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CYTOGENETIC STUDIES OF *LUPINUS MUTABILIS* Sweet. I. INFERTILITY DETERMINED BY DESYNAPSIS[1]

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Summary. The cause of recurring infertility in *Lupinus mutabilis* has been studied. As shown by cytological analyses, already at meiosis prophase, part of the chromosomes occur in the form of univalents. During metaphase I the number of univalents in the cells ranged from 2 to 48 and that of bivalents — from 1 to 9. There were on the average 2.67_{II} and 42.64_{I} per single pollen mother cell. The first and second divisions were irregular: lagging chromosomes, bridges and univalents dividing into chromatids were encountered during anaphase I: lagging chromosomes and bridges were also frequently found during telophase II. Microsporocytes in the process of microsporogenesis transformed into tetrads and polyads of microspores: sometimes monads and relatively frequently dyads of microspores were found. The nucleus in part of the pollen mother cells (about 1.2%) degenerated before the reduction division. Such cells did not divide. Among tetrads the microspores distinguished by the lack of nucleus or contained remains of scattered chromatin.

From the performed studies it follows that the revealed infertility in *Lupinus mutabilis* is caused by desynapsis. As shown by a genetic analysis, in the progeny of some plants it is caused by a single recessive gene, whereas in others these symptoms presumably depend on two acting genes. However, in view of a scarce material used for the described genetic analysis, the obtained results should be confirmed.

In the progeny of one of *Lupinus mutabilis* lines, reproduced and selected for many years, there have appeared plants, which had a single pod after shedding their flowers, though they flowered to late autumn. Such plants were found as single individuals with a certain regularity. This kind of infertility in the studied species has not been known so far (Nowacki 1958, Kazimierski, Nowacki 1961, Nowacki, Kazimierski 1961, Atabekova 1968, Pakendorf 1970, Maisuryan, Atabekova 1974). We have made an attempt to study this phenomenon and to determine the mode of its inheritance.

MATERIAL AND METHODS

Pollen was taken during vegetation from plants of the line with infertile individuals and its viability was determined in Belling's fluid. To analyse the course of meiosis the flower buds were fixed in Carnoy's fluid (3 parts of absolute ethanol + 1

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part of propionic acid). Chromosome conjugation and division, as well as microspore formation were analysed on squashed preparations stained in propiono-carmine. To determine the inheritance mode of infertility, fertile and infertile plants were counted, and, then, their interrelation was calculated.

Anthers from fertile and infertile plants may differ by shape and size. For such a comparison the anthers were taken from flowers and measured under a stereoscopic microscope; simultaneously their colour and dehiscence were determined.

RESULTS

Fertile and infertile plants did not differ from one another until the flowering period. After flowering, ovaries of fertile plants increased in size and transformed into pods; ovaries of infertile plants fell down after 7 - 10 days. In the first half of August fertile plants got matured — they lost their leaves and their stems got dried; infertile plants continued to develop their leaf-bearing lateral shoots, flowered till October — had intensively green, live, thick shoots. Only some of them set a single pod (Table 1, Phot. 1). The number of analysed plants given in Table 1 shows that

Table 1. The number of infertile individuals in the progeny of heterozygous *Lupinus mutabilis* Sweet. plants in the years 1982 - 1986

Year	Plant No.	Number of analysed plants			χ^2	P
		totally	including infertile			
			n	%		
1982	836	26	1	3.8		
1983	237	26	2	7.7		
1984	364	9	1	11.1		
1985	552	30	3	10.0		
1985	552/1	31	2	6.4		
1986	664	35	3	8.5		
1986	665	24	4	16.6	0.88	0.50 - 0.20
1986	666	31	8	25.8	0.0106	0.80 - 0.50

in the progeny of plants 665/1 and 3 the ratio of fertile to infertile plants was close to 3 : 1, whereas in the remaining plants it was rather similar to 15 : 1. Therefore, infertility in some plants of this lupin species is determined by a single recessive gene, whereas in others — by probably two similarly acting genes. In view of a limited material used for the analysis the inheritance mode of the studied character should be confirmed.

Anthers in the flowers of infertile plants developed normally. Their length and width (measured at the base) were averagely somewhat smaller than those of anthers in fertile plants. Anthers of infertile plants were more differentiated in the minimum and maximum length. Anthers of fertile plants were yellow, dehiscent, while those of infertile plants changed their initial yellow colour for grey, did not dehisce and got dried.

VIABILITY, DIAMETER AND SHAPE OF POLLEN GRAINS

Viability of pollen grains was analysed from June to September. Fertile plants had from 90 to 99% of viable pollen grains (Table 3, Phot. 2). Among infertile plants those with grains without plasma throughout the vegetative period (Phot. 3) as well as those with the pollen viability ranging from 0.0 to 57.0% were encountered (Table 3, Phot. 4). The percentage of grains with plasma was not similar in the stu-

Table 2. The length and width of anthers (at the base) in fertile and infertile plants of *Lupinus mutabilis* Sweet.

Plants	Length (mm)			Width (mm)		
	min.	max.	mean	min.	max.	mean
Fertile	2.4	3.0	2.65	0.9	1.2	1.05
Infertile	2.0	3.6	2.05	0.6	1.2	0.84

died plants. A higher percentage of such grains was found in some plants at the beginning of flowering, whereas in others — at full summer. By the end of the vegetation period — September, October — only single pollen grains with plasma were encountered in all the plants (Table 3). Irrespective of the percentage of pollen grains with plasma, infertile plants set a single pod only sporadically.

Table 3. Pollen viability in fertile and infertile plants of *Lupinus mutabilis* Sweet. during vegetation of plants.

Plants	Plant No.	Analysis						No. of analysed plants
		I		II		III		
		Date	Pollen grains with plasma (%)	Date	Pollen grains with plasma (%)	Date	Pollen grains with plasma (%)	
Fertile	552	16 VII	90.1 - 99.9	15 VIII	90.1 - 99.3			20
Infertile	552/1	24 VII	<0.1	31 VIII	0.0			
	4		<0.1		0.0			
	5		<0.1		0.1			3
Fertile	664 - 666	26 VI	91.3 - 98.4	10 VII	92.6 - 99.4			15
Infertile	664/3	16 VII	0.0	18 VIII	1.6	12 IX	0.0	
	5		0.0		<0.1		0.0	3
	2		0.0		<0.1		0.0	
Infertile	665/1	16 VII	0.0	18 VIII	0.0	12 IX	0.0	
	3		0.0		0.0		<0.1	
	6		57.1		1.5		0.0	
	8		0.0		4.3		<0.1	4
Infertile	666/1	16 VII	2.4	18 VIII	<0.1	12 IX	0.0	
	2		25.6		0.0		0.1	
	3		0.7		4.2		<0.1	
	4/1		0.0		0.0		0.0	
	4/3		4.9		0.0		<0.1	
	4/6		27.1		1.0		<0.1	
	4/8		11.7		1.1		0.1	
	5/5		<0.1		0.0		<0.1	8
Fertile	836	3 VII	93.4 - 98.6	13 VII	92.1 - 98.8			25
Infertile	836/1	6 VII	0.0	16 VII	<0.1	10 IX	0.0	1
Fertile	237	10 VII	92.3 - 99.9	25 VII	93.6 - 98.9			24
Infertile	237/1	12 VII	0.0			6 IX	0.0	
	2		<0.1				0.0	2
Fertile	364	25 VI	94.6 - 98.7	20 VII	93.4 - 98.9			8
Infertile	364/1	30 VI	0.0	15VIII	<0.1	5 IX	0.0	1

