

THE CAUSAL SEQUENCE OF MEIOSIS
III. THE EFFECT OF HYBRIDITY ON MALE AND
FEMALE CELLS IN *LILIUM*

BY C. D. DARLINGTON AND L. LA COUR

John Innes Horticultural Institution, Merton

(With Eighteen Text-figures)

CONTENTS		PAGE
1. Introduction		49
2. Technique		50
3. Embryo-sac mother cells		51
4. Pollen mother cells		55
5. Conclusion		63
6. Summary		64
References		64

I. INTRODUCTION

MEIOSIS in female cells, eggs or embryo-sac mother cells, inevitably differs from that in male cells on account of the difference in living space available for the chromosomes. Comparison, however, has been neglected in the past. The technical difficulties of studying meiosis in the solitary female cells have usually been a sufficient deterrent. Embryo-sac divisions now present several problems that call for solution. Zygotene pairing, crossing-over, co-orientation and the abnormal consequences of hybridity all demand comparison with the conditions in male cells, comparison at once of their mechanical conditions and their genetical consequences.

We have therefore examined meiosis in the embryo-sac mother cells of various species and hybrids, diploid and polyploid, in *Lilium*, *Fritillaria* and *Tulipa*, the group with which we are best acquainted. The results, in pre-anaphase stages, show a closer similarity of behaviour of male and female cells than we had expected. Particularly important seems to be the adjustment in localization of chiasmata to correspond in the two sexes in *Fritillaria*.

Our present object is to compare merely the metaphase configurations in embryo-sac and pollen mother cells of *Lilium testaceum*. This plant is reputed to be a first cross between *L. candidum* and *L. chalcedonicum*. Pollen meiosis has been described by Ribbands (1937). He has shown that this cross, like *L. Marhan*, is hybrid for numerous inversions. These

act on meiosis in two ways. Where they pair and cross-over bridges are formed at anaphase. Where they fail to pair chiasma formation is reduced. Statistical records show a combination of these two effects.

The development of the study however has led us to re-examine the pollen mother cells for special statistical treatment. This treatment is in several respects novel and perhaps requires some apology. The embryo-sac sections are remarkable in that, owing to the spacious arrangement of the chromosomes, every complete nucleus can be, and has been, analysed and recorded—thirty-two metaphases in twenty-four slides. The pollen smears on the other hand are subject to discrimination. A proportion of cells cannot be recorded owing to various conditions—damage, diagonal orientation or complexity of configuration. These conditions may be mixed, hence a discrimination may arise which will lead to an error of sampling. It is worth while comparing the perfect sample of female cells with Ribbands' slightly selected sample of male cells. On the other hand the enormous numbers of male cells available enable us to use discrimination for the special purpose of studying correlations. This is necessary where the range of variation of both variables falls in an extremely skew curve. The discrimination we have used consisted in taking pollen mother cells more evenly distributed over the range of their total chiasma frequency (and therefore having a lower mean) than a sample of natural distribution would allow. This enables us to make a significant comparison of the properties of the two types of chromosomes, *M* and *S*, at the two extremes of their behaviour. In this regard we are indebted to Dr K. Mather, Dr M. M. Richardson and Mr C. R. Ribbands for the use of their unpublished data on this and other species and hybrids.

2. TECHNIQUE

The embryo-sac presents a special problem of fixation on account of the thickness of the ovary wall. The wall itself can be satisfactorily prepared by the smear method for the study of mitosis, for which purpose it provides a useful substitute for root-tips. This method is not, however, practicable for the embryo-sacs. They are too few in number and too vulnerable by reason of their size.

A special method of dissection was therefore used. By taking advantage of the vascular fibres running lengthwise it is possible to peel rows of ovules off the larger liliaceous ovaries with a sharp scalpel. These ovules remain together until they are embedded. They can then be cut in suitable groups.

Even with this method it is necessary to use a fixative of high penetration. The following formula, a modification of solution 2BE with increased osmic and acetic acid, gave as fine a fixation as any used with pollen mother cells. We shall refer to it as 2BX:

18.0 c.c. 1% chromic acid.	9.0 c.c. water.
11.0 c.c. 2% osmic acid.	0.2 g. potassium bichromate.
10.0 c.c. 5% acetic acid.	0.61 g. saponine.

The sections need to be cut at 40μ . In doing this two precautions are necessary. First, the slide, smeared with albumen, should be heated before mounting to help the adhesion of the thick ribbon. Secondly, a weak solution of gentian violet, 0.1% or one-tenth saturated, should be used to allow of easier differentiation.

The pollen mother-cells were fixed in 2BE solution and hydrolysed 8 min. at 60°C . in *N* hydrochloric acid. They were stained by the Feulgen method.

