TULBAGHIA HYBRIDS

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THE analysis of chromosome behaviour in hybrids, especially at meiosis, can shed light on the genetic diversity of the species involved in the cross and on their phylogeny.

The chromosomes of *Tulbaghia* are amongst the largest in the plant kingdom and, therefore, very suitable for cytogenetic study. Their usually differentiated morphology makes it possible to observe and recognise them at all stages of the nuclear cycle.

1. MATERIAL AND METHODS

The plants used were: *Tulbaghia violacea, T. Dregeana, T. acutiloba, T. pulchella, T. leucantha, T. natalensis*, all diploids (2x = 12) and *T. alliacea* (4x = 24).

They were cultivated in pots in a heated greenhouse with a minimum winter temperature of 60-65° C., and under these conditions they flowered freely usually starting in early spring. *T. Dregeana* and *T. leucantha* start flowering somewhat later, and *T. pulchella* and *T. alliacea* which have a short flowering period may bloom either in spring or autumn. *T. pulchella, T. leucantha* and *T. acutiloba* possess chillable heterochromatic segments (Dyer, 1963, Vosa, 1966).

One to three flowers were cross pollinated at each time and a paper bag was placed over the inflorescence and secured. The ovary begins to show signs of swelling about four to seven days after pollination and, in the successful crosses, the seeds ripen after a month or a little longer. Germination takes ten to twenty days according to temperature.

Root tips were pretreated with 0·05 per cent. colchicine for up to five hours at the temperature of the growing plants. Fixation in 1:3 acetic-alcohol overnight was followed by Feulgen staining (Darlington and La Cour, 1962). The quick fixative suggested by Battaglia (1957) was also used, followed by a short hydrolysis and acetic-orcein staining especially for non-permanent preparations.

Anthers were squashed in 1·5 per cent. acetic-orcein (Vosa, 1961) and left to fix and stain for four or five hours and often overnight. All the preparations were made permanent according to the method of Darlington and La Cour (1962).

2. OBSERVATIONS

All the species studied are almost completely self-incompatible. The crosses attempted were reciprocally successful as follows:

$$T.\ violacea\ (2\text{x}) \times T.\ Dregeana\ (2\text{x})$$
$$T.\ violacea\ (2\text{x}) \times acutiloba\ (2\text{x})$$
$$T.\ violacea\ (2\text{x}) \times pulchella\ (2\text{x})$$
$$T.\ violacea\ (2\text{x}) \times alliacea\ (4\text{x})$$
$$T.\ Dregeana\ \ \ \times acutiloba\ (2\text{x})$$
$$T.\ Dregeana\ \ \ \times alliacea\ (4\text{x})$$

Any cross involving *T. leucantha* and *T. natalensis* resulted in very poor seed setting (table 1) and no viable progeny was raised.

TABLE I

Fertility in Tulbaghia *crosses*

	Crosses	No. of pollinations	Well developed seeds	Degenerated seeds	Germinated	No. of plants	Type of growth
1.	*viol.* × *Dreg.*	10	28	3	27	25 (5 self)	Very strong
	Dreg. × *viol.*	8	20	5	20	20	Very strong
2.	*viol.* × *acut.* (H)	12	26	6	20	17	Strong
	acut. (H) × *viol.*	8	18	11	12	9	Strong
3.	*viol.* × *pulch.* (H)	31	75	18	62	53†	Weak, with very poor root system
	pulch. (H) × *viol.*	10	30	5	23	17†	Weak, with very poor root system
4.	*Dreg.* × *acut.* (H)	8	12	8	10	10	Strong
	acut. (H) × *Dreg.*	8	9	6	5	4	Strong
5.	*Dreg.* × *pulch.* (H)	12	10	3	6	6†	Weak, with very poor root system
	pulch. (H) × *Dreg.*	5	8	2	2	2†	Weak, with very poor root system
6.	*viol.* × *nat.*	14	5	3	2	—	—
	nat. × *viol.*	9	4	2	1	—	—
7.	*viol.* × *all* (4x)	4	8	8	5	4	Very strong
	all. (4x) × *viol.*	3	8	4	5	3	Very strong

(H) Species possessing chillable heterochromatic segments.
† Plantlets with two or three leaves, weak, mostly succumbing to moulds or other diseases, but surviving up to two years.

The offspring of the reciprocal crosses *T. violacea* × *T. Dregeana*, *T. violacea* × *T. alliacea* (4x) and *T. violacea* × *T. acutiloba* are quite strong growing, and, in the case of the first, flowering started six months after sowing. In all other crosses, although there is a fair seed germination (table 1) the resulting plantlets develop very slowly with weak root formation and no plant, so far, has been raised to maturity. This deficiency in the root system and weakness of hybrid seedlings has been found previously in the hormone-induced pear-apple crosses (Crane and Marks, 1952: Brock, 1954) where also the reciprocal cross was unsuccessful, and in Lilac species hybrids (Sax, 1945). In both these cases the growth difficulty was obviated by grafting, a procedure which may be impossible to explore in the hybrid *Tulbaghia* because of the difficulty of grafting in monocotyledonous plants and also the very small size of the hybrid plantlets.

Several methods of improving growth were tried. Thus, crushed parental endosperm was applied to the roots (James, W. O. in Brock, 1954) of six month old plantlets growing on moist filter paper in petri dishes. Sugar solutions were tried as was the transplanting of the seedlings to mature compost taken from the roots of the parental plants. No success was achieved.

In hybrids between species with and without heterochromatin the chromosome complement of the former retains its allocycly.