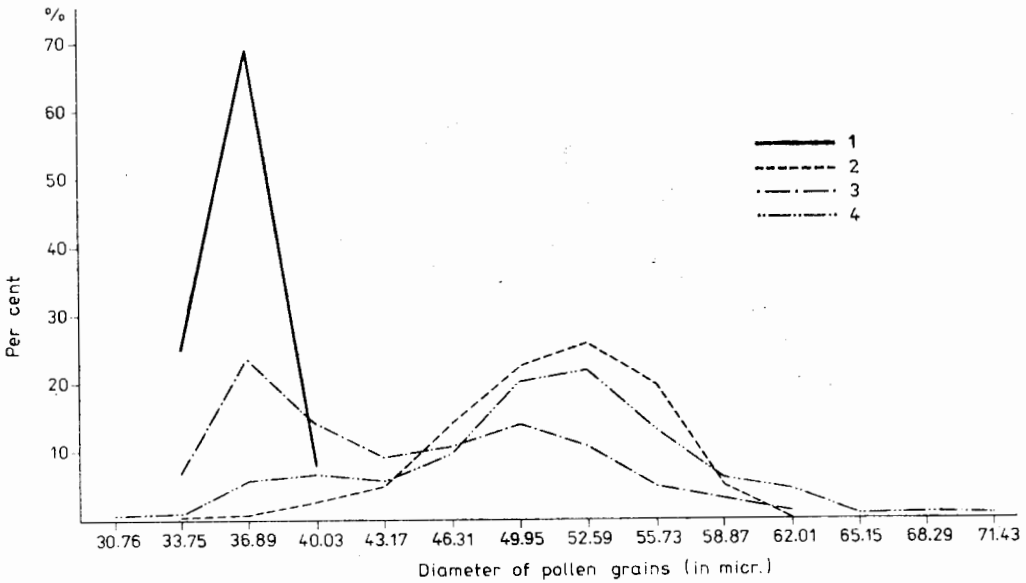


Fig. 1. Variation of the pollen grain diameter in fertile and infertile plants of *Lupinus mutabilis* Sweet.

1 — fertile plants, 2 — infertile plants No. 664, 3 — infertile plants No. 665, 4 — infertile plants No. 666

The mean diameter of pollen grains for fertile plants is 36.66 microns, ranging from 32.97 to 39.25 microns (Fig. 1). The diameter of the smallest pollen grains of infertile plants is 30.76 microns, that of the largest ones — 71.43 microns with the mean of 53.14 microns. The minimum and maximum diameter is 30.76 microns in infertile plants and 6.28 microns in fertile plants; pollen grains of infertile plants are averagely 16.48 microns larger than those of fertile plants.

Pollen grains of fertile plants have the shape of an equilateral triangle with three pores for the pollen tube; in infertile plants pollen grains with plasma are triangular, spherical and more or less rectangular, with 1 - 6 pores for the pollen tube.

MEIOSIS

The pollen mother cells of infertile plants, from leptotene to pachytene, usually contained two nucleoli of the same diameter, or one of which was larger than the other; the analysed cells from fertile plants had a single nucleolus in their nucleus (Table 4). It was also found, though rarely, that some pollen mother cells had two nuclei; there was even without nucleus. Binucleate cells were larger than uninucleate ones, and their shape and size suggest that during division preceding the formation and differentiation of microsporogenic cells following karyokinesis no cytokinesis occurred. It is, however, difficult to explain genesis of the cell without nucleus, since it does not differ in its shape and size from the surrounding cells with a nucleus (Phot. 5).

Only few out of the analysed pollen mother cells were found to have chromosome pairing at pachytene. There were cells coupled in pairs, as well as those with unlinked fragments or with partially coupled and uncoupled chromosomes.

At diplotene in some of the cells most of the chromosomes were coupled in bivalents (Phot. 6), whereas in others the majority were univalents. No cells were found to have only univalents, but there were cells, in which the chromosomes were paired in bivalents.

Table 4. The number of nucleoli in the pollen mother cell at the leptotene-pachytene phase in *Lupinus mutabilis* Sweet. plants with desynapsy

Plant No.	Number of nucleoli in nucleus			Number of cells without nucleus
	1-nucleate cells		2-nucleate cells	
	1	2	4	
486/5	24	1085	2	–
552/1	4	38	–	1
665/1	–	173	1	–
665/3	–	205	–	–

During diakinesis the bivalent number ranged from 0 to 24 (Phot. 7), but most frequently the analysed cells at this stage of meiosis prophase were found to have over 24 univalents.

The behaviour of the chromosomes in the pollen mother cells during the first metaphase was unusual (Table 5, Phots. 9 - 11). No division plate with chromosomes arranged in the equatorial plane of the cell like that in fertile plants (Phot. 8) was formed. The chromosomes, mostly in the form of univalents and in part of the cells only in the univalents, were distributed more or less chaotically. Few bivalents were in the central part of the cell (Phots. 9 - 11). Except only one plant, the cells of which did not have all the chromosomes in the form of univalents, all the remaining plants had only univalents in part of the cells. The bivalent number in the cells ranged from 1 to 9 and the univalent number — from 48 to 30 (Table 5). There were averagely 2.67_{II} and 42.54_{I} per PMC. Out of the total number of 66288 analysed chromosomes 7396 formed bivalents and 58892 occurred as univalents.

Table 5. Metaphase I in *Lupinus mutabilis* Sweet plants with desynapsy

Plant No.	Bivalent number in PMC										No. of analysed cells	Average number of		Percentage of cells with all the chromosomes in the form of univalents
	0	1	2	3	4	5	6	7	8	9		bivalents per PMC	univalents per PMC	
486/5	179	99	73	38	16	2					407	1.06	45.87	43.9
814/3				6	18	13	8	3		3	51	4.92	38.15	0.0
552/1	22	22	60	63	61	32	30	6	5	5	306	3.40	41.17	7.1
552/4	4	4	12	31	46	28	20	20	9	11	185	4.73	38.53	2.1
552/5	1	5	8	10	10	13	6	4	4	1	62	4.25	39.58	1.6
665/3	50	40	57	51	40	20	14				272	2.39	43.21	18.3
665/1	20	22	27	12	13	4					98	1.88	44.24	20.4
Totally											1381			
Averagely/1 PMC												2.67	42.64	

In part of the cells (2 - 3%) in two out of the studied plants, where all the chromosomes during diakinesis were in the form of univalents, nothing indicated that they underwent the stage of metaphase I; no karyokinetic spidle was formed, the chromosomes partially underwent despiralization, there appeared nucleolus-like spherical bodies connected with the chromosomes, or lying beyond them in the cell cytoplasm (Phots. 12 - 13).

Table 6. Chromosome segregation during anaphase I in infertile plants of *Lupinus mutabilis* Sweet.