TABLE I

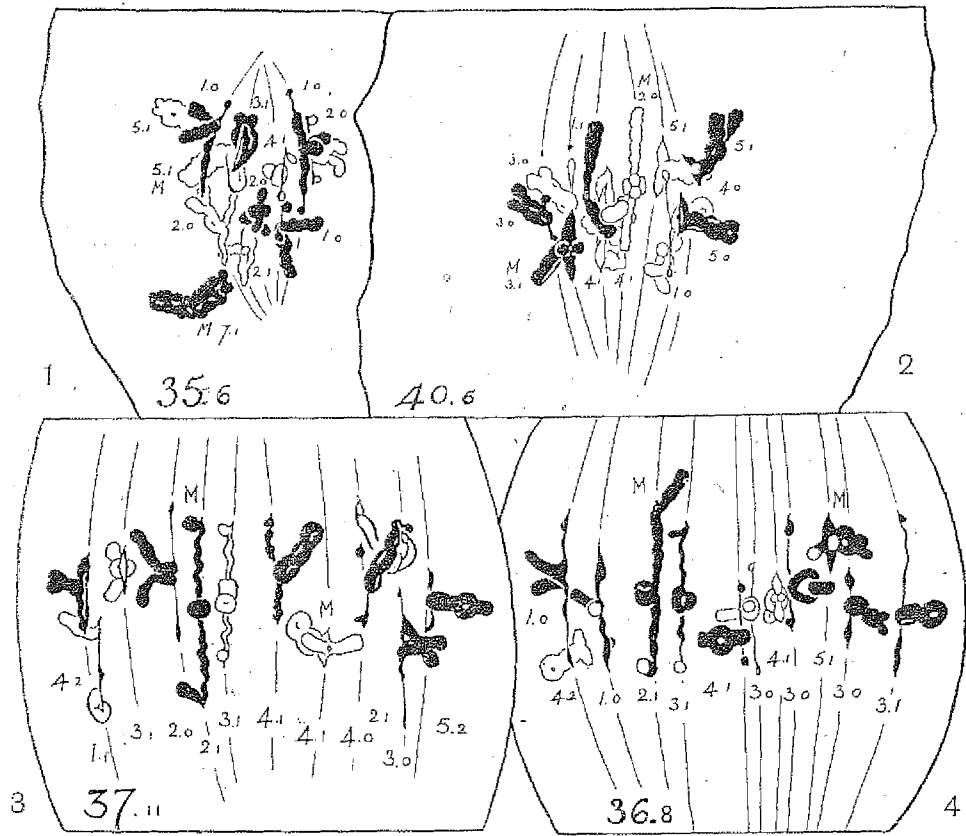
Chiasma-frequencies of Liliun testaceum, ♂ and ♀, and of L. candidum ♂ (Mather)

Chiasmata		0	1	2	3	4	5	6	7	II Xta	<i>M</i>	<i>V</i>	
<i>L. testaceum</i> , ♂	<i>M</i>	15	19	13	14	6	1	—	—	68	116	1.71	1.82
	<i>S</i>	58	85	92	58	34	11	1	1	340	647	1.90	1.92
<i>L. testaceum</i> , ♀	<i>M</i>	6	8	13	11	14	8	3	1	64	188	2.94	3.0
	<i>S</i>	28	49	92	79	62	8	2	—	320	770	2.41	1.7
<i>L. candidum</i> , ♂	<i>M</i>	—	—	—	1	5	4	1	1	12	56	4.67	1.15
	<i>S</i>	—	4	10	28	12	6	—	—	60	186	3.10	1.04

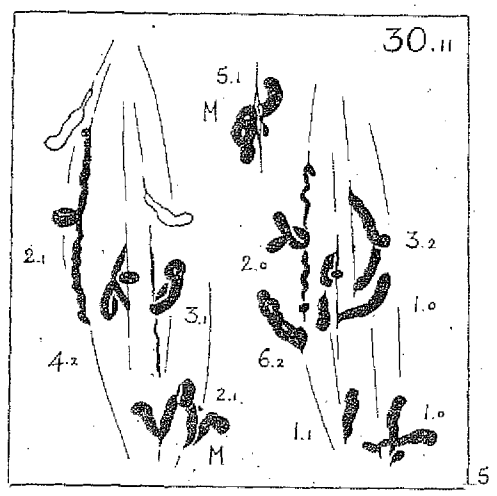
3. EMBRYO-SAC MOTHER CELLS

The arrangement of the bivalents on the first metaphase plate is slower than in the pollen mother-cells, no doubt on account of the cells being larger. We see bivalents lying far off the plate, unco-orientated when their fellows are already finally arranged. Nor is this always due to remoteness of the two centromeres (Figs. 1, 5). Another difference from the male cells is that the spindle is larger. Since the poles are farther apart it is not surprising that the co-orientated centromeres themselves move farther apart before the lapse of chromatid attractions which liberates the chromosomes for their anaphase movement. Hence the extreme tension of the proximal segments (Figs. 3, 4).

