EFFECT OF LOW TEMPERATURE ON DESYNAPSIS IN RICE[1]

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Since asynaptic gene in maize was found by Beadle and McClintock (1928), gene controlled asynapsis or desynapsis was discovered in many plant genera, such as rice (Ramanujam and Parthasarathy 1935, Chao *et al* 1960, 1961), *Pisum* (Koller, 1938), rye (Prakken, 1943), common wheat (Li *et al* 1945), *Lycopersicon esculetum* (Soost 1951), *Gossypium* (Beasley and Brown 1942), and others.

Since there is plenty of variation in the manifestation of this gene, many studies were made on the effect of temperature on this gene in many plant genera. Goodspeed and Avery (1939) made an assertion that high temperature decreased the pairing of chromosomes whereas low temperature favored the formation of bivalent in an asynaptic mutant of *Nicotiana sylvestris*. Li *et al* (1945) working with common wheat found that there was large number of univalents observed in the desynaptic plants when the material was fixed early in the season, in March in Chengtu, Szechuan, China when the temperature was rather cold. However, there were only few univalents or none observed of the same desynaptic plants when the materials were fixed later in the season when the weather warmed up. So in the second year, correlation studies were made between the frequencies of cells with bivalents for each desynaptic plant and the air temperature recorded at the time of fixation or of the previous day. From these studies it was found that low temperature would enhance the effect of the desynaptic gene. Soost (1951), working with *Lycopersicon* collected from the field at different time during the same day, found that increased temperature caused increased asynapsis. Recently, Chao *et al* (1960, 1961) made critical studies of the effect of temperature on the desynaptic gene in rice under controlled conditions and found that high temperature favored the formation of bivalents and low temperature induced more desynapsis. However, it seemed that the results of the two experiments carried out by them were not consistent

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with each other. Thus it would merit further experimentation so as to ascertain whether or not low temperature would favor desynapsis in rice.

Material and Methods

The desynaptic plants were found in the X_3 generation from the seeds of a highly sterile panicle found in the X_2 population, after the original seeds Chianung 242 were treated with X-ray of a dosage 20,000 r. There were 17 plants in all. All the plants were desynaptic except one which was supposed to be a natural hybrid. All the plants were subdivided so that every plant would be represented three or more times which were sufficient for the three different temperature treatments to be administered in the experiment. However, this did not work so well. Either there were not enough plants of each for all the treatments of the experiment. Or, even if enough plants of each were represented in all the treatments of experimentation but materials fixed from them would meet with failure in getting the right stage for cytological analysis.

As a result, the frequencies of univalents of all the plants under the same treatment were pooled together leaving the variance between plants unsolved.

The first experiment was done in May 1964, and the second was performed in September of the same year.

In experimentation for both experiments, the plants were treated just prior to the onset of meiosis and were put: 1), under natural conditions as checks, 2), in a room where temperature was kept at 25°C±1°C for the first experiment and 27°C±1° for the second experiment with artificial illumination for 12 hours a day, but the humidity was not controlled, 3), in a room where the temperature was kept at 15°C±0.5°C for the first experiment and 16°C± 1.5°C for the second experiment with good illumination. In both experiments, the outdoor temperature recorded were listed in table 1.

Table 1. *Outdoor temperature recorded in both experiments*

Experiment 1			Experiment 2		
Date	Maximum°C	Minimum°C	Date	Maximum°C	Minimum°C
May 16	35	27	Sept. 18	35	27
17	35	23	19	33	24
18	34	21	21	31	23
19	34	23			
20	34	22			
21	32	21			

The material were fixed in the Farmer's fluid and propiono-carmine was used exclusively for squash preparation.

Results

a. Genetics. Genetics of this mutant was yet to be worked out. The seeds of

the natural hybrid failed to produce any progeny, so another attempt is being tried. Presumably, this was a simple genic controlled mutant as many other asynaptic genes of many plant genera.

b. Cytology. Prakken (1943) classified the cases of probably genically caused asynapsis according to the intensity of the effect into three groups:

(1) Weak
(2) Medium strong, and
(3) Complete

In this mutant, the effect was only weak having about two univalents ordinarily.