3. MEIOSIS IN *TULBAGHIA VIOLACEA* × *DREGEANA*

The chromosome morphology of the parents has already been described and illustrated (Dyer, 1963, Smith and Flory, 1965, Vosa, 1966).

The first cross involving three flowers was made without proper emasculation and produced 17 seeds all of which germinated after two weeks. Five of the plants proved to be selfed, *T. violacea* and all the others hybrids. It is interesting that although the clone of *T. violacea* used as female parent is completely self-sterile, there was, on this occasion, a fair amount of selfing. It is probable that, once the foreign pollen has germinated on the stygma and the pollen tube has started growing down the style, there is a breakdown in the incompatibility system which allows for a certain amount of selfing.

Morphologically the F_1 plants are intermediate between the parents, robust and somewhat more floriferous than either of them. The differences in morphology and size of the parent chromosomes are preserved in the hybrid as, for example, in *Allium cepa* × *fistulosum* (Levan, 1936), *Crepis conyzifolia* × *capillaris* (Warner and Bruhin, 1949) and *Lolium* hybrids (Rees and Jones, 1967).

Chiasma frequencies and localisation in the parents and the hybrid are summarised in figs. 1 and 2. There is considerable variation in the amount and type of pairing between pollen mother cells in the hybrid. Chiasma position is affected by structural hybridity (figs. 2, 3, 4, 5, 6).
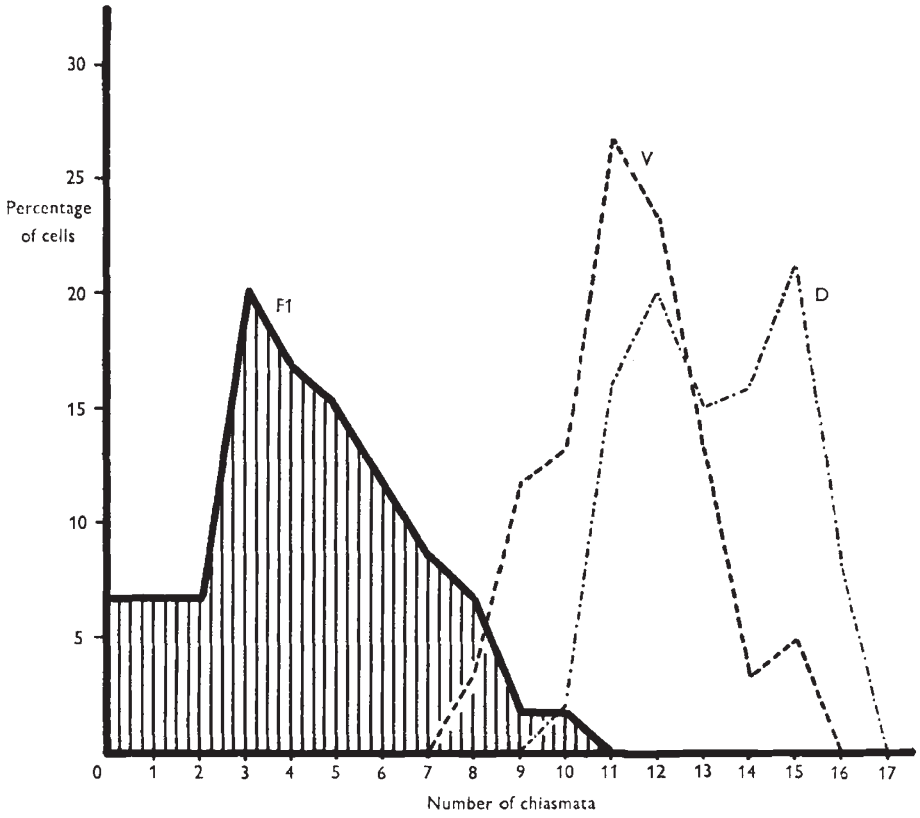
FIG. 1.—Chiasma frequency per cell in *Tulbaghia violacea* (V), *T. Dregeana* (D) and their F_1 hybrid in which a reduction in the mean number of chiasmata is associated with a greater range of frequency.
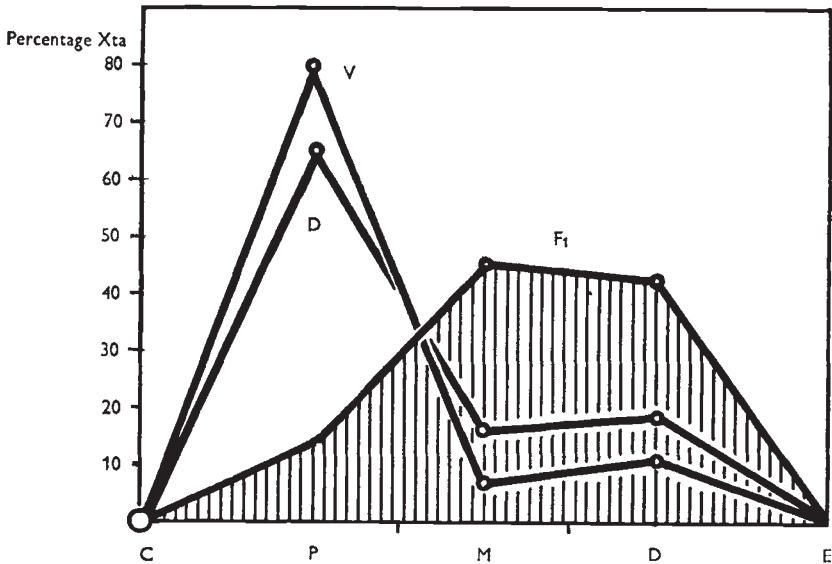

FIG. 2.—Chiasma localisation in *T. violacea*, *T. Dregeana* and their F_1 hybrid at metaphase I (60 cells each).

