed variances resulting. The agreement is of course more significant for the triplex tetraploid than for the duplex, on account of the greater frequency of unequally segregating configurations. The importance of these agreements is that they show the absence of selective elimination; pollen grains with all different chromosome numbers and combinations are equally viable up to the time of the first pollen grain mitosis.

TABLE VII

Frequency of unequal segregation of multivalents per 100 *cells in tetraploids*

	Pollen mother cells				
	No. of quadrivalents				
Species	Total	With 3 : 1 segregation	No. of (III + I) + (I + I)	½ previous value	No. of chromosomes above or below 18
P. kewensis, ffvv	240	20	40 + 0	20	40
P. kewensis, fffv	205	5	220 + 65	142	147

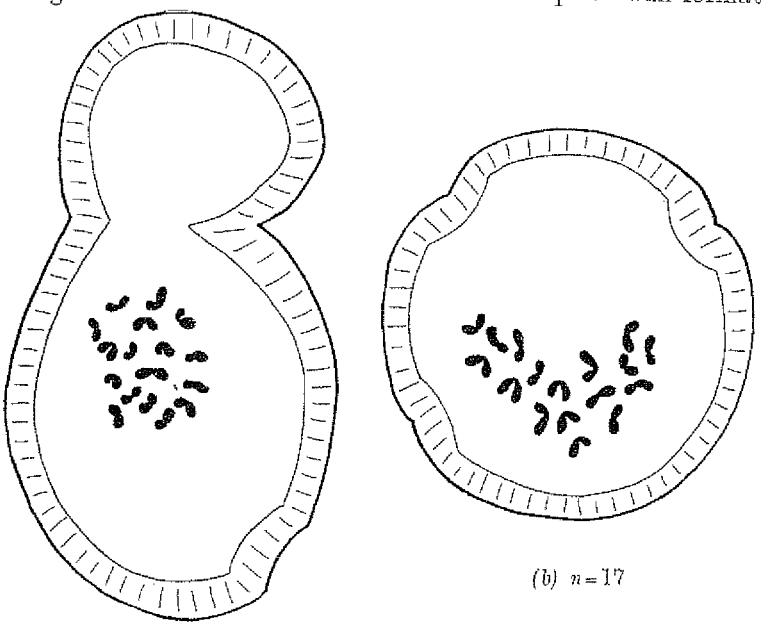
	Pollen grains					
	No. of pollen grains with					No. of chromosomes above or below 18
Species	$2x$	$2x \pm 1$	$2x \pm 2$	$2x \pm 3$	$2x \pm 4$	
P. kewensis, ffvv	54	—	2	—	—	48
P. kewensis, fffv	20	39	25	11	5	142

Apart from these expected properties of distribution, certain of the pollen grain mitoses show a new and surprising modification of the normal course of development which must have important genetic consequences. A number of abnormal pollen grains occur. In some of these, the plate is normal and the pollen grain is lobed (Fig. 12, *a*) showing that there had been a failure of wall formation at meiosis. In others the grain is normal, but the metaphase plate itself is abnormal in one of two ways.

In the first type, the plate is V-shaped (Fig. 12, *b*) or curved; in the second, one or more chromosomes lie off the plate (Fig. 13). If there are enough, these chromosomes form a secondary plate of their own

(Figs. 13, *b*, 14). In extreme cases, two equal plates are formed, each with 9 chromosomes. Equal plates are parallel (Fig. 14) unequal usually at right angles (Fig. 13). Each plate may separate into its two anaphase groups, so that four nuclei are formed instead of two (Fig. 15, *b*).