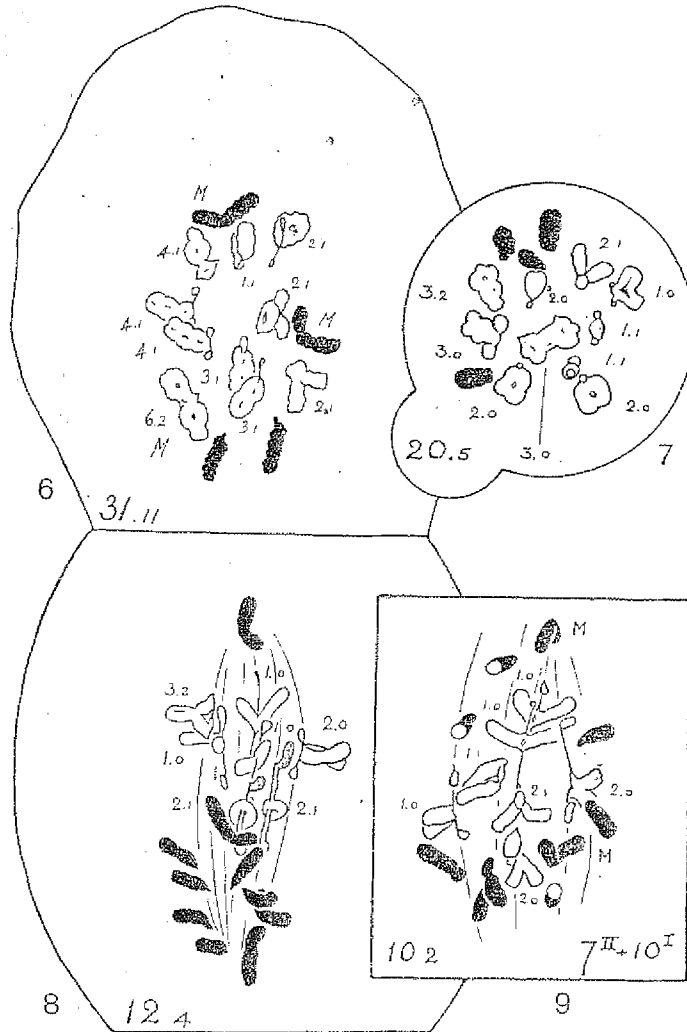
A first review of these cells shows two general properties distinguishing them from those of *Lilium* species: the lower mean chiasma frequency and its higher variance (Table I). Some chromosomes fail to



Figs. 1-4. First metaphase with twelve bivalents in the embryo sac of *Lilium testaceum*, giving total and terminal chiasmata under each bivalent and nucleus. *M*, the two bivalents with median centromeres. Figs. 1 and 2 *in situ*; Figs. 3 and 4 spaced. $\times 1600$.



form chiasmata and are unpaired at metaphase. These univalents lie distributed at random on either side of the plate. They do not show the position correlation which Ribbands described in the male cells.



Figs. 6-9. First metaphase in four embryo-sacs with decreasing numbers of chiasmata, increasing proximal localization and frequency of univalents. Fig. 7 shows one inversion chiasma. $\times 1700$.

A further study shows that the reduced numbers of chiasmata are distinctly grouped or localized. The two regions that are favoured are proximal and distal. An even distribution of chiasmata is reached only

when the longer chromosome arms have four or more chiasmata—and then they are always even. The long arm of the shortest *S* chromosomes can attain an even distribution with three chiasmata. This distribution corresponds with the “complete pairing” condition described by Frankel in *Fritillaria*.

If we turn to special cells, those with the fewest chiasmata, this impression is deepened. In the extreme examples illustrated (Fig. 9) we see in fact that every bivalent has one or two chiasmata close to the

