Short title:

A STUDY OF TRISOMY IN *LOTUS PEDUNCULATUS* (LEGUMINOSAE)

Chen

A CYTOGENETIC STUDY OF TRISOMY IN LOTUS PEDUNCULATUS (LEGUMINOSAE)

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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June 1967

ACKNOWLEDGEMENTS

The author wishes to express his deep appreciation to Dr. W. F. Grant, Department of Genetics, McGill University at Macdonald College for his guidance throughout the course of this study and for his help during the preparation of the manuscript; to Dr. J. Stanley and Mr. P. Jui, Department of Genetics, McGill University for their help in statistical analyses; to Mr. I. Ogilvie for his technical assistance during certain periods of this study; and to Mrs. M. Couture for typing the manuscript. This study was made possible through the financial assistance of the National Research Council of Canada which is gratefully acknowledged.

ABSTRACT

Ph.D. Chi-Chang Chen Genetics

A CYTOGENETIC STUDY OF TRISOMY IN LOTUS PEDUNCULATUS (LEGUMINOSAE)

Seventy-three simple trisomics, two double trisomics and one telosomic trisomic plant of Lotus pedunculatus ($2n = 12$) were obtained from crossing triploids (derived from $4x \times 2x$ and $2x \times$ trisomic crosses) with diploids and from selfing triploids. Two additional telosomic trisomic plants were obtained from crossing simple trisomics with diploids. The simple trisomic plants were classified into five distinct morphological groups; each group was identified cytologically as belonging to one primary trisomic type. Meiotic behaviour and rate of trisome transmission were studied for four primary trisomic types. The frequency of trivalents was positively correlated with the length of the trisomes whereas transmission rate was negatively correlated. From the cytological evidence and breeding data, it was considered that the main factor which determined the extent of transmission of the extra chromosome was the ability of the $2n+1$ zygotes to develop viable seeds. The telosomic trisomics closely resembled their diploid siblings in external morphology, but it was not possible to identify the extra telosome cytologically. The extra telosome formed trivalents less frequently than the primary trisomes but was transmitted to the offspring at a greater frequency.

CLAIM TO ORIGINAL CONTRIBUTION

This is to certify that the study reported in this thesis constitutes the original work of the author and is his contribution to the knowledge of trisomy in Lotus pedunculatus (Leguminosae).

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	i
CLAIM TO ORIGINAL CONTRIBUTION	ii
LIST OF TABLES	v
LIST OF FIGURES	vii
INTRODUCTION	1
LITERATURE REVIEW	7
I. Sources and Occurrence of Trisomics	7
1. Primary trisomics	7
2. Secondary trisomics	14
3. Tertiary trisomics	16
4. Telosomic trisomics	18
II. Morphological Characteristics of Trisomics	20
III. Cytological Studies of Trisomics	27
1. Identification of the extra chromosome in trisomics	27
2. Chromosome associations at diakinesis and metaphase I	32
IV. Transmission of the Extra Chromosome in Trisomics	38
V. Genetic Studies of Trisomics	50
VI. Conclusion	54
MATERIALS AND METHODS	56
RESULTS	62
I. Morphology of the Diploid, Triploid and Tetraploid Plants	62
II. Meiosis in the Diploid, Triploid and Tetraploid Plants	67
1. The diploid (B-193)	67
2. The triploids (HR-2 and 10327)	69
3. The tetraploid (B-124)	71

Page

III. Production of Aneuploids from Triploids . . 77
IV. Morphology of Trisomics 80
1. Primary simple trisomics 80
2. Double trisomics 89
3. Telosomic trisomics 89
V. Cytological Identification of Trisomics . . 93
1. Karyotype of the diploid 93
2. The extra chromosome of the primary trisomics 99
3. The extra chromosomes of the double trisomics 99
4. The extra chromosome of the telosomic trisomics 103
VI. Meiosis in Trisomics 103
1. Primary simple trisomics 103
2. Telosomic trisomics 109
VII. Transmission of the Extra Chromosome in Trisomics 121
1. Primary simple trisomics 121
2. Telosomic trisomics 132
DISCUSSION 135
I. Production of Aneuploids from Triploids . . 135
II. The Effects of Extra Chromosomes on Plant Morphology 136
III. Association and Segregation of Trisomes in Meiosis 141
IV. Transmission of the Extra Chromosome in Trisomics 143
V. Production of Unrelated Trisomic Types from Primary Trisomics 151
VI. The Telocentric Chromosomes in L. peduncu-latus 153
VII. The Stability of Telocentric Chromosomes . . 155
SUMMARY 159
LITERATURE CITED 167

LIST OF TABLES

Table | Page

1. Measurements of some morphological characters of the diploid, triploid and tetraploid plants 63
2. Meiotic chromosome behaviour of the diploid, triploid and tetraploid plants 72
3. Pod- and seed-set in reciprocal crosses between diploid and triploids and in triploids selfed 78
4. Production of aneuploids from two triploids . 79
5. Distribution of morphological types of simple trisomics from two triploids 82
6. Mean value of some morphological characters of the diploid and the four primary simple trisomics 83
7. t tests for ten phenotypic traits of the diploid and the four primary simple trisomics. 85
8. Diagnostic morphological features of the diploid and the five primary simple trisomics. 86
9. The karyotype of Lotus pedunculatus based on the measurements of ten cells at somatic metaphase 96
10. Chromosome associations at diakinesis and/or MI of the different types of trisomics 104
11. Meiotic chromosome behaviour at first and second division in anaphase and telophase of the different types of trisomics 107
12. Crosses of trisomic ♀ x diploid ♂ in Lotus pedunculatus 122

Table

Page

13. Crosses of diploid ♀ x trisomic ♂ in L. pedunculatus 123

14. Self-pollination of trisomic plants in Lotus pedunculatus 124

15. Transmission of the extra chromosome in trisomic ♀ x diploid ♂ crosses 128

16. Transmission of the extra chromosome in diploid ♀ x trisomic ♂ crosses 129

17. Transmission of the extra chromosome in self-pollinated trisomics 130

18. Transmission of the extra telosome 133

LIST OF FIGURES

Figure		Page
1-4	Mature diploid, triploid and tetraploid plants of Lotus pedunculatus	64
5-8	Leaves of diploid, triploid and tetraploid plants	65
9-12	Flowers of diploid, triploid and tetraploid plants	66
13	Pistils of diploid, triploid and tetraploid plants	66
14-17	Meiosis in the diploid plant	73
18-21	Meiosis in the two triploid plants	74
22-25	Meiosis in the two triploid plants	75
26-29	Meiosis in the tetraploid plant	76
30-35	Mature plants of diploid Lotus pedunculatus and five primary simple trisomic types . . .	90
36-41	Leaves of a diploid plant of Lotus pedunculatus and five primary trisomic types	91
42-46	Flowers of a diploid plant of Lotus pedunculatus and four primary simple trisomic types	92
47	Pistils of a diploid plant of Lotus pedunculatus and four primary simple trisomic types	92
48	Somatic chromosomes of Lotus pedunculatus .	95
49	Idiogram of the haploid chromosomes of Lotus pedunculatus..	97
50	Somatic chromosomes of the trisomic type Round showing chromosome 1 is in triplicate	100

Figure		Page
51	Somatic chromosomes of the trisomic type Round showing chromosome 1 is in triplicate	100
52a	Somatic chromosomes of the trisomic type Broad in which chromosome 3 is in triplicate	100
52b	An interpretive drawing of the chromosomes in Fig. 52a	100
53a	Somatic chromosomes of the trisomic type Pointed in which chromosome 4 is in triplicate	101
53b	An interpretive drawing of the chromosomes in Fig. 53a	101
54a	Somatic chromosomes of the trisomic type Pointed in which chromosome 4 is in triplicate	101
54b	An interpretive drawing of the chromosomes in Fig. 54a	101
55	Somatic chromosomes of the trisomic type Narrow showing chromosome 5 in triplicate .	102
56	Somatic chromosomes of the trisomic type Narrow showing chromosome 5 in triplicate .	102
57	Somatic chromosomes of the trisomic type Small showing chromosome 6 in triplicate . .	102
58	Somatic chromosomes of one double trisomic plant showing that a deficient chromosome 4 and probably a whole chromosome 6 are in triplicate	102
59-62	Meiosis in the trisomic type Broad	110
63-66	Meiosis in the trisomic type Broad	111
67-70	Meiosis in the trisomic type Pointed	112
71-74	Meiosis in the trisomic type Pointed	113
75-78	Meiosis in the trisomic type Narrow	114

Figure		Page
79-80	Meiosis in the trisomic type Narrow	115
81-84	Meiosis in the trisomic type Small	116
85-88	Meiosis in the trisomic type Small	117
89	Correlation between length of triplicated chromosome and frequency of trivalent formation of the four trisomic types, Broad, Pointed, Narrow, and Small	118
90-93	Meiosis in one telosomic trisomic, 00004 . .	119
94-97	Meiosis in one telosomic trisomic, 10458 . .	120
98	Correlation between transmission rate and length of triplicated chromosome of the four trisomic types, Broad, Pointed, Narrow, and Small	131

INTRODUCTION

Flowers in the crannied wall,
I pluck you out of the crannies,
I hold you here, root and all, in my hand,
Little flower--but if I could understand
What you are, root and all, and all in all,
I should know what God and man is.
--Tennyson

In 1919, following the genetic analyses by Morgan and his colleagues of mutants in Drosophila which provided much of the early information on the behaviour of the gene, a new type of mutation was reported in Datura stramonium by Blakeslee and Avery. This mutation was of a sudden, though rather rare, occurrence and the mutant plants were distinguished from normal ones not only by single visible trait differences, but also by a complex of characters which seemed to be transmitted as a whole to their offspring. When these mutants were selfed or used as female parents in crosses with normal plants about one fourth of the progeny were of the mutant type; when these mutants were used as male parents in crosses, however, few, if any, mutant offspring resulted. Therefore, the mutant characters seemed to be inherited in a manner different from that shown by simple Mendelian

ones. An approach considered for elucidating this problem was a study of the chromosomes of these plants to see if they could furnish a possible clue to the peculiar pattern of transmission of the mutant characters. This proved to be a most profitable one, as in addition to the normal complement of chromosomes an extra chromosome was found in each mutant plant.

As a result of Belling's cytological work, Blakeslee, Belling and Farnham (1920) discovered that each of the mutants in Datura stramonium is associated with the duplication of a different individual chromosome; thus the mutant plants are trisomics having one more chromosome than "wild type" plants with the normal diploid number. Different types of trisomics have been found in Datura and other organisms and they have been designated as primary (Blakeslee, 1921a), secondary (Blakeslee, 1924), tertiary (Belling and Blakeslee, 1926) and telosomic trisomics (Burnham, 1962). Primary trisomics have one extra chromosome which is homologous with one of the chromosome pairs of the diploid complement. In secondary trisomics the extra chromosome has the doubled half of one of the primaries. Tertiary trisomics have one extra chromosome which is made up of two different primary chromosomes as a result of an interchange. In telosomic trisomics, the extra chromosome

has only one arm of one of the primaries attached to a centromere.

The usefulness of trisomy in understanding cytogenetical problems was recognized soon after its discovery in Datura. Since the primary trisomic types can be distinguished from each other and from the normal diploids in several morphological characters, they have made it possible to analyze the influence of individual chromosomes on both the morphology and physiology of the plants. Comparisons in external and internal morphology between normal and different primaries, between normal, primary trisomics and tetrasomics, and between primaries and their two secondaries provide for the concept that character expression is a matter of balance between a large number of genes acting in different ways (Blakeslee, 1922; Sinnott, Houghtaling and Blakeslee, 1934). Almost every chromosome or part of a chromosome provides some specific effect on a given character, tending either to increase or decrease its relative development. The diploid condition seems merely to consist of an equilibrium which is established by these various tendencies. The recent finding that some multiple congenital anomalies and certain cases of mental retardation in humans are caused by the presence of single extra chromosomes has shown that the genic balance concept

applies equally well to humans.

Primary trisomics provide a means of localizing genes on specific chromosomes as well as identifying chromosomes with their respective linkage groups, because the presence of an additional chromosome to a complement modifies the expected genetic ratio for the genes on that chromosome. Blakeslee, Belling and Farnham (1920) first outlined the principle for this analysis and identified the gene "white" with trisomic type "Poinsettia" in Datura stramonium. By using this method, McClintock and Burnham were able to identify nine linkage groups with their respective chromosomes in maize. The value and efficiency of the trisome method for locating genes on specific chromosomes have been demonstrated now in various organisms and even in man. The secondary, tertiary and telosomic trisomics can be used further to determine which genetic factors are carried in each arm of a particular chromosome.

By using plants trisomic for chromosome 10, Brink (1959) was able to show that paramutation in the R locus in maize is not dependent upon conjugation of paramutable and paramutagenic alleles at zygonema in meiosis. The implication of Brink's work is that trisomics may be used as a powerful tool to investigate

genetic phenomena if experiments are carefully designed.

The tetraploid birdsfoot trefoil (Lotus corniculatus L., 2n = 24) is one of our leguminous species which has gained considerable importance in North America since its potentialities as a forage crop were first shown in 1934. In comparison with other polyploid crops such as wheat, cotton and tobacco, trefoil breeding has progressed much slower due to the lack of basic cytogenetic knowledge in Lotus. In order to ultimately obtain information on L. corniculatus which would provide both basic cytogenetic knowledge and also make it possible to establish effective breeding procedures, it is considered that, at the present time, further pertinent cytogenetic information is required on some of the closely related diploid species. In spite of the many uses of trisomy in understanding cytogenetical problems, to the author's knowledge, no study has been initiated on this aspect in Lotus to date. This is not surprising since interest in the genus as a whole has developed only in the last couple of decades. The study to be reported in this dissertation was planned as an attempt: first, to establish a complete series of primary trisomics in the diploid species L. pedunculatus (2n = 12), secondly, to define the morphological characteristics of these trisomics which might distinguish them from each other and

from normal diploids, thirdly, to identify the extra chromosomes by which the trisomic types may be distinguished, and finally, to study the rate of transmission of these trisomics. There were several reasons which led to the selection of L. pedunculatus for this study: (1) it is one of the diploid species closely related to L. corniculatus (Brand, 1898; Harney and Grant, 1964); (2) it has some useful characters to be transferred to L. corniculatus (Bent, 1958); (3) it has more than ten flowers per umbel and the flowers are larger than other diploid species, therefore emasculation can be performed more easily; (4) it is a perennial so that once the trisomics are established it would be possible to maintain them more easily.

LITERATURE REVIEW

In 1919 Blakeslee and Avery first reported the peculiar breeding behaviour of some mutant types in Datura. The next year (1920) Blakeslee, Belling and Farnham provided the chromosomal basis for these mutant types and identified one gene to a specific chromosome by means of trisomic ratios. The year 1920, therefore, forms the starting point for cytogenetic studies of trisomy. Since then, trisomics have been studied in an ever increasing number of plant species, and even in animals and man. A review of the pertinent literature in this field will be presented here.

I. Sources and Occurrence of Trisomics

1. Primary trisomics

Primary trisomics occur spontaneously, though with a rather low frequency, in the progeny of normal diploids. The "Globe" mutant in Datura stramonium, which was the first example of a primary trisomic found in plants, originated in this manner (Blakeslee and Avery, 1919). Blakeslee (1924) later reported the spontaneous occurrence of some 259 primary trisomics belonging to

11 different types among 32,792 offspring of normal diploid Datura. Other examples of primary trisomics that were found in the progeny of normal plants are Matthiola incana (Frost and Mann, 1924; Frost, 1927; Prakken, 1942), Crepis tectorum, C. setosa (Babcock and Navaschin, 1930), Antirrhinum majus (Stubbe, 1934), Secale cereale (Tagaki, 1935), Nicotiana sylvestris (Goodspeed and Avery, 1939) and Hordeum vulgare (Smith, 1941).

Belling and Blakeslee (1924) found eight cases of non-disjunction out of 1,137 pollen mother cells (PMC's) of normal diploids. This mechanism was calculated to produce about 0.4% of pollen grains with 13, rather than 12, chromosomes, which in turn accounted for the spontaneous occurrence of primary trisomics in Datura stramonium. Frost (1927) observed about 4-5% chromosome-mutant types in the variety Snowflake of Matthiola incana. Lesley and Frost (1927) reported that this variety, Snowflake, had "long" first metaphase chromosomes in the PMC's in comparison with other varieties which had shorter chromosomes at this stage. Chromosome abnormalities such as non-disjunction, fragmentation and non-reduction were always found in PMC's from Snowflake plants and would account for the production of trisomics. The appearance of long chromosomes at first metaphase

was found to be conditioned by a single recessive gene. However, one trisomic plant was later found in the short chromosome type of Matthiola (Prakken, 1942). In this case, the cytological causes underlying the production of the trisomic plant were not known.

Non-disjunction was also found responsible for the spontaneous origin of primary trisomics in animals. In Drosophila melanogaster, two cases, triplo-X (Bridges, 1922) and triplo-IV (Little, 1921), were discovered to be the result of non-disjunction. In humans, three of the 22 autosomal trisomies have been reported already (see Smith, 1964); they are Down's syndrome (Lejeune, Gautier and Turpin, 1959; quoted from Lejeune and Turpin, 1961), E trisomy syndrome (Edwards, Harnden, Cameron, Crosse and Wolff, 1960) and D_1 trisomy syndrome (Patau, Therman, Smith and DeMars, 1961). The general frequency of Down's syndrome was about 1.6 per 1,000 births, but the incidence varied with maternal age (Smith, 1964). Very limited data have been obtained on the frequency of the other two autosomal trisomics. From a recent report by Conen and Erkman (1966), estimates of the frequency of the E and D_1 syndromes vary considerably according to the individuals who made the study. A triplo-X human female was first reported by Jacobs, Baikie, Court-Brown, MacGregor, MacLean and Harnden(1959). Approximately

45 such individuals have been described (Miller, 1964).

In plants, most cases of primary trisomics have been obtained directly or indirectly from autotriploids. Blakeslee (1924) secured 12 primary trisomic types in Datura stramonium ($2n = 24$) by selfing triploids; or by crossing triploids as female parents with normal diploids. In Lycopersicon esculentum ($2n = 24$), Lesley (1928) was able to isolate 9 of the 12 primary trisomic types from the progeny of a triploid ♀ x diploid ♂ cross and added two more in 1932. Using a different variety of tomato, Rick and Barton (1954) obtained 342 trisomic plants among 783 offspring in the progeny of triploids (43.7%). These trisomic plants could be divided into 12 morphological types. Later, in a more primitive variety of tomato, Red Cherry, Rick and Notani (1961) secured 32 simple trisomics among 94 plants also from the progeny of triploids. Complete series of primary trisomics were established from triploids in other plant species such as Hordeum spontaneum var. transcaspicum ($2n = 14$, Tsuchiya, 1954, 1958, 1960, 1963), Spinacia oleracea ($2n = 12$, Tabushi, 1958; Janick, Mahoney and Pfahler, 1959), Antirrhinum majus ($2n = 16$, Rudorf-Lauritzen, 1958; Sampson, Hunter and Bradley, 1961), Secale cereale ($2n = 14$, Kamanoi and Jenkins, 1962), and Arabidopsis thaliana ($2n = 10$, Steinitz-Sears, 1963). Of the 12

primary trisomic types in Nicotiana sylvestris (2n = 24, Goodspeed and Avery, 1939, 1941) seven were obtained from progenies of triploid x diploid crosses. Triploids were used to produce trisomics in Zea mays (2n = 20, McClintock, 1929), Crepis capillaris (2n = 12, Babcock and Navaschin, 1930), Beta vulgaris (2n = 18, Levan, 1942; Butterfass, 1964), Hordeum aesitivum (2n = 14, Kerber, 1954), Clarkia unguiculata (2n = 18, Vasek, 1956), Collinsia heterophylla (2n = 14, Garber, 1964), Collinsia tinctoria (2n = 14, Chomchalow and Garber, 1964), Lolium perenne (2n = 14, Myers, 1944), Sorghum vulgare (2n = 20, Price and Ross, 1955), and Oryza sativa (2n = 24, Sen, 1965) although a complete series of primary trisomics was not established in any of these species.

Lesley (1926) secured the first primary trisomic plant in tomato from the selfing of a double trisomic. Blakeslee and Avery (1938) reported 39.87% simple trisomics in the progeny of various double trisomic types. The twelfth primary simple trisomic, "Stiff," of Nicotiana sylvestris appeared in the progeny of a double trisomic ♀ x diploid ♂ cross (Goodspeed and Avery, 1941). In Hordeum spontaneum var transcaspicum, all the seven primary trisomic types were obtained from double or triple trisomics (Tsuchiya, 1960).

Hypo- and hyper-triploids provide another source for the production of trisomic plants. Six simple primary trisomics were observed in progenies of a hypo-triploid barley plant ($2n = 20$) of a cultivated two-rowed variety (Tsuchiya, 1952). Jakob (1963) reported a trisomic male castor bean plant (Ricinus communis, $2n = 20$) in the offspring from open pollination of a monoecious hypotriploid. In Antirrhinum majus, 68 crosses made by Rudorf-Lauritzen (1958) between hypo- and hyper-triploids ($3x \pm 1$ or 2 chromosomes) and diploids resulted in only two seeds being produced. She did not mention, however, whether the seeds gave rise to normal or aneuploid individuals. Chomchalow and Garber (1964) obtained trisomic plants in progenies of hypotriploids ($3x-1$, $3x-2$) of Collinsia tinctoria.

Goodspeed and Avery (1939) reported that asynaptic plants of Nicotiana sylvestris gave rise to at least 134 primary trisomic plants of ten types among 2,873 plants. Burnham (1946) found one barley plant had long chromosomes at first metaphase of microsporogenesis similar to that reported in Matthiola incana (Lesley and Frost, 1927). Two univalents were frequently observed at metaphase I which resulted from failure of the two homologues to associate with each other. He predicted the occurrence of trisomics in the progeny of this

asynaptic plant which was later confirmed by McLennan (1947). Dyck (1964) reported obtaining six of the seven trisomic types in Avena strigosa ($2n = 14$) from one asynaptic stock. Shah (1964) obtained one trisomic plant in Dactylis glomerata ($2n = 14$) from crosses of an asynaptic plant with a normal diploid.

The frequency of chromosomal mutants increased after Datura seeds were subjected to radium treatment. Gager and Blakeslee (1927) obtained 15 primary trisomic plants of eight different types among 113 seedlings after such treatment. Goodspeed and Avery (1939) reported that in Nicotiana sylvestris, primary trisomics occurred at least twice (0.6%), and possibly six times (1.8%), as often in populations treated with X-ray as they did in progenies from untreated diploids (0.3%). Five morphological variants from X-ray treatment of embryo-sac mother cells, or of seeds, proved to be primary trisomics of three different types.

Trisomics were detected in the progenies of Collinsia heterophylla treated with colchicine (Soriano, 1957). Dhillon and Garber (1962) found that the trisomes obtained by colchicine treatment simulated supernumerary chromosomes in their cytogenetic characteristics. Garber (1964) postulated that C. heterophylla might have an unusual or pseudosupernumerary chromosome in its haploid

complement which could respond to colchicine treatment by undergoing non-disjunction.

Sutton (1939) obtained one primary trisomic in Pisum sativum ($2n = 14$) from selfing trisomic interchange heterozygotes. Catcheside (1954) reported seven primary trisomic types in Oenothera blandia ($2n = 14$) and Ramage (1960) also reported several different primary trisomics in barley, all from interchange heterozygotes. More recently, Garber (1964) observed one trisomic plant in Collinsia heterophylla in progeny from a self-pollinated plant with an interchange complex of four chromosomes.

2. Secondary trisomics

Secondary trisomics were first reported by Blakeslee (1924) and Belling and Blakeslee (1924) in Datura stramonium which occurred occasionally from the corresponding primaries or from triploids and also from diploids and other unrelated primaries. Only 14 of the 24 possible secondaries have been reported in Datura (Blakeslee and Avery, 1938; Avery, Satina and Rietsema, 1959). Philp and Huskins (1931) showed that the "Smooth" Matthiola plant reported by Frost (1927), which originated from the diploid variety Snowflake, was in reality a secondary trisomic. In maize, Rhoades (1933)

observed a secondary trisome of chromosome 5 among the progeny from a selfed plant trisomic for chromosome 6. Later, he (1940) obtained the same secondary trisome by crossing plants trisomic for the short arm of chromosome 5 to normal diploids. Goodspeed and Avery (1939) believed that they found several secondary trisomics in Nicotiana sylvestris. Their classification was based merely on the appearance of the plants and not on a cytological examination.

Several cases of secondary trisomics have been reported in tomato. One originated from a cross between a double trisomic (triplo-BC) and a normal diploid, and the other from a cross between a deficient diploid (one chromosome had 1/3 of the original length) and a normal diploid (Lesley and Lesley, 1941). Sen (1952) observed a secondary trisome of chromosome 9 of the tomato in F_1 mutants resulting from an experiment on chemical mutagenesis. Moens (1965) obtained a fourth secondary trisomic plant in tomato with two identical short arms of chromosome 2 from its corresponding primary. More recently, Rick and Khush (1966) reported obtaining several secondary trisomics in tomato after the pollen was submitted to X-ray treatment.

Belling and Blakeslee (1924) suggested that

secondary chromosomes might originate from an occasional reversed crossing-over, or interchange of non-homologous segments between two chromosomes of a trivalent. Upcott (1937), Koller (1938) and Darlington (1939) showed that the centromere of an univalent chromosome sometimes divided transversely at meiosis in such a way that the two short arms are joined together and the two long arms may become attached to the same centromere. Darlington (1940) stated that he observed the formation of iso-chromosomes in microspores of Fritillaria resulting from the delayed division of newly arisen telocentric chromosomes. Rhoades (1940) suggested that in maize, secondary chromosomes might arise in meiosis from misdivision of the centromere of the telocentric chromosomes. The resulting secondary chromosomes were occasionally incorporated into the generative nuclei during the first microspore division, whereas the vegetative nuclei contained the normal haploid number of chromosomes.