The proximal localisation of the parent species may depend on a genotypically controlled centric point of contact which ceases to be
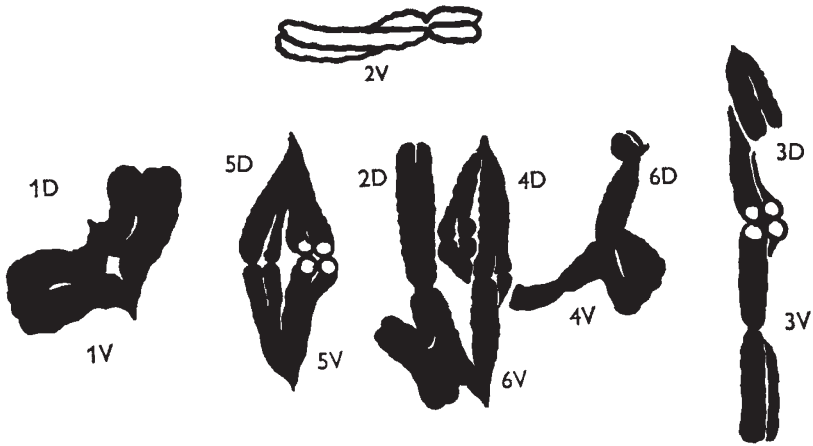

FIG. 3.—*T. violacea* × *Dregeana*. Metaphase I: showing the partial interchange chain: Chromosomes 2V is unpaired, while bivalents *1D*+*1V*, *3D*+*3V* and *5D*+*5V* have four, one and three chiasmata respectively. ×2100 ca.

effective in the hybrid or, alternatively, the change in localisation may have been caused by structural hybridity resulting from rearrangements in the parent chromosomes in segments near to the centromere.
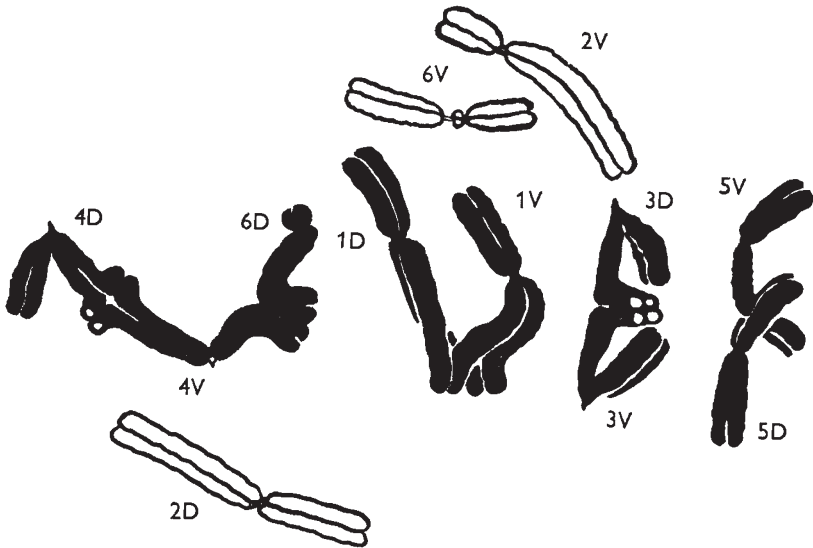

FIG. 4.—*T. violacea* × *Dregeana*. Metaphase I: with an association of three chromosomes (6D+4V+4D) with two chiasmata and pairs 1D+1V, 3D+3V and 5D+5V each with one chiasma. Chromosomes 2D, 2V and 6V are unpaired. ×2100 ca.

It is therefore possible that only relatively short, distally located regions in the chromosomes of the parents have retained their ancestral homology.
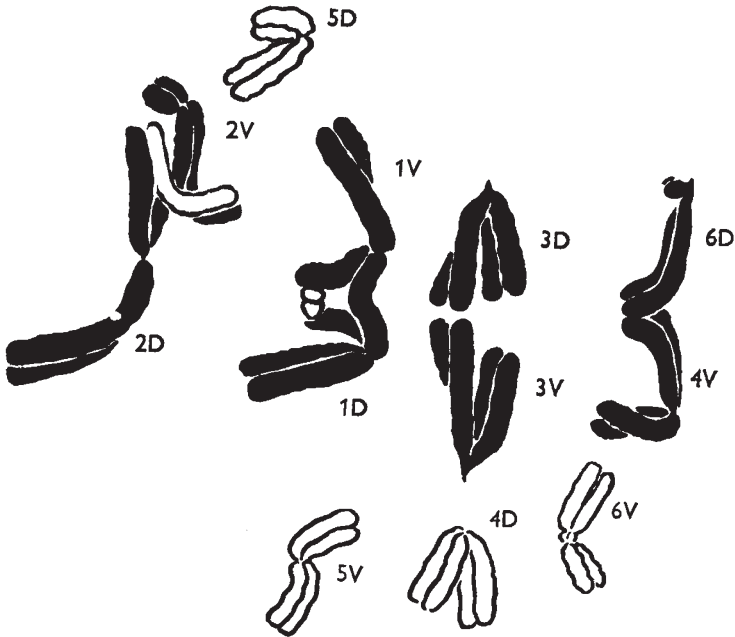
FIG. 5.—*T. violacea × Dregeana.* Early Anaphase I showing inversion bridges and fragment in pair 2V+2D, where a chiasma has occurred in the proximal inversion, and in pair 1V+1D. Pairs 6D+4V, and 3D+3V have separated but probably had one and two chiasmata respectively. All other chromosomes are univalent.