Plant No.	Number of cells with chromosome division:							Per cent of cells with disturbed division
	equal to 24	unequal	with lagging chromosomes	with lagging chromosomes and chromatids	with lagging chromosomes and bridges	without analysed groups	Totally	
486/5	3	15	12 (1–26)	3 (1–9) (2–16)*	1 (1) (2)**	–	34	91.2
814/3	5	–	7 (2–25)	–	–	12	24	79.1
552/1	1	14	34 (1–30)	4 (2–11) (4–10)*	2 (3–9) (1–2)**	1	56	98.2
552/4	–	2	21 (1–13)	1 (13) (2)*	5 (3–12) (1–3)**	–	29	100.0
552/5	1	20	15 (1–18)	4 (1–8) (2–6)*	–	–	40	97.5
665/1	–	11	1 (1)	–	–	–	12	100.0
665/3	–	8	18 (2–26)	–	–	–	26	100.0
Totally	10	70	108	12	8	13	221	
Per cent	4.52	31.68	48.87	5.43	3.62	5.88		

(from-to) – the number of lagging chromosomes
(from-to)* – the number of chromatids from divided univalents
(from-to)** – the number of bridges

During anaphase I the chromosomes of fertile plants numbering 24 migrated towards the poles (Phot. 14), in infertile plants their division was mostly unequal (Table 6, Phots. 15 - 16). The analysed cells may be divided into four groups:

a) without lagging chromosomes;
b) with lagging chromosomes;
c) with chromosomes dividing or divided into chromatids;
d) with lagging chromosomes and chromosome bridges;
e) with chromosomes numbering 48 localized in different places of the cell and forming no anaphase groups.

At telophase I (Table 7, Phots. 17 - 18) some of 63.2% analysed cells had: up to 24 lagging chromosomes; lagging chromosomes (to 10) and bridges (to 3); without lagging chromosomes but with bridges; with bridges and fragments; part of uni-

Table 7. Telophase I: lagging chromosomes and other deviations in infertile plants of *Lupinus mutabilis* Sweet.

Plant No.	Number of cells with: all chromosomes in telophase groups	chromosomes remained in cytoplasm between groups	remained chromosomes and a bridge	a bridge	a bridge and fragment	univalents divided into chromatids	Number of analysed cells
486/5	9	13 (1–12)	6 (5–10) (1–3)*	10 (1–5)*	4 (1–2)* ((1)**	4 (5–12)***	46
814/3	10	10	0	1 (2)*	0	0	21
552/1	27	5 (1–5)	0	0	1 (1)* (1)**	1 (3)***	34
552/4	11	14 (1–6)	1 (1) (1)*	2 (2)*	0	4 (1–2)***	32
552/5	18	19 (3–7)	3 (4–7) (1–2)*	6 (1)*	3 (1–2)* (1–2)**	0	49
665/1	4	12 (1–7)	0	0	0	0	16
665/3	9	26 (1–24)	5 (2–11) (1–2)*	1 (1)*	0	0	41
Totally	88	99	15	20	8	9	239
Per cent	36.8	41.4	6.3	8.4	3.4	3.7	

(from-to) – the number of lagging chromosomes
(from-to)* – the number of bridges
(from-to)** – the number of fragments
(from-to)*** – the number of univalents divided into chromatids

valents divided into chromatids. The number of univalents dividing and divided into chromatids ranged from 1 to 12. The percentage of cells with all the chromosomes in telophase groups was 36.8%.

Chromosomes, fragments and chromatin bridges between nuclei are encountered also at the interphase stage. The percentage of such cells is 4.1%. In the majority of dyads all the chromosomes were in the nuclei (Table 8). In part of the cells, at the

Table 8. The number of chromosomes in interphase nuclei and those remained in the cytoplasm in infertile plants of *Lupinus mutabilis* Sweet.

Plant No.	Chromosome number in dyad nuclei: 24	23	22	21	20	19	18	17	16	15	14	13	9	23	22	21	21	20	19	19	16	16	13	Number of analysed cels
	24	25	26	27	28	29	30	31	32	33	34	35	39	1 24	1 25	1 25	2 25	2+m 27	2ch 24	5 26	3 25	7 29	3 28	7	
486/5	2	2	2	1	4	2		2	1		1	1			1	1	1		1	1	1	1	1		26
814/3	2																								2
552/1	3	9	6	5		1	1	3		2				1				1							32
552/4	1	4	1	1										1											8
552/5		6	1			3	3	1			1														15
665/3	3	8	12	9	5	2	4	2	2	1	1	1	1												41
Totally	11	29	22	16	9	8	8	8	3	3	3	2	1	2	1	1	1	1		1	1	1	1		34

m – bridge
ch – chromatids

interphase stage, the chromosomes could be counted. It appeared that only a small part of the progeny nuclei had the same number of chromosomes i.e. 24 chromosomes each (Table 8), whereas the remaining nuclei had unsimilar chromosome numbers.

Mostly in both nuclei of dyad at the prophase of the second division the nucleolus disappeared simultaneously and the chromosomes underwent spiralization. There were, however, dyads with irregular synchronization (Phot. 19), where the chromosome in one nucleus looked like during interphase, whereas in another one — they were in an intermediate state between prophase and metaphase. In consequence, in one of the nuclei the chromosomes formed a metaphase plate, while the second one

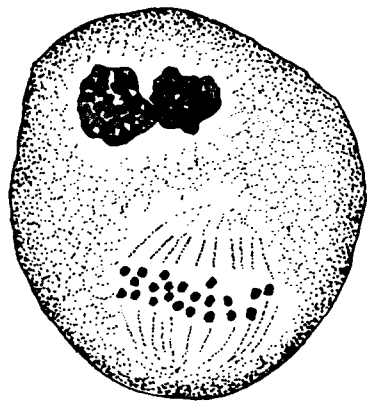
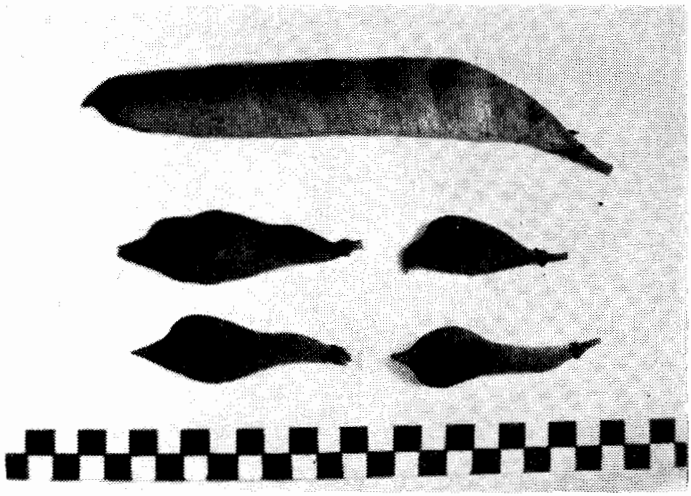


Fig. 2. Desynchronization of the second division in the PMC of the *Lupinus mutabilis* Sweet.plants with desynapsy

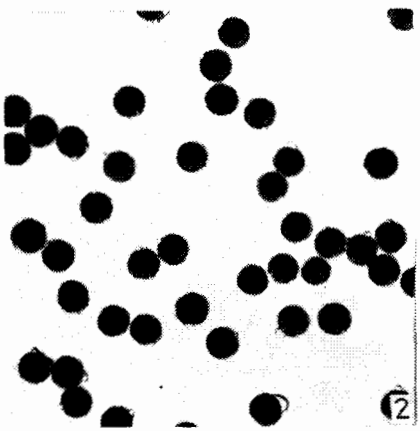
had been divided and the occurred nuclei were at the telophase stage (Fig. 2). In two groups the chromosomes were counted: their number in the metaphase plate was 22, and in one of the telophase nuclei — 26. In the second telophase group their number is likely also to be 26. Unsimilar chromosome number indicates that their separation towards the poles during anaphase I was unsimilar.