In pachytene stage, as shown in plate fig. 1, twelve completely paired chromosomes were found. This was the case in most of the sporocytes observed. However, in some cells, there were intercalary loops found in some chromosomes as shown in plate fig. 2. Whether these loops would occur more often on the long arm than on the short arm or vice versa as was found in maize (Miller 1963), or whether these loops would be found to be specifically localized on definite chromosomes or they would be randomly localized, no detailed studies were made to ascertain these points. Plate fig. 3. shows the opening up of the paired chromosomes in diplonema. It seemed that some chromosomes were dissociating. In early diakinesis

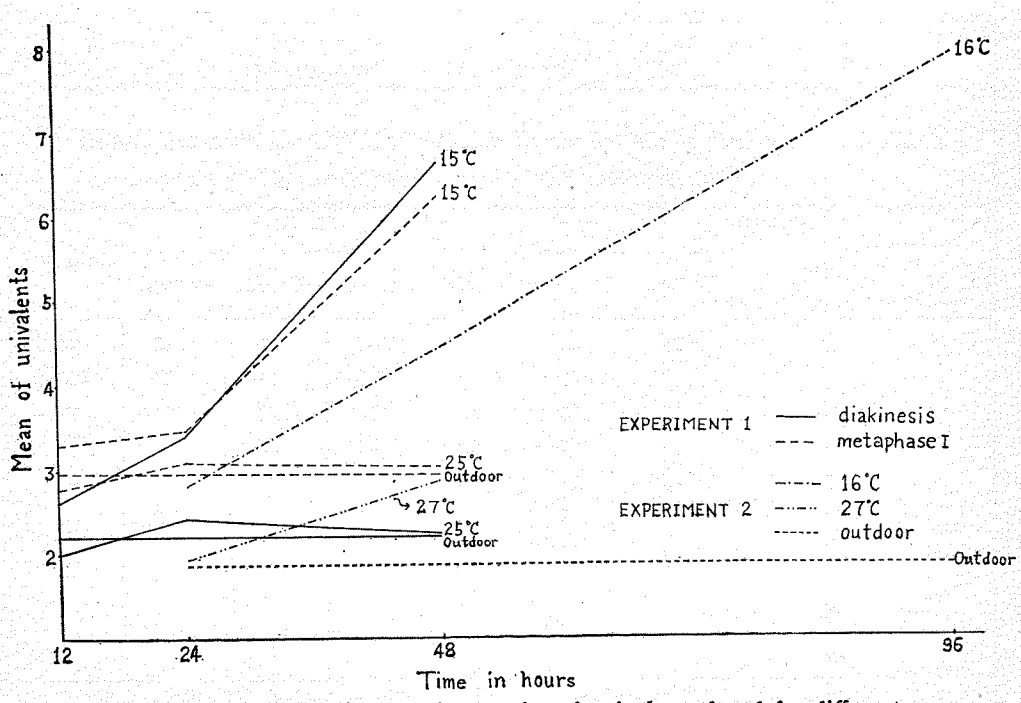

Fig. 1. Curves showing the frequencies of univalents found for different temperature treatments for both experiments

univalents were found and most of the chromosomes seemed to pair as rod or open bivalents. Plate fig. 6b shows that some chromosomes in MI would divide precociously. The significance of this was unknown.

c. Effect of temperature. The results of the first experiment are shown in tables 2 and 3, and those of the second experiment in table 4 and finally all the results are graphically represented in fig. 1.

Table 2. *Frequency of univalents found in diakinesis in desynaptic plants in different treatments of different duration. (First Experiment)*

Treatment / No. of cells (f) / No. of univalents (x)	15°C			25°C			outdoor
	12 hrs.	24 hrs.	48 hrs.	12 hrs.	24 hrs.	48 hrs.	
0	20	20	11	9	12	15	38
2	41	37	9	14	18	38	22
4	16	33	20	6	16	16	11
6	13	17	15	—	3	3	10
8	1	8	24	1		1	4
10	1	3	7				1
12			10				
14			5				
16			3				
$\sum f$	92	118	104	30	49	73	86
$\bar{x}$	2.63	3.40	6.61	2.00	2.41	2.26	2.21

Table 3. *Frequency of univalents in metaphase I of the desynaptic plants in different treatments of different duration. (First Experiment)*

Treatment / No. of cells (f) / No. of univalents (x)	15°C			25°C			outdoor
	12 hrs.	24 hrs.	48 hrs.	12 hrs.	24 hrs.	48 hrs.	
0	14	32	8	104	23	15	64
2	47	71	21	126	68	24	106
4	37	63	35	110	47	21	72
6	26	33	20	45	20	7	37
8	3	13	27	12	10	2	9
10		6	13	5		2	4
12			10	1		1	1
14			4				
16			3				
18			1				
$\sum f$	127	218	142	403	168	72	293
$\bar{x}$	3.32	3.46	6.26	2.77	3.12	3.08	2.9