3. Tertiary trisomics

The first report of a tertiary trisomic plant was the "Wiry" mutant in Datura which occurred in the offspring of a primary trisomic plant Poinsettia when the latter was crossed to another race with chromosomes modified by a segmental interchange (Belling and

Blakeslee, 1926). Occasionally the configuration of four chromosomes of an interchange heterozygote will undergo 3:1 disjunction at anaphase I, thus the resulting n+1 gametes may account for the origin of tertiary trisomics. Tertiary trisomics derived from interchange heterozygotes have been described in Datura (Belling and Blakeslee, 1926; Blakeslee and Avery, 1938; Avery, Satina and Rietsema, 1959), maize (Burnham, 1930, 1934), Pisum sativum (Sutton, 1939), Oenothera (Gates, 1923; Catcheside, 1933, 1954) and Hordeum vulgare (Burnham, White and Livers, 1954; Hagberg, 1954; Ramage, 1960).

Tertiary trisomics have appeared spontaneously only very rarely. According to an estimate of Avery, Satina and Rietsema (1959), from among approximately two million Datura plants, tertiary trisomics have arisen on only six instances spontaneously from untreated or homozygous parents. On the other hand, the occurrence of tertiary trisomics could be greatly increased by certain treatments. Garger and Blakeslee (1927) found two tertiary trisomics among 113 seedlings in the progeny of a normal Datura plant treated with radium. Goodspeed Avery (1939) obtained 12 tertiary trisomics in Nicotiana sylvestris from various sources including X-ray treatment of embryo-sac mother cells and seeds.

Rick and Barton (1954) and Rick, Dempsey and Khush (1964) obtained a tertiary trisomic plant in tomato in the progeny of a triploid. This plant could not be satisfactorily analyzed cytologically but was interpreted from genetic evidence as a tertiary trisomic in which the extra chromosome had arms of chromosomes 5 and 10.

Avery, Satina and Rietsema (1959) stated that of the 264 theoretically different tertiary trisomic types in Datura stramonium that are possible about 30 of them have been obtained.

4. Telosomic trisomics

Burnham (1962) first used the term telosomic trisomic to describe plants having an extra telosome (telocentric chromosome) in their normal complement. When a telosome consists of the short arm of a submetacentric or subtelocentric chromosome it is usually very small in size in comparison with a normal chromosome and as a result the telosome has been considered as a "fragment" by several authors (Lutz, 1916; Frost, 1927; Lesley and Lesley, 1929; Blakeslee and Avery, 1938).

Belling (1927) first recognized that the "fragments" he observed in Datura and those reported by Lutz (1916) in Oenothera are not fragments but consist of one

arm of a chromosome possibly attached to a centromere. He called plants with an extra half chromosome in their normal complement as "another kind of secondaries" to distinguish them from "true secondaries" in which the extra member has two identical arms. Lesley and Lesley (1929) adopted this terminology for two plants in tomato.

Chromosome fragmentation was thought to be one of the possible mechanisms for the production of telosomic trisomics. Gates and Thomas (1914) observed that the chromosomes in one trisomic plant of Oenothera lata sometimes fragmented. Lutz (1916) obtained two Oenothera mutants with an extra diminutive chromosome from trisomic O. lata x diploid O. Lamarkia. She postulated that the extra chromosome in the trisomic plant sometimes fragmented during meiosis which would occasionally produce eggs with an extra "fragment" in the normal haploid complement. Fragmentation has been observed in the PMC's of the Matthiola variety Snowflake (Lesley and Frost, 1927) and two chromosomal mutants, "Large" and "Slender," have been found to have a "fragment" in addition to the normal complement.

Lesley and Lesley (1929) reported in PMC's of two mutant tomato plants a "fragment" of about half the size of the normal chromosomes in addition to the normal 12

pairs. One of these mutants appeared in the progeny of double trisomics, the other in a triploid x diploid cross. Rhoades (1936) found one telosomic trisomic in maize among the progeny of a plant trisomic for chromosome 5. In this case, the telosome consisted of the short arm of chromosome 5 arising through misdivision of the centromere. Blakeslee and Avery (1938) obtained a telosomic trisomic 2n+.11 from a secondary 2n+11.11 in Datura. Smith (1947) secured one plant with an extra telocentric chromosome from X-ray treatment of dormant seeds of Triticum monococcum. The telosome arose as the result of breakage at the centromere.

II. Morphological Characteristics of Trisomics

The primary trisomics of Datura could be distinguished from each other and from normal diploids in several morphological characters. These included differences in plant size, growth habit, and leaf, flower, stigma, capsule and spine size and shape (Blakeslee, 1922, 1934; Blakeslee and Avery, 1938; Avery, Satina and Rietsema, 1959) and internal anatomy (Sinnott, Houghtaling and Blakeslee, 1934). Some differences were minute and inconspicuous, others gross and striking; some were qualitative and others quantitative. Each trisomic type represented a combination of several characters. Thus

the primary trisomics of Datura could be arranged into 12 distinct morphological groups corresponding to the haploid number of chromosomes of this genus (Blakeslee and Belling, 1924). The same general phenomenon has been reported in other genera of plants such as Matthiola (Frost and Mann, 1924; Frost, 1927; Prakken, 1942), Lycopersicon (Lesley, 1928, 1932; Rick and Barton, 1954), Nicotiana (Goodspeed and Avery, 1939, 1941), Beta (Levan, 1942; Butterfass, 1964), Oenothera (Catcheside, 1954), Antirrhinum (Stubbe, 1934; Rudorf-Lauritzen, 1958; Sampson, Hunter and Bradley, 1961), Hordeum (Tsuchiya, 1958, 1960; Ramage, 1960), Spinacia (Tabushi, 1958; Janick, Mahoney and Pfahler, 1959), Secale (Kamanoi and Jenkins, 1962), Arabidopsis (Steinitz-Sears, 1963) and Oryza (Sen, 1965).

Blakeslee (1921a, 1922, 1934) interpreted that each primary trisomic type in Datura contained one different extra chromosome, and that the morphological and physiological differences resulted from the change in genic balance brought about by the added chromosomal material to the normal complement. Support for this theory of genic balance was obtained by Frost (1927) from Matthiola, by Sinnott, Houghtaling and Blakeslee (1934) from Datura and by Goodspeed and Avery (1939) from Nicotiana. Frost (loc. cit.) observed that in

Matthiola, different trisomics differed in opposite directions from the normal type in various characters. Sinnott, Houghtaling and Blakeslee (1934) compared the mean value of the 12 primaries of Datura with the value for the diploid in various anatomical characters of the flower stalk. They found that individual trisomic types varied in different directions from the diploid, but the mean value of the 12 trisomic types was very close to the diploid value for most characters studied. Goodspeed and Avery (loc. cit.) examined and measured a number of qualitative and quantitative characters of ten trisomic types of Nicotiana. They found that the value of the diploid was also close to the average of the trisomics. In contrast to these positive findings, Sampson, Hunter and Bradley (1961) showed that most of the means of the quantitative characters for the Antirrhinum trisomics were slightly lower than the diploid means.

Another interesting trend of phenotypic variations in primary trisomics was observed by Goodspeed and Avery (1939). In each of the primary trisomic types of Nicotiana sylvestris, a change in a certain direction from the diploid in one organ was accompanied by a similar change in other organs. To this, Goodspeed and Avery wrote about the trisomics Enlarged, Narrow, Pointed and Stubby:

> Enlarged is enlarged not only in the length of the flower, but in the length of the internodes, the leaf, the pedicels, the capsule and the calyx; Narrow is narrow in leaf, flower and capsule; Pointed has leaves with pointed tips and flowers with pointed lobes, while Stubby has these same parts rounded.

Similar observations as found in Nicotiana were reported in the tomato (Rick and Barton, 1954) and Antirrhinum (Sampson, Hunter and Bradley, 1961). This phenomenon of correlated variation for several characters in primary trisomics has also been found in Datura and in several other plant genera although the authors have not specifically pointed out this fact in their papers.

In humans, for each of the three established autosomal trisomies, the process of embryo development was upset. This resulted in a pattern of multiple defects which allowed for a clinical diagnosis. Smith (1964) listed more than 20 common and 20 less common defects for Down's syndrome, 20 common and 14 less common defects for E trisomy syndrome, and 21 common and 20 less common defects for D_1 trisomy syndrome. Miller (1964) stated that there was no distinctive clinical picture for the triplo-X female, and most of them had no sexual abnormalities.

In contrast to those organisms just mentioned, trisomics of Sorghum derived from triploids did not show

any characteristic phenotype from which they could be distinguished from normal diploids (Price and Ross, 1957). Only two of the primary trisomics in maize were strikingly different from their diploid sibs. Plants trisomic for chromosome 5 had broader leaves with blunter tips (Rhoades, 1933), whereas those for chromosome 7 had much narrower and stiffer leaves (Burnham, 1962). Other trisomics tended to be only a little shorter and a little less vigorous, and were not recognizable phenotypically from diploids (McClintock, 1929). The trisomic plants of Clarkia unguiculata were variable but they fell within the range of variation of the diploid strain from which they were derived (Vasek, 1956). Vasek (1963b) attempted to identify them from a statistical analysis of 15 phenotypic traits without success. The simple trisomics of Collinsia heterophylla were usually less vigorous, slender and shorter than the diploids, but attempts to determine whether such phenotypic differences could be used to identify the trisomics were not successful (Dhillon and Garber, 1960; Garber, 1964). In another species of Collinsia, C. tinctoria, Chomchalow and Garber (1964) were able to arbitrarily put the trisomic plants into seven morphological groups based on plant height, size of leaves and flowers. But breeding experiments showed that only one trisomic type (Group I)

could transmit the mutant characters to the offspring. All the other trisomics produced either diploid progeny or some trisomic plants which could not be distinguished morphologically from the diploid sibs. They concluded that with the possible exception of the trisomics of Group I, different trisomics of C. tinctoria could not be distinguished from one another and from normal diploids.

In Datura, primary trisomics were usually found to be intermediate between their two corresponding secondaries in expression of most characters. Blakeslee and Avery (1938) observed that the secondary 2n+1.1 seedling had very narrow leaves due to factors in the .1 arm, those of its related secondary 2n+2.2 were relatively broad due to factors in the .2 arm, while those of their primary 2n+1.2 were intermediate in width due to factors in both arms acting in different directions. Sinnott, Houghtaling and Blakeslee (1934) observed that the shape of the vascular bundle in the flower stalk of the 2n+1.1 secondary was relatively narrow, in the 2n+2.2 relatively broad, and in the 2n+1.2 primary intermediate between them.

In maize and tomato, in which only one of the two secondaries of a primary has been established, the

primary was usually intermediate between the diploid and the secondary in character expression. Rhoades (1933) noted that certain of the characteristics which distinguished the primary trisome of chromosome 5 in maize were exaggerated in the secondary. Sen (1952) observed that in tomato, the primary trisome of chromosome 9 was intermediate in growth habit between the secondary and the normal. One exception to this rule was found in one secondary trisomic in tomato in which the extra chromosome consisted of two heterochromatic short arms of chromosome 2 (Moens, 1965). Phenotypically, this secondary trisomic could not be distinguished from normal diploids whereas the plant trisomic for chromosome 2 was distinct for several morphological characters.

It has been shown by Blakeslee and Avery (1938) that the phenotypical characteristics of some tertiary trisomics in Datura represented the influence of two parts of different chromosomes. In the 2n+2.17 tertiary, the leaves were depressed at the base like those of the 2n+17.17 but their width was increased due to the .2 arm. The "Sticky" mutant of Nicotiana sylvestris has been demonstrated cytologically to be a tertiary trisomic of two primary types Recurved and Compact by Goodspeed and Avery (1939). They observed that in many morphological characters Sticky was intermediate between Recurved and

Compact, or represented the sum of the character expressions in these two primaries.

Plants trisomic for a "fragment" or telosome in Oenothera (Lutz, 1916), Matthiola (Frost, 1927) and Triticum monococcum (Smith, 1947) could be distinguished from normal diploids in several morphological characters. Lesley and Lesley (1929) noted that the two tomato mutants with an additional "chromosome fragment" were not as easily distinguished morphologically from diploids as the mutants with a whole extra chromosome. Based on the gene balance hypothesis, they explained that a "chromosome fragment" should cause less disturbance of the normal balance than a whole extra chromosome. Rhoades (1936) also observed that one telosomic trisomic plant in maize with an extra short arm of chromosome 5 was intermediate in appearance between the primary trisomic for chromosome 5 and disomic individuals.

III. Cytological Studies of Trisomics

1. Identification of the extra chromosome in trisomics

It has been shown by Belling and Blakeslee (1922, 1926) that the meiotic chromosomes of Datura stramonium differed considerably in size, and they could be arranged into six classes. Applying special techniques in order

to study the chromosomes in pollen mitoses, Satina, Bergner and Blakeslee (1941) later found that the somatic chromosomes of Datura stramonium differed one from another not only in length but also in morphological structures. Adopting Blakeslee and Cleland's (1930) numerical terminology to describe Datura chromosomes, in which the largest chromosome was designated 1.2 and the smallest 23.24, Satina, Bergner and Blakeslee (1941) and Avery, Satina and Rietsema (1959) were able to identify and number the extra chromosomes of the 12 primaries, the 14 known secondaries and some tertiaries. The primary trisomic Rolled was called 2n+1.2 type, and its two secondaries Polycarpic and Sugarloaf were 2n+1.1 and 2n+2.2, respectively.

Goodspeed and Avery (1939) reported that the 12 chromosomes of Nicotiana sylvestris could be distinguished from each other at metaphase of the microspore mitosis. These chromosomes were named M^1, M^2, M^3, SM^1, SM^2_s, SM^3, SM^4, SM^5, ST^1_s, ST^2, ST^3, and ST^4 in the order of decreasing length. Enlarged was trisomic for SM^1, Late for M^3 and Compact for SM^3. They could not identify the extra chromosomes of Pointed and Stubby with certainty, the former was considered probably trisomic for SM^4 and the latter either for ST^2 or for ST^3.

Philp and Huskins (1931) reported that they were able to distinguish each of the seven chromosomes of Matthiola in root tip cells from their chromosome length and centromere position. These chromosomes were assigned letters A to G, A being the largest and G the smallest. They identified the triplicated chromosome in trisomic Crenate as chromosome A. Later, based on a study of chromosome morphology at mitosis and meiosis, Prakken (1942) identified the extra chromosome of another trisomic of Matthiola, Rosette, as probably F.

The somatic chromosomes of Spinacia oleracea were numbered from A to F by Tabushi (1958) and from 1 to 6 by Ellis and Janick (1960) on the basis of their length. Tabushi (loc. cit.) named the four trisomic types he obtained triplo-C, triplo-D, triplo-E and triplo-F. Ellis and Janick (loc. cit.) analyzed the karyotypes of the six morphological trisomic types and found that Reflex was trisomic for chromosome 1, Oxtongue for chromosome 2, Star for chromosome 3, Curled for chromosome 4, Wild for chromosome 5, and Savoy for chromosome 6.

The four largest chromosomes of barley are closely similar as to length and centromere position, therefore they have not been distinguished from each other in somatic metaphases. From karyotype analyses of root tip

cells, Tsuchiya (1959, 1960, 1963) was able only to identify the extra chromosomes of Pseudo-normal, Purple and Semi-erect as chromosomes 5, 6 and 7, respectively. He further analyzed the barley trisomics by the use of Burnham's interchange testers in which the chromosomes have already been identified (Tsuchiya, 1961). The seven trisomic types were crossed as females with interchange testers and meiosis of the trisomic F_1 hybrids was studied. His results showed that the extra chromosomes of Bush, Slender, Pale and Robust were chromosomes 1, 2, 3 and 4, respectively. At the same time he was also able to confirm the results from karyotype analysis for the trisomics Pseudo-normal, Purple and Semi-erect.

Tsuchiya's (1961) idea of using interchange testers to identify the extra chromosomes of barley trisomics was not new. In 1954, Catcheside already identified the extra chromosomes of the seven trisomic types of Oenothera blandina by crossing each trisomic with various standard interchange complexes. The numerical formula of the seven chromosomes of O. blandina were 1.2, 3.4, 5.6, 7.10, 8.13, 9.14 and 11.12. Lanceolate was found to be trisomic for 1.2, Whitish for 3.4, Broad for 5.6, Linear for 7.10, Glossy for 8.13 or 9.14, Blunt for 9.14 or 8.13, and Golden for 11.12.

It has been indicated by McClintock and Hill (1931), Rhoades and McClintock (1935), Emerson, Beadle and Fraser (1935) and Einset (1943) that the extra chromosomes of nine of the ten primary trisomics of maize have been determined from pachytene analysis. Barton (1950) showed that the pachytene chromosomes of tomato differed in chromatin patterns, in relative lengths of the different segments, and in arm ratios. Rick and Barton (1954) and Rick, Dempsey and Khush (1964) were able to identify the 12 trisomic types of tomatoes at prophase of meiosis. These trisomics were designated triplo-1 through triplo-12. Sen (1965) classified five trisomic types in rice as triplo-I, J, G, E, and B according to chromosome morphology at pachynema. Jakob (1963) assigned the extra chromosome of one trisomic castor bean plant to chromosome C from a pachytene study. Levan (1942) was able to identify the extra chromosomes of two trisomics in sugar beets from a meiotic analysis; Mosaic was trisomic for the longest chromosome, and Horseradish trisomic for the nucleolar chromosome.

Dhillon and Garber (1960), Garber (1964) and Chomchalow and Garber (1964) stated that the individual chromosomes of Collinsia heterophylla and C. tinctoria could not be identified by their morphology at any stage of mitosis and meiosis. Vasek (1961) observed that all

the chromosomes of Clarkia unguiculata were similar in morphology and that individual chromosomes could not be identified cytologically. No information has been reported on the cytology of the extra chromosomes of trisomics in Sorghum, Antirrhinum and Dactylis.

Although it is known that each of the three autosomal trisomy syndromes in man is due to a specific autosome in triplicate there has been difficulty in identifying the respective chromosomes. Lejeune, Gautier and Turpin (1959; quoted from Lejeune and Turpin, 1961) termed the trisomy of Down's syndrome as 21 trisomy. However, the cytological separation of the autosomes of the G group (21-22) still remains a problem (Patau, 1960). Edwards, Harnden, Cameron, Crosse and Wolff (1960) first identified the triplicated chromosome of the E trisomy syndrome as chromosome 17. But Smith, Patau, Therman and Inhorn (1960) considered the extra chromosome to be 18. Patau, Smith, Therman, Inhorn and Wagner (1960) found that the D_1 trisomy syndrome was due to one of the chromosomes of the cytologically indistinguishable D group (13-15).

2. Chromosome associations at diakinesis and metaphase I

From studies on chromosome configurations at diakinesis and metaphase I of various trisomics in

Datura, Belling and Blakeslee (1924, 1926) provided the cytological basis for the distinction of primary, secondary and tertiary trisomics. The triplicated chromosomes of primary and secondary trisomics formed either a trivalent or one bivalent and one univalent (Belling and Blakeslee, 1924). In primary trisomics, the trivalents were mostly frying-pan, Y, V or chain shapes, and the univalents were rod shape; whereas in secondary trisomics, a ring of three was predominant in trivalents, and most univalents formed a small ring. Philp and Huskins (1931) called the ring of three and ring univalent "secondary configurations" inferring that they occurred only in secondary trisomics and not in primaries. Tertiary trisomes produce a range of configurations at diakinesis and metaphase I including chains of five chromosomes which make it possible to distinguish the tertiary from primary and secondary trisomes (Belling and Blakeslee, 1926). Based on these observations and Belling's theory that only homologous ends of chromosomes are attached during meiosis (Belling, 1921), Belling and Blakeslee (1924, 1926) inferred that the extra chromosome in the primary trisomic was an unmodified chromosome, in the secondary trisomic it was a double half chromosome, and in the tertiary trisomic it was an interchange chromosome consisting of two arms

of nonhomologues. Further convincing support to show that the extra chromosome of the secondary trisomic was an isochromosome was later obtained from pachytene analysis of a secondary trisome of chromosome 5 in maize (Rhoades, 1933) and a secondary trisome of chromosome 9 in tomato (Sen, 1952).

The frequency of trivalents in primary trisomics varied considerably in different organisms. In Datura, Belling and Blakeslee (1924) observed as many as 109 cells with one trivalent among a total of 118 cells in ten primary trisomics, the rest of the cells (nine) had 12 bivalents and one univalent. McClintock (1929) studied meiosis in four trisomic plants of maize and found that the configuration of 1 III + 9 II was about twice as frequent as 10 II + 1 I at both diakinesis and MI. Lesley (1928) reported that more cells were found with 1 III + 11 II than cells with 12 II + 1 I at diakinesis in nine primary trisomics of tomato. According to Kamanoi and Jenkins (1962) the frequencies of cells with one trivalent in seven trisomics of rye ranged from 50.3 to 78.1% at diakinesis and from 30.8 to 66.1% at MI. These results are slightly different from an earlier report by Takagi (1935) who observed 50% of the cells with 1 III + 6 II and 50% with 7 II + 1 I both at diakinesis and MI in one spontaneously occurring trisomic

plant of rye. Price and Ross (1957) reported in Sorghum that cells having 10 II + 1 I were more frequent than cells having 1 III + 9 II. In one trisomic castor bean plant (Ricinus), the frequency of cells with one trivalent was 40% (Jakob, 1963). Shah (1964) observed that the frequency of univalents in one trisomic plant of Dactylis glomerata was 0.68 per cell at diakinesis and 0.73 at MI. In primary trisomics of Collinsia tinctoria, Chomchalow and Garber (1964) noted that the frequency of PMC's with a trivalent at MI was not constant ranging from 0 to 32%.

The trisomics of Collinsia heterophylla from different sources showed variations in the frequency of trivalents. In those obtained from colchicine treatment, the extra chromosome always occurred as an univalent (Dhillon and Garber, 1960), whereas in those trisomics from triploid progenies, the frequency of cells with a trivalent ranged from 21 to 74% (Garber, 1964).

According to Vasek's 1956 and 1961 papers, in trisomics of Clarkia the frequency of cells with a trivalent was less than 10%. However, he (1963a) later observed that the frequency increased to almost 43%. These differences were attributed to sampling variations and environmental differences.

Analyzing chromosome behaviour in meiosis of eight primary trisomics of maize, Einset (1943) found that the frequency of trivalent formation at MI was positively correlated with chromosome length, the longer the length of the trisome, the greater the tendency for it to associate in a trivalent. The percentages of microsporocytes with univalents at MI for trisomes of long, medium and short chromosomes were 20.6, 31.3 and 43.5, respectively. The same was for the primary trisomes of tomato (Rick and Barton, 1954) and spinach (Tabushi, 1958). The proportions of cells with 7 II + 1 I and cells with 1 III + 6 II at MI for two primary trisomics of Matthiola, Crenate and Rosette, were 22:78 and 40:41, respectively (Philp and Huskins, 1931; Prakken, 1942). Crenate was trisomic for chromosome A and Rosette for chromosome F, A being longer than F. On the other hand, Goodspeed and Avery (1939) did not observe any notable differences in trivalent frequency in four primary trisomics of Nicotiana sylvestris. In these trisomics, trivalents occurred in 56-60% of PMC's at MI, the average being 57%. They considered that the similarity in chromosome association was due to the lack of pronounced difference in chromosome length. Levan (1942) did not find any correlation between chromosome size and trivalent frequency in four primary trisomics of Beta. He

postulated that factors other than chromosome size might be operating. Tsuchiya (1960) reported that trisomic types in barley differed in their chromosome configurations at diakinesis and metaphase I, but no correlation between chromosome length and trivalent frequency was shown.

Chromosome configurations of secondary trisomics have been reported in several organisms. In Datura (Belling and Blakeslee, 1924), Matthiola (Philp and Huskins, 1931) maize (Rhoades, 1933) and the secondary trisome of chromosome 9 in tomato (Sen, 1952), most of the trivalents and univalents were found to be ring shape at diakinesis and MI which is characteristic of secondary trisomes. However, in the secondary trisome of chromosome 2 in tomato, Moens (1965) observed that the isochromosome had two heterochromatic arms and did not pair with a normal chromosome 2 during meiosis.

Belling (1927) observed that the trivalents in one telosomic trisomic of Datura were either of the frying-pan or the V shape. In one tomato plant trisomic for a "fragment," Lesley and Lesley (1929) noted that the behaviour of the "fragment" was similar to that of a whole extra chromosome. At metaphase I it might form a trivalent or an univalent. Rhoades (1936) studied meiosis of one telosomic trisomic in maize in which the extra

telesome consisted of the short arm of chromosome 5. At metaphase I, the telosome associated with the two normal chromosomes to form a trivalent in approximately 53% of the cells, a frequency which was considerably lower than the 85-90% found in plants trisomic for the whole chromosome 5. In one telosomic trisomic in Triticum monococcum, Smith (1947) observed 93 cells with 7 II + 1 I among a total of 273 cells studied. The telesome was almost always the unpaired chromosome, and appeared as a short rod.