In all anthers examined from each type of tetraploid, *double plates*, as I shall call them, occurred in 8 % of cases (Table VIII). The accompanying abnormalities of pollen formation offer what is perhaps the explanation of the origin of these double plates. The occurrence of lobed pollen grains shows that there has been an attempt at wall formation



(*a*) $n = 18$ (*b*) $n = 17$

Fig. 12. *a*, Lobed pollen grain and *b*, V-shaped plate in the duplex tetraploid (× 3000).

at meiosis between two nuclei which have later fused. The bent and narrow plates indicate that two nuclei were fusing at the time of metaphase. These two kinds of evidence point to the double plates being derived from binucleate pollen grains. But as the figures show, the numbers of the combined plates correspond with those of normally reduced nuclei. They are therefore such as can have arisen only from split spindles at the second anaphase of the type described by Darlington & Thomas (1937) in *Lolium* at first anaphase.

The consequences of the formation of these double plates are indicated by the anomalous breeding results of the tetraploid *P. kewensis*

as well as of other polyploids. As a result of selfing the tetraploid *P. kewensis* two anomalous plants have been obtained, one with 20 and the other with 26 chromosomes (Newton & Pellew, 1929). The plant with 20 chromosomes might have arisen apomictically, but the 26 chromosome plant was more difficult to explain. If, however, a pollen grain containing a *super-reduced* nucleus from a double-plate mitosis, that is

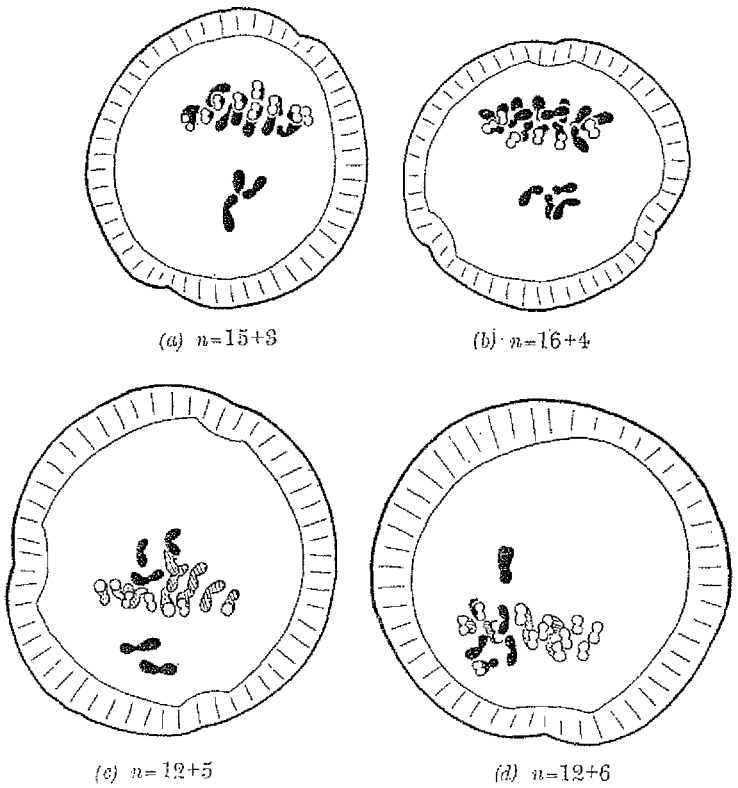


Fig. 13. Double plates showing 3–6 chromosomes forming the extra plate, *a*, duplex tetraploid, *b*, *c*, triplex tetraploid (× 3000).

one with a competent set of 9 chromosomes, had fertilized a normally reduced egg, a triploid or near-triploid plant would result.

There are other examples of a reduction of chromosome number occurring by other means than through normal meiosis (cf. Darlington, 1937, Table 27). Some of these are perhaps due to super-reduction (e.g. *Avena*). Again two species of *Rubus* with 56 and 28 chromosomes may exceptionally give offspring with 35 chromosomes (Crane & Thomas, unpublished). Similar spindle abnormalities occurring at the somatic