×2100 ca.

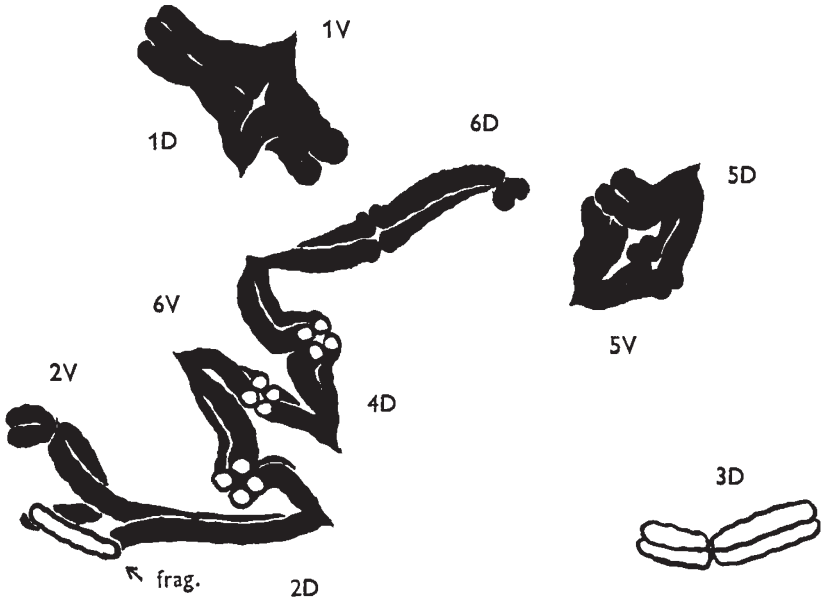
FIG. 6.—Camera lucida drawing of the incomplete cell illustrated on Plate I, fig. 2.

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Anaphase I is abnormal with persistent bridges and non-uniform segregation leading, after a short interphase, to a usually abortive second division. The pollen is completely sterile.
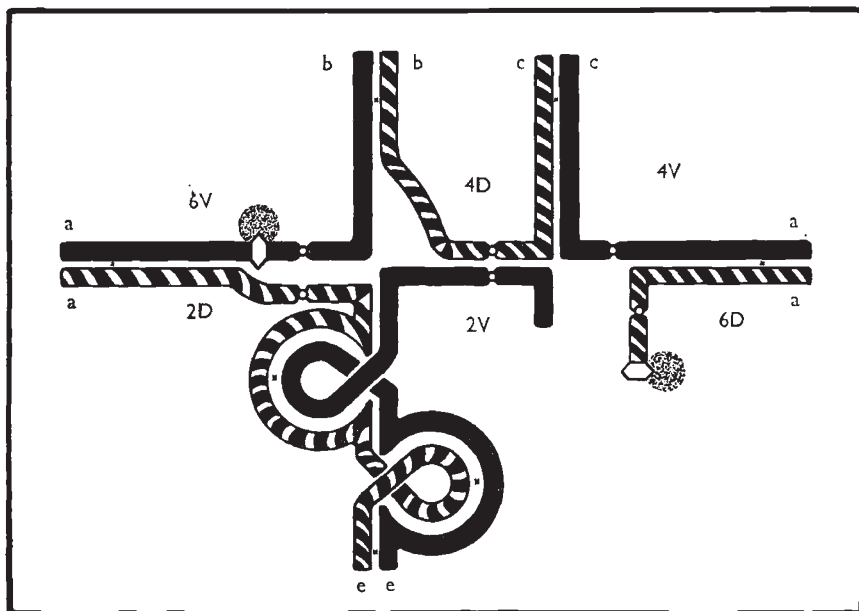

FIG. 7.—Pachytene configuration (assumed) in the interchange chain of six chromosomes in *Tulbaghia violacea* × *Dregeana* (not to scale).

The variance of the chiasma frequency per cell is slightly greater in the hybrid. A similar situation was found by Richardson (1936) in the hybrid *Lilium Martagon album* × *Hansonii* (fig. 1). Typical pollen mother cells are illustrated in figs. 3, 4, 5 and 6 and plates I and II.
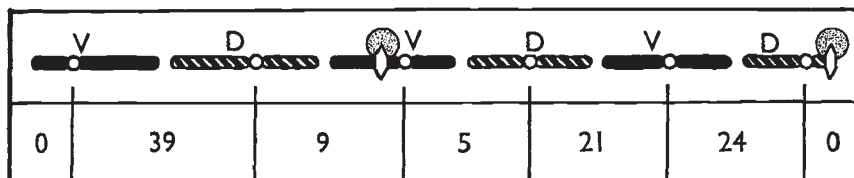

| 0 | 39 | 9 | 5 | 21 | 24 | 0 |

FIG. 8.—Pairing frequency in the interchange chain in *Tulbaghia violacea* × *Dregeana* (60 cells at M. I.).

4. INTERCHANGES

There are three interchange differences, giving rise to a chain of six chromosomes (plate I and figs. 7 and 8) which includes heterozygosity for two inversions in the longer arms of the two longest elements. The chromosomes involved are the pairs 2, 4 and 6 and the chiasma frequency is summarised in table 2. Only one complete chain, which includes both nucleolar chromosomes, was observed in the sample

2 X

scored.   The pairing frequency between the elements of the inter-
change chain is indicated in fig. 8 and the assumed pachytene con-
figuration is illustrated in fig. 7.

