THE EXPERIMENTAL CONTROL OF CHROMOSOME PAIRING IN *FRITILLARIA*

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(With Six Text-figures)

CONTENTS

1. Introduction

Normal meiosis depends on all the chromosome pairs being held together by at least one chiasma. Such a condition is necessary for regular segregation and hence for the stability of the reproductive cycle. Among the agents which are capable of reducing the chiasma frequency are. (*a*) hybridity, usually involving structural changes such as inversions, translocations etc. in the chromosomes; (*b*) heat or cold shocks during pairing; (*c*) genes causing asynapsis; (*d*) colchicine.

The first two generally seem to act by reducing the time available for pairing or its rate (Darlington & La Cour, 1940; Barber, 1941). This is shown by the fact that both these agents lead to a localization of chiasmata in those regions of the chromosomes which normally pair first (the contact points). These contact points are usually near the ends or near the centromeres of the chromosomes, i.e. in those regions which are most free to move or which owing to the orientation derived from the last premeiotic mitosis are situated closest together in the early leptotene nucleus. High temperatures probably reduce the absolute time available for pairing (Barber, 1941, on *Uvularia*), whilst structural hybridity means a more complicated type of pairing, which presumably takes a longer time (Darlington & La Cour, 1940, on hybrid lilies). The variation in

time limit of pairing can also be genotypically controlled, as has been inferred by comparison of species (Darlington, 1935; Frankel, 1940) in *Fritillaria*. In this genus, an early time limit to pairing leads to a pronounced proximal localization: distal ends rarely or never have time to pair.

Asynaptic genes usually seem to reduce chiasma frequency by a different means. Beadle (1933) has shown that in asynaptic *Zea*, pachytene pairing seems to be normal but chiasmata fail to form at diplotene, the previously paired chromosomes falling apart. A similar situation probably exists in *Pisum* (Koller, 1938) and *Crepis* (Richardson, 1935). Presumably here, the failure in chiasma formation is due to failure in the development of the torsion necessary for crossing-over.

The effect of colchicine on meiosis has been investigated by Dermen (1938) on *Rhoeo*, Walker (1938) on *Tradescantia* and Levan (1939) on *Allium*. In addition to its well-known effect inhibiting the development of the spindle (Levan, 1938; Barber & Callan, 1942) colchicine also leads to a failure in chromosome pairing at meiosis. Levan's figures also indicate that it reduces the characteristic localization, distal or proximal, of such chiasmata as do form. They appear in the wrong places.

In this paper I shall give a comparative account of the effect of high temperatures and colchicine on chromosome pairing in *Fritillaria Meleagris*, a species normally showing pronounced proximal localization. The object of the investigation was to find the mechanism by which colchicine reduces chromosome pairing and also to throw further light on the natural mechanism determining chiasma localization in this species.

2. Technique

The heat treatments were made by placing the plants in an incubator at $34 \pm 2°$ C. for 2 days in early spring.

Colchicine treatments were carried out in a cool greenhouse at about 15° C. The leaves round the bud were bent back slightly and drops of 0·25 and 0·5 % colchicine solution in water dropped in the cup so formed. With care the drop can be made to persist for several hours after application. Drops were applied twice a day for a week and the plants then allowed to come to meiosis, which may take place at intervals of up to 5 weeks after the first application. No differences were noted between the 0·25 and the 0·5 % treatments, so long as the colchicine penetrated well. Colchicine was still active in the plants for periods of up to 2 months. This was shown by the fact that typical colchicine mitoses could still be found in ovary walls after this time, sometimes with well

over 100 chromosomes. The plants were always stunted and the flowers malformed.

Acetocarmine smears were used throughout, as these were found to be more satisfactory than crystal violet or Feulgen.

With no spindle, the chromosomes tend to clump in the middle of the cell, which must be flattened in order to separate the bivalents for chiasma counting. Two-day fixation in acetic alcohol is necessary to harden the cells sufficiently for them to withstand the flattening.

For embryo sacs I am indebted to Mr L. La Cour's observations. His preparations were stained with lacmoid (Darlington & La Cour, 1942) after fixation of the ovules in acetic alcohol and softening with dilute hydrochloric acid. With care the embryo sacs can be pressed out entire.