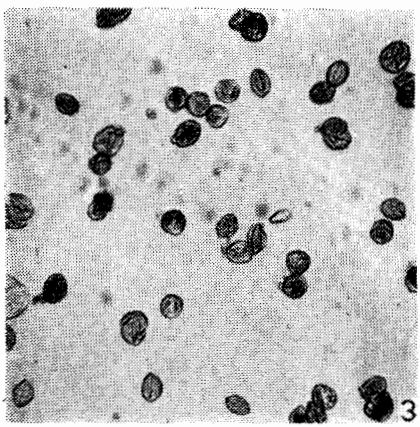
Few cells at metaphase II had the same chromosome number in their plates; it was mostly unsimilar and ranged from 7 to 25 in one and from 41 to 23, respectively, in another (Table 9). Nearly 9% of the analysed cells, besides unsimilar chromosome number in the metaphase plates, were found to have chromosomes beyond the plates — lagging and accelerating. In one of the cells 8 chromosomes formed a chain connecting the plates, and in another 2 chromatids remained beyond the plates.

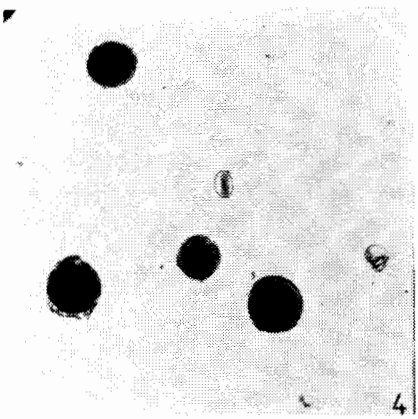
During anaphase II, 18 groups (10.4%) moving towards the poles had 24 chromosomes each out of the counted 172, and in the remaining groups the chromosome number significantly deviated, both in plus and in minus (Table 10). Eight out of the mentioned 18 anaphase groups with the chromosome number 24 originated from two pollen mother cells, where the chromosome division was equal; four next groups



Phot. 1. Pods of *Lupinus mutabilis*: at the top — from fertile plant, below — from plants with desynapsy

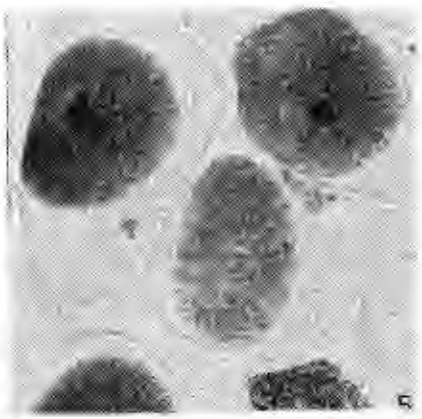


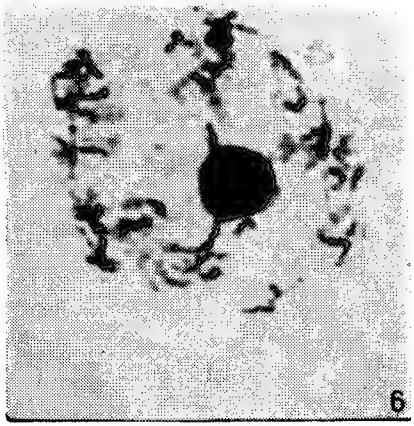


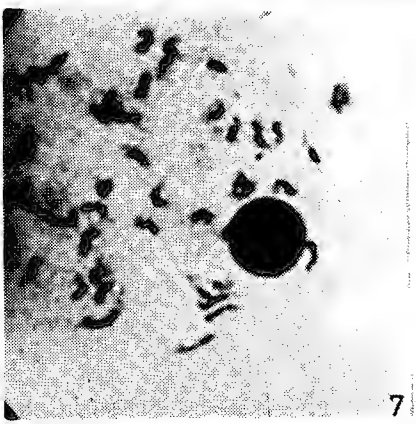


Phots 2 - 4. Pollen grains from *Lupinus mutabilis* plants

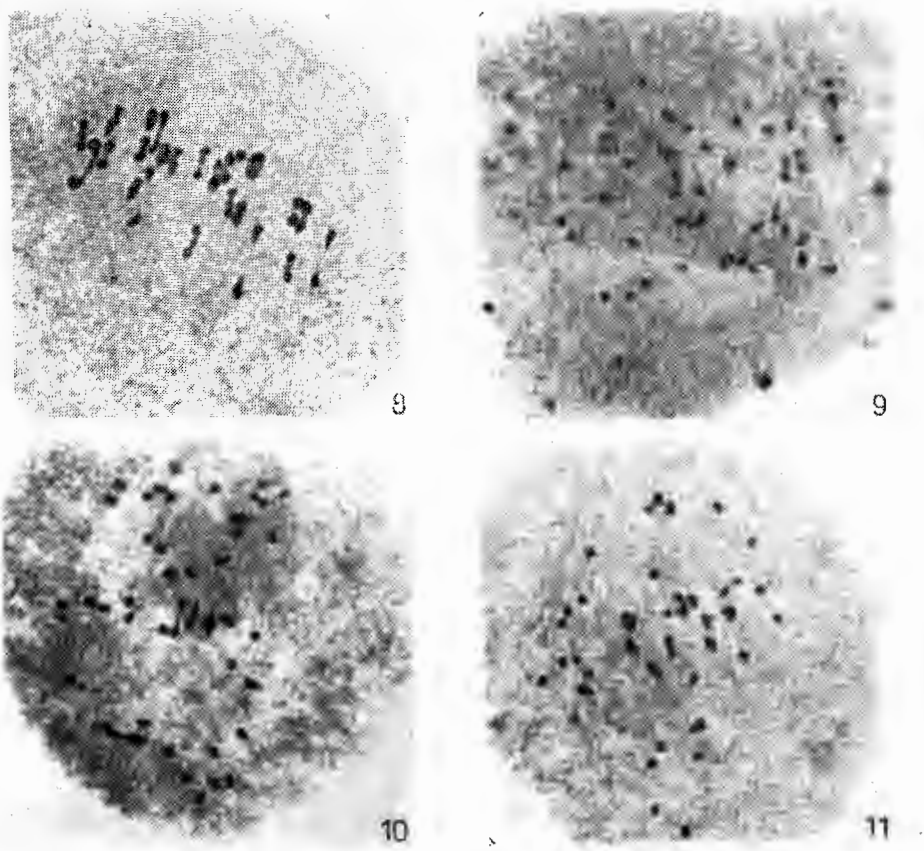
2 — of fertile plant, 3 — of infertile plant, grains without plasma, 4 — of infertile plant, grains without plasma and with plasma





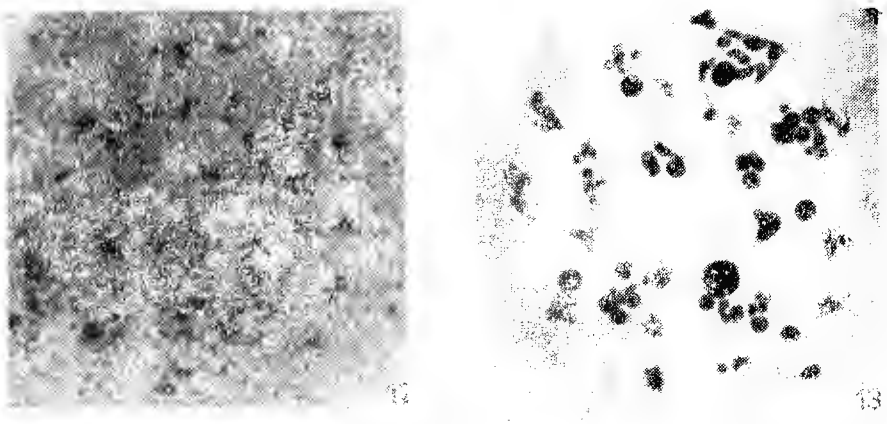


Phot. 5. Meiosis. Pachytene, one of the cells without nucleus; Phot. 6. Diplotene, $22_{II}4_{I}$; Phot. 7. Diakinesis, a larger part of the chromosomes are in the form of univalents