The chiasma frequency of the two longest elements (pair 2) of the
chain is high in spite of the structural differences between them.   As
Darlington (1958) has pointed out, chiasma frequency is usually

TABLE 2

Tulbaghia violacea × Dregeana

Chiasma frequency in the interchange chain in sixty cells at M.I.

	No. of Chiasmata					
	0	1	2	3	4	5
No. of cells	9	20	19	8	3	1

reduced by inversions which impair pachytene association, but in this
case and for the pairing situation of the whole interchange chain, we
must take into account the condition of hybridity and the actual
amount of linear homology between the chromosomes involved.   It is
very likely that in the interchanged chromosome the homologous
segments are short and this in itself may reduce the possibility of

TABLE 3

A comparison of pairing (Xta per biv.) between chromosome pairs 1, 3 and 5 in Tulbaghia
T. Dregeana and their hybrid

	Chromosome type		
	1	3	5
V	2·96	4·12	4·02
D	4·90	3·70	3·90
F_1	1·52	1·45	1·59

chiasmata.   This is illustrated by the low chiasma frequency between
translocated chromosomes $2^D+6^V+4^D$.   The interchange (4^V+6^D) and
the homologous association 4^D+4^V have a high pairing frequency.
No chiasmata are formed between the short arms of the chromosome
2V and chromosome 6D which form the end of the interchange chain.

The three chromosome pairs, 1, 3, and 5, are not involved in
interchange but the first two have relatively inverted segments.   A
comparison of the pairing behaviour between these hybrid pairs, and
the corresponding bivalents of the parents, is summarised in table 3.

Although there are differences in the chiasma frequency of the three bivalents, the association of the hybrid pairs is fairly constant around 1·5 chiasmata per bivalent. Except for chromosome pair 1, where chiasmata also occur in proximal positions, the localisation is mainly distal.
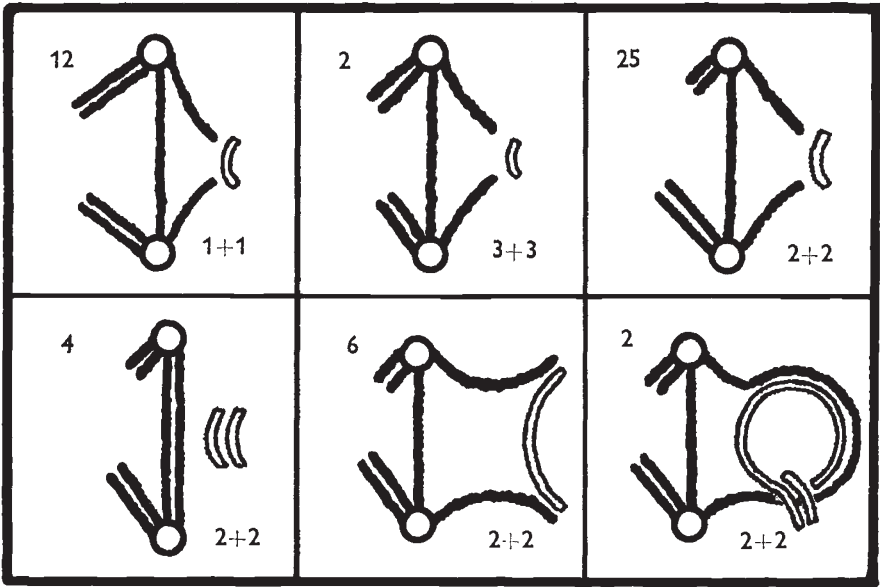

FIG. 9.—*Tulbaghia violacea* × *Dregeana*—Type and frequency of first division inversion bridges and fragments (125 cells at A. I.).
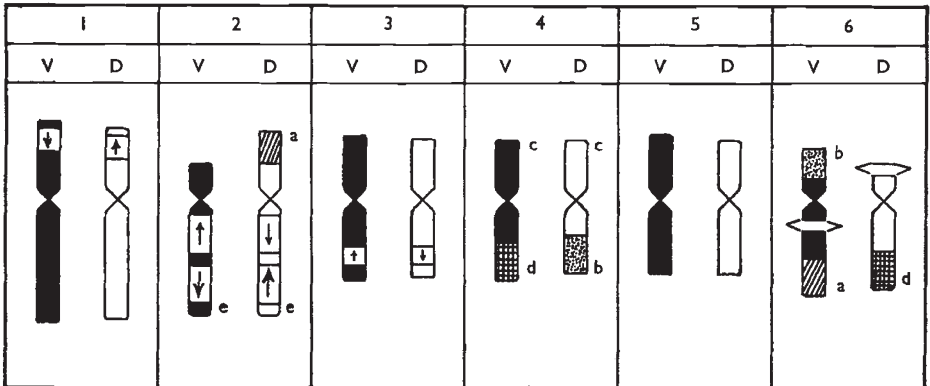

FIG. 10.—The segmental relationship of the two parental genomes in the Fr of *Tulbaghia violacea* × *Dregeana*.

5. INVERSIONS

There are four inversions, two in the long arm of pair 2, one in the short arm of pair 1 and one in the long arm of pair 3 (figs. 9 and 10). The type and frequency of crossing-over in the inversions, as indicated by the formation of bridges and fragments at Anaphase I, was scored

in 125 cells.   At the second division the chromosomes are generally very despiralised which makes the scoring of Anaphase II bridges very difficult and unreliable.   The highest frequency of crossing-over is found in the distal inversion of pair 2.   In this particular pair complementary crossing over, giving two bridges and two fragments of the same size was observed so also were two bridges and fragments of different sizes.   This involves a chiasma in each of the two inversions in disparate chromatids (fig. 9 and plate II).