3. TIMING

In *Fritillaria Meleagris* the last pre-meiotic mitoses probably take place late in December or early in January, and thereafter the pollen mother cells grow in size slowly until meiosis takes place, usually towards

Table 1. *Chiasma frequencies in M and S bivalents in* Fritillaria Meleagris *based on* 10 *cells* (2 *M bivalents*, 10 *S bivalents per cell*)

		M bivalents No. of chiasmata						*S* bivalents No. of chiasmata								
Treatment	Nuclear mean	0	1	2	3	4	Biv. mean	0	1	2	3	4	5	Biv. mean	Variance	*V/M*
Control	25·2	—	5	14	—	1	*1·85*	—	6	76	16	1	1	*2·75*	0·331	0·016
Heated 34° C. Early anther	9·4	9	11	—	—	—	*0·55*	35	48	16	1	—	—	*0·83*	0·526	0·634
Heated 34° C. Late anther	2·4	19	1	—	—	—	*0·05*	77	23	—	—	—	—	*0·23*	0·179	0·778
Colchicine 0·5%	15·7	8	8	1	2	1	*1·00*	32	29	21	9	6	3	*1·37*	1·791	1·308
Colchicine 0·25%	9·6	11	4	3	2	—	*0·80*	58	24	7	5	3	3	*0·80*	1·576	1·970
Colchicine 0·5%	8·0	16	3	1	—	—	*0·25*	60	17	13	8	2	—	*0·75*	1·179	1·572

the end of March. Bringing the plants into a warm greenhouse any time from the middle of February onwards leads to the onset of meiosis, a week or a fortnight later.

In the heat experiments, plants in which the pollen mother cells were just separating were used. After 2 days at 34° C., meiosis takes place 24 hr. after the return to normal temperature. In the controls it may occur a week or 21 days later. Thus high temperatures produce a great acceleration in the process of meiosis.

Within an anther, chiasma frequency is as a rule fairly constant between cells, but between anthers of the same flower wide variations exist. Table 1 gives data for two anthers of the same flower. The first

came to 1st metaphase 24 hr. after the treatment finished. It has a nuclear mean of 9·4 chiasmata. An anther of the inner whorl maturing 48 hr. after cessation of treatment had a nuclear mean of only 2·4 chiasmata. Presumably these results show that the process affected by temperature change, which as we shall see is the actual coming together ('synapsis') of the chromosomes, is of relatively short duration compared with time of treatment (probably not more than 2–3 days at normal temperatures). In the older anther pairing had begun before treatment started, whilst in the later anther it probably had not started. Later treatments, as for instance those described in my experiments (1940), give little if any decrease in chiasma frequency. In some of these cases, the anthers were actually undergoing meiosis before removal from the hot box. In *Uvularia* after heat treatment similar but not so extreme variations have been noted between anthers in the same flower. The temperature-sensitive period is therefore always short.

Colchicine shows a similar critical period. Thus in one experiment begun on 1 February in an unheated greenhouse (12–15° C.), plants maturing up to 3 weeks after the start of the experiment showed normal pairing of the chromosomes. The spindle was of course suppressed, and tetraploid cells in somatic tissues were common. Plants fixed during the fourth week showed abnormal pairing, the change-over being fairly sharp (within 2–3 days). Presumably in the earlier plants the chromosomes had paired before the colchicine had taken effect. The colchicined plants showed little if any delay as compared with normals in the time of 1st meiotic division. None of the plants were treated early enough to affect the pre-meiotic divisions, since all pollen mother cells and embryo sacs showed the diploid number of chromosomes.

These results show that to be effective in reducing chiasma frequency, high temperatures and colchicine must be applied at the same critical period. They are, therefore, affecting the same process of development, although as we shall see they affect it in a different way.

4. The effect of high temperature on chromosome pairing

Table 1 gives analyses of ten cells from two different anthers of the same plant subjected to 34° C. for 4 hr. In the earliest maturing anther the mean nuclear chiasma frequency is 9·4 chiasmata compared with a mean of 25·2 for a control. In the later anther the nuclear mean is only 2·4 chiasmata.

All the chiasmata in these heated plants are localized near the centromere in both submedian (*S*) and median (*M*) type chromosomes. The

maximum number observed in any one bivalent is three, but usually only one is formed (see Figs. 1 and 5). The results can most easily be interpreted as being caused by a reduction in the time available for pairing. Frankel has shown (1940) that pairing in *F. Meleagris* begins near the centromere, and only occasionally and later does pairing take place in distal regions. Distal pairing is absent in the heated plants because of the reduction in the time available. This also shows itself in another way. In the control plants, of 215 chiasmata in *S* bivalents, 89 or 24·2% are in the short arm. In the heated plant (N.M. 9·4), of 83 chiasmata in *S* bivalents, 32, or 38·5%, are in the short arm. This relative increase in number of chiasmata in short arms with reduction in chiasma frequency can only come about by pairing starting in the short arm itself or very near the centromere. Pairing stops before it can travel along the long arm to any extent.

These results are exactly parallel to those obtained by similar experiments on *Uvularia*, a species which normally shows complete pairing of chromosomes. The only difference lies in the fact that in *Fritillaria*, centric regions are favoured, whilst in *Uvularia* centric regions and ends are more nearly equal in time of coming into contact.