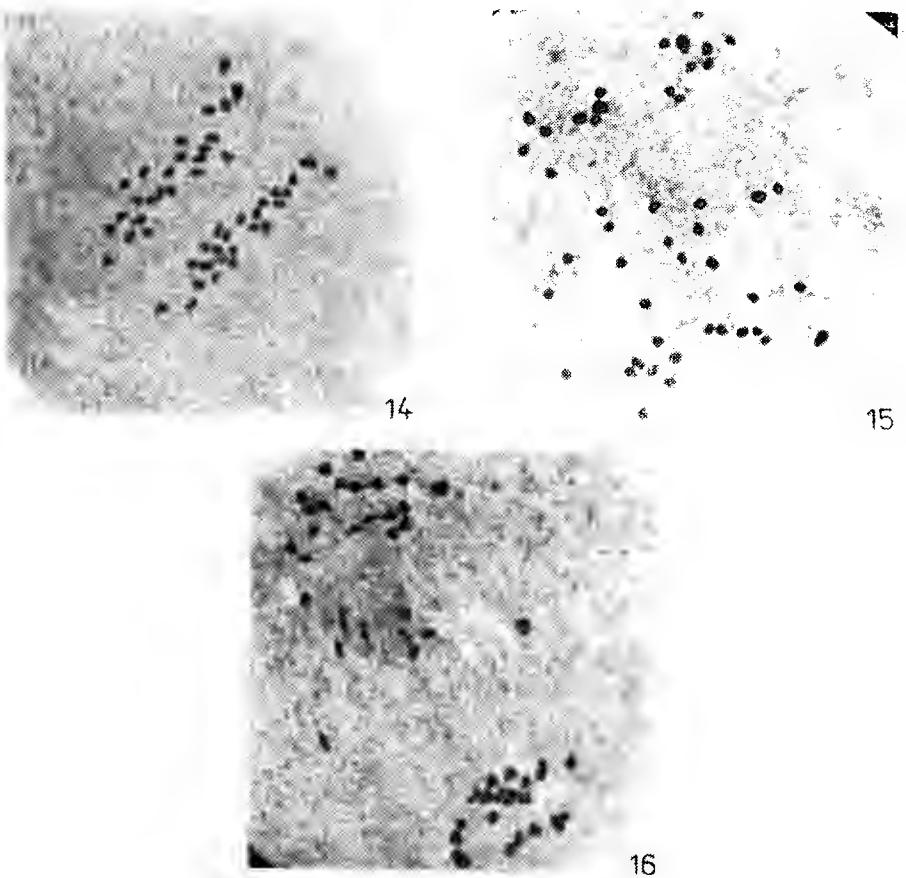


Phot. 8 - 11. Metaphase I.

Phot. 8. Fertile plant 24_{II}; Phots. 9 - 11. Infertile plants. Phot. 9 - $1_{II}46_{I}$; Phot. 10 - $3_{II}42_{I}$; Phot. 11 - $8_{II}32_{I}$.

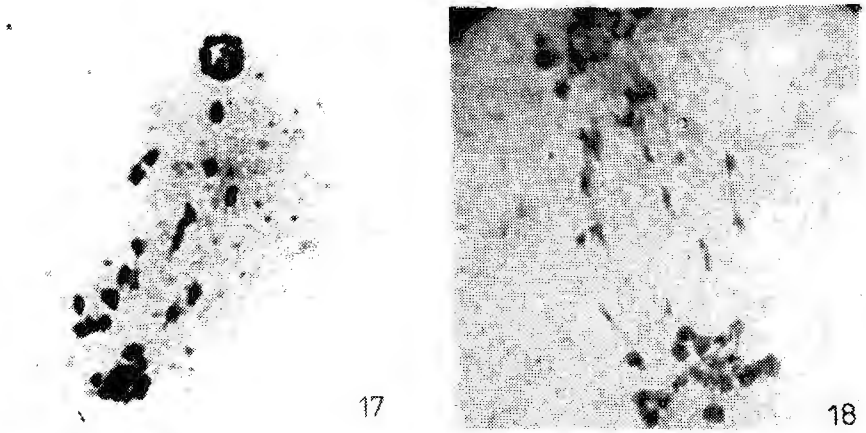


Phots 12 - 13. Divided chromosomes, do not move towards the poles, there are many spherical, carmine-stainable bodies in the cells (infertile plants)

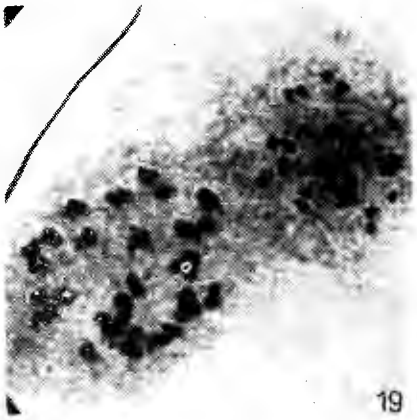


Phots 14 - 16. Anaphase I

14 a fertile plant, 15 and 16 present infertile plants, chromosome division: 15 16-11-18, 16 19-9-20



Phots. 17 - 18. Telophase I, lagging chromosomes and univalents divided into chromatids (infertile plants)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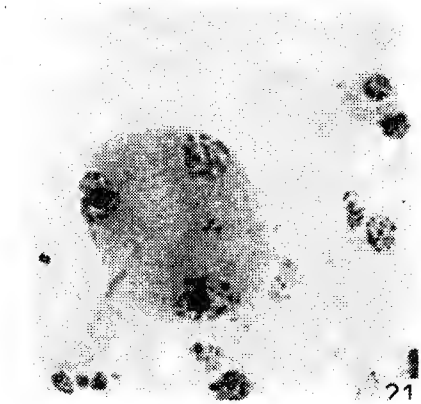
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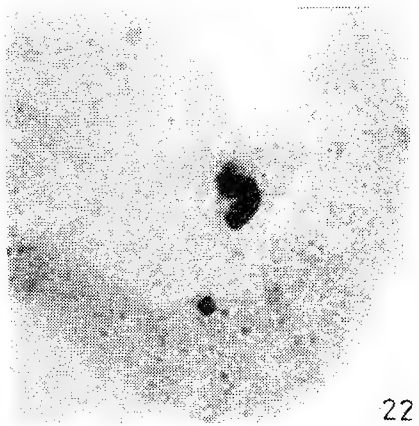
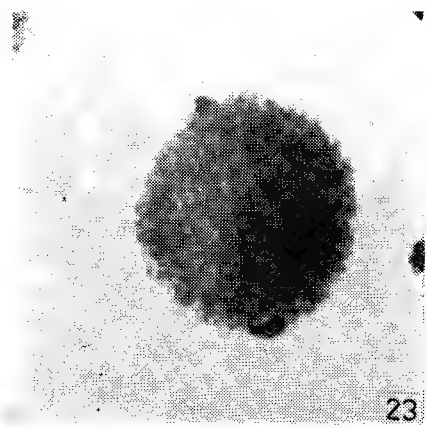