IV. Transmission of the Extra Chromosome in Trisomics

If reproduction were entirely regular in trisomics, half of their female gametes should carry an extra chromosome, and the other half should have the normal complement of chromosomes. The same should be true of the male gametes. Crossing of the normal plants should then give progeny consisting of about 50% trisomics and 50% normals. In the same way, selfing of trisomics should give about 25% tetrasomics, 50% trisomics and 25% normals. However, such a high proportion of parental types from trisomics has never been realized in almost all plant genera so far studied. In Datura (Blakeslee, 1921b; Blakeslee and Avery, 1938), Matthiola (Frost and Mann, 1924; Frost, 1927; Prakken, 1942),

tomato (Lesley, 1928; Rick and Barton, 1954), maize (McClintock and Hill, 1931; Einset, 1943), Nicotiana (Goodspeed and Avery, 1939), Antirrhinum (Rudorf-Lauritzen, 1958), spinach (Tabushi, 1958; Janick, Mahoney and Pfahler, 1959), barley (Tsuchiya, 1960) and rye (Kamanoi and Jenkins, 1962), transmission through the egg occurred with a frequency generally below 50%, and transmission through the pollen was usually less than 10% and often did not occur at all.

Buchholz and Blakeslee (1922, 1930) and Blakeslee and Avery (1938) observed that in reduction divisions of trisomic plants of Datura the chromosomes of PMC's segregated in such a way that equal numbers of n and $n+1$ pollen grains resulted, and it was suggested that the same proportions also were present among the megaspores of these plants. Blakeslee and Avery (1938), therefore, concluded that the deficiency of trisomic plants in the progeny was due to elimination of the extra chromosome, or of cells containing the extra chromosome, during some stages of development of the gametophyte or zygote. In Datura, selection against $2n+1$ zygotes has been shown by Buchholz and Blakeslee (1922) in which the zygotes of the trisomic type Globe had a much greater mortality than the normals in embryonic development. Frost (1927) reported that in Matthiola, zygotes with extra chromosomes

were less viable in the embryo stage. Lesley (1928) considered that a small number of viable seeds in fruits of trisomic tomato plants was caused by the presence of an extra chromosome in the embryos which created unbalance and disturbed normal developmental processes. Blakeslee and Avery (1938) found that trisomic seeds in Datura were slower to germinate than normal diploid seeds. Rick and Barton (1954) noted that in tomatoes the transmission of trisomics was correlated with the germination percentage, the relative yield of trisomics usually being higher in seed lots of those which germinate later. Tsuchiya (1960), Ramage and Day (1960) and Dyck (1965) found that the trisomic seeds were often smaller and lighter than diploid seeds.

Frost (1927), Lesley (1928), McClintock and Hill (1931), Goodspeed and Avery (1939), Einset (1943), Tabushi (1958), and Shah (1964) considered the failure of the extra chromosome to be included in the gametes in the meiotic divisions to be one of the main causes for the low rate of transmission of trisomics. In primary trisomics of tomato, Lesley (loc. cit.) observed that the univalent frequently lagged at anaphase, and was eliminated at telophase, therefore, less than half of the gametes contained the extra chromosome. Shah (1964) observed high frequencies of laggards at telophase I and

II in one trisomic plant of Dactylis and inferred that this could lead to a reduced frequency of n+1 gametes. McClintock (1929) and McClintock and Hill (1931) reported that in primary trisomics of maize, disjunction of the trisome was usually two from one when a trivalent was present at metaphase I. The univalent behaved very irregularly and often was not included in either nucleus. In this case, a loss of the univalent would occur in meiosis, with the formation of more n gametes, instead of half n and half n+1 gametes. Also working on maize, Einset (1943) observed that when a short chromosome was present in triplicate there were fewer trivalents and more univalents present at metaphase I. As a result of chromosome elimination at telophase, few microspores contained n+1 chromosomes and the frequency of trisomics in the progeny was low. On the other hand, when a long chromosome was present in triplicate, he observed more trivalents and fewer univalents and more microspores containing n+1 chromosomes. Consequently, in the latter case, the frequency of trisomics in the progeny was high. On the other hand, in Datura (Blakeslee and Avery, 1938), tomato (Rick and Barton, 1954), spinach (Tabushi, 1958; Janick, Mahoney and Pfahler, 1959), barley (Tsuchiya, 1960), and rye (Kamanoi and Jenkins, 1962), chromosome length has been shown to have no relation to the

transmission rate of trisomics. Blakeslee and Avery (loc. cit.) noted that the largest primary chromosome of Datura was transmitted to 10.51% of its offspring, the smallest was transmitted to 32.68% of its progeny, while the third smallest showed only a 2.99% transmission.

Pollen sterility has been postulated to explain the low rate of transmission through the pollen as compared with that through ovules (Lesley, 1928; Goodspeed and Avery, 1939). Lesley (loc. cit.) observed in tomato that some trisomics had fewer "bad" pollen grains than others and correspondingly transmitted the extra chromosome more readily through the pollen. Goodspeed and Avery (loc. cit.) considered that the decrease in transmission through the pollen in trisomics of Nicotiana was due to lower viability of the n+1 microspores as compared with the n. However, Blakeslee and Cartledge (1926) were of the opinion that there was no close relationship between the amount of pollen sterility and the extent to which the extra chromosome was transmitted through the pollen. They found in Datura that the primary trisomic Rolled had a low percentage of bad pollen grains and was one of the least frequent types in the offspring of triploids. The other trisomic Cocklebur had a relatively high percentage of bad grains but transmitted its extra chromosome through the pollen to a greater extent than

any of the other trisomics.

Non-germination and differential pollen tube growth of the n+1 pollen have been assumed to explain the low rate of transmission through male gametes in trisomics of Matthiola (Frost, 1927), tomato (Lesley, 1928), Nicotiana (Goodspeed and Avery, 1939), spinach (Tabushi, 1958; Janick, Mahoney and Pfahler, 1959) and Clarkia (Vasek, 1961) and have been demonstrated in Datura (Buchholz and Blakeslee, 1922, 1930, 1932; Buchholz, Doak and Blakeslee, 1932). It has been reported that in Datura the extra chromosome was transmitted through the pollen only in four primary and one secondary trisomic (Blakeslee and Avery, 1938). In the case of those trisomics not transmitted through the pollen, Buchholz and Blakeslee (1922, 1930, 1932) observed the following abnormal behaviour of the 2n+1 pollen grains: non-germination of the pollen, cessation of growth with swelling and bursting of pollen tubes, and slow growth of the pollen tubes. For those trisomics in which there was some pollen transmission, they found that some of the pollen tubes had normal growth but at a slower rate which ranged from 1/3 to 3/4 of the rate of the normal pollen tubes of the haploid complement. The competition in pollen tube growth was further demonstrated by Buchholz, Doak and Blakeslee (1932) by showing that

the rate of pollen transmission could be increased by some artificial means such as restricting the number of pollinations, or excising and discarding the longer n tubes. Conversely, if styles were cut off after the normal pollen tubes had entered the ovary, the offspring contained only normal plants (Buchholz and Blakeslee, 1930).

In tomato, Rick and Barton (1954) observed a positive correlation between the frequency of occurrence for each trisomic type in the progenies of triploids and the frequency of transmission in progeny of the same trisomic type. In general, those chromosomes that were transmitted most frequently in progenies of trisomic ♀ x diploid ♂ crosses were those trisomes which were present most frequently in the progeny of triploids. However, such a relationship did not exist in barley as Tsuchiya (1960) had pointed out that the trisomic type Pale appeared most frequently in the progeny of auto-triploids, and was not so frequently recovered in selfed progeny of Pale plants. The trisomic types Robust and Pseudo-normal appeared very frequently in their selfed progenies but they occurred only with low frequencies from autotriploids.

Lesley (1928) secured one triplo-E tomato plant

from a triplo-A ♀ x diploid ♂ cross and one triplo-C from a diploid ♀ x triplo-B ♂ cross, but these trisomics were not related to the parental types. Later, Blakeslee and Avery (1938) showed that all the 12 primary trisomics of Datura could produce some unrelated trisomic types in their offspring. The frequency of such trisomics ranged from 0.05% for the 2n+3.4 type to 2.3% for the 2n+19.20 type with the average being 0.8%. This frequency was higher than that for spontaneously occurring trisomics from normal diploids (0.16%). They suggested that the increased chromosomal mutation rate in the trisomics might be caused in some way by the presence of the extra chromosome which interfered with meiotic divisions. No close connection between the size of the extra chromosome and its ability to produce new trisomics was observed. Burnham (1962) postulated that a change in physiology in trisomic plants might cause a higher rate of chromosome non-disjunction. Goodspeed and Avery (1939) also found a high frequency of chromosomal variants in progenies of asynaptic trisomics in Nicotiana but not in progenies of trisomics from other sources.

Very few tetrasomics have been observed from selfing primary trisomics. Lesley (1928) stated that "tetrasomics probably occur, although rarely, in the progeny of certain simple trisomics of tomato." He did

not give actual figures on frequencies. In Datura, Blakeslee and Avery (1938) observed three $2n+(13.14)_2$ tetrasomics in 2,033 offspring, seven $2n+(15.16)_2$ tetrasomics in 2,278 offspring and 12 $2n+(21.22)_2$ tetrasomics in 2,340 offspring from selfing the respective primary trisomic types. Goodspeed and Avery (1939) obtained one tetrasomic plant in Nicotiana in the progeny of the trisomic type Late. All these authors agreed that the scarcity of tetrasomics was due to the low transmission rate of the extra chromosome through the pollen and the reduced viability of the zygotes with two similar extra chromosomes.

Dhillon and Garber (1960) reported that their Collinsia trisomics which originated from colchicine treatment produced as high as 65% tetrasomics in the progenies when crossed as female parents to diploids. Vasek (1961) also observed that one trisomic plant of Clarkia, used as a female plant, gave rise to 32% tetrasomics. To explain this phenomenon, Dhillon and Garber (loc. cit.) and Vasek (loc. cit.) suggested that non-disjunction or some sort of duplicating mechanism operated in these plants.

Miller (1964) observed the frequency of sex chromatin bodies and studied the karyotypes for about half of the 31 children from 11 triplo-X women and found

that they were all chromosomally normal individuals. He considered that the failure to observe XXX or XXY children among the progeny of triplo-X females was due to preferential segregation of the triplicated X chromosomes, with the XX-containing secondary oocyte becoming a polar body and the X-containing secondary oocyte giving rise to the ovum.

Transmission of extra chromosomes in secondary trisomics has been studied by Blakeslee and Avery (1938) in Datura and by Moens (1965) in tomato. Blakeslee and Avery (loc. cit.) calculated the theoretical proportion of various kinds of gametes expected from the random assortment of chromosomes in the trisome. However, the expected proportions have never been obtained in all the 14 secondary trisomics of Datura. They attributed this result to the non-random association of the three chromosomes in the secondary trisome in which they assumed the two normal chromosomes usually paired and separated to opposite poles whereas the secondary chromosome became attached to itself by its like ends to form a ring univalent which lagged and then went at random to one pole or another. This type of disjunction would tend to decrease the proportion of related primary trisomics and to increase the proportion of parental secondary trisomics in the progeny. Blakeslee and Avery (1938)

reported that the percentages of secondary trisomics in the progeny varied from 2.45% for the 2n+1.1 type to 31.42% for the 2n+5.5 type. The percentages of related primary trisomics ranged from 0.07% for the 2n+19.19 type to 8.46% for the 2n+9.9 type. In one secondary trisomic in tomato, Moens (1965) found that the isochromosome, with two identical heterochromatic short arms of chromosome 2, was transmitted through the male and female gametes to about 30% of the offspring. He concluded that the transmission rate was in agreement with the meiotic behaviour in which 60% of the PMC's did not eliminate the isochromosome at telophase II. Half of these gametes (30%) would be expected to transmit the isochromosome.

Blakeslee and Avery (1938) reported the breeding behaviour of five tertiary trisomics of Datura. When these tertiary trisomics were selfed or crossed as female parents to normal diploids they observed some related primaries and secondaries, in addition to the normal diploids and parental tertiary trisomics, in the progenies. The frequencies of parental tertiary trisomics ranged from 14.83% for the 2n+1.9 type to 30.07% for the 2n+4.6 type, the average being 22.09%. The average frequencies of related primary and secondary trisomics produced by the five tertiaries were 0.94% and

0.05%, respectively. The presence of related primaries in the progeny of tertiary trisomics was attributed to chromosome association at metaphase I and subsequent segregation at anaphase I (Blakeslee and Avery, 1938). Goodspeed and Avery (1939) found 4.8% related primaries in the progenies of one tertiary trisomic of Nicotiana. Rick and Barton (1954) reported that in a total of 415 offspring of "triplo-11" of tomato, 14.7% were "triplo-11," 0.7% were triplo-7 and 3.1% were triplo-10. Later, genetic evidence showed that "triplo-11" was not a primary but a tertiary trisomic in which the extra chromosome consisted of arms of chromosomes 5 and 10 (Rick, Dempsey and Khush, 1964).

Transmission of the extra telosome or "fragment" has been studied in tomato (Lesley and Lesley, 1929), maize (Rhoades, 1936, 1940), Datura (Blakeslee and Avery, 1938) and Triticum monococcum (Moseman and Smith, 1954). Blakeslee and Avery (loc. cit.) observed 7.69% Datura plants containing two extra telosomes from selfing a telosomic trisomic 2n+.11 type. Lesley and Lesley (loc. cit.) also secured some tomato plants with two extra telosomes in the progenies of two telosomic trisomic types. Rhoades (1936) reported that about 1.5% of the progeny were primary trisomics when a maize plant having an extra short arm of chromosome 5 was crossed as the

female parent to normal diploids. Primary trisomics did not occur in the progeny of telosomic trisomics of tomato (Lesley and Lesley, loc. cit.) and of Triticum monococcum (Moseman and Smith, loc. cit.), however, they did occur in Datura but only very rarely (Blakeslee and Avery, loc. cit.).

Belling (1927) and Rhoades (1936) observed that the configurations of the trivalents involving the telosome were either the ring-and-rod type, the telosome being the rod; or V shape, the telosome being at the end of the V. Consequently, the telosome would go to the same pole as one of the normal chromosomes, and only rarely the two normal chromosomes passed together to one pole while the telosome went to the other pole. The rare occurrence of primary trisomics in the progeny of telosomic trisomics substantiated the idea of a non-random segregation of members of the trivalent group.

V. Genetic Studies of Trisomics

The value of primary trisomics for localizing genes on specific chromosomes and for identifying chromosomes with their respective linkage groups was first recognized by Blakeslee, Belling and Farnham in Datura in 1920 when they observed that the presence of an extra chromosome in a trisomic modified the genetic ratios for

the genes in that chromosome. They outlined the principle for trisomic analyses, and tentatively identified the gene "white" with the trisomic type Poinsettia which was later shown to be correct (Blakeslee and Farnham, 1923). Other types were then identified; "swollen" was identified with the trisomic type Ilex (Garger and Blakeslee, 1927), "curled" with Poinsettia (Blakeslee, Morrison and Avery, 1927) and "tricarpel" with Reduced (Buchholz and Blakeslee, 1927). In 1941 Blakeslee and Avery reported that they had located 72 genes on the chromosomes of Datura and 29 of these had been placed in specific arms.

The trisome method was soon utilized for localizing genes in the tomato by Lesley (1928) who demonstrated that the gene d in the first linkage group resided in the satellite chromosome in which triplo-A was the trisomic. He later (1932) identified r with triplo-I and l with triplo-B. Rick and Barton (1954) confirmed Lesley's earlier results and located seven more genes on five chromosomes. They also disproved the independence of linkage groups VI and VIII and of X and XII. More recently, Rick, Dempsey and Khush (1964) reported that they had assigned 30 genes on 11 of the 12 chromosomes of tomato by trisomic ratio tests.

In maize, extensive data were presented by McClintock and Hill (1931) in which they successfully associated the r-g linkage group with chromosome 10 and five other linkage groups with specific chromosomes. It was reported later by Emerson, Beadle and Fraser (1935) that McClintock and Burnham by the trisome method had identified nine linkage groups in maize with their respective chromosomes. Rhoades (1936) assigned six genes on the long arm and two genes on the short arm of chromosome 5 in maize by using a telosome consisting of the short arm of chromosome 5.

In Oenothera blandina Catcheside (1954) identified the gene br on chromosome 11.12 by using primary trisomics. The use of the tertiary trisomic 2n+4.12 (Subwhitish) enabled him to place br in the distal euchromatic region of arm 12. Rudorf-Lauritzen (1958) stated that she had associated six of the eight linkage groups of Antirrhinum majus with particular trisomics. Janick, Mahoney and Pfahler (1959) were able to associate the locus in spinach responsible for sex-determination with the trisomic type Reflex. Later, cytological analyses of Reflex plants showed them to be trisomic for chromosome 1 (Ellis and Janick, 1960). Working on a telosomic trisomic plant, Moseman and Smith (1954) showed that from segregation ratios the genes y and js of linkage group D

of Triticum monococcum were not located on the extra telosome. Tsuchiya (1959, 1960) and Tsuchiya, Hayashi and Takahashi (1960) established the seven linkage groups of barley with six of the seven trisomic types. They showed that what had been considered two genetic linkage groups properly belonged to the trisomic Bush and that the extra chromosome of Purple did not carry either of these linkage groups. In Collinsia heterophylla, Rai and Garber (1960) were unable to assign genes to specific chromosomes on the basis of segregation ratios by the trisome method. They suggested that the unusual cytological behaviour of the extra chromosomes in Collinsia made this method ineffective.

In humans, the leucocyte alkaline-phosphatase activity (Trubowitz, Kirman and Masek, 1962) and galactose-1-phosphate uridyl-transferase activity (Brandt, Frøland, Mikkelsen, Nielsen and Tolstrup, 1963) were found to be significantly higher in trisomy 21 than in normal individuals suggesting that the loci for these enzymes were situated on chromosome 21. Similarly, Huehns, Hecht, Keil and Motulsky (1964) postulated that the genes responsible for the synthesis of the ϒ and e chains of human hemoglobin were located on the triplicated chromosome in the D_1 trisomy.

VI. Conclusion

As may be seen from this review of literature the classic investigations of *Datura* by Blakeslee and associates have set the pattern for the cytogenetic study of trisomy in plants. It has been followed with little modification and supplementation by workers in the studies of trisomics in *Matthiola*, *Lycopersicon*, *Zea*, *Crepis*, *Antirrhinum*, *Oenothera*, *Secale*, *Nicotiana*, *Hordeum*, *Sorghum*, *Clarkia*, *Triticum*, *Spinacia*, *Collinsia*, *Ricinus*, *Dactylis*, *Avena*, *Arabidopsis*, and *Oryza*. In these genera, trisomics have been demonstrated to be particularly useful in understanding the phenotypic effects of individual extra chromosomes, in localizing genes on specific chromosomes and in identifying individual chromosomes with their respective linkage groups. In *Lotus*, however, in the past few years, attention has been focused on those subjects such as cytotaxonomy, chemotaxonomy, interspecific hybridization and inheritance of certain qualitative characters. No study on trisomy has been initiated.

The present investigation of trisomics in *Lotus pedunculatus* has been stimulated by information recently obtained in this laboratory from the continuing study of the cytogenetics of *Lotus*. In the first place, the effective emasculation and pollination techniques as

described by Grant, Bullen and Nettancourt (1962) have enabled the author to establish a large number of trisomics and to study the transmission of the extra chromosomes. In the second place, Zalite (1966) has revealed that the somatic chromosomes of L. pedunculatus can be distinguished morphologically in root tip cells, thereby permitting the identification of the extra chromosomes in trisomics.

MATERIALS AND METHODS

Both the diploid ($2n = 12$) and tetraploid ($2n = 24$) plants of *Lotus pedunculatus* utilized in this study were grown from single seed. They were accessioned as B-193 and B-124, respectively, and were maintained and propagated by cuttings. The seed of the diploid was received from the Service de la Recherche Agronomique et de l'Experimentation Agricole, Rabat, Morocco, as *L. uliginosus* var. *decumbens* Poir and originally accessioned as B-110. The seed of the tetraploid was descendant from a colchicine-induced tetraploid obtained from the Department of Scientific and Industrial Research of New Zealand under their accession number ST155. Two triploid plants ($2n = 18$), produced by the writer, were given accession numbers HR-2 and 10327 and were used for establishing trisomics; HR-2 resulted directly from the cross between the diploid and tetraploid, and 10327 was obtained from the cross of diploid and one plant of the trisomic type Narrow when the latter was used as the male parent (see Table 16). The triploids HR-2 and 10327 were selfed and back-crossed to diploid plants in 1964 and 1965 in order to produce aneuploids.

Emasculation and pollination were made in a growth chamber in which the temperature was maintained at 24°C ± 2°, the relative humidity between 80-90% and photoperiod 16 hours. The procedures followed the techniques described by Grant, Bullen and Nettancourt (1962). Since L. pedunculatus is a self-incompatible species and self-fertilization has to be accomplished by some artificial means (Bubar, 1958), a small piece of fine sandpaper was used to break the stigmatic membrane for deliberate self-pollination.

Seeds were scarified for better and quicker germination. They were germinated on moist filter papers in Petri dishes, usually 20 seeds per dish. The Petri dishes containing seeds were placed in the dark in an incubator maintained at a constant temperature of 22°C for 2 days, after which the germinated seeds were moved to another incubator containing fluorescent lights and were gradually moved to an increased intensity of light. About 5-7 days later, young seedlings were transplanted to 2-inch pots and were kept in a growth chamber. Because of the limitation of space, seeds were germinated in different batches with about a one-month interval between lots. After the chromosome numbers of the young plants had been determined in root tip cells by routing procedures, the diploids, with the exception

of those used for morphological studies, were discarded. The aneuploid plants were then transferred and maintained in 4-inch pots. During the winter months the plants were kept in a greenhouse.

Morphological investigations were made on field plants in the summers of 1965 and 1966. The potted plants were moved from the greenhouse to a coldframe in May. The qualitative characters of the diploids and aneuploids were examined and recorded. The quantitative characters were measured of the plant parts to the nearest millimeter, as nearly as possible at the time when they reached particular stages of development. Thirty central leaflets were taken at random from the upper half branches of each plant as soon as the first flowers appeared. The length and width of each leaflet were measured and the width/length ratio was calculated. From each plant ten florets from different umbels were taken and the following parts were measured: floret length, standard width, style and ovary length, and the style/ovary ratio was calculated. The florets per umbel were counted from an average of 20 umbels per plant.

Estimates of pollen fertility were based on the number of pollen grains stained with fast green in lacto-phenol. Only those pollen grains which were plump and

full and could take up the stain were counted as viable. Grains that did not stain green and were shriveled, shrunken and devoid of cytoplasm were counted as inviable. For each plant samples of more than 1,000 pollen grains from five florets of different umbels were counted.

For all the trisomic types the means and the standard errors of the means were calculated for each phenotypic trait. The significance of the differences for all the phenotypic traits was tested by means of a t test. An IBM 650 data processing machine was used for statistical analyses.

The routing determination of chromosome numbers and karyotype analyses were made from root tip squashes. Root tips were collected from seedlings grown in the growth chamber. They were pretreated for two hours in 0.002M 8-hydroxyquinoline and then were fixed in a mixture of one part of concentrated acetic acid and three parts of 95% alcohol for 24-48 hours. After a minimum of 24 hours the root tips were washed thoroughly with 70% alcohol and stored in the same fluid in a refrigerator until needed. Before staining, root tips were hydrolyzed for 8 minutes in N HCl at 60°C and rinsed in distilled water. Root tips were stained in leuco-basic fuchsin (Feulgen) for 2 hours and then squashed in 45% acetic acid.

Chromosome measurements were made from drawings with the aid of a camera lucida. A total of ten metaphase cells in which the chromosomes were very well spread were measured for the diploid plant and an idiogram was constructed based on these measurements. For each primary trisomic type two plants were chosen as representatives for karyotype studies.

Meiosis was studied in pollen mother cells (PMC's) as described below. Flower buds were collected from plants grown in the coldframe and in the growth chamber at different times of the day. They were fixed in a mixture of one part of propionic acid and three parts of alcohol for 24 hours and then were stored in 70% alcohol until needed. Anthers were smeared in a drop of propionocarmine. Three to four representative plants were chosen from each trisomic type for meiotic studies. Attention was paid to chromosome behaviour at diakinesis, metaphase I, anaphase I and II, and telophase I and II.

Slides for meiosis and mitosis were examined from temporary mounts. It was possible to keep temporary slides sufficiently long enough for a complete examination, measurement and photographing of appropriate figures.

Studies on the transmission rate of trisomics

were conducted during 1965 and 1966. Plants which had been used for meiotic studies were crossed to diploids both as male and female parents and also selfed. The progenies of trisomics were classified as diploids, parental trisomics and in other categories according to their morphology; chromosome numbers were also verified by routing counting.

RESULTS

I. Morphology of the Diploid, Triploid and Tetraploid Plants

Lotus pedunculatus is a self-incompatible species which seldom sets seeds in the greenhouse or in a growth chamber without artificial pollination. Under open pollination in the field, however, the diploid plant (B-193) set 15 to 25 seeds per pod, whereas the tetraploid (B-124) and the two triploid plants (HR-2 and 10327) were essentially largely sterile and set seeds only occasionally. The tetraploid had 84.40% stainable pollen grains, a frequency which was lower than that of the diploid (98%). There was considerable variation in pollen fertility between the two triploids; HR-2 had 77.80% good pollen, whereas the pollen fertility of 10327 was 37.13%.