Variation	F	
	Diakinesis	Metaphase I
Treatment	32.917**	41.062**
A (Temp.)	50.341**	76.798**
B (Time)	28.825**	62.351**
AB	28.296**	1.815

** significant at 1% level

Table 4. *Frequency of univalents in diakinesis and metaphase I of the desynaptic plants in different treatments of different duration. (Second Experiment)*

Treatment / No. of cells (f) / No. of univalents (x)	16°C			27°C		outdoor
	24 hrs.	48 hrs.	96 hrs.	24 hrs.	48 hrs.	
0	92	68	21	182	44	16
2	92	59	41	195	59	47
4	88	44	27	90	32	27
6	40	18	19	19	20	10
8	11	14	7	6	7	3
10	5	10	10		4	
12	1	4	2		2	
14		1	2			
16			1			
18			—			
20			2			
22			3			
24			27			
$\sum f$	329	202	162	492	168	153
$\bar{x}$	2.81	4.48	7.91	1.85	2.89	1.87

F=87.79**

Significant test for outdoor vs. treatments.

		d. f.	t
Experiment I	Diakinesis	545	30.435 **
	Metaphase I	1,416	3.3611**
Experiment II		1,633	4.3641**

From table 5, the univalent frequencies of the natural hybrid of this population of desynaptic plants (C20-14-18) were found to be rather constant, after being subjected to different temperature treatments together with other desynaptic

plants. This of course would indicate that the variations in the frequencies of univalents observed in desynaptic plants of different treatments—was really induced by the change of temperature. Whereas in the natural hybrid, it was not affected whatsoever by the low temperature treatment even at long duration; Pao and Li (1948) subjected different plant material under high temperature and found out that the effect of high temperature would induce desynapsis as well as other abnormalities. Certainly, the natural hybrid in this case was not affected by the temperature treatment.

Table 5. *Freqnency of univalents found in the natural hybrid in different treatments at different duration.*

Treatment / Stage observed / No. of cells / No. of univalents(x)	15°C			25°C	outdoor	
	48 hrs.	96 hrs.		24 hrs.		
	Diakinesis and Metaphase I combined	Diakinesis	Metaphase I	Diakinesis	Diakinesis	Metaphase I
0	36	15	9	35	15	21
2	4	3	2	2		4
$\bar{x}$	0.20	0.33	0.37	0.11	0	0.32

From variance analysis, it was found that F values for treatments, both for temperature and time were significant at 1% level. So was the F value for the interaction of temperature and time for diakinesis, but was not significant for metaphase I.

The frequencies of univalents for desynaptic plants left outdoors as checks for both experiments were scored only once each time for each temperature treatment for comparison. In this case, time duration was not considered.

In general, with 25°C treatment (27°C for 2nd experiment), irrespective of the time used in the treatment, the average frequency of univalents had no appreciable difference from each other nor from the checks. With low temperature treatment, 15°C for the first experiment and 16°C for the second, with short duration of 12 and 24 hours, the average univalent frequency did not differ significantly from those of 25°C treatments. However, with longer duration, 48 hours at 15°C in the first experiment and 48 and 96 hours at 16°C for the second experiment, the average number of univalents were significantly higher than those treatments of shorter duration with same temperature treatment. Also the univalents of these treatments with low temperature at a longer duration, were significantly higher than the treatments with higher temperature 25°C and those of the checks. It seemed that the effect of low temperature treatment was cumulative. The longer the treatment, the more effecive was the

Table 6. *Comparison of $\bar{x}$'s of different treatments by using Least Significant Difference [LSD] method. (from data in Table 2)*

$LSD_{.05}=0.982$ $LSD_{.01}=1.293$

Treatment	$\bar{x}i$					
15°C, 48 hrs.	$6.61=\bar{x}_3$					
15°C, 24 hrs.	$3.40=\bar{x}_2$	3.21**				
15°C, 12 hrs.	$2.63=\bar{x}_1$	3.98**	0.77			
25°C, 24 hrs.	$2.41=\bar{x}_5$	4.20**	0.99*	0.22		
25°C, 48 hrs.	$2.26=\bar{x}_6$	4.35**	1.14*	0.37	0.15	
25°C, 12 hrs.	$2.00=\bar{x}_4$	4.61**	1.40**	0.63	0.41	0.26