22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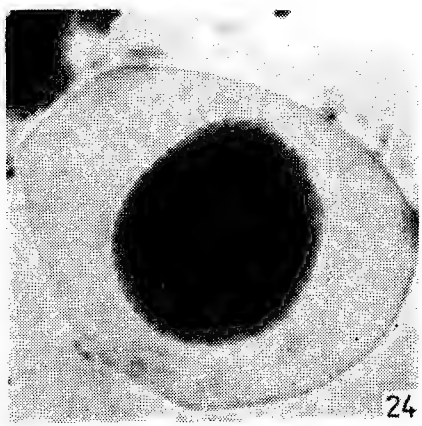
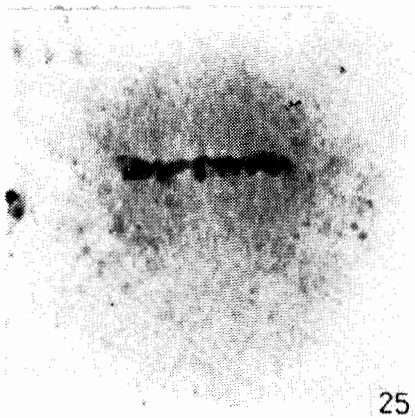
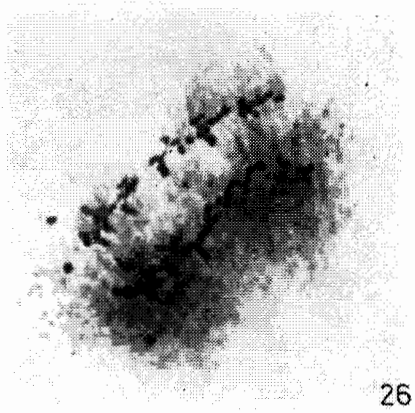

24



25



26

Phots 19 - 26. Infertile plants

19 — lack of synchronization into dyad nuclei, 20 — linked nuclei after telophase II; 21 — two nuclei with a nucleolus, one without nucleolus, three chromosomes are beyond the nuclei, 22-24 — Pollen mother cells with desintegrated nucleus, 25 — metaphase — there are 48 chromosomes in the plate, 26 — anaphase — 48 chromosomes are migrating to each pole

Table 9. Metaphase II: the number of chromosomes in plates, lagging and accelerated chromosomes in infertile plants of *Lupinus mutabilis* Sweet.

Plant	Chromosome number in division plate lagging and accelerated chromosomes																																		Totally
No.	24	23	22	21	20	19	18	17	16	15	14	13	12	11	10	7	22	22	23	23	24	24	25	25	25	26	26	26	26	27	27	28	30	32	
																	6+	8+2)	3+	5+	1+	4+	2+	3+	2+1	1+	11+	4+	1+3)	1+	4+	1+	1+	2+	48+4)	
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	41	20	18	22	20	23	20	21	20	22	21	11	18	22	20	17	19	17	14		
468/5	9	29	28	16	15	17	12	4	5	6	5	1			1	1		1		1			2	1	1	1	1			1	1	1		1		161
814/3	7				1																1															9
552/1	2	6	6	2	1	1	1			1													1									1	1			23
552/2		2	1	1									1				1					1														7
552/5	3	6	7	3	4	3	2	2						1							1		1	1				1		1		1				37
665/1	3	14	21	8	4	3	5			2		1							1		1		1						1				1			66
665/3	5	17	6	13	6	11	1	5	3	1	1		1	1					1		1			1			1								1	76
Totally	29	74	69	43	31	35	21	11	8	10	6	2	2	2	1	1	1	1	2	1	4	1	5	3	1	1	2	1	1	2	1	3	2	1	1	379

+ chromosomes beyond the plates lagging or accelerated
+1) chromatids
+2) chromosomes form a chain linking the plates
+3) fragment
+4) all the chromosomes are in one metaphase plate

— from two cells, in which the division was equal; six others — with the chromosome number 24 were found in 5 cells, in which 24 chromosomes moved to one of the sister groups and more or less than 24 chromosomes to the other. Accelerating and lagging chromosomes, as well as bridges, were encountered during that stage.

Table 10. Telophase II in sister nuclei A and B: the number of cells with lagging chromosomes and bridges in infertile plants of *Lupinus mutabilis* Sweet.

Sister nuclei B ↓	→ A	Disturbances: t	1-o	2-o	3-o	4-o	1 mb	1mb +2-o	1mb +f	1mb +1-o	2mb +5-o	linked nuclei	Totally
t		193	18	18	4	2	14	2					251
1-o			11	5			3			1	1		21
2-o			1	2	1								4
3-o					2								2
4-o			2										2
5-o						1							1
1mb							10		1			2	13
Totally		193	32	25	7	3	27	2	1	1	1	2	294

t — without bridge and lagging chromosomes
-o — the number of lagging chromosomes
mb — bicentric bridge
f — fragment

The second telophase in the cells of the studied plants proceeded also with different abnormalities (Table 10). In most of the analysed cells (65.6%) all the chromosomes were in the progeny nuclei. In the remaining cells — in the space between the telophase groups — from 1 to 5 lagging chromosomes, 1 - 2 bicentric bridges, sometimes lagging chromosomes and bridges were encountered. The last ones happened to be between both one and two groups of telophase nuclei. In two cells the telophase nuclei were linked in two (Phot. 20), and in the next two cells — with a tripolar spindle — there occurred three telophase nuclei.

MICROSPOROGENESIS

On the average 69.5% of microsporocytes transformed into tetrads of microspores (Table 11); the percentage of such microsporocytes in individual plants ranged from 55.8 to 94.8%. Part of the microsporocytes (21.5%), besides four microsporocytes of a more or less similar size were found to have from 1 to 6 minimicrospores (spores with a small diameter with a single or several chromosomes). The remaining microsporocytes transformed into monads, diads, triads with minimicrospores or without them, and one of them gave a pentad of microspores.

Among the analysed microspores, tetrads with a single nucleus containing no nucleolus and with three nuclei with a nucleolus, as well as triads with no nucleolus in a single nucleus, were found (Phot. 21). The lack of a nucleolus in the nucleus of one of the microspores permits to suggest that after the second division the nucleolus-producing chromosome was not in the nucleus of such a microspore.

Table 11. Microsporogenesis in infertile plants of *Lupinus mutabilis* Sweet.

Plant No.	Number of microspores in microsporocyte																										Number of analysed micro-sporocytes
	4	4+1	4+2	4+3	4+4	4+5	4+6	3	3+1	3+2	3+3	3+4	3+5	2	2+1	2+2	2+3	2+4	2+5	1	1+1	1+2	1+3	1+4	1+5	5	
231/1	596	205	122	26	3			9	11	5	1		1	26	13	12	3	1	1								1035
468/5	698	188	66	9	1			25	20	14	7			88	51	55	13	8		4	1	1	1			1	1251
814/3	1010	28	25	1	1																						1065
552/1	829	135	52	2	2			10	1																		1031
552/4	725	119	109	20	8			5	1	2		1		6	7	5	3										1011
552/5	914	79	64	7	5			4	3	1				2	1	2											1082
665/1	643	171	114	18	6			13	4	12	1	2		12	6	4					1						1007
665/3	1258	233	195	43	7	2	1	36	26	19	4		1	133	61	60	17	9	2				2		1		2110
Totally	6673	1158	747	123	33	2	1	102	66	53	13	3	2	267	139	138	36	18	3	4	2	1	3		1	1	9589
%	69.5	21.5						2.5						6.2						0.1						0.001	

+1..n — the number of minimicrospores

Amongst other irregularities in the process of microsporogenesis tetranucleate cenocytes and then tetrads of microspores with degenerating 1 - 4 nuclei should be mentioned. Microspores with a degenerating nucleus in the tetrad diminished and died, the remaining ones looked typical. In case, when nuclei degenerated in all the microspores, the entire cenocyte died.