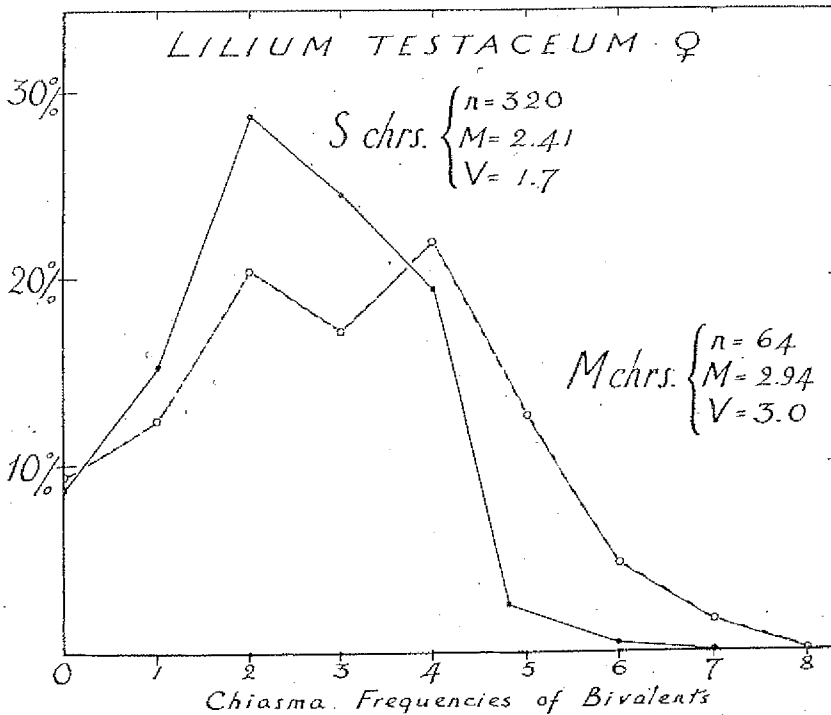


Fig. 10. Chiasma frequencies in the sample of thirty-two embryo-sac mother cells.

centromere. These bivalents might be taken from a typical cell of *Fritillaria Meleagris* showing procentric localization.

Following the argument developed elsewhere (Darlington, 1940*b*) that different degrees of chiasma frequency correspond to different stages at which pairing has been interrupted, the conclusion must be reached that in this plant most of the chromosomes begin to pair near the centromere while the rest begin to pair near the distal end of the chromosome.

In order to test this view more thoroughly it is necessary to turn to the pollen mother cells, where we can pick out a larger sample of cells

with the lower chiasma-frequencies which are the more significant for this purpose.

TABLE II

Comparison of pairing and chiasma frequencies in ♀ *L. testaceum*, in selected samples and unselected (Ribbands) of ♂ *L. testaceum*, in ♂ *L. Marhan* (Richardson) and in ♂ *L. candidum* (Mather)

Species	Mean no. of Xta per bivalent			Mean no. of univalents per cell			Chance of pairing	
	M	S	Total	M (2)	S (10)	Total	M	S
<i>L. testaceum</i> , ♂*	1.71	1.90	1.87	0.44	1.71	2.15	0.78	0.83
<i>L. Marhan</i>	1.88	2.26	2.20	0.18	0.52	0.70	0.91	0.95
<i>L. testaceum</i> , ♀*	2.94	2.41	2.50	0.18	0.88	1.06	0.91	0.91
<i>L. testaceum</i> , ♂	—	—	2.67	—	—	0.68	—	—
<i>L. candidum</i>	4.7	3.1	3.30	—	—	0.60	1.00	1.00

* Present observations.

4. POLLEN MOTHER CELLS

The kind of sample taken for study can be seen by the comparison of univalent and chiasma frequencies (Table II and Fig. 11). In the natural

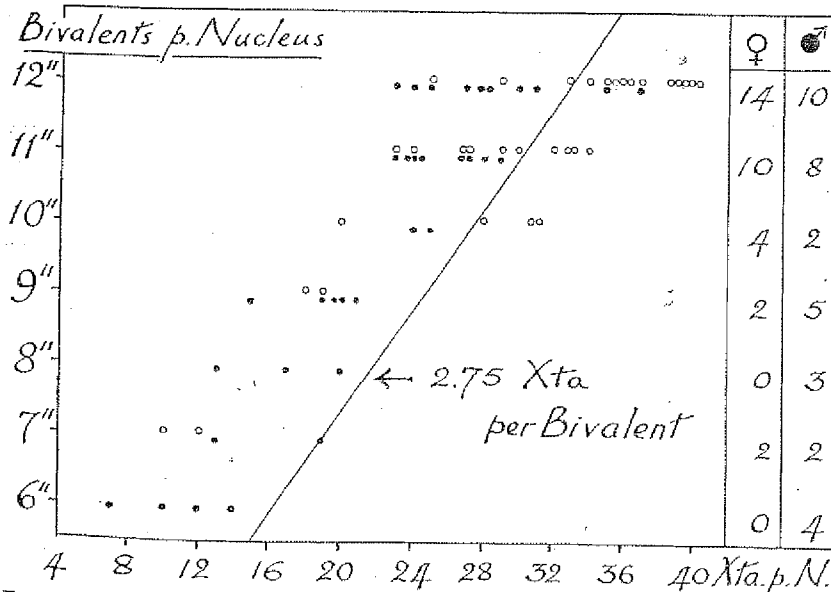


Fig. 11. Record of the chiasma frequency of nuclei with different numbers of bivalents in the two samples of *L. testaceum*, showing lower frequency per bivalent in the lower cells.

sample of female meiosis nearly half the cells have twelve bivalents. Further the chiasma frequency of these bivalents is higher than that in

the cells with fewer bivalents. This follows the natural law that univalents, being in origin bivalents-with-no-chiasmata, the frequency of this class is related to the frequency of the classes with one, two or more chiasmata. In the selected male sample the frequency of chiasmata in bivalents remains nearly the same in all classes and we have chosen disproportionately many cells with large numbers of univalents.