All these experiments show that high temperatures, whilst leading to a general acceleration of meiotic processes, accelerate some of the reactions going on more than others. Presumably if we wished to use physico-chemical or physiological terminology we should say that the process of chromosome pairing has a lower Q_{10} than other concurrent nuclear processes which determine when pairing shall end, e.g. the rate of synthesis of new chromosomal material in preparation for division.

5. THE EFFECT OF COLCHICINE

(*a*) *General*

Colchicine has the same general effect on meiosis in the pollen mother cells as that described by Levan (1939) on *Allium*. The development of the meiotic spindle is completely suppressed soon after application. There is, however, one difference: the chromosomes spiralize normally. Levan has shown in *Allium* that colchicine can prevent the meiotic bivalents from developing the major coil. Such an effect has not been observed in *Fritillaria*.

The fully spiralized bivalents and univalents usually come together in the middle of the cell. After a delay lasting a few hours the chromatid attraction lapses, the centromeres divide and a single tetraploid nucleus

is formed. There is no second division, both divisions being telescoped into one abortive metaphase.

Wall formation round the tetraploid pollen grain takes place normally in the absence of a spindle. In my experiments the colchicine was still active when the pollen grain division took place a fortnight or 3 weeks later. No spindle develops, and a single octoploid nucleus is formed in the pollen grain. Such pollen grains have proved ineffective in pollination.

The effect on the embryo-sac meiosis is similar. No spindle is developed. The division of the centromere is delayed with regard to the lapse of chromatid attraction, so that there is a persistent stage in development in which there are 24 univalent chromosomes with the two chromatids widely separated and only joined by the undivided centromere. Finally its division takes place and a single tetraploid nucleus is formed. Both nuclear divisions, as in the pollen mother cell, are telescoped into one.

(*b*) *Chromosome pairing*

In one respect the effect of colchicine is similar to that of high temperature. It causes a reduction in chiasma frequency. Thus in three experiments the nuclear mean in normal plants of from 25 to 30 chiasmata was reduced to between 8 and 15 chiasmata. The effect differs from that of heat in that colchicine leads to a reduction of localization, whilst, as we have seen, heat increases the proximal localization. In colchicine, chiasmata form in unusual positions (cf. Figs. 2, 3 and Fig. 6).

Let us first consider the distribution of chiasmata in the normal *Fritillaria Meleagris*. Table 2 gives an analysis of ten cells from a control plant. The nuclear mean is 25·2. Of these 215 or 85·5% are in the

Table 2. *Distribution of chiasmata in S bivalents in* Fritillaria Meleagris

Treatment	Percentage no. of chiasmata in			Total chiasmata
	P	*M*	*D*	
1	92·2	6·9	0·9	215
2	100	—	—	83
3	100	—	—	23
4	62·0	25·5	12·5	137
5	70·0	17·5	12·5	80
6	72·0	12·0	16·0	75

S chromosomes. Fig. 6 gives a graphic representation of the distribution of these chiasmata in the *S* chromosomes. 92% are proximally localized (*P*), only 7% are in the median (*M*) region of the long arm and 1% in

the distal region (*D*). No cases were observed in which the *M* or *D* regions were paired without the *P* regions possessing at least one chiasma. Frankel (1940) has made a similar analysis and these results agree with his. His plant had a slightly higher chiasma frequency and a slightly greater distal pairing. These results show, as Frankel pointed out from a comparison of several *Fritillaria* species with different degrees of localization, that in *F. Meleagris D* and *M* regions pair only after the *P* regions have paired. Heat, by reducing the time available for pairing, prevents the *D* and *M* regions from pairing at all (see Fig. 1).

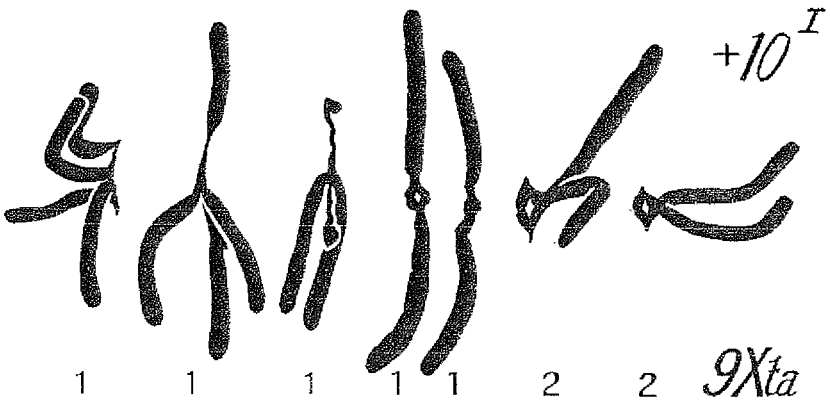

Fig. 1. Bivalents from plant kept at 34° C. for 2 days. Note the proximal localization of chiasmata. (×approx. 2000.)