Measurements of some phenotypic traits of the diploid, triploid and tetraploid plants are presented in Table 1. For most of the plant parts measured, the two triploids were intermediate in size between those of the tetraploid and diploid (Figs. 1-13). Thus when they were grown together in the coldframe, the tetraploid plants were readily distinguished by their larger leaves

TABLE 1.--Measurements of some morphological characters of the diploid, triploid and tetraploid plants

Ploidy	Acc. no.	Central leaflet length (mm)	Central leaflet width (mm)	Leaflet index (w/l ratio)	Florets per umbel	Flore lengt (mm)
2x	B-193	11.55	5.87	0.50	6.10	11.15
3x	HR-2	12.75	6.85	0.54	7.80	13.05
3x	10327	13.32	7.22	0.54	7.75	13.02
4x	B-124	13.31	8.20	0.62	4.70	13.57

Floret length (mm)	Standard width (mm)	Ovary length (mm)	Style length (mm)	Style/ovary (ratio)	Pollen fertility (%)
11.15	5.95	5.63	5.47	0.97	98.00
13.05	8.00	6.47	6.18	0.96	77.80
13.02	7.81	6.98	6.37	0.91	36.13
13.57	8.51	6.83	6.79	0.99	84.40

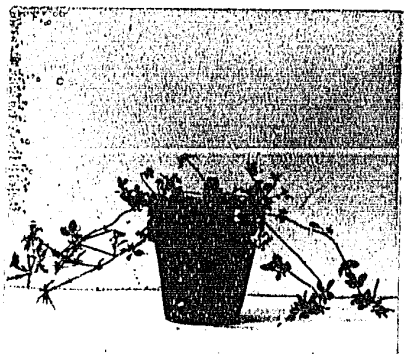

Fig. 1.--Diploid (B-193)

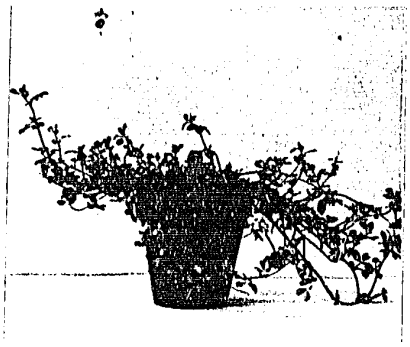

Fig. 2.--Triploid (HR-2)

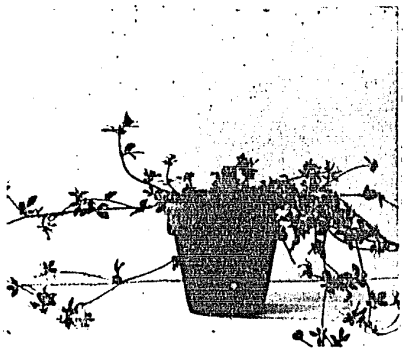

Fig. 3.--Triploid (10327)

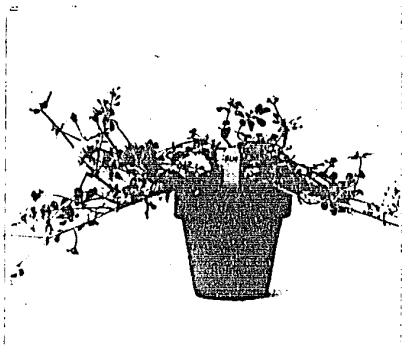

Fig. 4.--Tetraploid (B-124)

Figs. 1-4.--Mature diploid, triploid and tetraploid plants of Lotus pedunculatus.

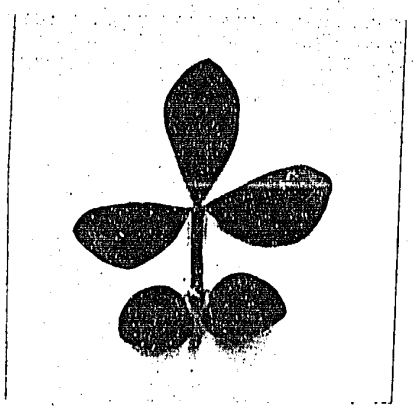

Fig. 5.--Diploid (B-193)

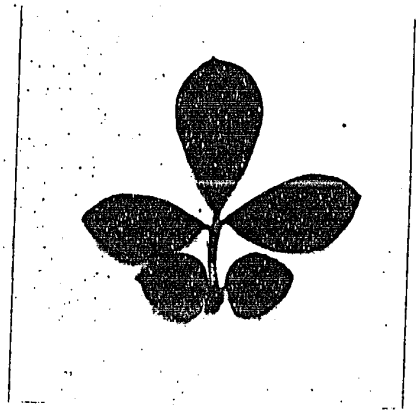

Fig. 6.--Triploid (HR-2)

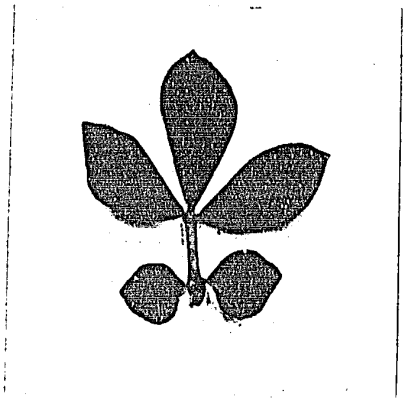

Fig. 7.--Triploid (10327)

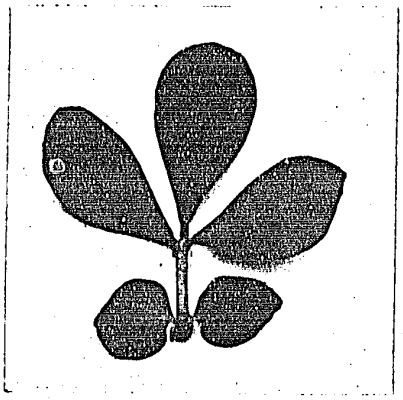

Fig. 8.--Tetraploid (B-124)

Figs. 5-8.--Leaves of diploid, triploid and tetraploid plants. x ca. 1.5

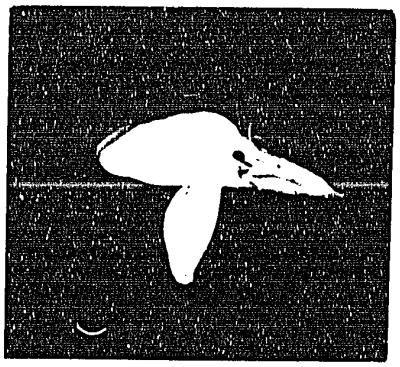

Fig. 9.--Diploid (B-193)

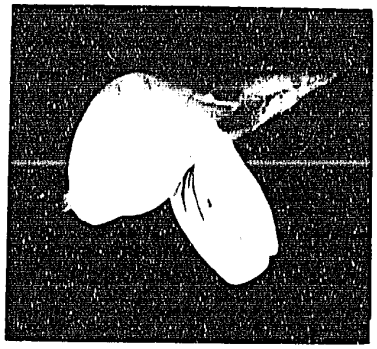

Fig. 10.--Triploid (HR-2)

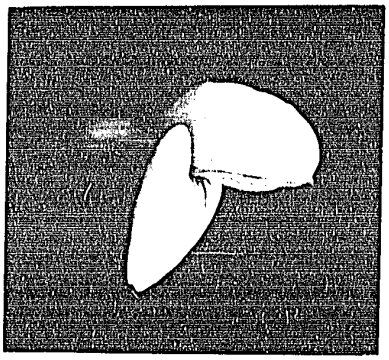

Fig. 11.--Triploid (10327)

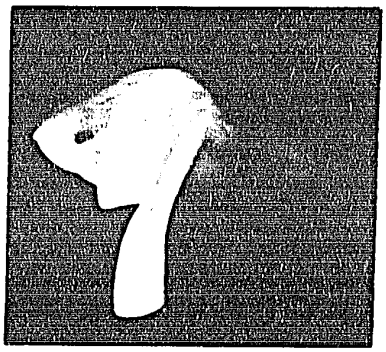

Fig. 12.--Tetraploid (B-124)

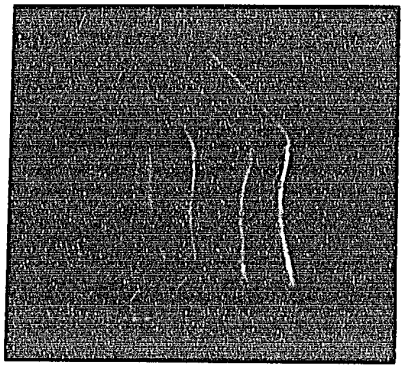

Fig. 13.--Pistils. From left to right: diploid (B-193), triploid (HR-2), triploid (10327), tetraploid (B-124).

Figs. 9-12.--Flowers of diploid, triploid and tetraploid plants. x ca. 2

Fig. 13.--Pistils of diploid, triploid and tetraploid plants. x ca. 2

and flowers and thicker stems, in contrast to the diploid plants with their smaller leaves and flowers and thinner stems, and the intermediate size of these plant parts which characterized the triploids. The central leaflet indexes (w/l ratio) calculated for the diploid, triploid and tetraploid were 0.50, 0.54 and 0.62, respectively. This would indicate that although there are increases both in leaflet length and in leaflet width from lower to a higher level of ploidy, the increase in width is proportionally greater than the increase in length. The diploid had an average of 6.10 florets per umbel, while the two triploids had more (7.80 and 7.75). In contrast, the tetraploid had less florets (4.70) than the diploid.

II. Meiosis in the Diploid, Triploid and Tetraploid Plants

1. The diploid (B-193)

The chromosomes of L. pedunculatus were always clumped or very closely associated at pachynema, metaphase I and anaphase I, and they could not be separated from one another at these stages of meiosis by any of the cytological techniques used. This made it impossible to study the chiasma frequency and to distinguish clearly the chromosome configurations at metaphase I. Similarly, observations on chromosome segregation at anaphase I

were also difficult since the individual chromosomes could not be distinguished from one another with any certainty.

At diakinesis and prometaphase I, six bivalents were usually observed although occasionally five bivalents and two univalents were found. From an examination of 84 microsporocytes, the frequencies of bivalents and univalents per cell were 5.93 and 0.14, respectively (Table 2). From the morphological appearance of the chromosomes at diakinesis and prometaphase I, the meiotic chromosomes of L. pedunculatus were arranged into four classes according to size: one large (L), one medium large (M), two medium (m) and two small (S) (Fig. 14).

At anaphase I, disjunction of the six bivalents was not always simultaneous; the largest bivalent occasionally separated later than the others in some of the PMC's. But the two dyads of this bivalent were able to reach the two respective poles in time to be included in the telophase nuclei. Consequently, no lagging chromosomes were observed at telophase I.

The largest chromosome was also observed occasionally to separate later than the other chromosomes at anaphase II (Fig. 16). At telophase II, only a single

cell was observed with a lagging chromosome from a total of 67 examined. Late separation of the largest chromosome in the first and second meiotic divisions also occurred in the trisomic plants of L. pedunculatus (Figs. 63, 64, 73, 79, 92) and in some interspecific hybrids of Lotus as reported by Grant, Bullen and Nettancourt (1962).

In general, meiotic behaviour of the diploid L. pedunculatus was quite regular. The homologous chromosomes usually formed bivalents as seen in diakinesis and prometaphase I, and no lagging chromosomes were observed at telophase I and II. The characteristic feature of this species was the clumping or very close association of the pachytene and metaphase chromosomes and the late separation of the largest chromosome at anaphase.

2. The triploids (HR-2 and 10327)

The 18 chromosomes of the two triploids generally occurred in associations of from two to six trivalents at diakinesis and prometaphase I. The association of 6 III was most frequent and the mean number of trivalents per cell was found to be 4.57 in the triploid HR-2, whereas in the triploid 10327, the association of 4 III + 2 II + 2 I was most frequent and the mean number of trivalents

per cell was 3.85. It was observed that the large and the medium large chromosomes formed trivalents more frequently than the smaller chromosomes. When the trivalents involved the small chromosomes they usually formed chains with two homologous chromosomes closely associated and the third one only loosely paired with them (Figs. 18, 19). The triploid 10327 had a higher frequency of univalents (mean 2.85 per cell) in the PMC's than HR-2 (mean 1.36 per cell)(Table 2).

At telophase I, only seven out of 23 cells of the triploid 10327 contained lagging chromosomes, whereas the number of cells with lagging chromosomes was 80 out of the 88 cells examined for HR-2. Lagging chromosomes ranged from zero to six per cell for HR-2 and from zero to two for 10327; they were either divided (monads) or undivided (dyads) (Figs. 22, 23). It was interesting to note that in one PMC the chromosomes of the two separated univalents misdivided so that partial chromosomes were formed of unequal size (Fig. 22).

At telophase II, the number of cells containing lagging chromosomes for the two triploids HR-2 and 10327 was 37 out of 53 cells examined and eight out of 11, respectively. Lagging chromosomes ranged from zero to five per cell for HR-2 and from zero to three for 10327.

3. The tetraploid (B-124)

An examination of 15 PMC's at diakinesis and prometaphase I showed three cells with five quadrivalents, three cells with two quadrivalents, six cells with one quadrivalent and three cells without any quadrivalents. The mean number of quadrivalents per cell (1.80) was much lower than the basic chromosome number of this species ($\underline{x} = 6$). The frequencies of trivalents, bivalents and univalents were 0.13, 7.53 and 1.33, respectively (Table 2). The large and the medium large chromosomes tended to form quadrivalents more frequently than the medium and the small chromosomes. Thus, when one or two quadrivalents were present in a PMC, they were always composed of the large or the medium large chromosomes; the medium and the small chromosomes usually formed bivalents (Figs. 26, 27).

The number of cells with at least one lagging chromosome at telophase I and telophase II was 18 out of the 44 cells examined and 33 out of 73, respectively. The telophase I cells contained up to seven lagging chromosomes; they were either divided into monads or were undivided as dyads. Lagging chromosomes ranged from zero to six per cell at telophase II.

TABLE 2.--Meiotic chromosome behaviour of the diploid, triploid and tetraploid plants

Ploidy	Acc. no.	Diakinesis and/or Metaphase I					
		No. cells	IV* Mean (Range)	III* Mean (Range)	II Mean (Range)	I Mean (Range)	N ce
2x	B-193	84	0	0	5.93 (5-6)	0.14 (0-2)	3
3x	HR-2	67	0	4.57 (2-6)	1.36 (0-4)	1.36 (0-4)	8
3x	10327	20	0	3.85 (2-6)	1.80 (0-4)	2.85 (0-8)	2
4x	B-124	15	1.80 (0-5)	0.13 (0-1)	7.53 (0-11)	1.33 (0-6)	4

*Quadrivalents, trivalents, etc.

Anaphase I--Telophase I			Anaphase II--Telophase II		
No. cells	Cells without laggards	Cells with laggards	No. cells	Cells without laggards	Cells with laggards
39	39	0	67	66	1
88	8	80	53	16	37
23	16	7	11	3	8
44	26	18	73	40	33

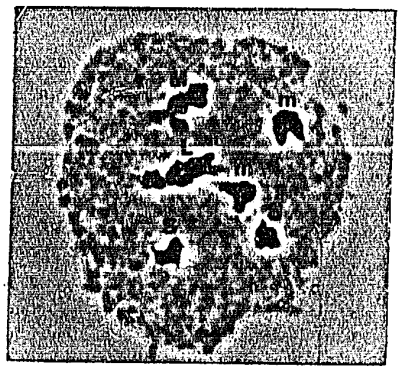

Fig. 14.--Diakinesis, showing 6 II.
x ca. 1320

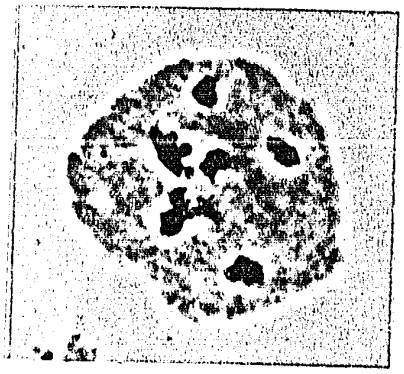

Fig. 15.--Diakinesis, showing 6 II.
x ca. 1320

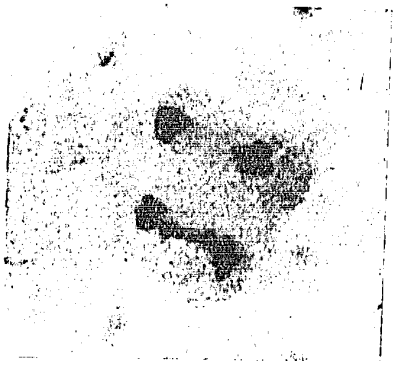

Fig. 16.--AII, showing late separation of the largest chromosome.
x ca. 1200

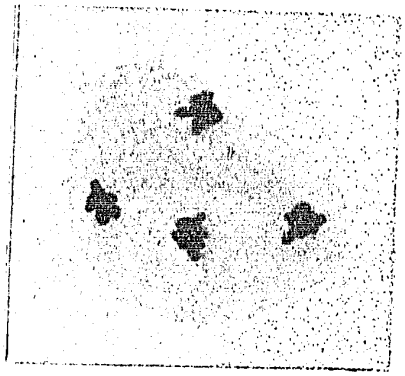

Fig. 17.--TII, normal.
x ca. 1200

Figs. 14-17.--Meiosis in the diploid plant.

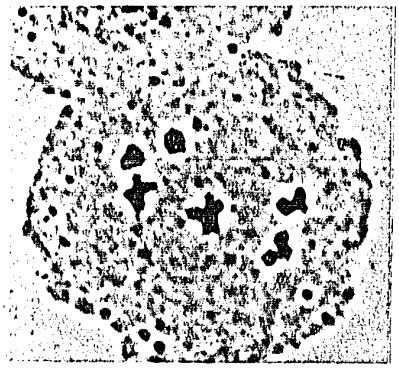

Fig. 18.--Diakinesis, showing 6 III. x ca. 945

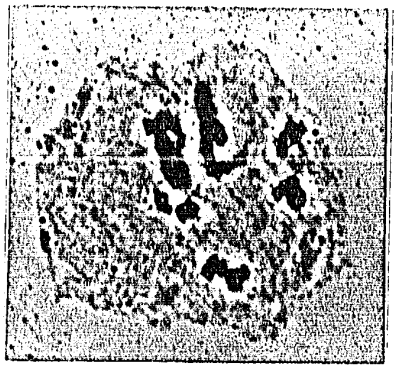

Fig. 19.--Diakinesis, showing 5 III + 1 II + 1 I. x ca. 1100

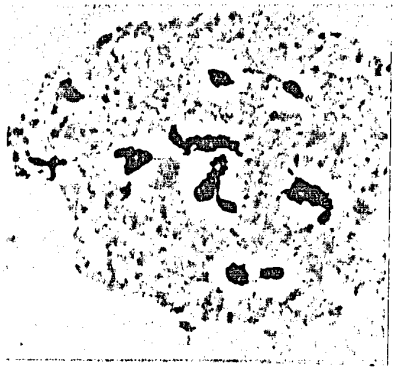

Fig. 20.--Diakinesis, showing 4 III + 2 II + 2 I. x ca. 1150

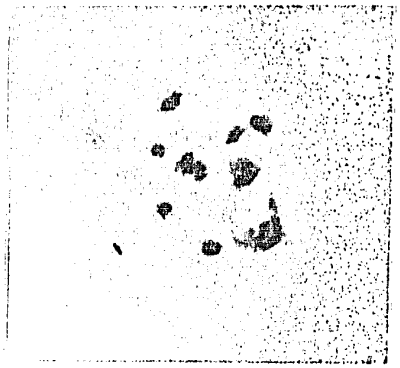

Fig. 21.--Diakinesis, showing 3 III + 3 II + 3 I. x ca. 1150

Figs. 18-21.--Meiosis in the two triploid plants.

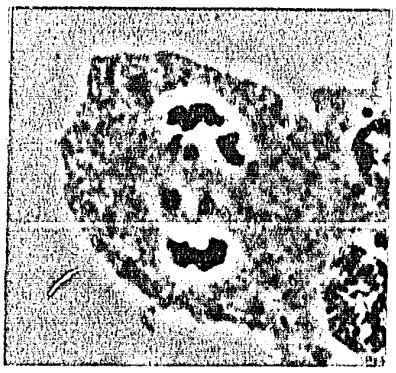

Fig. 22.--TI, showing one undivided and two dividing laggards. Note that the daughter chromosomes of the dividing univalents are unequal in size. x ca. 1260

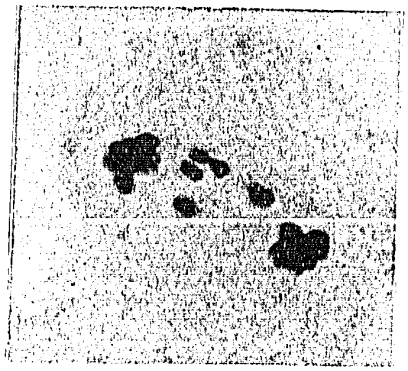

Fig. 23.--TI, showing 3 undivided laggards and a single dividing one. x ca. 1700

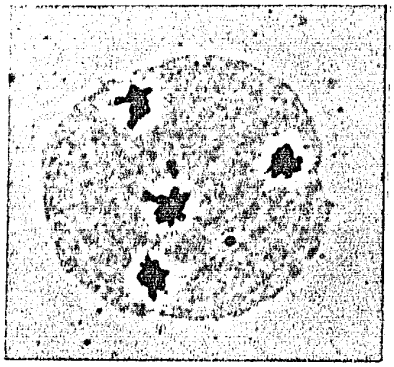

Fig. 24.--TII, showing 2 laggards. x ca. 1050

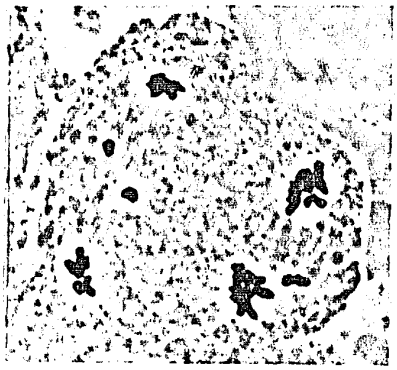

Fig. 25.--TII, showing 3 laggards. x ca. 1260

Figs. 22-25.--Meiosis in the two triploid plants.

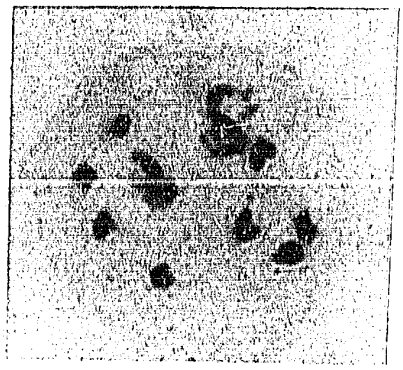

Fig. 26.--Diakinesis, showing 2 IV + 8 II.
x ca. 1386

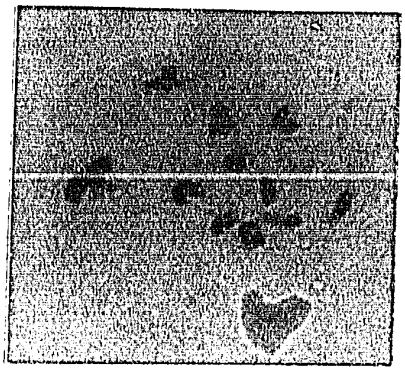

Fig. 27.--Diakinesis, showing 1 IV + 10 II.
x ca. 1100

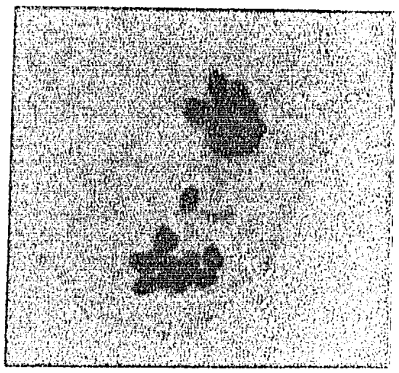

Fig. 28.--TI, showing one undivided laggard.
x ca. 1368

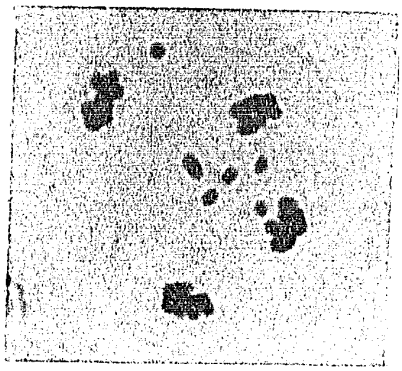

Fig. 29.--TII, showing 6 laggards.
x ca. 1386

Figs. 26-29.--Meiosis in the tetraploid plant.

III. Production of Aneuploids from Triploids

The result of selfing the two triploids (HR-2 and 10327) and crossing the triploids to the diploid B-193 are shown in Table 3. The triploid 10327 had a much lower percentage of pollen fertility than triploid HR-2 (36.13% versus 77.80%), but the former set pods more frequently and the pods contained more plump seeds. The frequency of pod-set was higher when the triploids were used as female parents (28.23%) than when they were used as male parents (10.53%) in the crosses. However, there was little difference in the number of plump seeds per pod obtained between reciprocal crosses (1.38, when triploids were used as female parents and 1.17 when used as male parents). Selfing triploids resulted in only 1% pod set; each pod contained an average of 0.29 seeds.

A total of 202 mature plants were raised from the triploids. The distribution of chromosome numbers found in the progenies is shown in Table 4. Among these 202 plants, 126 (62.38 %) were normal diploids, 73 (36.14%) were simple trisomics, two (0.99%) were double trisomics, and one (0.49%) was a telosomic trisomic. Plants with more than two extra chromosomes were not found in the progenies of the triploids.