In the thirty-four cells selected we see at once the same principle of localization, both proximal and distal, as in the female cells (Fig. 12).

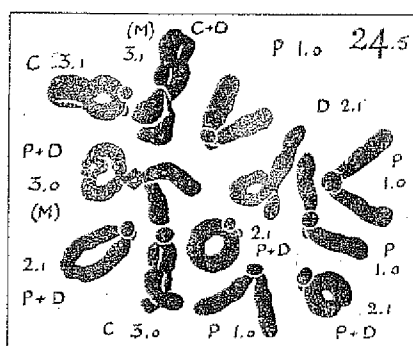


Fig. 12.

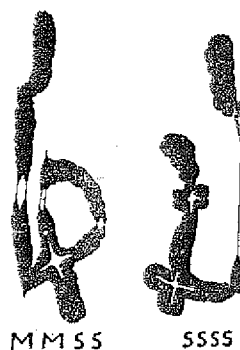


Fig. 13.

Fig. 12: Pollen mother cell of *L. testaceum* in polar view, showing frequency and localized distribution of chiasmata; *P*, proximal; *D*, distal; *C*, complete or even. $\times 1600$.

Fig. 13: Two translocation groups, each showing an inversion chiasma giving acentric and dicentric chromatids. Parallel co-orientations. $\times 1600$.

When these cells are arranged in order of diminishing chiasma-frequency we see the effects of this localization increasing in a diagrammatic way (Figs. 14, 15). In the higher cells a few bivalents are found with chiasmata grouped in the middle of an arm (intermediately) but this type disappears in the lowest cells, as does the type with complete pairing, having four to seven chiasmata.

In order to make the most of these observations we need, however arbitrarily, to record the points of pairing and examine their relation to the total chiasma frequency and to the special behaviour of the *M* chromosomes (with median centromeres). We can divide thirty-three of our cells into three equal samples with different numbers of total chiasmata. We then arrive at the classification shown in Figs. 16 and 17.

It now appears that these classes can be represented without exception as corresponding to stages in the completion of pairing. No-pairing passes into one-point pairing—proximal, distal or rarely intermediate—and this in turn passes, perhaps directly, into complete-pairing,

or more probably into two-point pairing which itself can later become complete.

Between the *M* and *S* chromosomes two differences appear. In the first place the *S* series seem to represent later stages than the corresponding *M* series. It is as though the *S* chromosomes were quicker in getting to work and in consequence reached completion earlier on the average and consequently at each corresponding stage more frequently. In the second place the *S* chromosomes show more proximal and less distal pairing than the *M* chromosomes.

These last observations must be considered together and in relation to the different effects of proximal contact on *M* and *S* chromosomes. In *Fritillaria*, where the pairing regularly begins near the centromere, the *S* chromosomes always have a chiasma frequency equal to, or higher than, that of the longer *M*'s (Frankel, 1940). *L. testaceum* is intermediate in this respect between *Lilium* and *Fritillaria* species (Darlington, 1940*a*). Apparently so far as the pairing begins at the centromere the *M* chromosomes suffer from its interruption more than the *S*'s. And for this mechanical reason that the centromere of an *M* is in the middle of the chromosome and therefore doubly tethered, that of an *S* is near an end and therefore only singly tethered.

Returning to our *Lilium*, where we see that the point of first contact is optional, we should expect (a) that the *M*'s would suffer less in pairing than those of *Fritillaria*, where proximal contact is obligatory, and (b) that the distal contact should be relatively more important in the *M*'s than in the *S*'s. Both of these expectations are borne out. *Lilium testaceum* is intermediate between the *Lilium* and *Fritillaria* species in the *M/S* chiasma frequency proportions (as shown by the true female sample), and its *M* chromosomes have relatively more distal contacts than its *S* (as shown by all classes of the male sample).

Before considering this any further, let us note that the absolute as well as the relative frequency of distal contacts is less in the *S* chromosomes than in the *M*. This may be due in part to some distal contacts having been lost in the two-point and complete classes. But in part it must also be due to a real reduction. Pairing at one end has reduced the chance of pairing at the other. The low frequency of the two-point class in all groups supports this view.

The relative frequencies of chiasmata in *M* and *S* chromosomes may be profitably analysed in another way. We can co-ordinate the total *M* and *S* chiasma frequencies of separate nuclei in a single graph (Fig. 18). For this purpose the combination of the male and female samples is



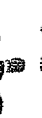























































0	1	2	3	4	5	6	7
		  	  	 			
	P D	P I D	D PD C	PD PC	C	C	38
		 	 				
	P I D	P P I	P I DD		C	C	28
		 					
	I D D	P PD DD	PD D		C	C	24
	 	   					
	P PD	P P P P PD D D		DD		C	24
	  						
	P P P D	P	C C	C C			20
 	  						
	I D D D	P	P	C	C		17

Fig. 14.