An exact analysis of chromosome distribution in colchicined plants is difficult. Owing to the suppression of its action, it is often impossible to be sure where the centromere is situated, but in cases of doubt, the chiasma has always been scored as proximal. This means that the *D* class is certainly larger than it is shown in the tables and graphs. It is also impossible, for the same reason, to decide whether *P* chiasmata are situated in the short arm or in the long arm in many cases.

Table 2 and Fig. 6 show immediately the effect of colchicine in increasing the pairing in *M* and *D* regions relative to the *P* regions in the *S* bivalents. The *M* type bivalents show a similar trend, but the data are insufficient for an exact analysis. This difference also shows itself in the abnormal types of bivalent produced under colchicine (see Figs. 2, 3). Bivalents with a single chiasma in the middle or at the end of the long arm are frequent. Ring bivalents and (most remarkable of all) bivalents with complete pairing also occur. This happens even with a nuclear mean

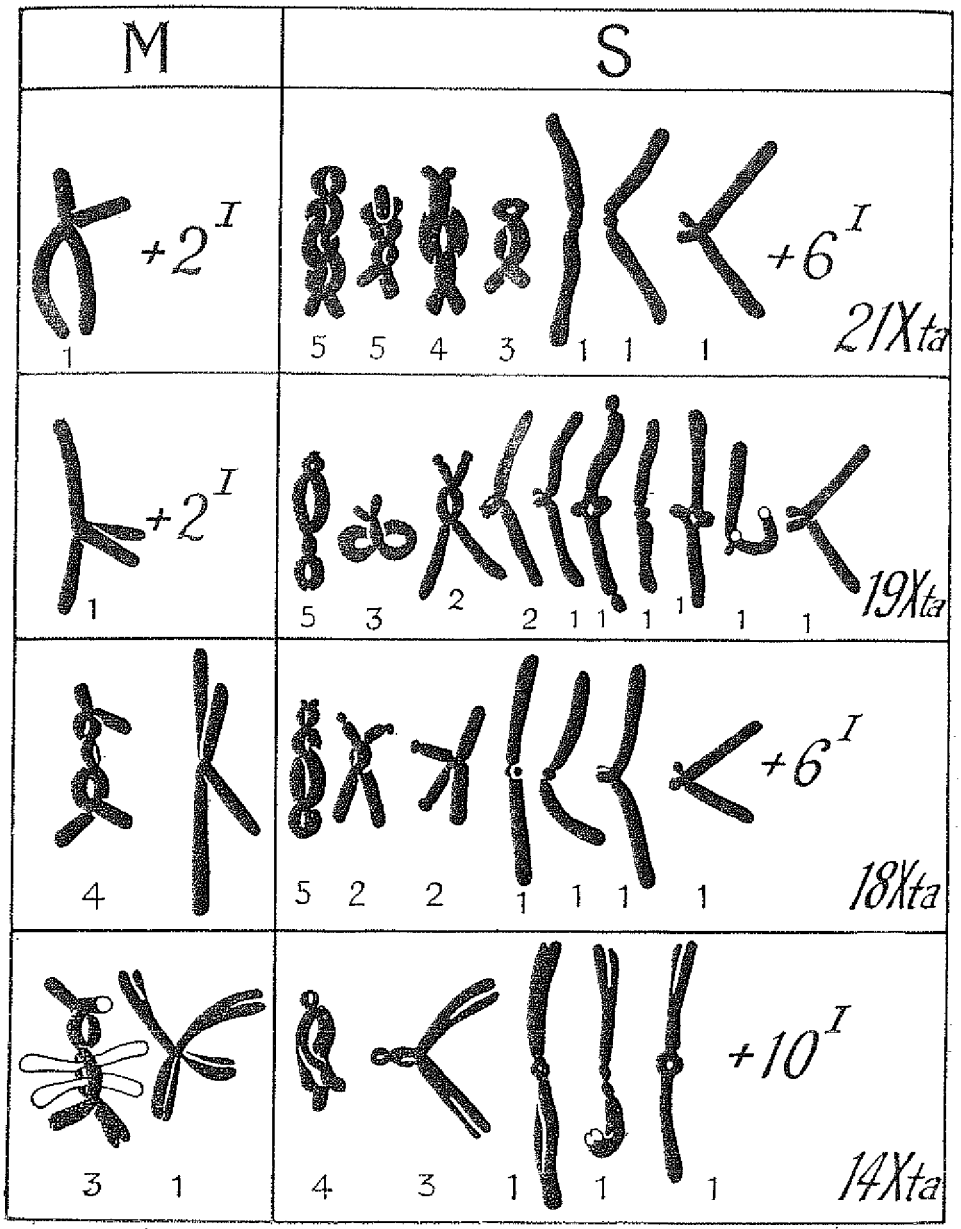

Fig. 2. Bivalents from plants treated with colchicine. The first cell received treatment 5, the others treatment 4 (see Table 1). Note the abnormal types of chiasma distribution. In cell 2 there is an example of self-interlocking. (× approx. 2000.)