Bridges and fragments at Anaphase I may result either from crossing-over in relatively inverted segments or from sister reunion following chromosome breakage which is known to occur frequently in hybrids and inbred species (Lewis and John, 1965; Rees and Thompson, 1955; Haga, 1953), and is no doubt due to genotypic unbalance or an adverse environment.   This type of behaviour is expected to involve different chromosomes and loci more or less at random and also the occurrence of various kinds of abnormalities including fragments without bridges. These conditions have never been observed in the subject of this study. A demonstration of the existence of true paracentric inversions is given by the constancy of the length of both bridges and fragments for a given inversion.

A diagrammatic representation of the segmental relationship of the two parental genomes is indicated on fig. 10.

6. *TULBAGHIA VIOLACEA* $(2x = 12) \times$ *ALLIACEA* $(4x = 24)$

The seed setting of this reciprocal cross is good and the seedlings, which are strong-growing, flowered six months after sowing.   The resulting hybrid plants, all $2n = 3x = 18$, are somewhat intermediate morphologically, but with a certain preponderance of characters from the tetraploid parent.

The chromosome size and morphology are preserved in the hybrid complement: the chromosomes of *T. alliacea* are larger than those of *T. violacea* (Vosa, 1966).

At meiosis there is complete autosyndesis of the *T. alliacea* complement while the chromosomes of *T. violacea* lie scattered about the *p.m.c.'s* without orientation.   At Anaphase I the bivalents of *T. alliacea* segregate regularly while the univalents of *T. violacea* tend to be swept into two unequal groups.   Occasionally one or two univalents fail to be included in the telophase daughter cells and form micronuclei.   After a short interphase all the chromosomes reappear at the second division very much despiralised but they orientate correctly and divide normally.   Apart from the micronuclei which persist at least for a while in a degenerate, highly pycnotic stage, the other chromosomes form daughter nuclei, and the tetrad cells develop into pollen grains of varying sizes.   The pollen was observed in all its stages of development, but no pollen grain mitoses were found.   At anthesis the pollen grains are shrivelled with degenerate nuclei.

The pairing frequency and localisation in 18 *p.m.c.'s* are summarised in table 4. The chiasma localisation is proximal as in both parents (Vosa, 1966).

The condition of autosyndesis of the chromosomes of *T. alliacea* may indicate that there is a strong intragenomic preferential pairing. Alternatively it may be considered as the result of a lack of homology between the two complements, taking into account also the difference in size of the chromosomes.

TABLE 4

Tulbaghia violacea (2x = 12) × alliacea (4x = 24)

Chiasma frequency and localisation in 18 p.m.c's at Metaphase I. The chromosomes of
T. violacea *are always unpaired*

	P	M	D	Total Xta.
Xta. per cell	10·9	2·9	1·8	282

7. DISCUSSION

One of the principal causes of hybrid inviability and weakness is the unbalanced interaction between the two genomes as they combine in the hybrid nuclei. This may lead to the death of the embryo at a very early stage of development, and this accounts for the low fertility and the many shrivelled seeds found in some crosses. Conversely there might be a normal growth of the embryo and of the endosperm, resulting in good seeds which germinate regularly but the unbalance may show in the seedlings. They may be chlorotic, dwarf, or their leaf or root development may be impaired. In many cases, however, the seed set, germination and growth of the seedlings are normal, but the resulting plants are sterile on account of the structural diversity of their chromosomes which usually result in pollen and egg sterility. This is well illustrated by the hybrids *T. violacea* × *Dregeana*, and *T. violacea* × *alliacea*. Interchanges, inversions and other chromosome rearrangements play an important role in plant speciation. Any chromosome mutation is unique and must arise in a single cell. If such a mutation can be inherited, its survival and propagation normally depend on its establishment in the homozygous condition, which must involve inbreeding. These new chromosome mutants may have a better chance of survival in the small marginal populations where the basic type is not completely suited to the environment. The mutation itself will probably disturb the chromosome balance of the plant carriers and still may induce yet new mutations. The occurrence of inversions is a step toward the development of reproductive isolation, based on segregational sterility, which is very important in evolution.

Crossing over in an inversion produces an acentric fragment which is lost, and a dicentric chromatid bridge which may break anywhere

2 X 2

along its length. In this way the primary structural variation, brought about by inversion, by reduplication and deficiency, becomes the basis of variability (Darlington, 1937). An inversion in itself effectively restricts or altogether abolishes crossing over and therefore recombination in the segments involved. The gene mutations occurring within these segments are thus kept together establishing the basis of a genetic divergence which results in discontinuity within the species, and may eventually lead to the creation of new species. The significance of structural hybridity in hybrids has been fully discussed by John and Lewis (1965).

It is evident that structural mutation including interchange and inversion has played an important role in the speciation of the genus *Tulbaghia*.

8. SUMMARY

1. Experimental hybridisation has been carried out in seven species of *Tulbaghia*.

2. The reciprocal crosses *T. violacea* × *Dregeana*, *T. violacea* × *alliacea* (4x) and *T. violacea* × *acutiloba* produced robust well developed plants which, in the case of the first two, flowered within the first year from sowing.

3. The crosses *T. violacea* × *pulchella* and *T. Dregeana* × *pulchella* gave very weak plantlets with poorly developed root system.