O ₂	1	2	3	4	5
	 P P D D M	 P P D I M	 C	-	-
					15
	 P D	 PD PD M	 PD	-	
					14
	 P I D D	 P	 PD		-
					13
	 P P D I M	 D	-	-	
					12
	 P P P	 D PD	 P	-	-
					10
	 P P P P D M	 PD	-	-	-
					6

Fig. 15.

Figs. 14 and 15. Series of complete cells of male *L. testaceum* with decreasing eiasma frequencies, corresponding to Figs. 6-9 for the embryo sacs. These figures show the method of scoring used in Table III and Figs. 16 and 17.

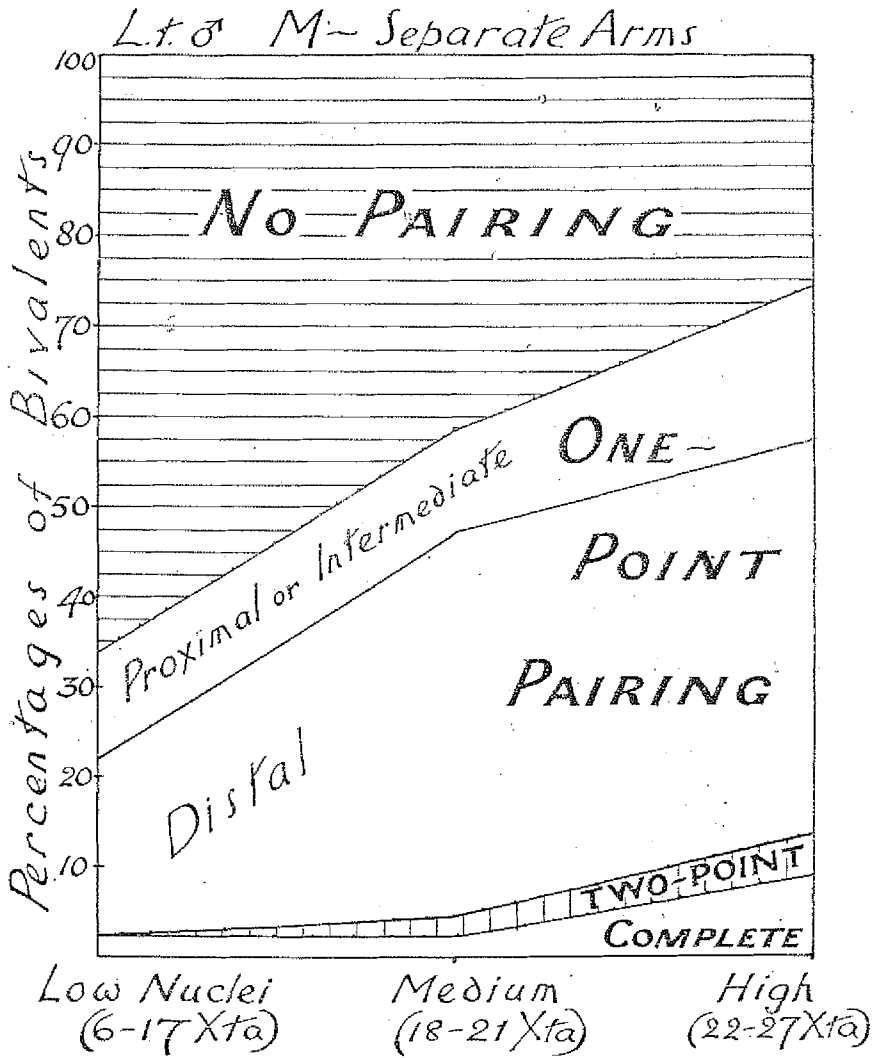


Fig. 16.

useful, for although the male sample was selected it was not selected for differential properties of *M* and *S*. We find in fact that the two samples agree in demonstrating the expected principle, namely that the *M* chiasmata fall away more rapidly than the *S* chiasmata. The regression line of *M* on *S* evidently crosses the direct proportionality line given in the figure at a considerable angle.