Table 12. The number of nuclei in the pollen grain 3 - 4 days prior to flowering in infertile plants of *Lupinus mutabilis* Sweet.

Pollen grains	Number of nuclei						Totally
	1	2 (not differing by diameter and structure)	1 and micronuclei 1	1 and micronuclei 2	without nucleus	2 (vegetative and generative)	
Number	1030	5	14	3	35	27	1114
Per cent	92.47	0.45	1.25	0.27	3.14	2.42	100.0

Three-four days before flower opening, nuclei in the pollen grains were counted (Table 12). Pollen grains with a single nucleus constituted the most, then were grains with two nuclei of a similar structure and diameter, with a single large nucleus and a single and sometimes two micronuclei. There were grains without nucleus and few grains (2.4%) with two nuclei — vegetative and generative. It should be added that pollen grains at the flower stage showed a far-advanced vacuolization proceeding from wall towards the center of a cell.

Table 13. Nucleus degradation in the pollen mother cell in prophase of meiosis in infertile plants of *Lupinus mutabilis* Sweet.

Plant No.	Number of analysed cells at the phase of 4-nucleate cenocytes	Cells with degraded nucleus	
		number	%
232/1	1035	20	1.93
468/5	1251	15	1.22
814/3	1484	15	1.01
552/4	1011	11	1.08
552/5	1082	10	0.92
552/1	1031	11	1.06
665/1	1021	20	1.96
665/3	2132	22	1.03
Totally	10047	124	1.23

Among the formed tetrads and polyads of microspores, pollen mother cells with undivided nucleus undergoing degradation and degraded were encountered (Table 13). Such cells differed from one another by the internal structure: some of them had a still visible, small, undivided nucleus (Phot. 22), others — slightly carmine-stainable surfaces in a different part of the cell (Phot. 23), at least some cells had no nucleus with a bright, evenly stained cytoplasm (Phot. 24). The percentage of that type of cells in the studied sterile plants ranged from 0.9 to 2.0%.

DISCUSSION

The studied infertile plants of *Lupinus mutabilis* Sweet. at the early prophase of meiosis were found to have pollen mother cells with the chromosomes coupled in pairs and with part of coupled and uncoupled chromosomes, as well as pollen mother cells with unlinked fragments. The number of cells in which the way of linkage of all the chromosome could be precisely determined was small. Only at diplotene and diakinesis conjugation was determined on a larger material. From diplotene to diakinesis the number of chromosomes not coupled into bivalents increased. Disjunction of chromosomes coupled in pairs at prophase of meiosis was called desynapsy by Sharp (1934, cited after Rieger, Michaelis and Green, 1968). When there is no conjugation or when it is incomplete during the first meiotic division, which is a frequent phenomenon in interspecific hybrids, we have to do with asynapsy. The studied plants of *Lupinus mutabilis* Sweet. were not of hybrid origin. Since at pachytene, at least in part of the cells, the chromosomes coupled in pairs were observed, it is accepted that infertility in these plants is induced by desynapsy.

The infertility of that kind in *Lupinus mutabilis* Sweet., like in other species of that genus, has not been known so far. But it was revealed in the following plants belonging to other genera, as a feature, which occurred spontaneously: *Pennisetum orientale* (Jauhar, Singh 1969), *Fritillaria japonica* (Ito, Takegami, Noda 1983), *Stenbergia fisheriana* (Karihallo, Koul 1983), wheat (Li, Pao, Li 1945); as a result of recombination and segregations in intraspecific hybrids of wheat (Li, Pao, Li 1945), *Crytesion violaceum* (Wang 1984); under the influence of agents inducing mutation in *Allium cepa* (Konvicka, Fischbeck 1983), pea (Gottschalk, Baquar 1971), *Penisetum americanum* (Subba Rao, Sukhaden, Murty 1982), soyabean (Palmer, Kaul 1983) and induced by temperature in *Avena strigosa* (Rajhathy, Fedak 1971) and pearl millet (Dhesi 1980). Therefore, agents inducing desynapsy may be different. In plants with incomplete desynapsy (partially fertile) its intensity may be increased and decreased by exposing pollen mother cells at prophase of meiosis to the action of a certain temperature (Li, Pao, Li 1945, Rajhathy, Fedak 1971, Dhesi 1980). It appears that in some plants a temperature increase causes an increase in the number of univalents, for instance, in tomatoes, pearl millet, *Avena strigosa*, whereas in others, vice versa, the number of bivalents is reduced with decreasing temperature, for instance, in rice, wheat. It should be noticed here that deviations from optimal temperature for meiosis in a given species are meant. The gene conditioning desynapsy was designated with the symbol *ds* (desynapsis) by Li, Pao and Li (1945).

In plants with desynapsis originating from different sources this character, though stable, undergoes certain fluctuations in meiosis, which are explained by incomplete *ds* gene penetration, modified by factors of internal and external environment. It appears that in individual pollen mother cells from a single flower, and even from a single anther, the number of bivalents and univalents is not stable and fluctuates within certain limits.

In the case of *Lupinus mutabilis* Sweet. desynapsy occurred spontaneously. The gene, the action of which has a definite consequence, shows incomplete penetration, as the number of univalents in the cells ranges from 30 to 40 and that of bivalents — from 5 to 9. In only one out of the studied plants, amongst the analysed pollen mother cells no one had all the chromosomes in the form of univalents. In the remaining plants the percentage of cells with only univalents was from 1.6 to 43.9. In our opinion, a large differentiation of plants with regard to the number and percentage of cells with different intensity of desynapsis may result from several reasons. The ratio of fertile to infertile plants shows that in one case we have to do with a single recessive gene, whereas in others it may be assumed that there are two similarly acting genes. Thus, in monomeric, as well as in dimeric inheritance, a full phenotypic expression of the gene may not be similar. Besides that, at the pachytene stage, cells with all the chromosomes conjugated, part of the cells with non-conjugated chromosomes, as well as cells with at least certain unlinked fragments were encountered. Presumably disjunction of chiazmata and centromers in such cells does not proceed similarly rapidly, and therefore in part of the cells, as well as within a single cell, differentiation begins already at the early prophase of meiosis and increases with its progress. It is difficult to explain the presence of two nucleoli in the pollen mother cell at the phase from leptotene to pachytene in plants with desynapsis, as in fertile plants the nucleolus is always single. It may only be presumed that a supernumerary nucleolus is a specific way of expression of the *ds* gene activity at the early stage of meiosis prophase.

A disorderly distribution of univalents during the first metaphase and the lack of typical division spindle cause uneven chromosome division during the first anaphase, the formation of irregular division plates at metaphase II and in consequence — the formation of microspores with unsimilar chromosome number. It should be added that even those microspores, in which after the second division the chromosome number is 24 and is haploid, do not settle the question, since not every chromosome in such a microspore may be single in number.

A disorderly migration of chromosomes during the first and second divisions is also indicated by the formation of tetranuclear cenocytes without nucleolus in one of the nuclei; thus there is no nucleolus-organizing chromosome in the chromosome set of one of the nuclei as a result of irregular division.