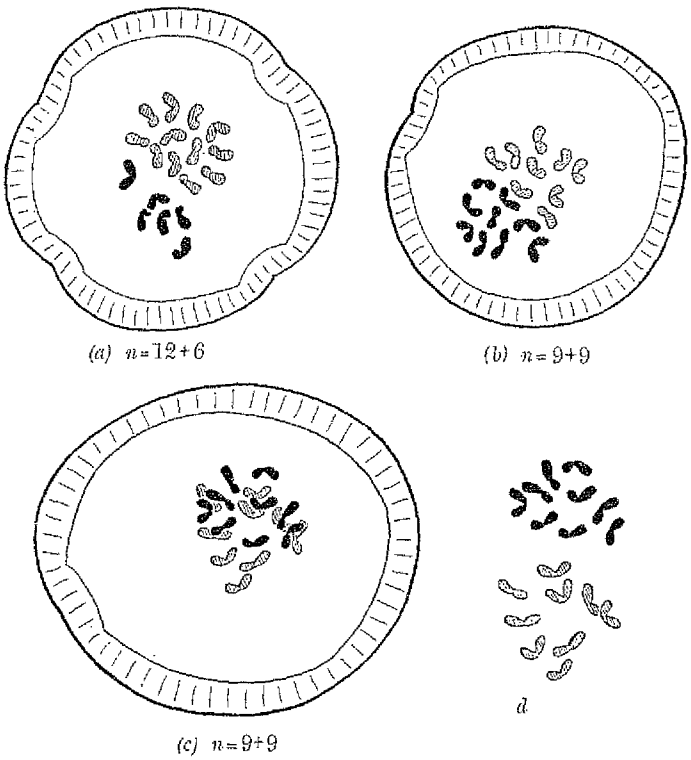


Fig. 14. Double plates showing *a*, 12 + 6 chromosomes and *b*, *c*, 9 + 9. The two plates in *c* are shown separately in *d*. They are 4 μ apart (× 3000).

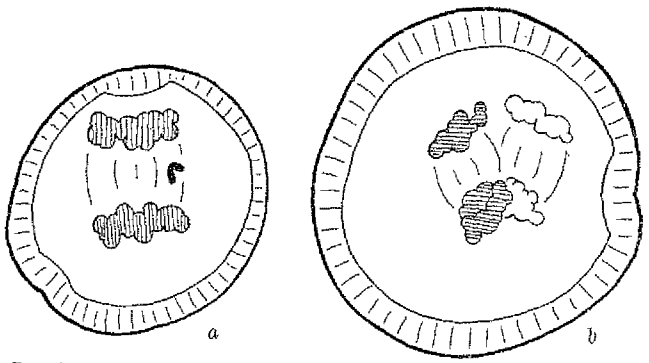


Fig. 15. *a*, Lagging chromosome at anaphase. This is possibly an iso-chromosome resulting from misdivision (Darlington, 1939). *b*, Anaphase with 4 groups of chromosomes, presumably resulting from a double plate (× 3000).

mitoses would account for the production of triploid pollen mother cells in otherwise hexaploid *Triticum vulgare* (Love, 1936) and for the