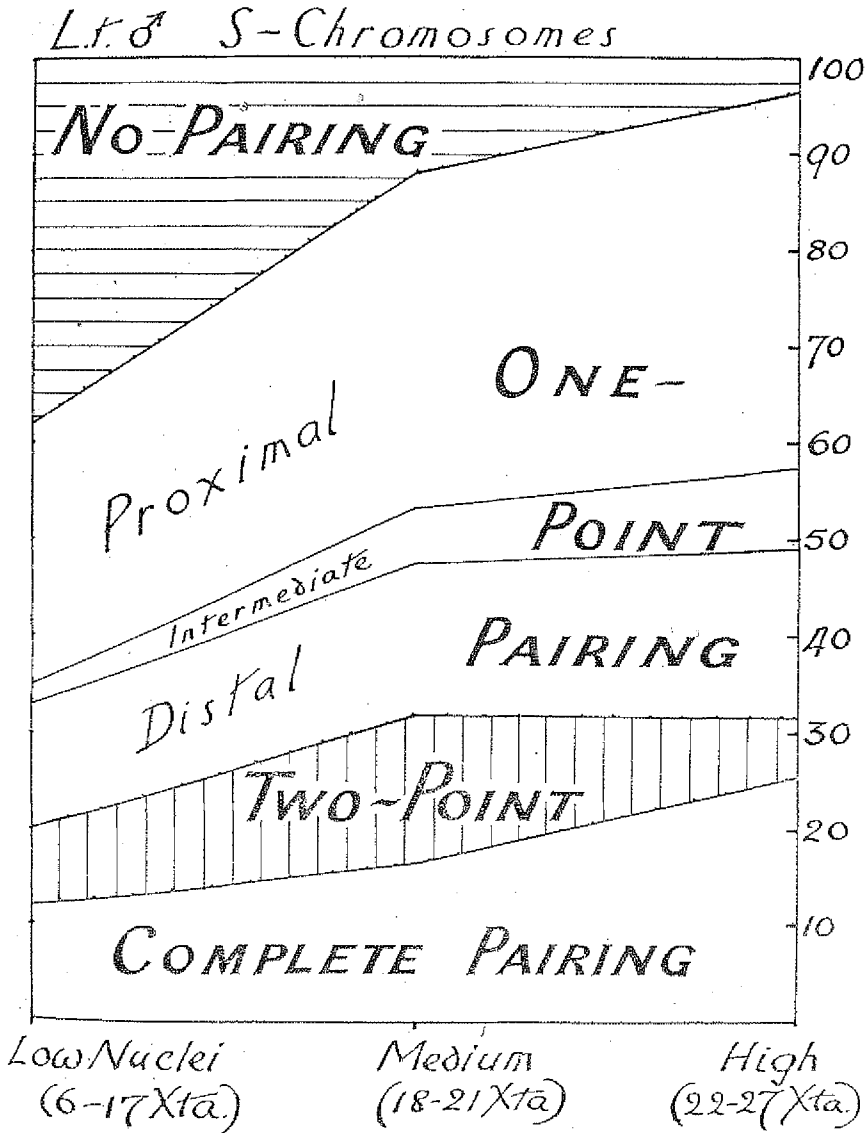


Fig. 17.

Figs. 16 and 17, Graphs representing Table III and showing developmental relationship of *M* and *S* chromosomes in low and high chiasma nuclei.

This relationship enables us to understand another of the differences between the *Lilium* hybrid and its parent species. If we take those cells of the hybrid which correspond in total chiasma frequency with the

species we find that the *M* chromosomes have a higher chiasma frequency than the *S*. The upper end of the hybrid sample corresponds with a

TABLE III

Analysis of pairing points in Lilium testaceum ♂ bivalents taking low (6-17), medium (18-21) and high (22-27) chiasma nuclei separately (cf. graphs, Figs. 16, 17)

	Pairing points	None	One <i>P</i> , <i>D</i> or <i>I</i>	Two <i>P</i> + <i>D</i>	Three (2 arms)	Com- plete	Total
<i>S</i>	Low	42	46	9	—	13	110
	Medium	13	62	17	—	18	110
	High	3	73	6	—	28	110
<i>M</i> , one arm	Low	29	14	—	—	1	44
	Medium	17	25	1	—	1	44
	High	11	28	1	—	4	44
<i>M</i> , both arms	Low	9	11	2	—	—	22
	Medium	5	7	9	1	—	22
	High	1	9	10	1	1	22

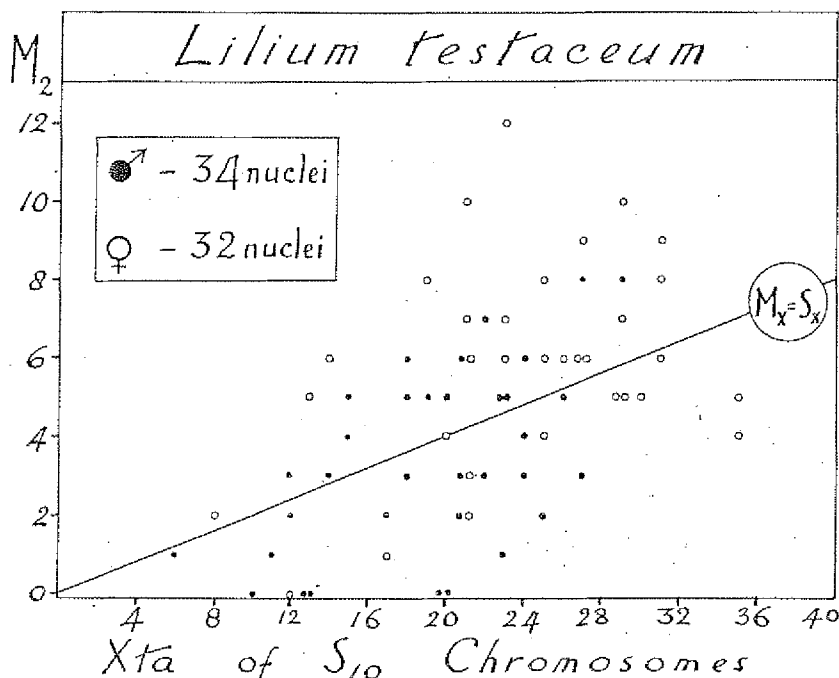


Fig. 18. Chiasma frequencies of *M* and *S* chromosomes co-ordinated in the same nuclei of male and female cells, showing indirect variation.