However, uneven division as well as the fact that part of chromosomes remain beyond anaphase and telophase groups, give certain chances for formation of gametes and then zygotes with hyper- and hypoploid chromosome number. Such plants were obtained by Li, Pao et Li (1945) in wheat and by Palmer and Kaul (1983) in soyabean. In the case of such individuals in the progeny of desynaptic plants, this phenomenon could and can play a definite role in the evolution of species, for instance, in karyotype differentiation.

Attention should be also paid to a possible occurrence of triploid and polyploid individuals in the progeny of desynaptic plants (if they will produce a progeny). A frequently encountered phenomenon that all the chromosomes remain on their place between diakinesis and anaphase I, which is followed by the formation of a

single spindle and a single division plate (Phot. 25 and 26), leads to the occurrence of dyads of microspores with diploid and aneuploid chromosome number. In some cells, where the chromosome after diakinesis remained on the spot, division did not occur and they transformed into monads with tetraploid or aneuploid number of chromosomes. Aneuploid chromosome number in the case, when part of the chromosomes, usually small, without forming a division spindle, become separated by the cell wall, forming a minimicrospore or minimicrospores. As a matter of fact, the formation of aneuploids and polyploids of various types in the progeny of desynaptic plants is possible only then, when their few pollen grains are with plasma and possibly formed female gametophytes are physiologically efficient.

The occurrence of huge pollen grains from dyads and intact pollen mother cells indicates that the second division became arrested in part of the microsporocytes. This rarely encountered phenomenon in plants with desynapsy was revealed by Jauhar and Sing (1969) in *Pennisetum orientale*. These authors consider that the cause that the chromosomes remain on their place is the lack of the spindle and the loss of centromers activity. In the mentioned authors the chromosomes behaved in the described way in the majority of cells; but in the studied plants of *L. mutabilis* Sweet. a similar behaviour of chromosomes was observed only in part of the pollen mother cells. We think, that in our case the elimination of the first division in part of the cells is induced by an additional gene, which has not been identified.

In all the analysed plants the nucleus of about 1% pollen mother cells undergoes degradation between the late stage of microsporocyte formation and early prophase of meiosis. Such cells, encountered among tetrads contained residues of degradated chromatin or a shapeless, small, degenerating nucleus. We presume that also in this case we have to do with incomplete penetration of an unidentified gene.

Most of the pollen grains had a single nucleus; binuclear pollen grains were of a double kind: with two nuclei of the same diameter and structure and with a larger — vegetative and a smaller — generative nucleus. There were also grains with a large nucleus and micronuclei, as well as with no nucleus. They all displayed a far-advanced vacuolization, which proceeded from the grain wall towards the middle. It is, therefore, doubtful, whether even that small per cent of grains (2.4%) of a normal structure (with vegetative and generative nucleus) in connection with the progressing vacuolization is capable of physiological functioning.

CONCLUSIONS

1. Infertility regularly repeating in the progeny of one of the lines of *L. mutabilis* Sweet. is determined by desynapsy (*ds*). This phenomenon induces recessive gene in some plants, whereas others, presumably have two similar genes.

2. In the pollen mother cells of different individuals part of the chromosomes did not pair, while in others they were linked. This may indicate incomplete penetration of the *ds* gene or modification of its action through an unidentified additional gene.

3. A further consequence of desynapsy are irregularities during the first and second divisions, abnormalities in the process of microsporogenesis, the occurrence of uncapable of functioning pollen grains.

4. Though the process of megasporogenesis and megagametogenesis were not studied in plants with desynapsy, it may, however, be suggested that disturbances in the development of sporophyte and female gametophyte were similar to those in microsporogenesis, and that viable egg cells occurred exceptionally rarely. For that reason plants appeared to be mostly infertile.

5. A sporadic setting of a single seed permits, however, to suggest that sometimes there may occur a viable female gamete, which after fertilization with a normal male gamete will give rise to aneuploid zygote.

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BADANIA CYTOGENETYCZNE ŁUBINU ZMIENNEGO *LUPINUS MUTABILIS* SWEET. I. NIEPŁODNOŚĆ WARUNKOWANA DESYNAPSJĄ

Streszczenie

Badano przyczynę powtarzającej się nieplodności u łubinu zmiennego (*Lupinus mutabilis*). Jak wykazały analizy cytologiczne, już w profazie mejozy część chromosomów występuje w formie uniwalentów. Podczas metafazy pierwszej liczba uniwalentów w komórkach wynosila 2 - 48, a biwalentów 1 - 9. Przeciętnie na jedną komórkę macierzystą pylku przypadało: $2{,}67_{II}$ i $42{,}64_{I}$. Podziały pierwszy i drugi były nieregularne: podczas anafazy pierwszej spotykano opóźnione chromosomy, mostki oraz uniwalenty dzielące się na chromatydy; opóźnione chromosomy i mostki znajdowano także często podczas telofazy drugiej. W procesie mikrosporogenezy mikrosporocyty przekształcaly się w tetrady i poliady mikrospor, niekiedy znajdowano monady i stosunkowo często diady mikrospor. W części komórek macierzystych pyłku (około 1,2%) jądro degenerowało przed podziałem redukcyjnym. Komórki takie nie dzieliły się. Wśród tetrad mikrospory wyróżnialy się brakiem jądra albo zawierały resztki rozproszonej chromatyny.

Z przeprowadzanych badań wynika, że stwierdzoną niepłodność u łubinu zmiennego powoduje zjawisko desynapsji. W potomstwie jednych roślin, jak wykazała analiza genetyczna, powoduje ją pojedynczy gen recesywny, natomiast u innych objawy te zależą przypuszczalnie od dwóch działających genów. Jednak materiał do analizy genetycznej był szczupły i otrzymane wyniki wymagają potwierdzenia.

ЦИТОГЕНЕТИЧЕСКИЕ ИССЛЕДОВАНИЯ *LUPINUS MUTABILIS* SWEET. I. БЕСПЛОДИЕ, ОБУСЛОВЛЕННОЕ ДЕСИНАПСИЕЙ

Резюме

В настоящей работе исследовалась причина повторяющегося бесплодия у *Lupinus mutabilis*. Как показали цитологические анализы, уже в профазе мейоза часть хромосом имеет форму унивалентов. В метафазе I число унивалентов в клетках колеблется от 2 до 48, а число бивалентов — от 1 до 9. В среднем было $2{,}67_{II}$ и $42{,}64_{I}$ на одну материнскую клетку пыльцы. Первое и второе деления были нерегулярны: опоздывающие хромосомы, мосты и униваленты, делящиеся на хроматиды, встречались во время анафазы I; опаздывающие хромосомы и мосты также часто встречались во время телофазы II. Микроспороциты в процессе микроспорогенезы преображались в тетрады и полиады микроспор; иногда встречались монады и относительно часто диады микроспор. Ядро у части материнских клеток пыльцы (около 1,2%) дегенерировалось перед редукционным делением. Такие клетки не делились. Среди тетрад микроспоры отличались отсутствием ядра или содержали отстатки разбросанного хроматина.

Из проведённых исследований следует, что обнаруженное бесплодие у *Lupinus mutabilis* вызвано десинапсией. Как показывает генетический анализ, в поколении некоторых растений оно вызвано одним рецессивным геном, в то время как у других растений эти симптомы предположительно зависят от двух действующих генов. Однако, ввиду скромного материала, использованного для описанного генетического анализа, полученные результаты должны быть подтверждены.