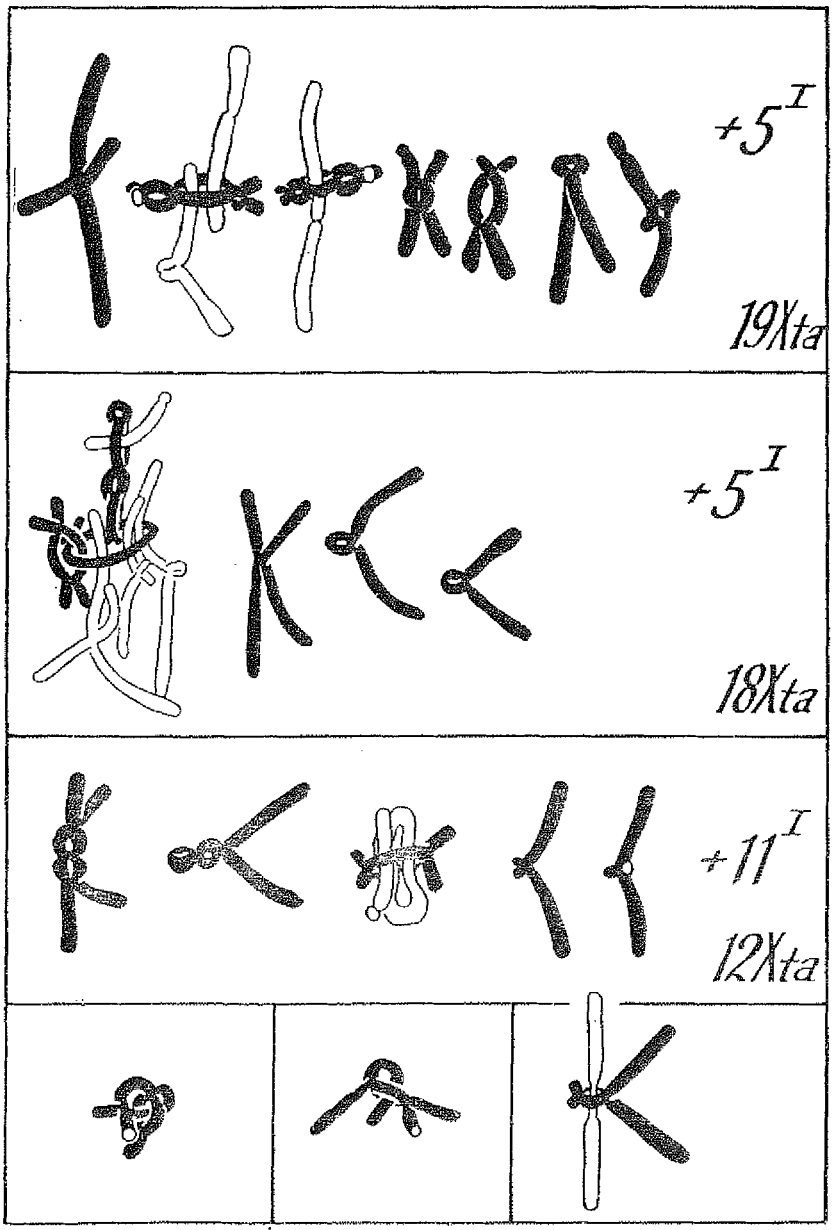

Fig. 3. Bivalents from plant treated with colchicine (treatment 4). There are three complete cells showing various types of interlocking. The last three bivalents are from different cells and give two examples of self-interlocking and a case of false interlocking of two bivalents. (× approx. 2000.)

of less than half normal. Such bivalents never occurred in the heat experiments.