species sample. The lower end is responsible for the divergence between the two at once in the absolute frequency, relative frequency, and spatial

distribution of chiasmata. Expressing these differences in terms of the rate of pairing, we may say that pairing proceeds at a more variable rate in the hybrid and its average rate is lower.

Why should these differences go together? Variations in rate of pairing and chiasma frequency in the species are no doubt due to chance variations in the spacing of the leptotene chromosomes. In the hybrid a second source of variation is added. The homologous chromosomes differ in regard to their genetic structure, chiefly owing to inversions but partly also owing to translocations (Fig. 13; cf. Ribbands, 1937). These dislocations must interrupt the zip-process of pairing and, demanding a new point of contact, they will introduce a new obstacle and a new source of variation to the process of pairing depending on the choice of end at which the first contact is made. The obstacle and the variation are in fact what our statistical analysis and its timing interpretation require.

In *Lilium testaceum*, as in the triploid *Fritillaria*, we can now see the results of applying a more searching statistical analysis to the study of the frequencies of chiasmata. By taking these frequencies not as an undifferentiated mass but separated in different classes of bivalents and different classes of nuclei, we can represent a sample of cells not as a static picture of the results of undefined processes of development but as a dynamic picture which by its own movement defines these processes and reveals causal relationships between them that would evade any more direct mode of attack.

5. CONCLUSION

The first and least disputable conclusion to be drawn from this treatment is that the samples on which our ordinary observations of meiosis depend consist of cells which differ in a series of correlated respects affecting different types of chromosomes differently.

The second conclusion which seems to follow is that these correlations are due to a unitary control in the co-ordination of the chromosomes in each cell; further that the differences in properties between the different types of chromosomes (*M* and *S*) are such as would arise if they had different rates of movements in the cells, while the differences between the different classes of nuclei are such as would arise if different periods were available for movement.

The third conclusion, which follows immediately from the last, is that the co-ordination is a co-ordination of the time during which the pairing of the chromosomes can take place. The pairing is slower or the time shorter in the crosses than in the species.

Our knowledge of the structural differences between the chromosomes that have to pair in *Lilium testaceum* leaves no doubt that their failure is due to the process being retarded by these obstacles. This is probably true of most species crosses, but in those where the pairing is largely suppressed we may well suppose that the time limit has come into play earlier. We have no reason to suppose that the new balance of a species cross will give the same accurate adjustment as allows for the regular meiosis of its parents.

6. SUMMARY

1. The frequency of chiasmata and of univalents is similar in pollen and embryo sac mother cells of *Lilium testaceum*.

2. Cells with fewest chiasmata have those chiasmata most strongly localized either proximally or distally.

3. Hence pairing must have begun either near the centromere or near an end. The contact point is optional.

4. *M* chromosomes come into contact more readily near the ends, *S* chromosomes near the centromere.

5. But *M* chromosomes pair more slowly, so that the frequency of their chiasmata falls away more rapidly in low chiasma cells than that of *S*'s.

6. Samples of cells used for the study of meiosis in species and crosses must therefore be regarded as representing cross-sections of the process of pairing secured by a variable interruption of the process. Statistical treatment can be used to indicate the order of pairing and the means of interruption.

REFERENCES

- DARLINGTON, C. D. (1940*a*). "The causal sequence of meiosis. II. Contact points and crossing-over potential in a triploid *Fritillaria*." *J. Genet.* **41**, 35-48.
- (1940*b*). "The prime variables of meiosis." *Biol. Rev.* (in the Press).
- FRANKEL, O. H. (1940). "The causal sequence of meiosis. I. Chiasma formation and the order of pairing in *Fritillaria*." *J. Genet.* **41**, 9-34.
- LA COUR, L. (1937). "Improvements in plant cytological technique." *Bot. Rev.* **5**, 241-58.
- MATHEE, K. (1935). "Meiosis in *Lilium*." *Cytologia, Tokyo*, **6**, 354-80.
- RIBBANDS, C. R. (1937). "The consequences of structural hybridity at meiosis in *Lilium × testaceum*." *J. Genet.* **35**, 1-24.
- RICHARDSON, M. M. (1936). "Structural hybridity in *Lilium Martagon album × L. Hansonii*." *J. Genet.* **32**, 411-50.