TABLE 3.--Pod- and seed-set in reciprocal crosses between diploid and triploids and in triploids selfed

Cross	Flowers pollinated	Pods	% Pod-set	Seeds	Seeds/pod
		3x ♀ x 2x ♂			
HR-2 x B-193	720	198	27.50	232	1.17
10327 x B-193	77	27	35.06	79	2.93
Total	797	225	28.23	311	1.38
		2x ♀ x 3x ♂			
B-193 x HR-2	36	3	8.33	1	0.33
B-193 x 10327	21	3	14.29	6	2.00
Total	57	6	10.53	7	1.17
		3x selfing			
HR-2	464	6	1.29	2	0.33
10327	236	1	0.42	0	0.00
Total	700	7	1.00	2	0.29

TABLE 4.--Production of aneuploids from two triploids

Cross	Chromosome number				Total
	12	13	14	12+1t*	
		3x ♀ x 2x ♂			
HR-2 x B-193	95	51	1	1	148
10327 x B-193	26	18	1	0	45
		2x ♀ x 3x ♂			
B-193 x HR-2	1	0	0	0	1
B-193 x 10327	3	3	0	0	6
		3x selfing			
HR-2	1	1	0	0	2
10327	0	0	0	0	0
Total	126	73	2	1	202
%	62.38	36.14	0.99	0.49	100

* t = telocentric chromosome

IV. Morphology of Trisomics

1. Primary simple trisomics

When the simple trisomic plants, which were acquired from the progenies of the two triploids, were grown under greenhouse conditions in the winter months, it was difficult to distinguish the trisomics from each other and from normal diploid plants. But, when the trisomics were grown in the coldframe during the summer, a number of phenotypic differences were readily observed. These included growth habit, thickness of stems, size, shape and colour of leaves, and size and shape of flowers. For example, the growth habit of the diploid plants was decumbent as were some trisomic plants (Figs. 30, 32, 33, 34), but other trisomics were ascending (Fig. 31), or semi-erect (Fig. 35). Some trisomic plants had smaller leaves (Fig. 41) than those of the diploid, others had larger leaves (Fig. 37). Some trisomic plants had narrow (Fig. 40) or pointed leaves (Fig. 39), others had broad (Fig. 38) or round leaves (Fig. 37). The leaf colour of some trisomics was darker than those of the diploid, other trisomics had pale or yellow green leaves. The leaves of some trisomic plants had prominent midribs and veins (Fig. 39) which made it possible to distinguish them at an early seedling stage. The flowers of some trisomic plants

were deformed and contained few pollen grains in the anthers. Some trisomic plants had smaller than normal sized flowers (Fig. 46). The styles of some trisomic plants protruded from the corolla tubes (Fig. 44). Thus, by visual observation, the 73 simple trisomic plants could be classified into five distinct morphological groups, each representing one trisomic type with a unique combination of characters. These five simple trisomic types were named Round, Broad, Pointed, Narrow and Small according to their distinguishing leaf characters. The frequency of the five morphological trisomic types which arose in the progenies of the two triploids is shown in Table 5.

To further analyze differences between the diploid and the trisomic types, pollen fertility was estimated and nine quantitative phenotypic traits were measured. The means and standard errors that were calculated for the diploid and for each of the four trisomic types are listed in Table 6. The flower parts of Round were not available for measuring during the period of this study, and therefore, this trisomic type is not included in the table. From this table it may be seen that some differences occur in the mean value for each phenotypic trait from one trisomic type to another. The significance of these differences was tested by means of a $\underline{t}$ test, and

TABLE 5.--Distribution of morphological types of simple trisomics from two triploids

Cross	Morphological type of simple trisomics					Total
	Round	Broad	Pointed	Narrow	Small	
			3x ♀ x 2x ♂			
HR-2 x B-193	2	8	8	24	9	51
10327 x B-193	0	4	2	3	9	18
			2x ♀ x 3x ♂			
B-193 x HR-2	0	0	0	0	0	0
B-193 x 10327	0	0	0	3	0	3
			3x selfing			
HR-2	0	0	0	1	0	1
10327	0	0	0	0	0	0
Total	2	12	10	31	18	73
%	2.74	16.44	13.74	42.46	24.65	100

TABLE 6.--Mean value of some morphological characters of the diploid and the four primary simple trisomics

Characters	Diploid	Broad	Pointed	Narrow	Small
Central leaflet length (mm)	12.33±0.70	12.71±0.49	10.90±0.34	11.14±0.22	9.53±0.21
Central leaflet width (mm)	6.43±0.43	7.72±0.22	5.12±0.22	4.49±0.10	5.23±0.11
Leaflet index (w/l ratio)	0.52±0.01	0.61±0.01	0.47±0.02	0.40±0.01	0.54±0.01
Florets per umbel	8.49±0.22	5.74±0.43	6.83±0.28	6.61±0.26	5.66±0.18
Floret length (mm)	11.66±0.14	11.00±0.07	11.21±0.17	10.85±0.07	10.48±0.11
Standard width (mm)	6.80±0.27	6.28±0.08	6.47±0.12	5.74±0.05	5.50±0.16
Ovary length (mm)	6.17±0.18	5.46±0.09	6.38±0.14	5.55±0.05	5.18±0.07
Style length (mm)	5.76±0.02	5.72±0.10	5.35±0.08	5.46±0.06	5.09±0.04
Style/ovary (ratio)	0.94±0.03	1.05±0.03	0.84±0.01	0.99±0.01	0.98±0.01
Pollen fertility (%)	94.54±1.43	88.21±2.42	89.57±2.59	89.91±0.97	83.00±1.88
No. of plants	5	7	6	20	7

the results are presented in Table 7. Inspection of Table 7 shows that each trisomic type differs significantly from the diploid and from the other types in four to eight (average 6.6) phenotypic traits. It may be seen that Broad has larger and broader leaves, and a greater style/ovary ratio, that Pointed has a greater ovary length and a lower style/ovary ratio, that Narrow has narrower leaves and standards, that Small has smaller central leaflet length, floret length, standard width, and style and ovary length. These phenotypical traits represent statistically proven differences.

The most conspicuous and diagnostic morphological characters of the five simple trisomic types which differentiate them from each other and the diploid are presented in Table 8. The following is a brief description of these trisomic types based on visual observations of qualitative characters and on measurements of quantitative ones.

Round.--The most striking character of Round, to which its designation refers, is the large and round leaves (Fig. 37). Plants of this trisomic type have an ascending growth habit (Fig. 31). Stems are thicker than those of the diploid and other trisomic types. Leaves are extremely large, round, thick, slightly pale

TABLE 7.--t tests for ten phenotypic traits of the diploid and the four primary simple trisomics

Characters	Broad vs. Diploid	Broad vs. Pointed	Broad vs. Narrow	Broad vs. Small	Pc Di
Central leaflet length (mm)	0.413	2.711*	3.173**	5.541**	1.
Central leaflet width (mm)	2.581*	7.491**	>10**	9.118**	2.
Leaflet index (w/l ratio)	5.088**	6.548**	>10**	3.900**	2.
Florets per umbel	4.629**	1.870	1.659	0.142	4.
Floret length (mm)	4.253**	1.131	1.199	3.845**	1.
Standard width (mm)	1,939	1.185	5.269**	4.059**	1.
Ovary length (mm)	3.474**	5.123**	0.794	2.269*	0.
Style length (mm)	0.356	2.535*	2.087*	5.144**	4.
Style/ovary (ratio)	2.552*	6.017**	2.684*	2.046	3.
Pollen fertility	1.857	0.352	0.751	1.573	1.
d.f.	10	11	25	12	
T.05	2.228	2.201	2.060	2.179	2.
T.01	3.169	3.106	2.787	3.055	3.

* Significant
** Highly significant

id

d l	Pointed vs. Diploid	Pointed vs. Narrow	Pointed vs. Small	Narrow vs. Diploid	Narrow vs. Small	Small vs. Diploid
**	1.754	0.524	3.193**	2.016	3.893**	3.985**
**	2.561*	2.817**	0.402	6.376**	3.968**	2.831*
**	2.001	4.753**	3.577**	9.298**	>10**	1.518
	4.078**	0.421	3.289**	3.424**	2.024	9.304**
**	1.801	2.274*	3.467**	5.219**	2.855**	6.220**
**	1.097	6.159**	4.379**	6.114**	1.847	4.084**
*	0.871	6.699**	7.260**	4.463**	3.753**	5.262**
**	4.387**	0.932	2.843*	2.454*	3.505**	>10**
	3.191*	7.667**	7.883**	1.994	0.131	1.594
	1.439	0.145	1.922	2.145*	3.355**	4.157**
	9 2.262 3.250	24 2.064 2.797	11 2.201 3.106	23 2.069 2.807	25 2.060 2.787	10 2.228 3.169

TABLE 8.--Diagnostic morphological features of the diploid and the five primary simple trisomics

Trisomics	Growth habit	Leaf characters	Leaflet index (w/l ratio)	Flower characters	Style/ ovary (ratio)	Pollen fertility (%)
Round	Ascending	Extremely large, round; Thick; Less pubescent; Pale green	*	*	*	*
Broad	Decumbent	Large, broad; Less pubescent; Yellow green	0.61	-	1.05	88.21
Pointed	Decumbent	Narrow with pointed tips; More pubescent; Dark green; Midribs and veins prominent; A few leaves deformed, with 6 leaflets	0.47	Styles protrude from corolla tubes	0.84	89.57
Narrow	Decumbent	Extremely narrow; Thick and rigid; Pubescent; Dark green	0.40	A few flowers deformed; Fewer pollen grains in anthers	0.99	89.91
Small	Semi-erect	Small with blunter tips; Pubescent; Green	0.54	Small	0.98	83.00
Diploid	Decumbent	Pubescent; Green	0.52	-	0.94	94.54

* No information

green in colour and less pubescent. It is considered that the developmental process of flower formation in this trisomic type was greatly disturbed as after two years only three flowers formed on one of the two plants, and the flowers are a little smaller than those of the diploid.

Broad.--Broad is distinct from the diploid and the other trisomic types in many respects, but its name is derived from the large and broad leaves (Fig. 38). The growth habit is decumbent like that of the diploid (Fig. 32). Young stems and the midribs and margins of the young leaves are purple. Leaves are large, broad, less pubescent and yellow green in colour. The central leaflet index (width/length ratio, 0.61) is the largest of the four trisomic types measured. The style is slightly longer than the ovary with the ratio of style/ovary being 1.05 (Fig. 47). Pollen fertility is 88.21%.

Pointed.--The name Pointed refers to the conspicuously acuminate leaf tip (Fig. 39). Plants of this trisomic type are decumbent in growth habit (Fig. 33). Leaves are slightly thinner with pointed tips, greater pubescence, and a dark green colour. Midribs and veins are raised from the leaf surface and are more prominent

than those of the diploid and the other trisomic types (Fig. 39). Central leaflet index is 0.47. A few deformed leaves have six leaflets. Styles usually protrude from the corolla tubes (Fig. 44). The style is shorter than the ovary with the ratio of style/ovary being 0.84 (Fig. 47). Pollen fertility is 89.57%.

Narrow.--This trisomic type has extremely narrow leaves which distinguish it readily from the diploid and from other trisomic types at the early seedling stage (Fig. 40). Growth habit is decumbent like that of the diploid (Fig. 34). Stems are slender; young stems and the midribs and veins of young leaves are purple. The leaves are thick and rigid, usually a little curved and wavy, dark green, and pubescent. The central leaflet index (0.40) is the smallest among the diploid and for all the trisomics described. Flowers are usually normal in shape, but a few of them were deformed. The style/ovary ratio is 0.99, which is near to that of the diploid (Fig. 47). Anthers are usually degenerated before anthesis, with relatively few pollen grains within them. Nevertheless, pollen fertility as indicated by pollen stainability amounted to 89.91%.

Small.--The small leaves and flowers, characteristic for this trisomic type, as compared with those of the

diploid and the other trisomic types, is the basis upon which the name is derived. This trisomic type is also distinct in its semi-erect growth habit (Fig. 35). Leaves are smaller with blunter tips, green in colour, and pubescent (Fig. 41). The central leaflet index is 0.54. Flowers are normal in shape but smaller in size than those of the diploid and the other trisomic types (Fig. 46). Style/ovary ratio is 0.98 (Fig. 47). Pollen fertility (83%) is the lowest of all the trisomic types. Small cannot be easily distinguished from the diploid at the early seedling stage.

2. Double trisomics

The two double trisomic plants, 00109 and 00185, derived from the cross triploid ♀ x diploid ♂, were closely similar in external morphology. Their leaves were dark green in colour and more pubescent than the diploid. The midribs and veins of the leaves were raised from the leaf surface. In these aspects the double trisomics resembled the simple trisomic type Pointed, but differed from it by not having pointed and acuminate leaves. These two double trisomics have never flowered.

3. Telosomic trisomics

Three telosomic trisomic plants have been obtained

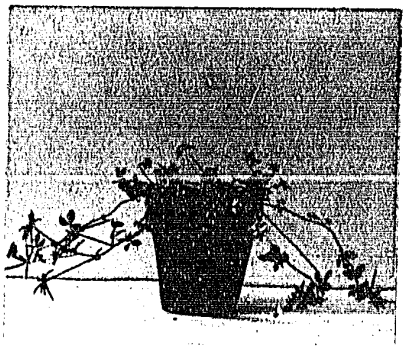

Fig. 30.--Diploid.

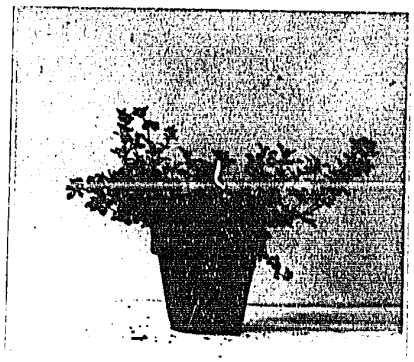

Fig. 31.--Round.

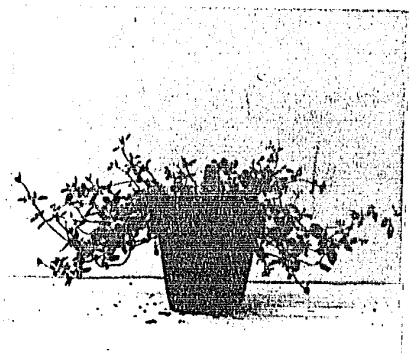

Fig. 32.--Broad.

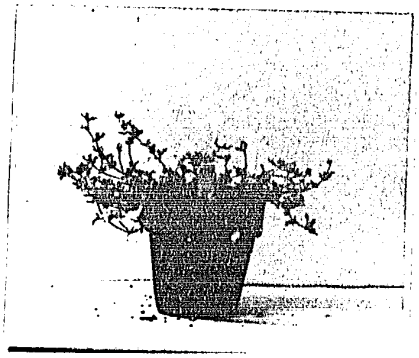

Fig. 33.--Pointed.

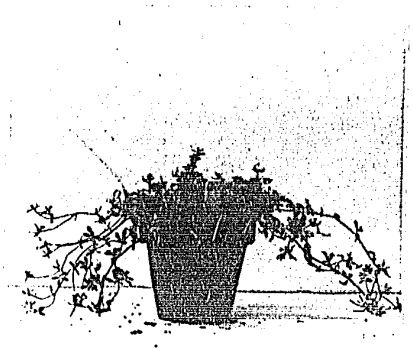

Fig. 34.--Narrow.

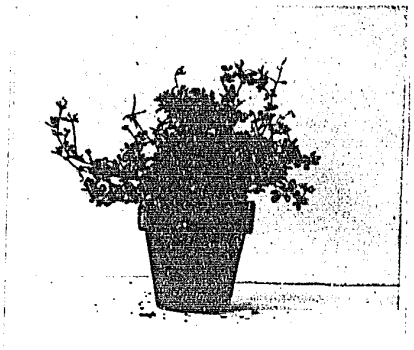

Fig. 35.--Small.

Figs. 30-35.--Mature plants of diploid Lotus pedunculatus and five primary simple trisomic types.

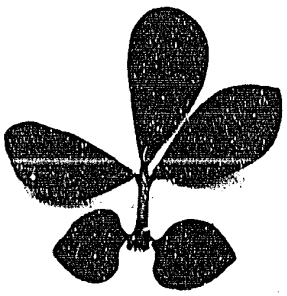

Fig. 36.--Diploid.

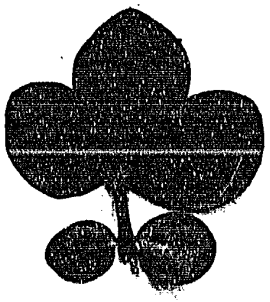

Fig. 37.--Round.

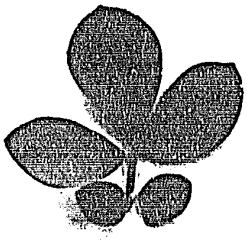

Fig. 38.--Broad.

Fig. 39.--Pointed.

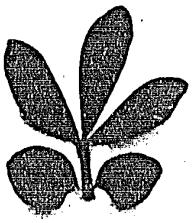

Fig. 40.--Narrow.

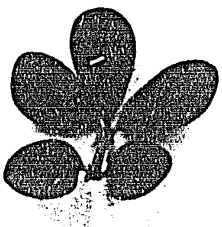

Fig. 41.--Small.

Figs. 36-41.--Leaves of a diploid plant of Lotus pedunculatus and five primary trisomic types. x ca. 1.5.

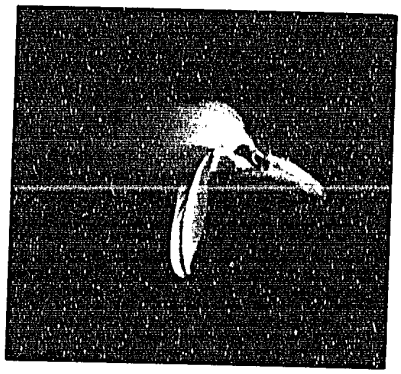

Fig. 42.--Diploid.

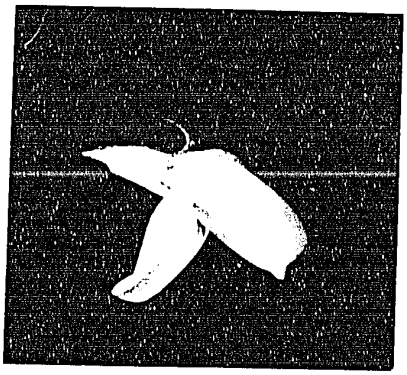

Fig. 43.--Broad.

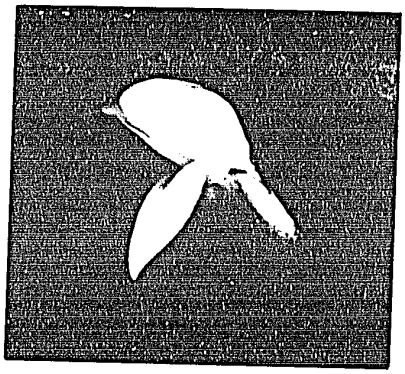

Fig. 44.--Pointed.

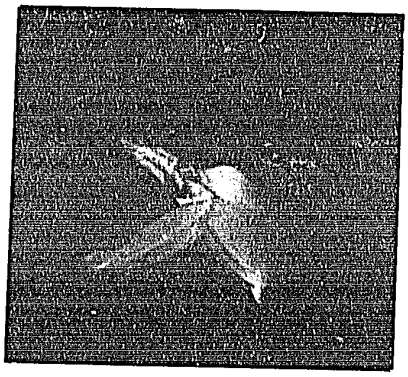

Fig. 45.--Narrow.

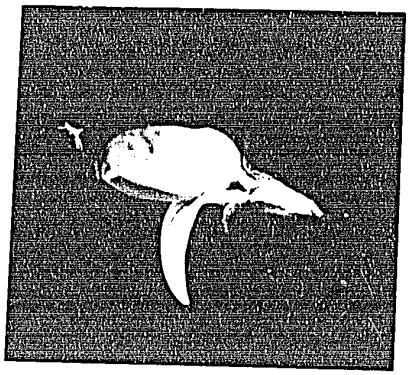

Fig. 46.--Small.

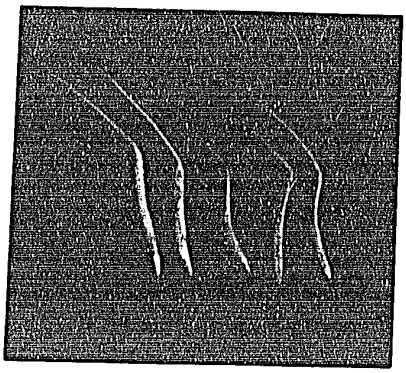

Fig. 47.--Pistils. From left to right: Pointed, Broad, Small, Narrow, diploid.

Figs. 42-46.--Flowers of a diploid plant of Lotus pedunculatus and four primary simple trisomic types. x ca. 2

Fig. 47.--Pistils of a diploid plant of Lotus pedunculatus and four primary simple trisomic types. x ca. 2

in L. pedunculatus. The telosomic trisomic 00004 derived from the cross triploid ♀ x diploid ♂, 10458 from diploid ♀ x Broad ♂, and 11947 from Pointed ♀ x diploid ♂. Only two of them, 00004 and 10458, have been studied morphologically. The telosomic trisomic 00004 differed from the diploid by having a semi-erect growth habit. Leaf colour of 10458 was slightly yellower than that of the diploid but slightly darker than the trisomic type Broad. In all other morphological aspects they closely resembled their diploid siblings, and even quantitative measurements did not help in distinguishing them.

V. Cytological Identification of Trisomics

1. Karyotype of the diploid

Measurements of ten well spread metaphase chromosome plates from root tip cells (Fig. 48) for the diploid L. pedunculatus are presented in Table 9. As a standard measure for chromosome length, a relative value was used which was expressed as chromosome length in percentage of the total complement length (TCL). The ratio of the long to short arm of the chromosome (L/s) was used as an index to indicate the centromere position. An idiogram was constructed based on the measurements which is shown in Fig. 49.

The actual average length of the chromosomes of L. pedunculatus varied from 3.11 u for the longest chromosome to 1.54 u for the shortest chromosome. The chromosomes were numbered from 1 to 6 in the order of decreasing length, chromosome 1 being the longest and chromosome 6 the shortest. According to length alone, the somatic chromosomes of L. pedunculatus could be arranged into four classes: one large, one medium large, two medium and two small. Although all the chromosomes are metacentric, chromosome 1 and 2 could be readily distinguished from the rest and from each other by their length. In addition, chromosome 2 has a small satellite on the short arm. The size difference between chromosomes 3 and 4 was slight. However, chromosome 4 has a tiny satellite on the short arm which was visible under good preparations and could be used as a character to distinguish it from chromosome 3. It is rather difficult to identify chromosomes 5 and 6 since they did not differ appreciably in length (0.1 u difference) and they did not have any particular cytological markers. The only notable difference between these chromosomes seemed to be the arm ratio in which chromosome 5 had a value of 1.49 and chromosome 6, 1.76. Again, it could not be considered as a reliable criterion for distinguishing these two chromosomes as they are too small to allow accurate measuring.

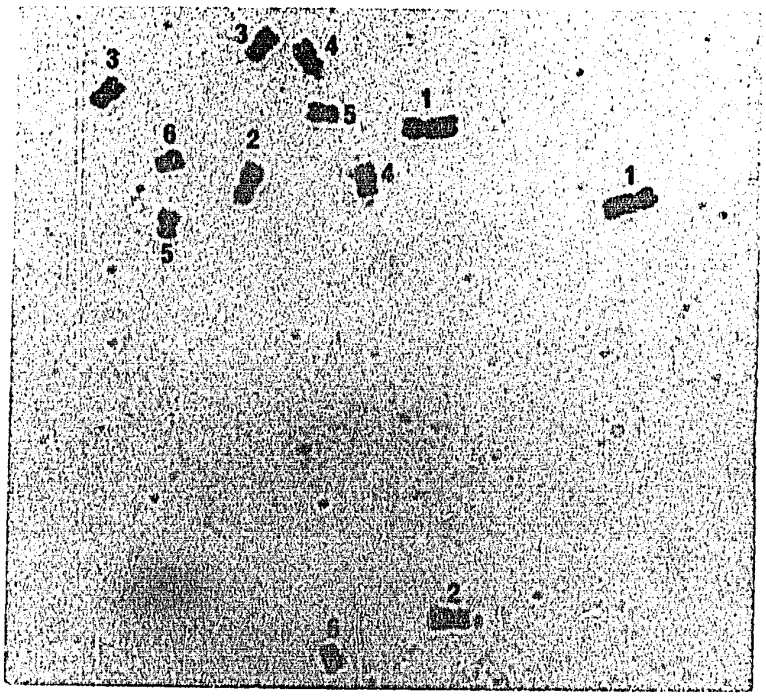

Fig. 48.--Somatic chromosomes of Lotus pedunculatus. 2n = 12. x ca. 2500

TABLE 9.--The karyotype of Lotus pedunculatus based on the measurements of ten cells at somatic metaphase

Chromosome	Chromosome length in u			Arm ratio L/S	Chromosome length in %		
	Long arm	Short arm	Total		Long arm	Short arm	Total
1	1.98	1.13	3.11	1.76	15.84	9.01	24.85
2	1.48	0.87	2.35	1.70	11.86	6.96	18.82
3	1.26	0.76	2.02	1.67	10.10	6.08	16.18
4	1.19	0.65	1.84	1.83	9.54	5.23	14.77
5	0.98	0.66	1.64	1.49	7.84	5.25	13.09
6	0.98	0.56	1.54	1.76	7.84	4.45	12.29

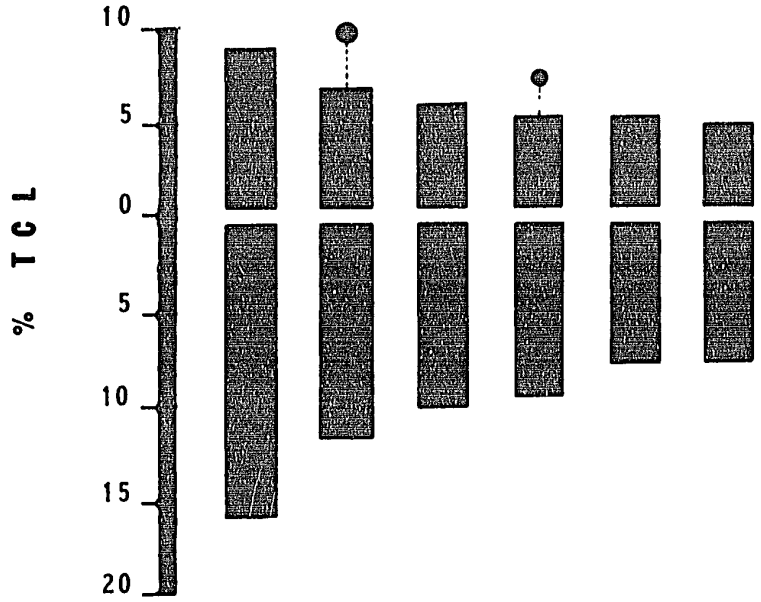

Fig. 49.--Idiogram of the haploid chromosomes of Lotus pedunculatus.