4. All crosses involving *T. leucantha* and *T. natalensis* resulted in no seed set or a few shrivelled seeds which did not germinate.

5. Parental differences with regard to chromosome size and morphology including the allocycly of heterochromatic segments are preserved in all the hybrids.

6. The hybrid *T. violacea* × *Dregeana* is structurally heterozygous. Interchanges give rise to a chain of six chromosomes which includes two inversions both in the long arms of the longest chromosomes. Two other inversions are present: one in the short arm of the longest chromosomes of the complement and the other in the long arm of the third chromosome pair. Both parents have proximal chiasma localisation but in the hybrid localisation is mainly distal. The chiasma frequency of the hybrid is about a third of the parents.

7. Meiosis in the F_1 of *T. violacea* × *alliacea* (4x) results in the complete autosyndesis of the chromosomes of the tetraploid parent.

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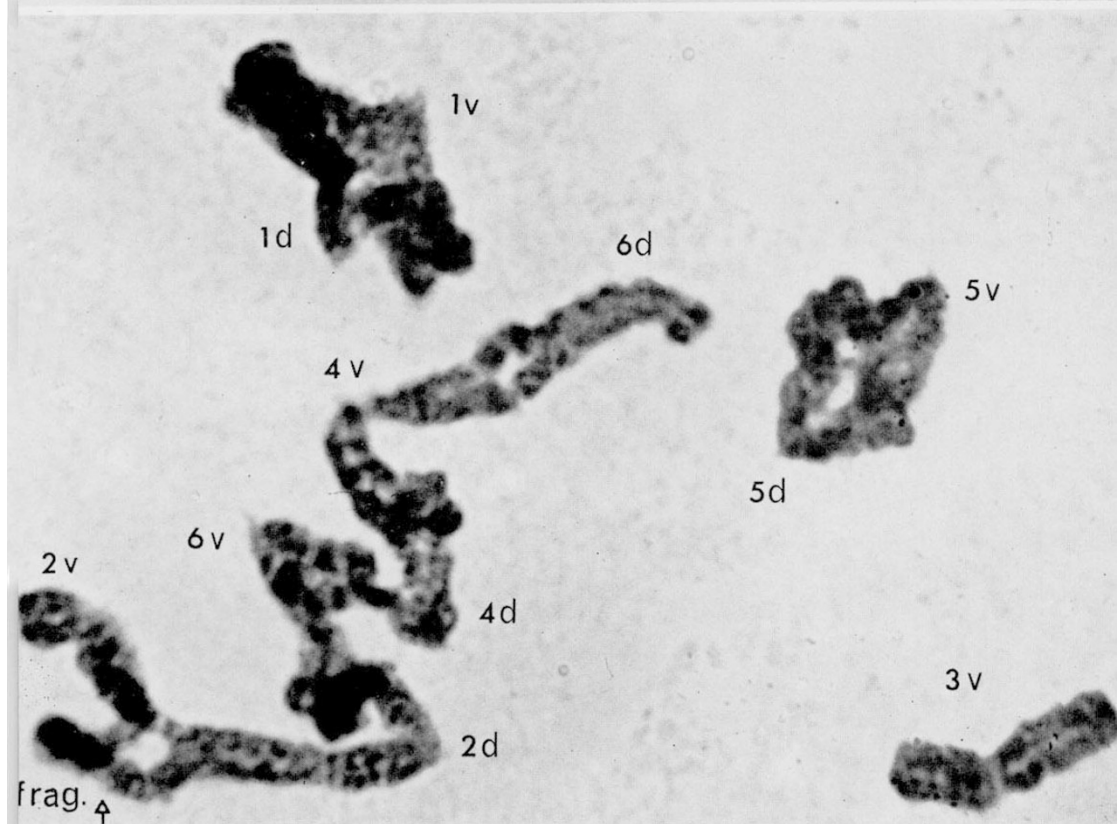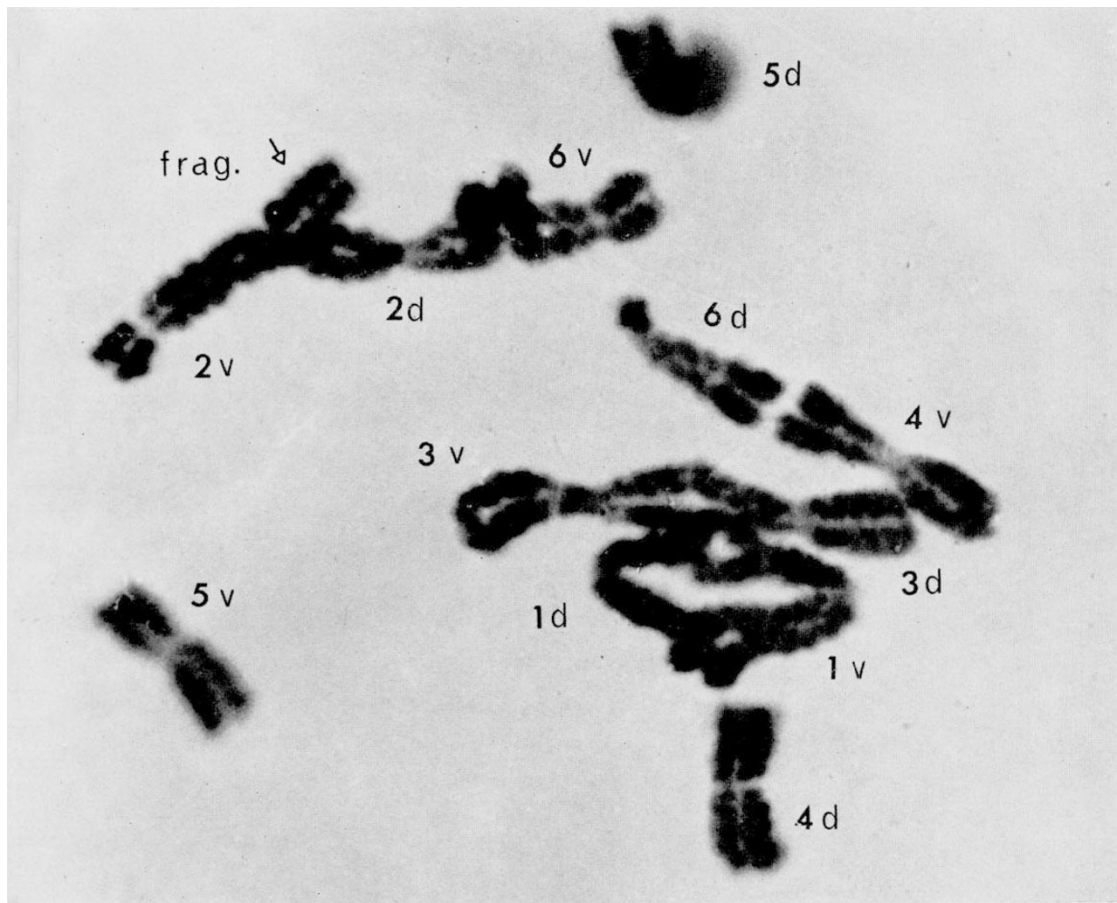DARLINGTON, C. D. 1937. The biology of crossing over. *Nature, 140*, 759-761.