Outdoor vs, different treatments

$LSD_{.05}=0.958$ $LSD_{.01}=1.261$

$\bar{x}_{outdoor}\sim\bar{x}_{i\,outdoor}=2.21$	$\bar{x}_1=2.63$	$\bar{x}_2=3.40$	$\bar{x}_3=6.61$	$\bar{x}_4=2.00$	$\bar{x}_5=2.41$	$\bar{x}_6=2.26$
	0.42	1.19*	4.40**	0.21	0.20	0.05

* significant at 5% level
** significant at 1% level

Table 7. *Comparison of $\bar{x}$'s of different treatments by using LSD method. (data from Table 3)*

$LSD_{.05}=0.587$ $LSD_{.01}=0.772$

Treatment	$\bar{x}_i$					
15°C, 48 hrs.	$6.26=\bar{x}_3$					
15°C, 24 hrs.	$3.46=\bar{x}_2$	2.80**				
15°C, 12 hrs.	$3.32=\bar{x}_1$	2.94**	0.14			
25°C, 24 hrs.	$3.12=\bar{x}_5$	3.14**	0.34	0.20		
25°C, 48 hrs.	$3.08=\bar{x}_6$	3.18**	0.38	0.24	0.04	
25°C, 12 hrs.	$2.77=\bar{x}_4$	3.49**	0.69*	0.55	0.35	0.31

Outdoor vs. different treatments

$LSD_{.05}=0.555$ $LSD_{.01}=0.730$

$\bar{x}_{outdoor}\sim\bar{x}_{i\,outdoor}=2.9$	$\bar{x}_1=3.32$	$\bar{x}_2=3.46$	$\bar{x}_3=6.26$	$\bar{x}_4=2.77$	$\bar{x}_5=3.12$	$\bar{x}_6=3.08$
	0.42	0.56	3.36**	0.13	0.22	0.18

* significant at 5% level
** significant at 1% level

Table 8. *Comparison of $\bar{x}$'s of different treatments by using LSD method. (data from Table 4)*

$LSD_{.05}=0.685$ $LSD_{.01}=0.901$

Treatment	$\bar{x}_i$					
16°C, 96 hrs.	$7.914=\bar{x}_3$					
16°C, 48 hrs.	$4.485=\bar{x}_2$	3.429**				
27°C, 48 hrs.	$2.893=\bar{x}_5$	5.021**	1.592**			
16°C, 24 hrs.	$2.815=\bar{x}_1$	5.099**	1.670**	0.078		
27°C, 24 hrs.	$1.854=\bar{x}_4$	6.060**	2.631**	1.039**	0.961*	

Outdoor vs. different treatments

$LSD_{.05}=0.661$ $LSD_{.61}=0.869$

$\bar{x}_{outdoor}\sim\bar{x}$	$\bar{x}_{i\,outdoor}=1.87$	$\bar{x}_1=2.815$	$\bar{x}_2=4.485$	$\bar{x}_3=7.914$	$\bar{x}_4=1.854$	$\bar{x}_5=2.893$
		0.945 *	2.615**	6.044	0.016	1.032**

* significant at 5% level
** significant at 1% level

effect of treatment and the results of these two experiments were very consistent with each other. (See detailed analysis in tables 6, 7 and 8).

Discussion

Asynapsis vs. Desynapsis.

Prakken (1943) classified the cases of probably genically caused asynapsis according to the intensity of the effect into 3 groups: 1), weak, 2), medium strong and 3), complete. In the completely asynaptic plant of maize, Miller (1963) found no pairing in diakinesis and metaphase I. Early prophase observations also revealed essentially an absence of pairing from leptotene to diplotene.

With medium asynaptic plants of maize each showed 15% metaphase I sporocytes with 10 bivalents, of which several were rod pairs. Cells with up to 20 univalents were also observed. Stages from leptotene to pachytene in these plants showed that the amount of pairing ranged from cells with no paired threads similar to those found in the highly asynaptic plants to cells in which all the threads appeared to be paired.

In our asynaptic mutant it would be classified as a weak type and we did find perfect pairing in pachytene stage. The desynaptic condition found in diakinesis and metaphase I would be the result of dissociation of some of the chromosomes after pachytene.

It seems evident, therefore, that the complete asynaptic type can be called true asynapsis having no synapsis as early as leptotene. The weak type, on the other hand, can be called the desynaptic type and the medium type can be called either asynaptic or desynaptic depending on the amount of synapsis found in pachytene.

Crossing Over and Desynapsis.