The karyotype of L. pedunculatus can be summarized as follows:

Chromosome 1: The longest chromosome in the complement. Actual average length 3.11 u, relative length 24.85%. Arm ratio 1.76, submetacentric.

Chromosome 2: The second longest chromosome in the complement. Actual average length 2.35 u, relative length 18.82%. Arm ratio 1.70, submetacentric. This chromosome has a small satellite attached to the terminal end of the short arm.

Chromosome 3: This chromosome is of medium size. Actual average length 2.02 u, relative length 16.18%. Arm ratio 1.67, submetacentric.

Chromosome 4: Medium size. Actual average length 1.84 u, relative length 14.77%. Arm ratio 1.83, submetacentric. This chromosome has a very small satellite attached terminally to the short arm.

Chromosome 5: Small. Actual average length 1.64 u, relative length 13.09%. Arm ratio 1.49, submetacentric.

Chromosome 6: The shortest chromosome in the complement. Actual average length 1.54 u, relative length 12.29%. Arm ratio 1.76, submetacentric. This chromosome is very difficult to distinguish from chromosome 5.

2. The extra chromosome of the primary trisomics

Since the somatic chromosomes of L. pedunculatus could be distinguished from each other by length, by the presence and absence of a satellite and by arm ratio, an attempt was made therefore to identify the extra chromosomes of the five morphological simple trisomic types. It was found that Round was trisomic for chromosome 1 (Figs. 50, 51), Broad trisomic for chromosome 3 (Figs. 52a, 52b) and Pointed trisomic for chromosome 4 (one of the satellite chromosomes, Figs. 53a, 53b, 54a, 54b). The extra chromosomes of Narrow and Small could not be identified with certainty. Narrow was probably trisomic for chromosome 5 (Figs. 55, 56), and Small trisomic for chromosome 6 (Fig. 57).

3. The extra chromosomes of the double trisomics

Only one double trisomic plant, 00185, was studied karyologically to determine which chromosomes it possessed in triplicate. One of the extra chromosomes had its two arms equal in length and it was identified as a deficient chromosome 4 since it possessed a tiny satellite attached to the terminal end of one arm (Fig. 58). This deficient chromosome could have originated from breakage of the terminal portion of the long arm of chromosome 4. The

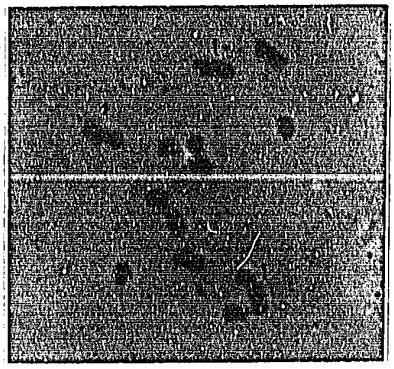

Fig. 50.--Somatic chromosomes of the trisomic type Round showing chromosome 1 is in triplicate.
x ca. 2000

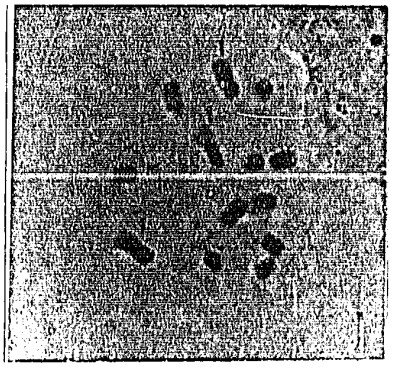

Fig. 51.--Somatic chromosomes of the trisomic type Round showing chromosome 1 is in triplicate.
x ca. 1800

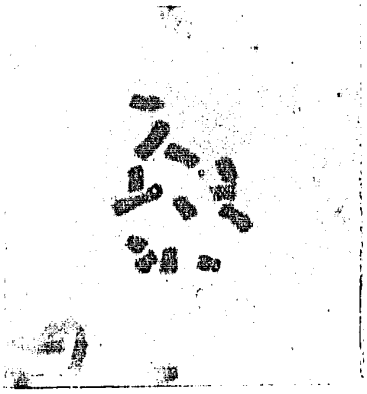

Fig. 52a.--Somatic chromosomes of the trisomic type Broad in which chromosome 3 is in triplicate.
x ca. 2000

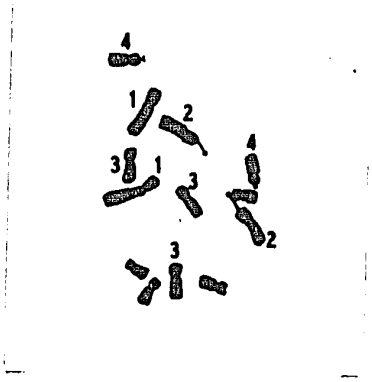

Fig. 52b.--An interpretive drawing of the chromosomes in Fig. 52a.

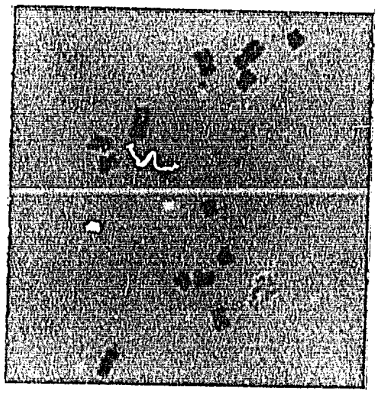

Fig. 53a. Somatic chromosomes of the trisomic type Pointed in which chromosome 4 is in triplicate. x ca. 1700

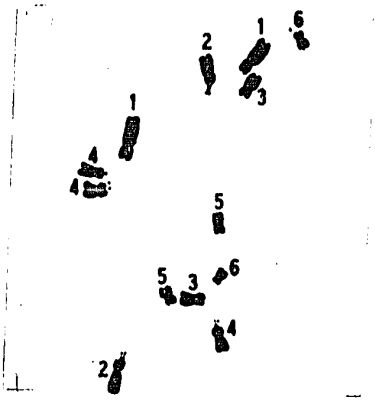

Fig. 53b.--An interpretive drawing of the chromosomes in Fig. 53a.

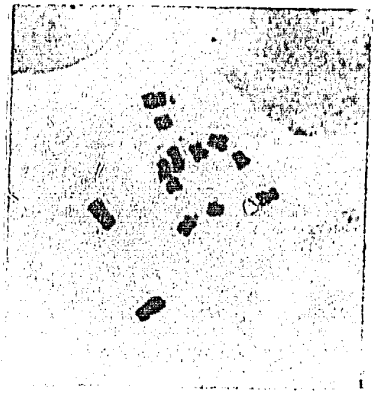

Fig. 54a.--Somatic chromosomes of the trisomic type Pointed in which chromosome 4 is in triplicate. x ca. 1700

Fig. 54b.--An interpretive drawing of the chromosomes in Fig. 54a.

Fig. 55.--Somatic chromosomes of the trisomic type Narrow showing chromosome 5 in triplicate.
x ca. 1500

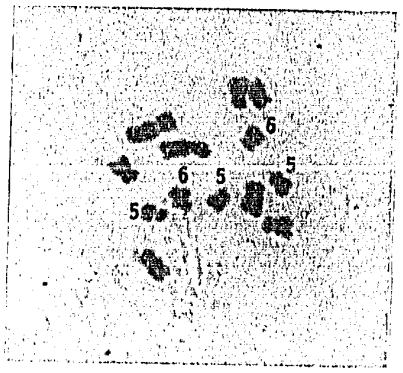

Fig. 56.--Somatic chromosomes of the trisomic type Narrow showing chromosome 5 in triplicate.
x ca. 2200

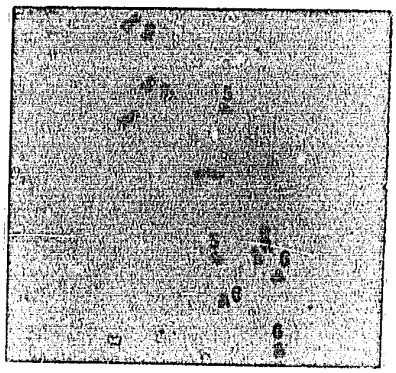

Fig. 57.--Somatic chromosomes of the trisomic type Small showing chromosome 6 in triplicate.
x ca. 1280

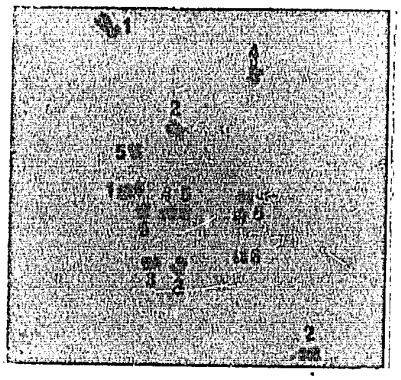

Fig. 58.--Somatic chromosomes of one double trisomic plant showing that a deficient chromosome 4 (indicated by arrow) and probably a whole chromosome 6 are in triplicate. x ca. 1500

other extra chromosome of this double trisomic was considered probably to be a normal chromosome 6.

4. The extra chromosome of the telosomic trisomics

The extra chromosomes of the three telosomic trisomic plants, 00004, 10458 and 11947, could not be identified with certainty. These telosomes ranged from 0.75 u to 0.55 u at the metaphase stage of mitosis. Judging from their length, they could be any one of the short arms of chromosomes 3 to 6 (see Table 9). Although the short arm of chromosome 4 is distinct in having a tiny satellite, in some of the cells studied this satellite was not visible and also could be easily confused with the terminal centromere of the telosomes.

VI. Meiosis in Trisomics

1. Primary simple trisomics

At diakinesis and prometaphase I in the microsporocytes of the four trisomic types the chromosome association of 6 II + 1 I was more frequent than the association of 1 III + 5 II (Table 10). In some PMC's, univalents were found to replace one or two bivalents to form associations such as 5 II + 3 I (Figs. 69, 70, 77, 83), 1 III + 4 II + 2 I (Fig. 61) and 4 II + 5 I. In the association of 5 II + 3 I, the three univalents were

TABLE 10.--Chromosome associations at diakinesis and/or MI of the different types of trisomics

Acc. no.	No. cells	6II+1I	5II+3I	4II+5I
Broad				
00032	98	58	12	1
00088	62	45	4	0
00100	33	19	9	1
Total	193	122	25	2
Pointed				
00015	74	49	9	0
00108	65	45	3	0
00142	36	32	0	0
Total	175	126	12	0
Narrow				
00010	79	65	10	0
00028	67	52	5	0
00075	46	34	5	0
Total	192	151	20	0
Small				
00037	52	40	8	0
00070	47	35	3	1
00097	52	44	7	0
00144	37	31	1	0
Total	188	150	19	1
Telosomic				
00004	65*	60	3	0
10458	37	31	4	0

* One asynaptic cell with 13 I is not included

of

	3II+7I	2II+9I	1III+5II	1III+4II+2I	% cells with one trivalent
	0	0	26	1	27.55
	0	0	11	2	20.97
	0	0	3	1	12.12
	0	0	40	4	22.80
	0	0	16	0	21.62
	0	0	16	1	26.15
	0	0	4	0	11.11
	0	0	36	1	21.14
	0	0	4	0	5.06
	0	0	10	0	14.93
	0	0	7	0	15.22
	0	0	21	0	10.94
	0	0	4	0	7.69
	2	3	3	0	6.38
	0	0	1	0	1.92
	0	0	5	0	13.51
	2	3	13	0	6.91
	0	0	2	0	3.08
	0	0	2	0	5.41

in a trivalent. Fig. 89 clearly shows such a positive correlation between the length of the triplicated chromosome and the frequency of trivalent formation.

Chromosome configurations at diakinesis and prometaphase I were not studied in detail. Preliminary observations showed that most of the trivalents formed chains usually with two chromosomes closely associated together and the third one only loosely paired with them (Figs. 76, 78, 82). Other less frequent types of trivalent associations found in the trisomic types Broad and Pointed were the frying-pan (Fig. 60) and V shapes (Fig. 62). These trivalent configurations were not found in the trisomic types Narrow and Small. All the univalents were rod shape; ring univalents were never observed in any of the four trisomic types. Pentavalents were not observed in a single cell.

Most univalents congressed and became oriented on the equatorial plate although they did so somewhat later than the bivalents. Univalents either divided later than the bivalents (Figs. 63, 65, 66, 71) or remained undivided (Figs. 64, 65, 72, 85) at anaphase I and telophase I. No particular trend was apparent as to which extra chromosome, when present as an univalent, tended to divide more frequently than others (Table 11).

usually equal in size. But in the PMC's of Broad, occasionally two univalents were found smaller than the third one, and also in the PMC's of Narrow and Small, occasionally two univalents were larger than the third one (Fig. 83). One plant of the trisomic type Small, 00070, seemed to be primarily asynaptic, since as many as nine univalents were found in the PMC's (Fig. 84). The frequency of cells with one trivalent varied between plants of the same trisomic type (Table 10). For example, the frequencies for the three plants of Broad were 27.55%, 20.97% and 12.12%. Variation between the four plants of Small for the presence of one trivalent was even greater, the highest frequency was 13.51% and the lowest 1.92%. However, when the data for the same trisomic type were combined together and the frequencies of trivalent were compared between different trisomic types, an interesting trend of variation was observed. The frequency of cells with one trivalent was 22.80% for Broad, 21.14% for Pointed, 10.94% for Narrow and 6.91% for Small. The length of the triplicated chromosome of Broad, Pointed, Narrow and Small was 2.02 u, 1.84 u, 1.64 u, and 1.54 u, respectively. It appears that the type of chromosome association of the trisome is correlated with chromosome length, the longer the triplicated chromosome, the greater is the tendency for it to associate

TABLE 11.--Meiotic chromosome behaviour at first and second division in anaphase and telophase of the different types of trisomics

Acc.	Anaphase I--Telophase I					
	No. cells	% cells with laggards	Laggards per cell (mean)	Laggards per cell (range)	% dividing univalents	un un
Broad						
00032	42	64.28	0.67	0-2	75.00	
00088	139	35.97	0.47	0-3	66.15	
00100	58	43.10	0.43	0-1	24.00	
Total	239	42.68	0.47	0-3	61.06	
Pointed						
00015	50	42.00	0.48	0-2	62.50	
00108	63	20.63	0.21	0-1	15.38	
00142	103	28.16	0.28	0-1	41.38	
Total	216	29.17	0.31	0-2	43.94	
Narrow						
00010	55	32.73	0.33	0-1	100.00	
00028	70	44.29	0.53	0-3	91.89	
00075	45	80.00	1.24	0-3	75.00	2
Total	170	50.00	0.65	0-3	84.68	1
Small						
00037	29	79.31	0.79	0-1	13.05	8
00070	30	50.00	0.57	0-3	94.12	
00097	52	59.62	0.60	0-1	96.77	
00144	59	59.32	0.59	0-1	88.57	1
Total	170	61.18	0.62	0-3	75.47	2
Telosomic						
00004	18	61.11	0.72	0-3	15.38	8
10458	69	43.48	0.43	0-1	96.67	

. second
.t types

		Anaphase II--Telophase II			
% iding alents	% undivided univalents	No. cells	% cells with laggards	Laggards per cell (mean)	Laggards per cell (range)
5.00	25.00	45	33.33	0.53	0-2
6.15	33.85	58	55.17	1.03	0-6
4.00	76.00	53	32.08	0.43	0-2
1.06	38.94	156	41.03	0.69	0-6
2.50	37.50	48	58.33	0.96	0-2
5.38	84.63	31	9.68	0.16	0-2
L.38	58.62	54	51.85	0.91	0-4
3.94	56.06	133	44.36	0.75	0-4
).00	0.00	37	48.65	0.76	0-3
..89	8.11	39	61.54	1.40	0-6
;.00	25.00	63	50.79	0.89	0-4
..68	15.32	139	53.24	0.95	0-6
.05	86.95	36	52.78	0.86	0-4
.12	5.88	37	37.84	0.46	0-2
.77	3.23	40	62.50	1.30	0-6
.57	11.43	31	41.93	0.55	0-2
.47	24.53	144	49.31	0.81	0-6
.38	84.61	16	68.75	1.31	0-2
.67	3.33	36	69.44	1.19	0-4

After the bivalents had completed their anaphase movement, those univalents which congressed and oriented on the equatorial plate separated equationally and the two halves migrated toward opposite poles. It should be noted, therefore, that the univalents, whether divided or undivided, were always behind the bivalents in their anaphase movement. For descriptive purposes, these univalents will be referred to as "laggards" when they were observed remaining either on the equatorial plate or delayed on their way to the poles at anaphase I and telophase I after the other chromosomes had already moved poleward or had reached the poles. Some of the univalents were not laggards in the true sense since their daughter chromosomes were able to complete their journey to their respective poles in time to be included in the interphase nuclei. The frequency of cells with "laggards" and the average number of "laggards" per cell varied within and between trisomic types (Table 11). No specific pattern of variation was observed. The frequency of "laggards" per cell at anaphase I and/or telophase I for the four trisomic types (0.47 for Broad, 0.31 for Pointed, 0.65 for Narrow, 0.62 for Small) was not correlated with the frequency of univalents at diakinesis and/or prometaphase I (1.11 for Broad, 0.94 for Pointed, 1.09 for Narrow, 1.34 for Small).

Only those univalents which remained undivided and became incorporated into the interphase nuclei by chance in the first division divided at anaphase II. If the monads of the divided univalents succeeded in reaching the respective poles in the first division, they lagged at anaphase II and telophase II. The frequency of cells containing laggards and the average number of laggards per cell varied within and between trisomic types (Table 11). No specific pattern of variation could be seen.

As in the normal diploid, the largest chromosome of the trisomic plants always separated later than other chromosomes in the first and second meiotic divisions (Figs. 63, 64, 73, 79).

2. Telosomic trisomics

Meiotic behaviour of the two telosomic trisomics was essentially similar to that of the primary trisomic types. At diakinesis and prometaphase I, the extra telosome formed trivalents less frequently than the primary trisomes (Table 10). The frequencies of cells with one trivalent for the telosomic trisomics 00004 and 10458 were 3.08 and 5.41, respectively. In the microsporocytes of 10458, it was found that the trivalent consisted of a telosome and two primary chromosomes

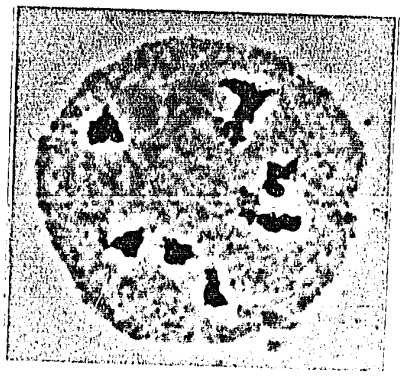

Fig. 59.--Diakinesis, showing 6 II + 1 I.
x ca. 1400

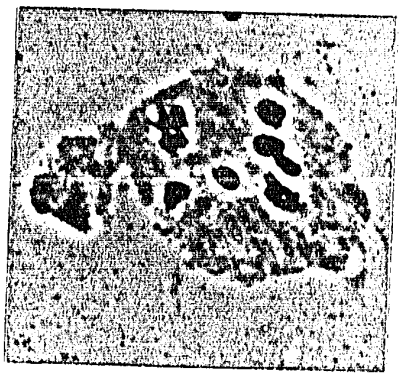

Fig. 60.--Prometaphase I, showing 1 III + 5 II.
x ca. 1400

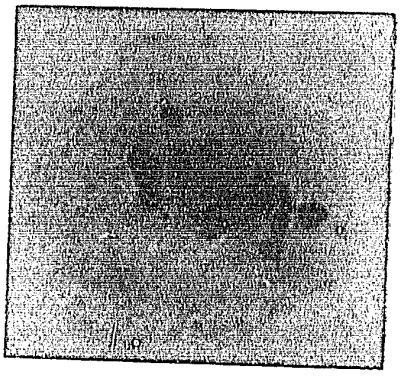

Fig. 61.--MI, showing 1 III + 4 II + 2 I.
x ca. 1260

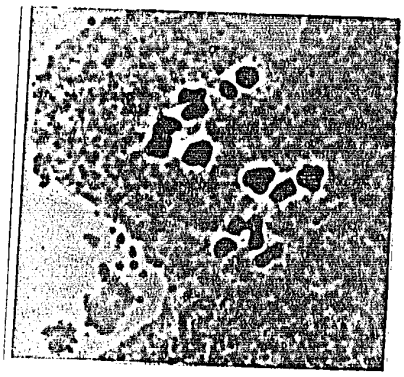

Fig. 62.--MI, showing 1 III + 5 II. (lower right)
x ca. 1740

Figs. 59-62.--Meiosis in the trisomic type Broad.

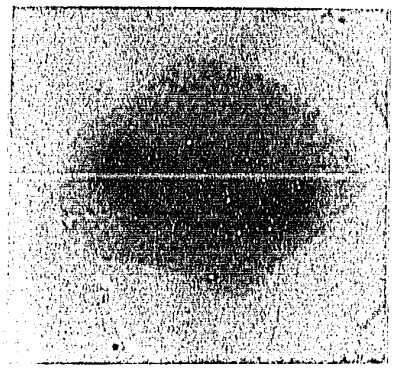

Fig. 63.--TI, showing late separation of the largest bivalent and one dividing laggard.
x ca. 1600

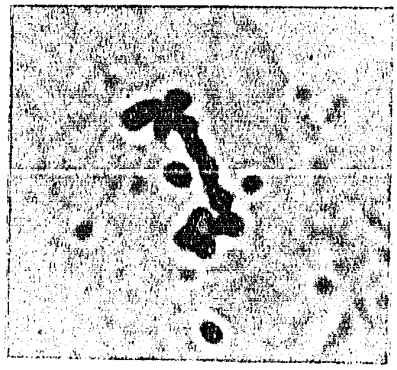

Fig. 64.--AI, showing late separation of the largest bivalent and one undivided laggard.
x ca. 1600

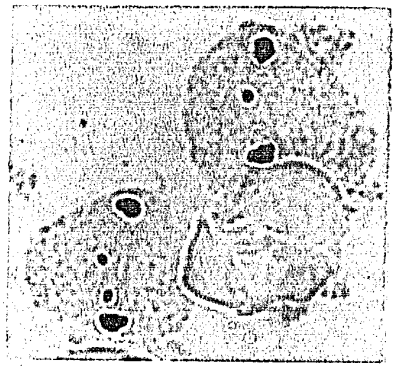

Fig. 65.--Two TI cells, one with a dividing laggard and the other with an undivided laggard.
x ca. 800

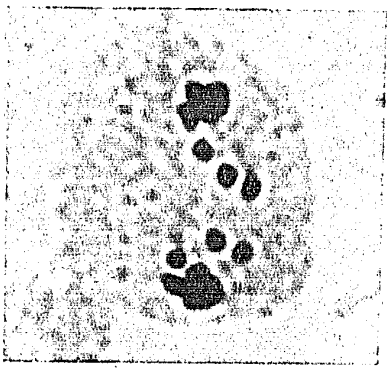

Fig. 66.--TI, showing 3 dividing laggards.
x ca. 1450

Figs. 63-66.--Meiosis in the trisomic type Broad.

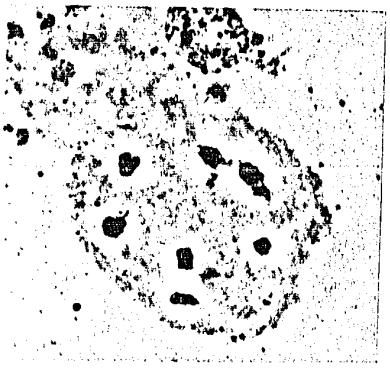

Fig. 67.--Diakinesis, showing 6 II + 1 I. x ca. 1000

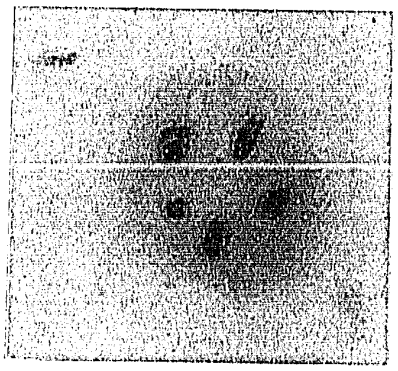

Fig. 68.--Diakinesis, showing 1 III + 5 II. x ca. 1000

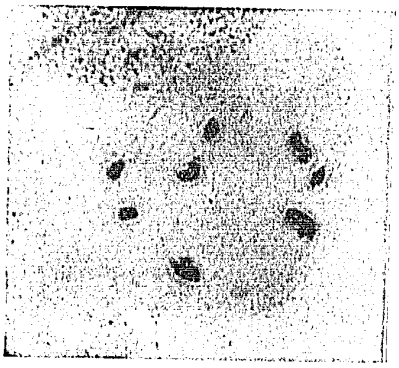

Fig. 69.--Diakinesis, showing 5 II + 3 I. x ca. 1000

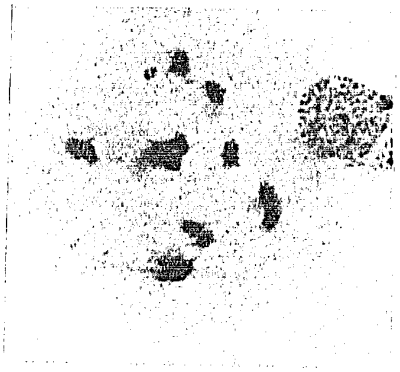

Fig. 70.--Diakinesis, showing 5 II + 3 I. x ca. 1260

Figs. 67-70.--Meiosis in the trisomic type Pointed.