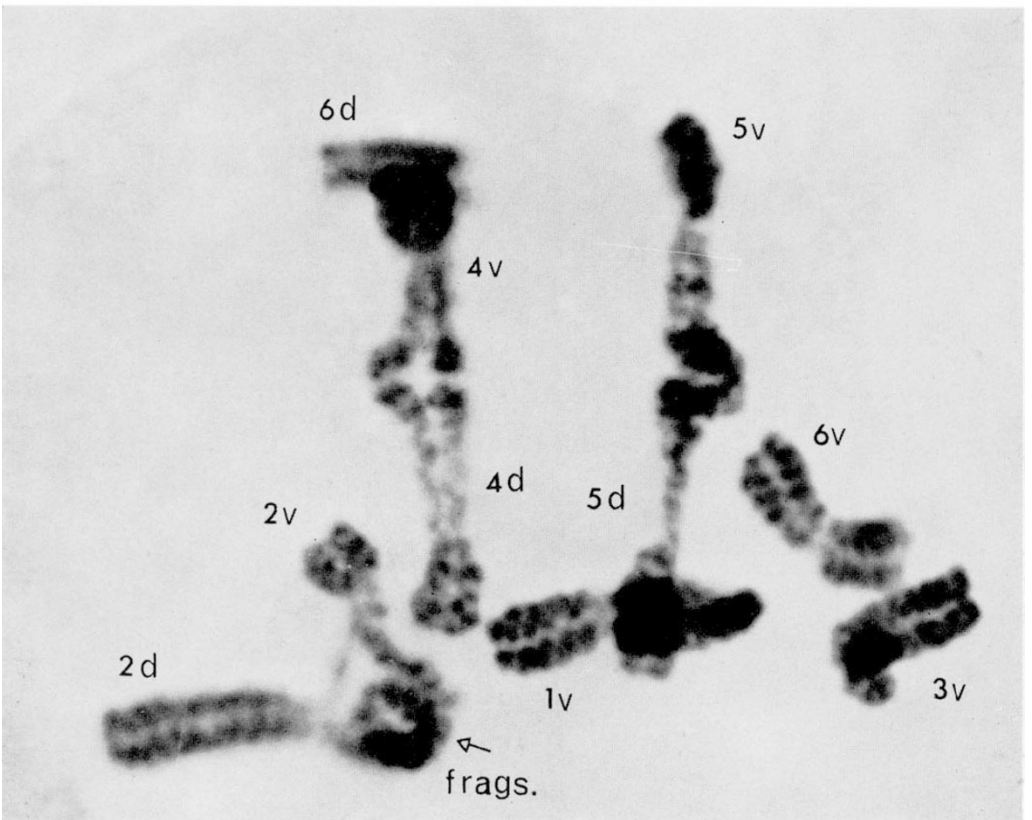
Plate II

FIG. 1.—*Tulbaghia violacea* × *Dregeana*. Incomplete cell at early A.I. showing an association of three chromosomes, $6V+2D+2V$. The double bridge and two fragments between chromosome 2D and 2V indicate the occurrence of complementary crossing-over in one of the reciprocally inverted segments present in their long arm. Chromosome pairs $3D+3V$ and $5D+5V$ show one and two chiasmata respectively.

× 2800 ca.

FIG. 2.—Incomplete cell at early anaphase I, showing pair $2D+2V$ with two bridges and two fragments both of different size. This indicates that one chiasma in each of the inversions has taken place involving disparate chromatids. Chromosome $5D+5V$ and $4V+4D$ are paired each with one chiasma. All other chromosomes are unpaired.

× 2800 ca.

frag. ↘

5d

6 v

2d

2 v

6 d

4 v

3 v

3 d

5 v

1 d

1 v

4 d

1v

1d

6d

4 v

5 v

5 d

2 v

6 v

4 d

3 v

2 d

frag. ↑

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