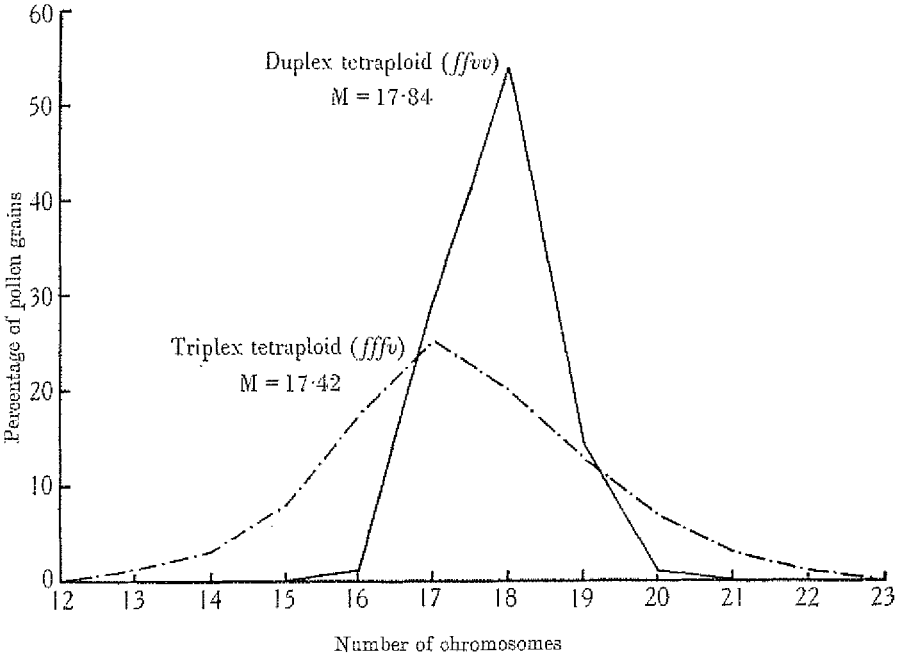


Fig. 16. Distribution of chromosomes in the pollen grains of the two tetraploid types of *P. kewensis*. The triplex tetraploid shows the type of curve to be expected from a triploid. 150 pollen grains of each were counted.

TABLE VIII

Distribution of chromosomes in double plates

Total no.	No. in smaller plate									*T*
	1	2	3	4	5	6	7	8	9	
14	.	.	.	2	.	.	.	.	.	2
15	.	.	.	.	.	.	.	.	.	.
16	.	.	.	.	.	.	.	.	.	.
17	3	.	.	.	2	.	.	.	.	5
18	5	.	1	.	.	1	1	.	3	11
19	2	.	.	.	.	.	.	.	.	2
20	.	.	.	1	.	.	.	.	.	1
T	10	.	1	3	2	1	1	.	3	21

Note. The proportion of pollen grains with double plates is 8·2% (21 out of 256). One preparation of the duplex tetraploid differed from all others in having 15% with double plates (10 out of 76).

occurrence of haploid tissue in *Drosophila* (Bridges, 1925; MacKnight, 1937). This tissue showed recessive characters which happened to be

derived from the father only. Presumably the elimination of paternal chromosomes carrying recessive genes is as frequent.

These results seem to indicate that we may look for apparently determinate abnormalities in inheritance to follow the selection of rare competent nuclei from frequent incompetent results of spindle abnormalities at mitosis and meiosis.

5. SUMMARY

1. The chiasma frequency of *Primula kewensis* (1·36), the diploid hybrid between *P. floribunda* (2·00) and *P. verticillata* (2·13) is lower than that of either of the parents. Univalents are occasionally present at the first metaphase of meiosis.

2. The chiasma frequency of the duplex tetraploid hybrid (1·90) is nearly as high as that of the parental species. The slight reduction ($\times 0{\cdot}92$) can be explained by the larger size of the cell and the consequently slower pairing. The triplex tetraploid with three sets of *floribunda* chromosomes and one set of *verticillata* has the chiasma frequency expected of it, namely the mean of the *floribunda* and the *kewensis* frequencies multiplied by a similar factor (0·94). Other tetraploids, equally allo and auto, obey the same reduction rule.

3. The frequency of quadrivalents in the duplex tetraploid is 2·4 per cell, together with 0·4 trivalents-and-univalents. The triplex tetraploid has a similar number of quadrivalents, 2·0 per cell, together with 2·2 trivalents-and-univalents and 1·3 pairs of univalents.

4. The distribution of chromosomes in the pollen grains agrees with the observed co-orientations of multivalents at meiosis. In the duplex tetraploid the variance is small, over half the pollen grains having 18 chromosomes, the greatest deviation being ± 2. In the triplex tetraploid the variance resembles that to be found in a triploid, less than 20 % of pollen grains having 18 chromosomes, and the greatest deviation being ± 5.

5. A new type of *double* metaphase plate is formed in 8 % of the pollen grains. The extra plates, presumably derived from split spindles at meiosis, contain 1–9 chromosomes. At anaphase 4 nuclei may result. This behaviour explains certain *super-reduced* progeny derived from the tetraploid *P. kewensis* and from other polyploids.

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