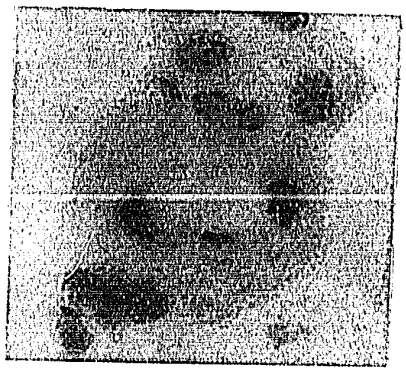

Fig. 71.--TI, showing one dividing laggard.
x ca. 1000

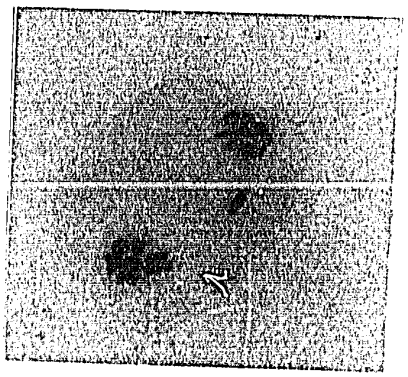

Fig. 72.--TI, showing one undivided laggard.
x ca. 1260

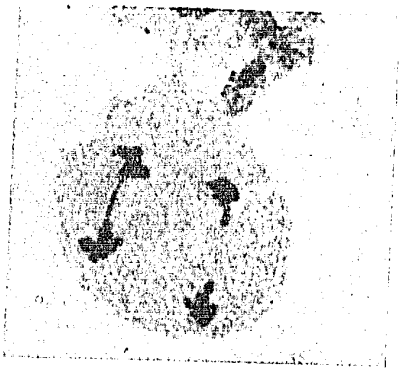

Fig. 73.--TII, showing late separation of the largest chromosome.
x ca. 1260

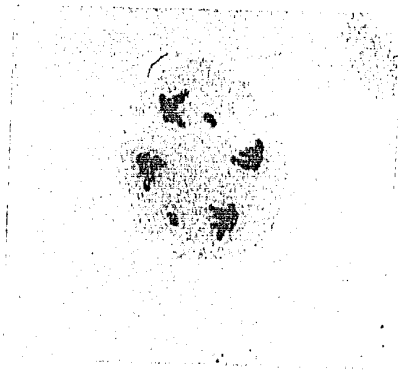

Fig. 74.--TII, showing 2 laggards. x ca. 1000

Fig. 71-74.--Meiosis in the trisomic type Pointed.

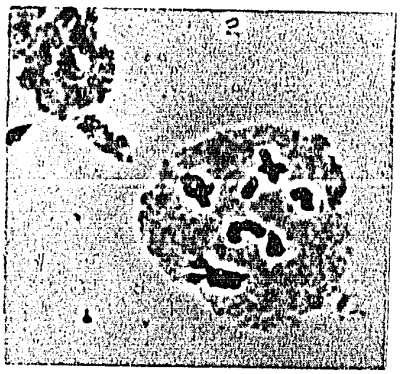

Fig. 75.--Diakinesis, showing 6 II + 1 I. x ca. 1100

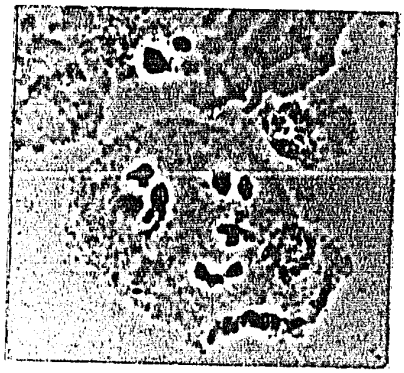

Fig. 76.--Diakinesis, showing 1 III + 5 II. x ca. 950

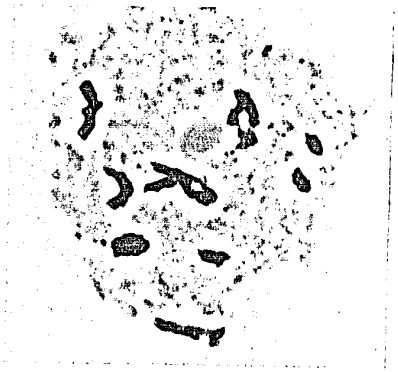

Fig. 77.--Diakinesis, showing 5 II + 3 I. x ca. 1260

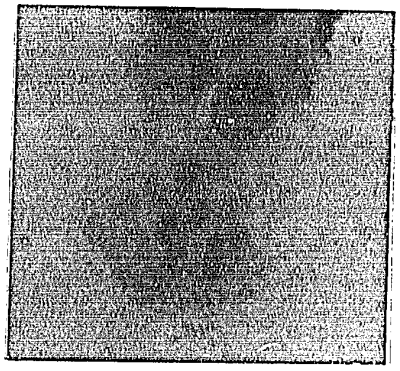

Fig. 78.--Diakinesis, showing 1 III + 5 II. x ca. 950

Figs. 75-78.--Meiosis in the trisomic type Narrow.

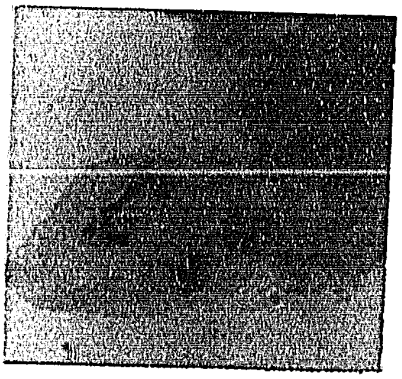

Fig. 79.--AII, showing late separation of the largest chromosome. x ca. 1050

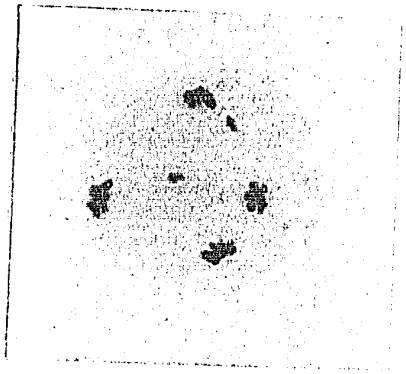

Fig. 80.--TII, showing 2 laggards. x ca. 1050

Figs. 79-80.--Meiosis in the trisomic type Narrow.

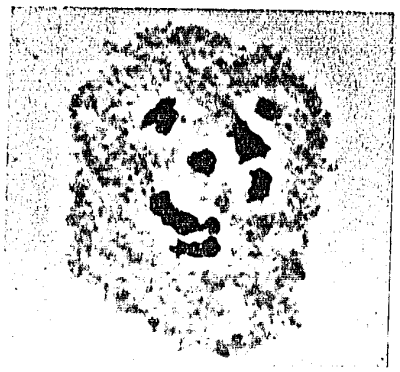

Fig. 81.--Diakinesis, showing 6 II + 1 I.
x ca. 1500

Fig. 82.--MI, showing 1 III + 5 II.
x ca. 1300

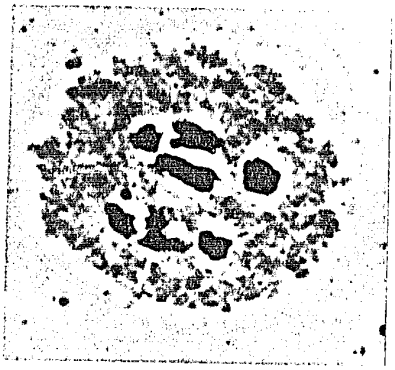

Fig. 83.--Diakinesis, showing 5 II + 3 I.
x ca. 1500

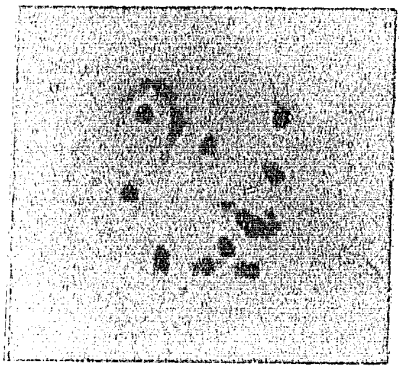

Fig. 84.--Diakinesis, showing 2 II + 9 I.
x ca. 1260

Figs. 81-84.--Meiosis in the trisomic type Small.

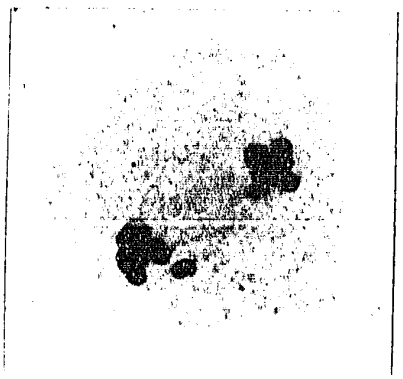

Fig. 85.--TI, showing one undivided laggard.
x ca. 1400

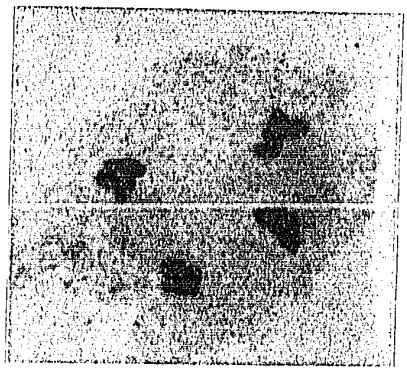

Fig. 86.--One cell with normal TII.
x ca. 1400

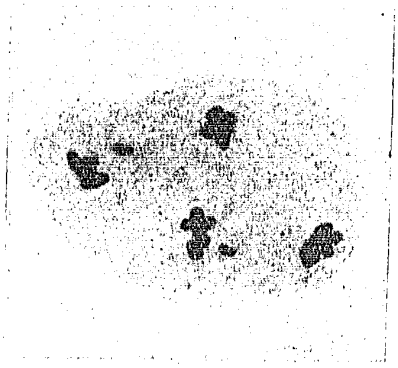

Fig. 87.--TII, showing 2 laggards. x ca. 1400

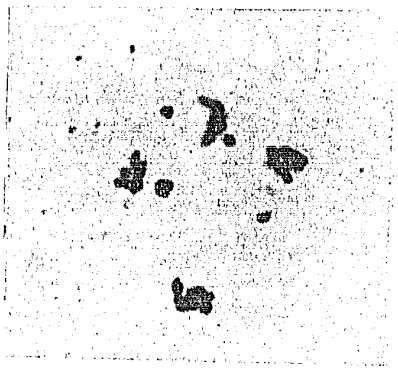

Fig. 88.--TII, showing 4 laggards. x ca. 1400

Figs. 85-88.--Meiosis in the trisomic type Small.

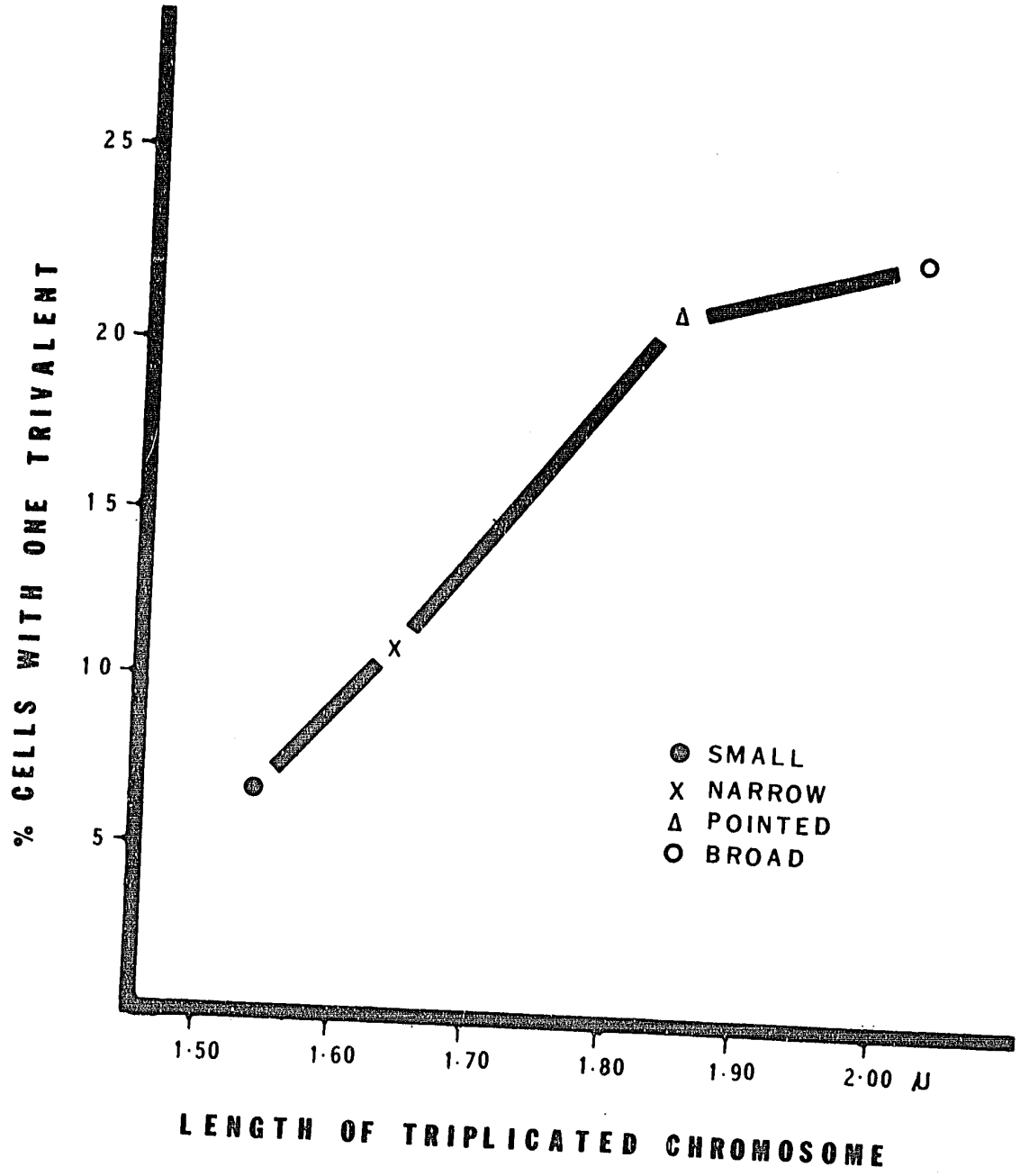

Fig. 89.--Correlation between length of triplicated chromosome and frequency of trivalent formation of the four trisomic types, Broad, Pointed, Narrow, and Small.

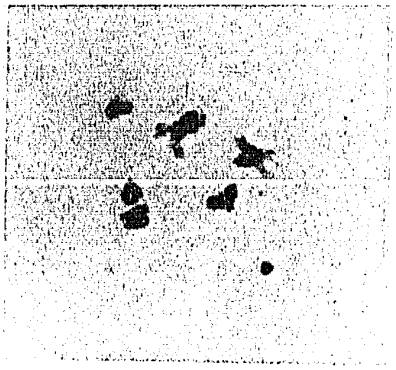

Fig. 90.--Diakinesis, showing 6 II + 1 I. x ca. 1260

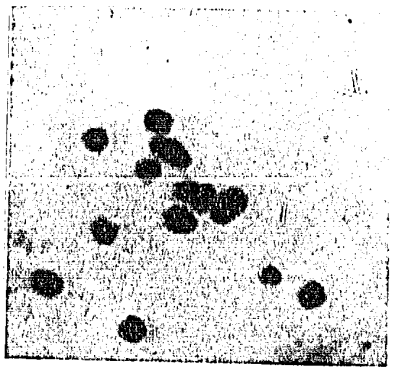

Fig. 91.--An asynaptic cell with 13 I at prometaphase I. x ca. 1900

Fig. 92.--AI, showing late separation of the largest bivalent and one undivided laggard. x ca. 1900

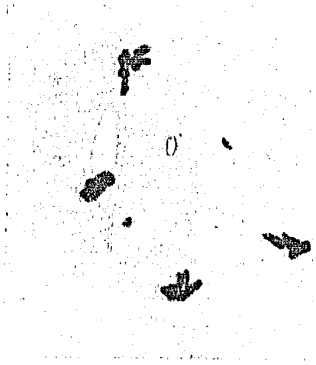

Fig. 93.--TII, showing 2 laggards. x ca. 1260

Figs. 90-93.--Meiosis in one telosomic trisomic, 00004.

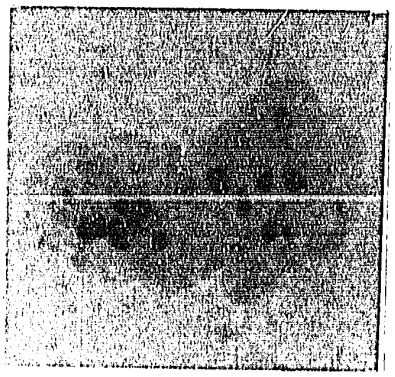

Fig. 94.--Prometaphase I, showing 6 II + 1 I (right).
x ca. 1100

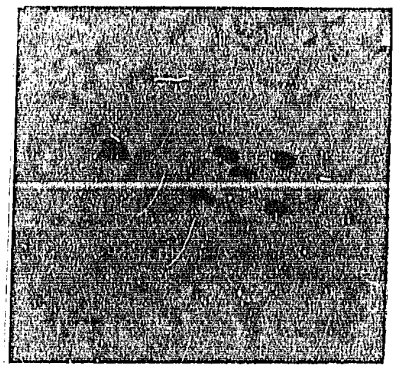

Fig. 95.--MI, showing 1 III + 5 II.
x ca. 1150

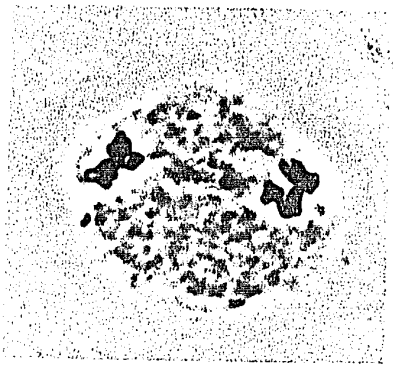

Fig. 96.--TI, showing one dividing laggard.
x ca. 1500

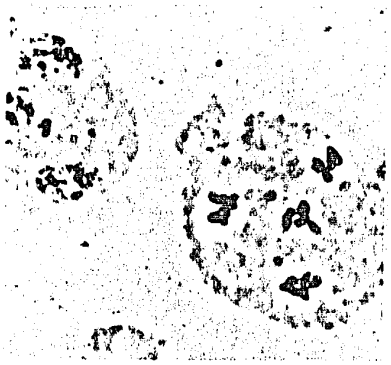

Fig. 97.--TI, showing 2 laggards (right).
x ca. 1100

Figs. 94-97.--Meiosis in one telosomic trisomic, 10458.

belonging to the small group (presumably chromosome 5 or 6; Fig. 95). One asynaptic microsporocyte of 00004 contained 13 I at prometaphase I (Fig. 91). The telosome usually lagged behind the bivalents in its anaphase movement (Figs. 92, 96). Laggards were observed at anaphase II (Figs. 93, 97).

VII. Transmission of the Extra Chromosome in Trisomics

1. Primary simple trisomics

The rate of transmission was studied in reciprocal crosses of trisomics with diploids and also after selfing trisomics; the results of the crosses are shown in Tables 12, 13, and 14. The percentage of pod-set seemed lower when the trisomics were selfed than when they were crossed to the diploids. Reciprocal crosses of trisomics with diploids did not show any significant differences. There was no indication that any one particular trisomic type tended to produce pods more frequently than any other trisomic type. When trisomic plants were used as females in crosses with diploids the percentage pod-set in crosses involving the trisomic type Broad (58.73%) was the highest among the four trisomic types studied. In the reciprocal crosses Narrow ranked first position in pod-set (71.43%). When trisomics were selfed the

TABLE 12.--Crosses of trisomic ♀ x diploid ♂ in *Lotus pedunculatus*

Acc. no. of ♀	Flowers pollinated	Pods	% Pod-set	Seeds	Seeds/pod
Broad					
00032	21	8	38.10	34	4.25
00088	20	11	55.00	72	6.55
00100	22	18	81.82	122	6.78
Total	63	37	58.73	228	6.16
Pointed					
00015	46	10	21.74	53	5.30
00132	48	30	62.50	173	5.77
00142	64	13	20.31	96	7.38
Total	158	53	33.54	322	6.08
Narrow					
00010	24	13	54.17	95	7.31
00028	26	21	80.77	125	5.95
00075	37	14	37.84	45	3.21
Total	87	48	55.17	265	5.52
Small					
00070	9	6	66.67	53	8.83
00097	25	11	44.00	104	9.45
00144	42	15	35.71	125	8.33
Total	76	32	42.11	282	8.81
Telosomic					
00004	68	46	67.65	537	11.67
10458	25	14	56.00	149	10.64

TABLE 13.--Crosses of diploid ♀ x trisomic ♂ in L. pedunculatus

Acc. no. of ♂	Flowers pollinated	Pods	% Pod-set	Seeds	Seeds/pod
Broad					
00032	45	24	53.33	145	6.04
00088	31	4	12.90	45	11.25
00100	31	6	19.35	53	8.83
Total	107	34	31.78	243	7.14
Pointed					
00015	31	16	51.61	155	9.69
00132	28	16	57.14	75	4.69
00142	25	6	24.00	49	8.17
Total	84	38	45.24	279	7.34
Narrow					
00010	25	21	84.00	157	7.48
00028	16	13	81.25	67	5.15
00075	15	6	40.00	41	6.83
Total	56	40	71.43	265	6.62
Small					
00070	9	8	88.89	101	12.63
00097	43	14	32.56	106	7.57
00144	42	5	11.90	68	13.60
Total	94	27	28.72	275	10.19
Telosomic					
00004	46	25	54.35	202	8.08
10458	6	4	66.67	43	10.75

TABLE 14.--Self-pollination of trisomic plants in Lotus pedunculatus

Acc. no.	Flowers pollinated	Pods	% Pod-set	Seeds	Seeds/pod
Broad					
00032	87	18	20.69	56	3.11
00088	103	30	29.13	123	4.10
00100	71	26	36.62	95	3.65
Total	261	74	28.35	274	3.70
Pointed					
00015	59	7	11.86	11	1.57
00132	82	13	15.85	39	3.00
00142	91	14	15.38	35	2.50
Total	232	34	14.66	85	2.50
Narrow					
00010	101	40	39.60	97	2.43
00028	70	18	25.71	12	0.67
00075	91	4	4.40	6	1.50
Total	262	62	23.67	115	1.85
Small					
00070	73	21	28.77	41	1.95
00097	77	40	51.95	226	5.65
00144	87	46	52.87	175	3.80
Total	237	107	45.15	442	4.13
Telosomic					
00004	158	50	31.65	245	4.90
10458	123	51	41.46	160	3.14

percentage of pod-set for Small (45.15%) was higher than any of the other trisomic types.

Pods contained more viable seeds when the cross was diploid ♀ x trisomic ♂ than in the reciprocal one. The average number of seeds per pod was greatly reduced when trisomics were selfed (cf. Tables 12, 13, 14). Different trisomic types also differed in the average number of seeds per pod. Small produced more seeds than any other trisomic type in all of the crosses, and Narrow produced the least seed in comparison with the other trisomic types. When the trisomics were selfed or crossed as female parents to diploids, Broad set more seeds per pod than Pointed. But when the trisomics were used as male parents, the situation was just reversed; Broad and Pointed produced an average of 7.14 and 7.34 seeds per pod, respectively.

The frequency of parental trisomics in progenies from the cross trisomic ♀ x diploid ♂ is presented in Table 15. The data suggest variation within and between trisomic types. One plant of Broad (00088) and two plants of Pointed (00132 and 00142) did not transmit the extra chromosome to their offspring through the eggs. Of the four trisomic types, the lowest frequency in which parental trisomics were found in progeny was 2.91% for

the trisomic Pointed and the highest was 15.51% for Small, the average being 9.07%. One telosomic trisomic (0.97%) was found in the offspring of Pointed. Small produced one unrelated trisomic type, Narrow, among a total of 129 offspring (0.77%).

Each of the four trisomic types transmitted the extra chromosome through the pollen, and the rate of transmission was not significantly lower than through the eggs. The frequencies of parental trisomics in the cross diploid ♀ x trisomic ♂ for the four trisomic types Broad, Pointed, Narrow and Small were 5.11%, 6.07%, 11.02% and 13.54%, respectively. Narrow produced one triploid (0.85%) and two unrelated trisomic plants (1.69%), all of them morphologically identified as Broad. One telosomic trisomic (0.73%) was obtained in the progenies of diploid ♀ x Broad ♂ crosses.

The frequency of parental trisomics was higher in the offspring when trisomics were selfed than when they were crossed to diploids. The data presented in Table 17 indicate that there was some variation within trisomic types. No notable differences were observed in the frequency of parental trisomics in the offspring between the four trisomic types. Tetrasomics and unrelated trisomics were not obtained from selfing trisomics.

The rate of transmission of the extra chromosome through male and female gametes in the four trisomic types is correlated with the length of the triplicated chromosome, the shorter the chromosome, the greater the tendency for it to be transmitted to the offspring (Tables 15, 16). Such a negative correlation between transmission rate and length of the extra chromosome is shown in Fig. 98.