This variable pairing leads to another difference. The graphs in Figs. 4 and 5 give the chiasma frequency histograms together with the type

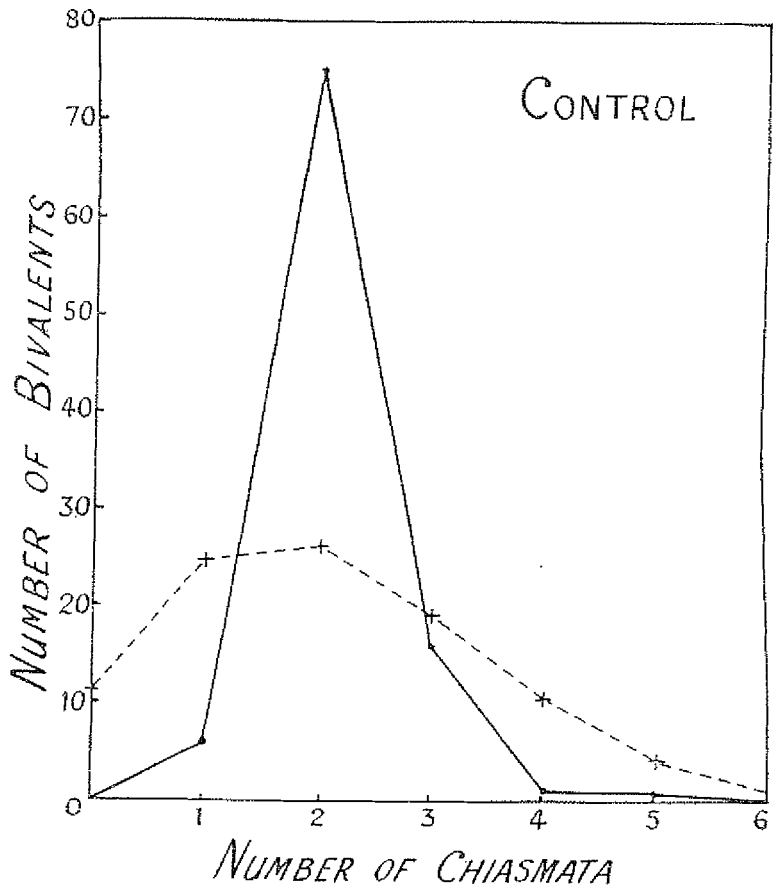

Fig. 4. Chiasma frequency histograms of S bivalents of ten cells from control. The curve with broken line gives the distribution expected on a random formation of chiasmata in the chromosomes.

of distribution expected on a random formation of chiasmata (Poisson distribution). It will be seen that both the normal and the heated plants give the distribution which Haldane (1931) showed to be characteristic of nearly all sexually reproducing organisms. The variance is less than the mean, the bivalents tending to form a standard number of chiasmata and only occasionally forming more or less than this number. In the

colchicined plants, on the other hand, extremes seem to be favoured. There are more bivalents with a high and low number of chiasmata than would be expected on random grounds, so that the relative variance in contrast to the controls and heated plants is greater than one. There

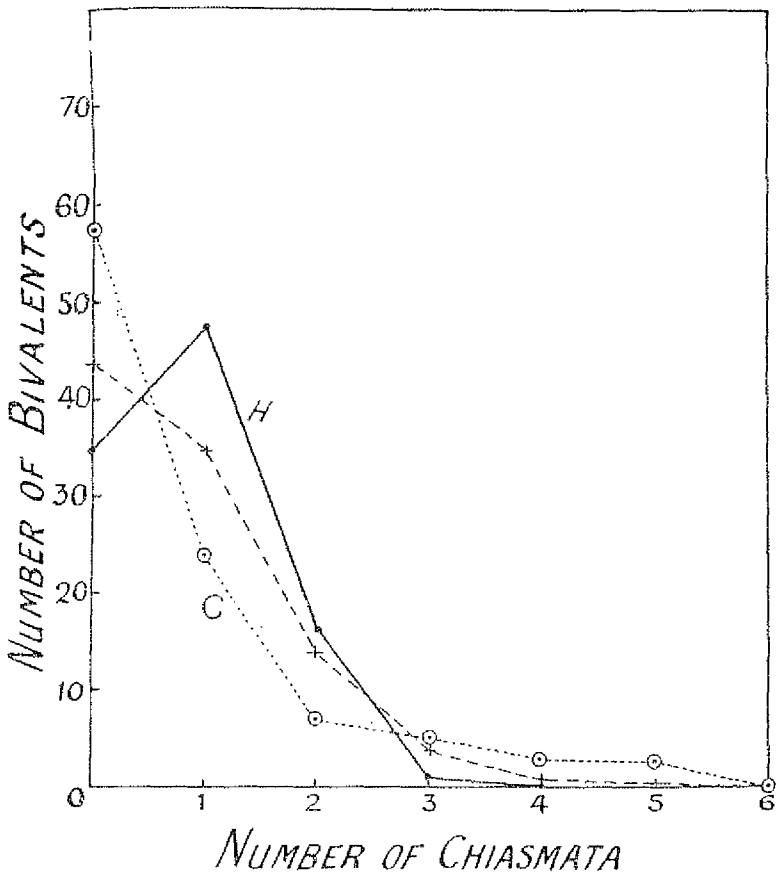

Fig. 5. Chiasma frequency distributions of S bivalents in a colchicined (C) and heated (H) plant. Both samples of ten cells have nearly the same bivalent mean ($C=0{\cdot}80$, $H=0{\cdot}83$). Note the opposite type of deviations produced by the two treatments, from the distribution expected on random chiasma formation (broken line).