Working with low desynapsis, Miller (1963) incorporated three different heterochromatic knobs in heterozygous condition. Although genetic data from normal stocks indicate that chiasmata frequently occur proximal to the knob locations, but no equational disjunction of the knobs was observed in 287 chromosome pairs present at diakinesis as univalents or as rod bivalents with chiasmata in the knobless arm. It was concluded that chromosomes showing desynapsis at diakinesis or metaphase I have not undergone cytological crossing over. This would imply that even though the chromosomes would pair almost perfectly at pachytene, cytological crossing over is prevented, thus resulting in the reduction of chiasmata paving the way for the dissociation of some of the paired chromosomes.

Effect of Desynaptic Gene on Desynapsis.

With weak desynapsis in rice there is almost perfect pairing of all the chromosomes at pachytene. The dissociation of some of the chromosomes takes

place, it seems, from diplotene and terminates at metaphase I. From our studies, there are more univalents encountered at metaphase I than at diakinesis when the conditions are equal. Chao *et al* (1961) suggested that since there is consistent higher content of RNA present in the desynaptic florets than in the normal ones, this desynaptic gene might have partially blocked the synthesis of certain substance(s) necessary for normal pairing of chromosomes. It seems to us, however, that the effect of this gene is to create some disturbance in the biochemical and biophysical condition of the chromosomes so that crossing over is partially prevented, even though all the chromosomes are fully paired in pachytene. This is substantiated by Beadle's (1932) observation of his asynaptic maize.

	Diplotene		Diakinesis	
	Normal	Asynaptic	Normal	Asynaptic
Average no. of chiasmata per bivalent	3.7	1.8	1.8	1.1

Chiasmata are overwhelmingly reduced in asynaptic plants as compared with those of the normal ones. With reduction in chiasmata formation, naturally coupled with terminalization, some of the paired bivalents would fall apart from each other and exist as univalents. This effect is greatly enhanced with low temperature treated at a longer duration, two days or more.

The Same Desynaptic Gene?

The desynaptic mutants of Chao's (Chao *et al* 1960, 1961) and ours are all obtained from irradiation. Genetical studies are attempted to find out the identity of these mutants.

Summary

A desynaptic mutant strain was obtained in X_3 after the original seeds were treated with X-ray irradiation.

There was complete pairing of all the chromosomes at pachytene. Dissociation started from late pachytene and terminated at diakinesis and metaphase I.

Presumably this was gene controlled even though the genetical studies were not made.

Desynapsis was enhanced when the desynaptic plants were placed in low temperature 15°C at a longer duration, two days or more and the difference was found to be statistically significant as compared with the check and treatments with higher temperature 25°C. Treatment with low temperature at a shorter duration had small or no effect.

The natural hybrid of this mutant strain was not affected by the temperature treatments whatsoever.

This desynaptic gene seemed to control the biochemical and biophysical state of the chromosomes after pachytene, causing great reduction in the formation of chiasmata and facilitating the falling apart of the paired chromosomes from mid to late prophase.

低溫對水稻 Desynapsis 因子之影響

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嘉農242號種子經X光線之處理，在第三代（X_3）得到一個 desynapsis 突變系統，共有17株，其中一株爲天然雜種。

由觀察染色體之配對情形，發現所有染色體在粗絲期(pachytene)之配對完全正常，至粗絲期末了（late pachytene）配對的染色體開始分離，到了肥厚期（diakinesis）及第一中期（metaphase I），完全分開成單價體，此乃係一由因子所支配的現象。

將 desynapsis 植株加以不同溫度及時間的處理（第一次試驗：15°C，25°C，配合12, 24, 48小時共六種處理；第二次試驗 16°C，27°C 配合 24, 48, 96 小時共六種處理），計算其肥厚期及第一中期每個細胞平均單價體數。試驗結果經統計分析，證明低溫處理可以促進 desynapsis 之發生，尤其低溫配合較長時間的處理，有顯著的效果。又雖然以同樣的低溫，而處理時間較短，則對此因子的促進效果較小或無效果(即與戶外對照處理無差異)。此突變型的天然雜種（C20—14—18）並不受溫度處理之影響。

總之，此 desynapsis 因子控制染色體粗絲期末了以後的生物化學及生物物理狀況，以致減少染色體交叉（chiasma）之形成，並使得配對的染色體在分裂前期稍後一段時期有分離的趨勢。