are two reasons for this difference. There is a much greater intercell variability in the colchicined plants than in the others. For example, in one experiment the nuclear total varied between 3 and 21 chiasmata, whilst in the heated plants and controls corresponding figures are 6–13 and 23–27. This effect is probably due in part to a variable penetration of the colchicine. There is, however, a further cause for the increased

variability of pairing under colchicine. As we shall see, pairing within the cells is a much more variable process in the presence of colchicine than under normal conditions.

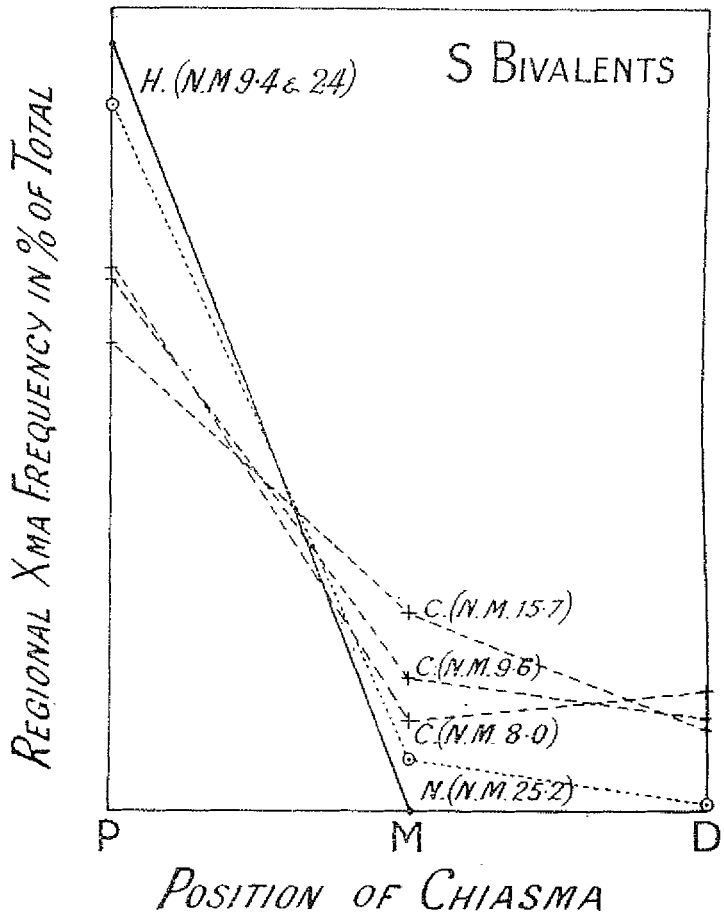

Fig. 6. Chiasma distribution in proximal (*P*), median (*M*) and distal (*D*) regions of *S* bivalents. The five curves are: *N*, control; *H*, heated; *C*, three separate colchicine treatments. Colchicine increases the pairing in *D* and *M* regions whilst heat decreases such pairing.

Colchicine has a similar effect on pairing in the embryo sac, but only a few cells which showed abnormal types of bivalent were available for analysis.

6. THE FACTORS PRODUCING LOCALIZATION IN *FRITILLARIA MELEAGRIS*

Previous analysis (see Darlington, 1940) has indicated that to produce a localization like that in *Fritillaria Meleagris* two conditions are necessary. These are (*a*) a favouring of certain regions in the beginning of pairing, and (*b*) a relatively early time limit to the continuance of pairing. Pairing is usually initiated at certain characteristic *contact points*. In a species or race or hybrid with localization, pairing never has time to spread from these contact points to the whole length of chromosome.

It is unlikely that colchicine abolishes localization by removing the time limit to pairing. In the first place the treatment causes a reduction in chiasma frequency, and in the second it is impossible to interpret the observations serially. Bivalents occur with a single chiasma at any position along the chromosome. Thus we must assume that it is by interfering with the initiation of pairing that the localization is abolished Under colchicine, pairing can begin at any point along the chromosome.

The large chromosomes in those species of the Liliaceae with freely distributed chiasmata usually seem to pair by two or more contact points in the chromosomes. This is equally clear from the observations of Darlington on hybrid lilies, of Frankel on *Fritillaria imperialis* and of Barber on *Uvularia*. The position of these contact points is just what we might expect from a consideration of the mechanical and spatial properties of the chromosomes in the leptotene nucleus. First, contact evidently takes place as a rule at the ends and at the centromeres in these long chromosomes. Ends close to centromeres are specially favoured, but even distal ends have a priority over middles in these species. The determination of the contact point depends on the greater ease of movement of ends as compared with middles and on the proximity of all centromeres and of all ends in the leptotene nucleus, this arrangement persisting from the last pre-meiotic mitosis. With an early time limit to pairing this type will give both proximal and distal localization. Middles will usually fail to pair.

There are thus two problems before us. The first is: How is the procentric localization in *Fritillaria Melcagris* brought about? or in other words, What hastens the pairing of centric parts? The second is: How does colchicine suppress this procentric pairing? It is difficult to suppose that colchicine affects the spatial distribution of the chromosomes in the resting nucleus, although possibly it does affect the growth of the lepto-

tene nucleus so that the chromosomes cannot space themselves out properly in preparation for pairing. In *F. Meleagris* a fourfold increase in nuclear volume occurs between the last pre-meiotic mitosis and pachytene.

We are assisted in this consideration by one analogy. Second-generation hybrids between two species of *Allium* with proximal and distal localization show the occasional breakdown of localization of any kind correlated with a breakdown of regular distribution. Bivalents with complete pairing and evenly distributed chiasmata occur in the same nuclei with others having one or both kinds of localization—just as after colchicine in *Fritillaria* (Maeda, 1937; Darlington, 1940).

The simplest explanation of these results appears to be that the centromere has some specific action in controlling localization of pairing. An early time limit to pairing is not enough to produce a strict procentric pairing. Colchicine has some specific action on the centromere which delays pairing in its neighbourhood relative to that of the rest of the chromosome. It is highly significant that the other effect of colchicine, namely, spindle suppression, is largely to be accounted for by its specific effect on centromeres and centrosomes.

The immediate result of chromosome pairing in colchicine is thus to allow the chromosomes to come together at any point, often in two or three places at once, along their length. Hence pairing, instead of being a strictly controlled process, becomes a catch-as-catch-can scramble.

This scramble for partners shows itself in the extraordinary amount of interlocking after colchicine treatment. Interlocking in the normal plant is rare. There is only one contact point for pairing, so we should not expect interlocking to occur. In heat-treated plants, again, interlocking has not been observed. On the other hand, in one of the three colchicine treatments, interlocking was especially common. In 10 analysed cells 20 bivalents out of 80 showed interlocking. Both true and false types occurred. The most complex case (Fig. 3) involved six bivalents and one univalent. Several cases of self-interlocking were also observed, proving conclusively that pairing was started independently at two or three contact points in these chromosomes (see Figs. 2, 3).

The existence of this interlocking might at first sight be thought to show that colchicine upsets the spatial arrangement of chromosomes in the leptotene and thus destroys the localization. But this assumption is unnecessary. The interlocking is probably a direct result of pairing beginning at more than one contact point in a species not adapted for this mode of pairing. Perhaps the wide variation in frequency of inter-

locking in the colchicined plants is symptomatic of the wide variation possible in the arrangement of the distal parts of the chromosomes. In the normal plant these regions rarely pair, and therefore their arrangement in the nucleus will not be critical. But when an agent such as colchicine upsets the localization in pairing by inhibiting the action of the centromere, interlocking will set in. A somewhat similar situation has been shown by Gairdner & Darlington (1931) to exist in *Campanula persicifolia*, a plant with contact points at both ends of the chromosomes. Interlocking in this species was shown to be correlated to some extent with the number of structural changes present. Such changes, involving the formation of rings, etc., will obviously require a special arrangement of its double-contact chromosomes in the leptotene nucleus if interlocking is to be avoided.

SUMMARY

The application of high temperatures or colchicine at early prophase reduces the metaphase chiasma frequency in *Fritillaria Meleagris*. The reduction by heat is correlated with an increased proximal localization of such chiasmata as do form, whereas under colchicine, this localization is destroyed. It is inferred that heat acts by advancing the time limit to pairing, leaving the strongly procentric pairing type unchanged. Colchicine, on the other hand, by destroying the advantage that the centric regions have in pairing, leads to a slower, uncontrolled and highly variable pairing. Contact takes place at any point along the chromosome, sometimes at two or three independent points. This latter type of pairing leads to frequent interlocking. The action of colchicine is probably due to its specific inhibitory effect on the centromere.

The normal localization in *Fritillaria* therefore seems to depend on two factors:

(*a*) An early time limit to pairing.

(*b*) A centromere control of the contact points of the pairing chromosomes.

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