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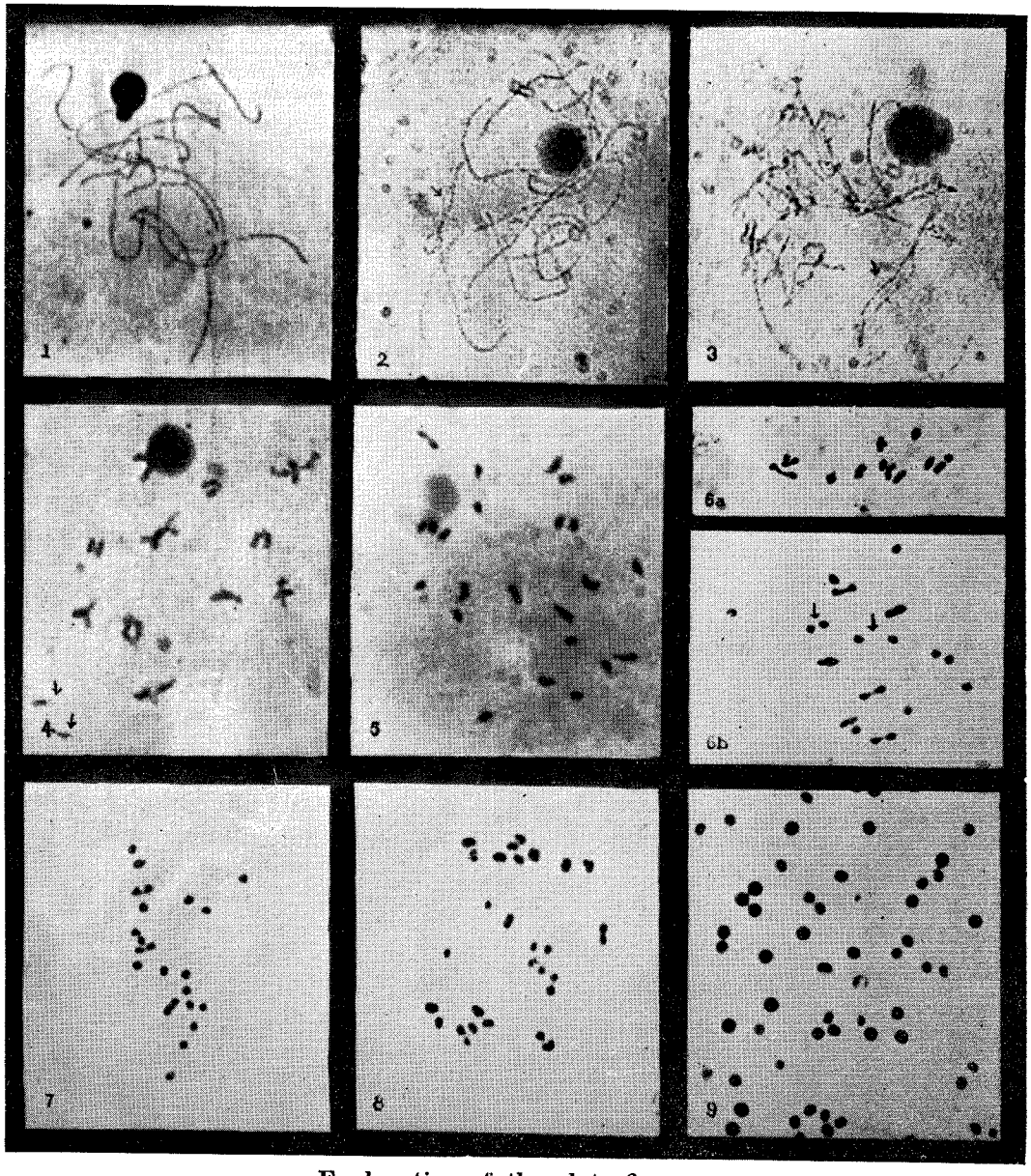

Explanation of the plate figures

Fig. 1. Pachytene stage of desynaptic plant showing twelve closely paired chromosomes.
Fig. 2. Pachynema, arrow showing loops in the intercalary region of one of the chromosomes.
Fig. 3. Diplonema, showing further opening up of the chromosome.
Fig. 4. Early diakinesis, showing eleven bivalents and two univalents.
Fig. 5. Diakinesis, showing many univalents, result from treatment with cold temperature at long duration.
Fig. 6a. Metaphase I, showing two univalents and eleven bivalents.
6b. Metaphase I, arrow pointing to chromosomes precociously divided.
Fig. 7. Metaphase I, showing three bivalents and eighteen univalents.
Fig. 8. Anaphase I, showing lagging univalents on the equator after the bivalents have migrated to the poles.
Fig. 9. Pollen grains of desynaptic plant under outdoor culture showing about 50% sterility.