Also observed was some rough correlation between transmission rate and the average number of viable seeds per pod. Small contained more seeds per pod than all the other trisomic types, and it was Small which transmitted the extra chromosome more frequently to the offspring in all the crosses. In the cross diploid ♀ x trisomic ♂, the average number of seeds per pod was higher in Pointed than in Broad, and Pointed was found to transmit the extra chromosome more frequently than Broad. Broad set more seeds and transmitted the extra chromosome with a higher frequency than Pointed in the reciprocal cross. A contradiction to these findings was shown by Narrow. This trisomic type set fewer seeds than all other trisomics but it transmitted the extra chromosome through male and female gametes to the offspring more frequently than Broad and Pointed.

TABLE 15.--Transmission of the extra chromosome in trisomic ♀ x diploid ♂ crosses

Acc. no.	Seeds sown	Seeds germinated	% Seed germination	Mature plants	% Survival of seedlings
Broad					
00032	33	33	100.00	25	75.76
00088	35	35	100.00	34	97.14
00100	70	64	91.43	35	54.69
Total	138	132	95.65	94	71.21
Pointed					
00015	42	38	90.48	36	94.74
00132	57	47	82.46	41	87.23
00142	29	27	93.10	26	96.30
Total	128	112	87.50	103	91.96
Narrow					
00010	94	81	86.17	50	61.73
00028	60	56	93.33	27	48.21
00075	33	27	81.81	16	59.26
Total	187	164	87.70	93	56.71
Small					
00070	53	47	88.68	43	91.49
00097	50	43	86.00	37	86.05
00144	60	54	90.00	49	90.74
Total	163	144	88.34	129	89.58

* $2n = 12 + 1t$
** $2n = 13$, "Narrow"

in trisomic ♀

% Survival of seedlings	Diploid		Parental trisomics		Other types	
	No.	%	No.	%	No.	%
75.76	23	92.00	2	8.00	0	0
97.14	34	100.00	0	0.00	0	0
54.69	31	88.57	4	11.43	0	0
71.21	88	93.62	6	6.38	0	0
94.74	33	91.67	3	8.33	0	0
87.23	41	100.00	0	0.00	0	0
96.30	25	96.15	0	0.00	1*	3.85
91.96	99	96.12	3	2.91	1	0.97
61.73	44	88.00	6	12.00	0	0
48.21	25	92.59	2	7.41	0	0
59.26	15	93.75	1	6.25	0	0
56.71	84	90.32	9	9.68	0	0
91.49	38	88.37	5	11.63	0	0
86.05	28	75.68	8	21.62	1**	2.70
90.74	42	85.71	7	14.29	0	0
89.58	108	83.72	20	15.51	1	0.77

TABLE 16.--Transmission of the extra chromosome in diploid ♀ x trisomic ♂ crosses

Acc. no.	Seeds sown	Seeds germinated	% Seed germination	Mature plants	% Survival of seedlings
Broad					
00032	100	97	97.00	58	59.79
00088	37	35	94.86	32	91.42
00100	49	49	100.00	47	95.92
Total	186	181	97.31	137	75.69
Pointed					
00015	50	47	94.00	45	95.74
00132	63	57	90.48	32	56.14
00142	28	27	96.43	22	84.48
Total	141	131	92.91	99	75.57
Narrow					
00010	100	97	97.00	58	59.79
00028	64	63	98.44	34	53.96
00075	35	28	80.00	26	92.86
Total	199	188	94.47	118	62.77
Small					
00070	90	77	85.56	49	63.63
00097	50	49	98.00	19	38.77
00144	34	30	88.24	28	93.33
Total	174	156	89.66	96	61.54

* $2n = 12 + 1$ t
** One triploid ($2n = 18$) and one $2n = 13$, "Broad"
*** $2n = 13$, "Broad"

in diploid ♀

re ts	% Survival of seedlings	Diploid		Parental trisomics		Other types	
		No.	%	No.	%	No.	%
	59.79	54	93.10	3	5.17	1*	1.72
	91.42	32	100.00	0	0.00	0	0
	95.92	43	91.49	4	8.51	0	0
	75.69	129	94.16	7	5.11	1	0.73
	95.74	41	91.11	4	8.89	0	0
	56.14	31	96.88	1	3.13	0	0
	84.48	21	95.45	1	4.55	0	0
	75.57	93	93.93	6	6.07	0	0
	59.79	53	91.38	3	5.17	2**	3.45
	53.96	31	91.18	3	8.82	0	0
	92.86	18	69.23	7	26.92	1***	3.85
	62.77	102	86.44	13	11.02	3	2.54
	63.63	45	91.84	4	8.16	0	0
	38.77	16	84.21	3	15.79	0	0
	93.33	22	78.57	6	21.43	0	0
	61.54	83	86.46	13	13.54	0	0

road"

TABLE 17.--Transmission of the extra chromosome in self-pollinated trisomics

Acc. no.	Seeds sown	Seeds germinated	% Seed germination	Mature plants	% Survival of seedlings
Broad					
00032	23	18	78.26	11	61.11
00088	52	39	75.00	25	64.10
00100	88	76	86.36	39	51.32
Total	163	133	81.60	75	56.39
Pointed					
00015	11	9	81.82	3	33.33
00132	37	27	72.97	20	74.07
00142	33	26	78.79	17	65.38
Total	81	62	76.54	40	64.52
Narrow					
00010	71	38	53.52	21	55.26
00028	11	5	45.45	1	20.00
00075	6	4	66.67	1	25.00
Total	88	47	53.41	23	48.94
Small					
00070	37	32	86.49	22	68.75
00097	50	33	66.00	18	54.55
00144	60	39	65.00	29	74.36
Total	147	104	70.75	69	66.35

)some in self-

ıre ıts	% Survival of seedlings	Diploid		Parental trisomics		Other types	
		No.	%	No.	%	No.	%
l	61.11	10	90.91	1	9.10	0	0
5	64.10	22	88.00	3	12.00	0	0
9	51.32	35	89.74	4	10.26	0	0
5	56.39	67	89.34	8	10.66	0	0
3	33.33	3	100.00	0	0.00	0	0
)	74.07	16	80.00	4	20.00	0	0
7	65.38	16	94.12	1	5.88	0	0
)	64.52	35	87.50	5	12.50	0	0
.	55.26	21	100.00	0	0.00	0	0
.	20.00	0	0.00	1	100.00	0	0
.	25.00	0	0.00	1	100.00	0	0
	48.94	21	91.30	2	8.70	0	0
	68.75	21	95.45	1	4.55	0	0
	54.55	16	88.89	2	11.11	0	0
	74.36	25	86.21	4	13.79	0	0
	66.35	62	89.86	7	10.14	0	0

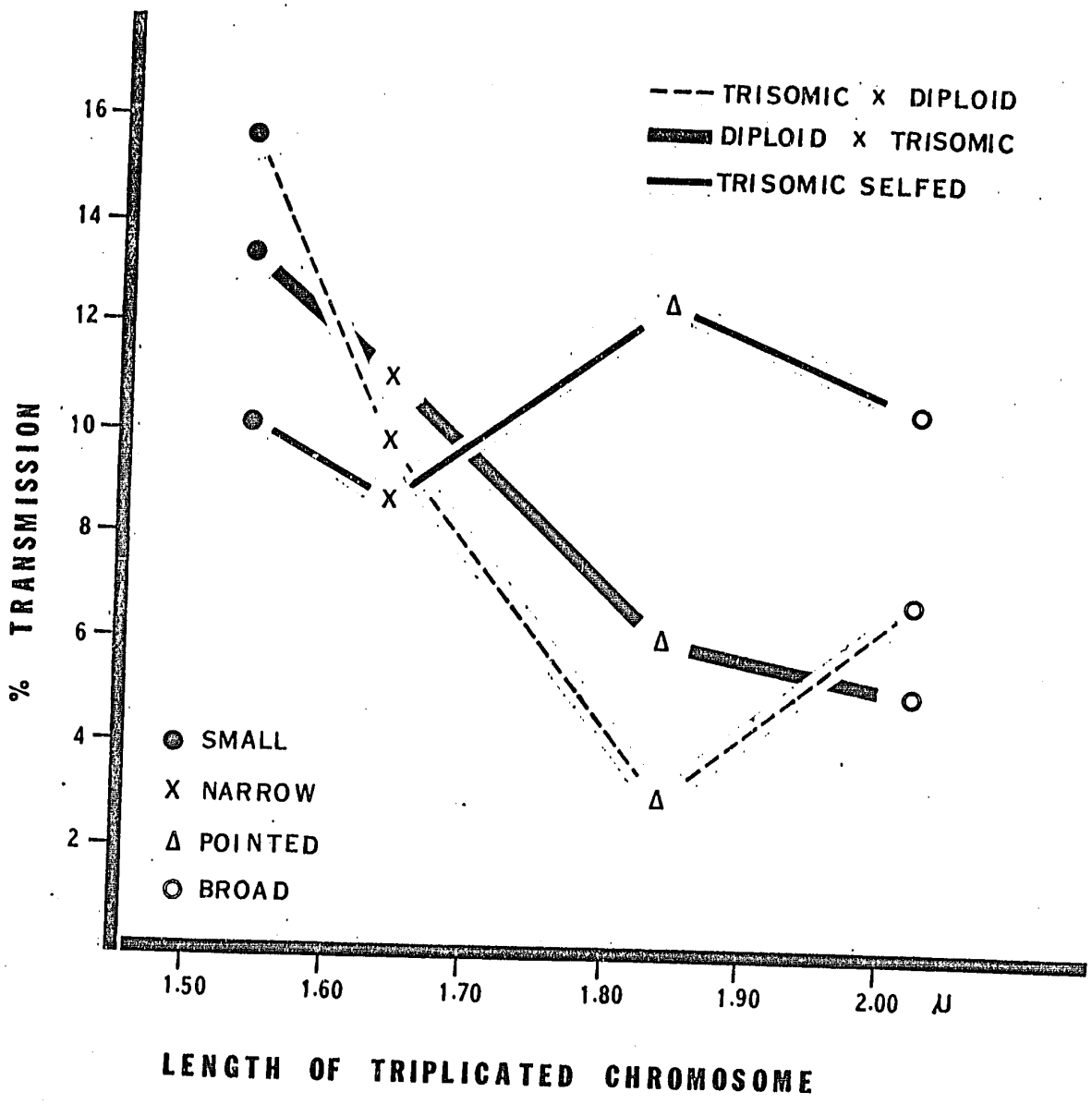

Fig. 98.--Correlation between transmission rate and length of triplicated chromosome of the four trisomic types, Broad, Pointed, Narrow, and Small.

The rate of transmission of trisomics is neither correlated with the percentage of seed germination nor with the percentage of seedlings surviving after germination (Tables 15, 16, 17).

Transmission through the pollen is not correlated with pollen fertility. The trisomic type Small had a relatively high percentage of bad (unstained) pollen grains but transmitted the extra chromosome through the pollen to a greater extent than any of the other trisomic types.

The two trisomic types Narrow and Small occurred more frequently in the progenies of triploids and also tend to show a higher rate of transmission than the two less frequent types, Broad and Pointed, which likewise had a lower transmission rate. However, the correlation was not consistent. The trisomic type Narrow appeared most frequently in the progenies of triploid ♀ x diploid ♂ crosses, but Narrow was below Small in its transmission rate.

2. Telosomic trisomics

Two telosomic trisomics, 00004 and 10458, were crossed as male and female parents to diploids and also selfed. The results of the crosses are shown in Tables 12, 13 and 14. The percentage of pod-set and the

TABLE 18.--Transmission of the extra telosome

Acc. no.	Seeds sown	Seeds germinated	Mature plants	Chromosome number in progeny							
				12		12+1t		12+2t's		Other types	
				No.	%	No.	%	No.	%	No.	%
Trisomic ♀ x diploid ♂											
00004	160	148	130	96	73.85	33	25.38	0	0.00	1*	0.77
10458	30	28	22	18	81.82	4	18.18	0	0.00	0	0.00
Diploid ♀ x trisomic ♂											
00004	186	165	85	66	77.65	19	22.35	0	0.00	0	0.00
10458	40	35	33	26	78.79	6	18.18	0	0.00	1**	3.03
Trisomics selfed											
00004	106	75	55	33	60.00	17	30.91	4	7.27	1***	1.82
10458	48	43	24	14	58.33	8	33.33	2	8.33	0	0.00

* $2n = 13 + 1$ t
** $2n = 13$, "Small"
*** $2n = 18 + 3$ t's

average of viable seeds per pod were higher in the two telosomic trisomics than in the primary trisomics.

The rates of transmission of the telosomes are shown in Table 18. The data are not sufficient to establish significant differences between the two telosomic trisomics. However, Table 18 clearly shows that the telosomes were transmitted with higher frequencies than the primary trisomes. In both telosomic trisomics, transmission through the male gametes was as frequent as transmission through the female gametes. About 8% of the plants contained two extra telosomes in the selfed progenies of the two telosomic trisomics. One plant was found to be a triploid with three extra telosomes ($2\underline{n}$ = 18+3t's) in the selfed progeny of 00004 and another plant contained 13 normal chromosomes and one telosome ($2\underline{n}$ = 13+1t) when 00004 was crossed as the male parent to the diploid. Telosomic trisomic 10458 produced one primary trisomic plant (3.03%), which was morphologically identified as Small, in the cross 10458 ♀ x diploid ♂.

DISCUSSION

I. Production of Aneuploids from Triploids

The distribution of chromosome numbers in the progenies of triploids usually deviates from the binomial distribution that would be expected if only statistical factors were involved. Satina and Blakeslee (1937a, 1937b) and Satina, Blakeslee and Avery (1938) have shown that elimination of extra chromosomes during meiosis and elimination of gametes and zygotes containing extra chromosomes at various stages of development were responsible for the relatively greater proportion of individuals with smaller chromosome numbers in the offspring of triploid Datura. In the present investigation of Lotus pedunculatus, diploids and simple trisomics occurred almost exclusively in the progenies of the triploids, whereas double trisomics appeared only twice. The observation of lagging chromosomes at telophase I and II in the microsporocytes of the two triploids suggests that certain extra chromosomes might be eliminated from the gametes during meiosis. The presence of a large number of inviable seeds in the crosses shows

that zygotes with extra chromosomes tend to be eliminated through their inability to develop fully viable seeds. The weakness of the two double trisomic offspring furnishes further evidence for zygotic elimination. No attempt has been made to demonstrate gametic selection and, therefore, whether gametic selection also plays a role in distorting the distribution curve in the progenies of the triploids is not known. However, it is considered that such a factor might be involved.

II. The Effects of Extra Chromosomes on Plant Morphology

In studies on the effects of extra chromosomes on plant morphology, trisomic plants of a certain species are usually provided with a uniform genetic background through the use of a triploid which is homozygous or through repeatedly backcrossing the available stock of trisomics to some selected inbred line until the residual genotype of the original stock has been eliminated. When such a condition has been attained one can conclude that the similarities within the same trisomic type are due to the presence of the same specific extra chromosome and the differences between types are under the influences of different extra chromosomes. Most of the trisomic plants used in the present study were derived from the triploid HR-2, which was the

immediate offspring from a cross tetraploid x diploid belonging to two different strains, hence these trisomics are highly heterozygous. The morphological variation in the 73 trisomic plants would, therefore, consist of the effects of the extra chromosome and the effect due to the heterozygosity of the material. Despite this fact, the greater variation between types and the relative uniformity within types as has been revealed from the morphological investigations indicate that the five morphological groups correspond to the five simple trisomic types. This classification has been verified from the karyotype analyses of representative plants from each of the five groups. Chromosome configurations at diakinesis and breeding behaviour of four of the five trisomic types further indicate that these trisomic plants are primaries and not secondaries or tertiaries.

There are two explanations for the failure to obtain plants trisomic for chromosome 2. A trisomic of this type might have been present but was undetected. If this was the case, it must have been grouped into one of the other trisomic types due to their morphological similarity. On the other hand, a trisomic of this type might be inviable or less viable than the other types, and therefore, it may not occur, or if so, only rarely

in the progenies of the triploids.

Several investigators (Blakeslee, 1922; Frost, 1927; Sinnott, Houghtaling and Blakeslee, 1934; Goodspeed and Avery, 1939; Sampson, Hunter and Bradley, 1961) have tried to use the genic balance theory (Bridges, 1921) to explain the morphological variations in trisomics. According to this theory, character expression is a matter of balance between a large number of genes acting in different ways. The diploid phenotype consists of an equilibrium which is established by these various tendencies. Support for this theory of genic balance has been obtained in several plant genera. Frost (loc. cit.) observed that in Matthiola the trisomics differed in opposite directions from the diploid in various characters. Similarly, Sinnott, Houghtaling and Blakeslee (loc. cit.) found that individual trisomic types in Datura varied in different directions from the diploid in several anatomical characters of the flower stalk, but the mean value of the 12 trisomic types was very close to the diploid value for most characters studied. Goodspeed and Avery (loc. cit.) measured a number of quantitative characters for ten of the 12 trisomic types of Nicotiana and found that the value of the diploid was also close to the average of the trisomics. In Antirrhinum, Sampson, Hunter and Bradley (loc. cit.)

observed that almost every character was exaggerated in some trisomic types, similar to the diploid in some, but reduced in others. In the present study, comparisons in some morphological characters between the five trisomic types and the diploid revealed that the diploid L. pedunculatus also represents a balance between the variations occurring in trisomic types. For example, the leaf colour of Pointed and Narrow is darker, whereas that of Round and Broad is lighter than the diploid; there is no difference in this character between Small and the diploid. The leaves of Round and Broad are larger and broader than those of the diploid, whereas Narrow and Pointed have narrower, and Small has smaller leaves. These observations provide evidence for the genic balance hypothesis.

Morphological comparisons between aneuploids in the progenies of the triploid L. pedunculatus provide further evidence for the genic balance theory. If the morphological changes reflect the degree of internal genic unbalance of the aneuploids, and if unbalance is caused by duplication of chromosome material in the normal complement, we would expect to observe a positive correlation between the length of the duplicated chromosome and the degree of the phenotypic alterations in aneuploids. Such a relationship does exist in the

progenies of the triploid L. pedunculatus. The two double trisomic plants are weaker and they show greater morphological alterations than the simple trisomics. The simple trisomics do not show any weaknesses but they are morphologically distinct from the diploid. The telosomic trisomics closely resemble their diploid siblings in almost all morphological aspects and even quantitative measurements did not help in distinguishing them.

In the primary trisomics of L. pedunculatus, several characters show correlated variations, that is, in each of these primary trisomic types a change from the diploid in a certain direction for one organ is accompanied by a similar change in other organs. For example, Small is not only small in leaf size, but also in flower parts such as floret length, standard width and ovary and style length. Narrow is narrow in leaf width and slender in diameter of the stem. Two hypotheses have been postulated to explain this phenomenon of correlated variation of characters in trisomics. According to Sampson, Hunter and Bradley (1961), different characters might reflect the same genetic processes and therefore, they are influenced by the same genes. On the other hand, Goodspeed and Avery (1939) suggested that the physiological action of a chromosome as a whole

would account for the accumulation in each trisomic type of characters all varying from their diploid in the same direction. Data obtained from the present investigation do not distinguish between these two alternatives.

III. Association and Segregation of Trisomes in Meiosis

In triploid L. pedunculatus, the longer chromosomes tend to form trivalents more frequently than the shorter ones. A more direct indication of such a relationship between chromosome length and trivalent formation is shown in the four trisomic types in which there is a positive correlation between the length of the triplicated chromosome and the frequency of trivalents. Similar observations have also been reported by Belling (1925) in triploid hyacinthus, by Darlington and Mather (1932) in triploid Tulipa, by Einset (1943) in trisomics of maize, and by Rick and Barton (1954) in trisomics of tomato. This result can be explained when we realize that homologous chromosomes are held together by chiasmata at diakinesis and metaphase I. The longer chromosomes would have a greater physical chance to synapse, form trivalents and to have chiasmata than the shorter chromosomes.

The mean number of trivalents per extra chromosome

for the two triploids is 0.76 and 0.64 which is calculated by dividing the mean number of trivalents per cell (4.57 and 3.85) by the basic chromosome number of this species (6.0). This frequency is much higher than that for chromosomes 3, 4, 5, and 6 (0.23, 0.21, 0.11, and 0.07, respectively) obtained from individual trisomics. Unfortunately, the author did not obtain plants trisomic for chromosome 2 and could not study chromosome associations in the trisome of chromosome 1 since the plants have not flowered up to the present time. However, even if we assume that these two chromosomes form trivalents with a frequency of 1.00 per extra chromosome in their respective trisomics, the mean number of trivalents per extra chromosome calculated from the two triploids (0.76 and 0.64) would still be higher than that for the individual trisomics (0.44). It appears that the same trisomes form trivalents more frequently in triploids than in simple trisomics. Vasek (1963a) has observed that in multiple trisomics of Clarkia unguiculata ($2n = 18$), the number of trivalents per extra chromosome increases from 0.43 at $2n+1$ to 0.59 at $2n+7$; the frequency for triploids is 0.61. He therefore suggests that extra chromosomes have a greater effect in promoting trivalent formation at higher than at lower chromosome numbers. It seems that an increase in the

number of trivalents per extra chromosome between 2n+1 and a triploid might also be expected in L. pedunculatus if a series of multiple trisomics were available. The mechanism of this phenomenon is not known, and a discussion on this subject is beyond the scope of the present data.

If we assume that the chromosomes of a trivalent separate in such a way at anaphase I that two go to one pole and one to the other pole and that only univalents, when present, remain on the equatorial plate, as has been reported in the case of maize trisomics (Einset, 1943), we would expect to observe at telophase I more cells containing lagging chromosomes in trisomics having a lower frequency of trivalents than in trisomics having a higher frequency of trivalents. Lack of such correlation in the four trisomic types of L. pedunculatus suggests that the two chromosomes of a trivalent may go to opposite poles and one remains at the equator or lags behind in division. The frequency of one chromosome lagging might not be related to chromosome size.

IV. Transmission of the Extra Chromosome in Trisomics

Several reasons have been suggested for the failure of the extra chromosome to be transmitted to 50% of the

progeny in trisomics. These include elimination of the extra chromosome during meiosis and elimination of gametes and zygotes containing the extra chromosome at various stages of development. One, or a combination, of these factors must be involved in causing low transmission of the trisomics of L. pedunculatus.

Einset (1943) observed that in trisomics of maize, when a short chromosome was present in triplicate there were fewer trivalents and more univalents present at metaphase I. As a result of elimination of these univalents at telophase I, few microspores contained n+1 chromosomes and the frequency of trisomics in the progeny was low. On the other hand, when a long chromosome was present in triplicate, he observed more trivalents and fewer univalents and more microspores containing n+1 chromosomes. Consequently, in this case, the frequency of trisomics in the progeny was high. Therefore, he believes that in maize, elimination of the extra chromosome during the meiotic divisions is the main cause which determines the extent of transmission of the extra chromosome.

In the present study no determination of chromosome numbers has been made in microspores to determine the presence or absence of an extra chromosome in

addition to the normal complement. However, the fact that there were lagging chromosomes at anaphase and telophase suggests that some of the univalents would be eliminated at later stages of meiosis, and this would account, at least in part, for the low transmission of the trisomics of L. pedunculatus.

The four trisomic types of L. pedunculatus transmit the extra chromosome at different rates. There is a tendency for the shorter chromosomes to be transmitted more frequently than the longer ones. That the difference in transmission rate between the four trisomic types is not due to elimination of the extra chromosome during meiosis is suggested by the absence of a negative correlation between the transmission rate and the frequency of lagging chromosomes per cell at anaphase and/or telophase (cf. Tables 11, 15, 16 and 17). The inference here is that while chromosome elimination during meiosis might be responsible in part for the general decreased transmission in trisomics of L. pedunculatus, it is not the main reason for the difference in transmission rate between trisomic types. Apparently, other factors are involved.

Almost in all plant genera studied, transmission of trisomics usually occurs with a greater frequency

through female gametes than through male gametes. Pollen sterility has been postulated to explain the low rate of transmission through the pollen as compared with that through ovules in trisomics of Lycopersicon (Lesley, 1928) and Nicotiana (Goodspeed and Avery, 1939). Non-germination and differential pollen tube growth of the n+1 pollen have also been assumed to explain the low rate of transmission through male gametes in trisomics of Matthiola (Frost, 1927), Lycopersicon (Lesley, 1928), Nicotiana (Goodspeed and Avery, 1939), Spinacia (Tabushi, 1958; Janick, Mahoney and Pfahler, 1959) and Clarkia (Vasek, 1961) and have been demonstrated in Datura (Buchholz and Blakeslee, 1922, 1930, 1932; Buchholz, Doak and Blakeslee, 1932). According to this hypothesis, the n+1 pollen either is unable to germinate or its tubes grow at a reduced rate in competition with pollen having the normal haploid complement.

In L. pedunculatus, although pollen fertility is lower in the four primary trisomic types than in the diploid, there is no correlation between transmission rate through pollen and pollen fertility. Apparently, failure of the extra chromosome to be transmitted to 50% of the progeny through pollen is not due to the lower viability of the n+1 microspore.

Except in Pointed, there is no significant difference in transmission rate through male and female gametes in the other trisomic types. The average transmission rate for the four trisomic types is 9.07% through the female gametes and 8.67% through the male gametes. This would suggest either that there is no differential selection against the male and female gametes containing n+1 chromosomes, or that selection does not occur at the gametophytic stage, that is, the n and n+1 gametes (both male and female) are able to function equally well in fertilization. The data obtained from this study do not distinguish between these two alternatives. However, in view of the fact that the male and female gametes function so differently in fertilization in that the pollen has to be disengaged from the pollen sac, to germinate on the stigma, and to grow down through the style, it would seem that an equal selection strength against both the n+1 male and female gametes would be unlikely. This leaves the second explanation as the most likely one, that is, selection against the n+1 male and female gametes does not occur.

The data so far discussed exclude elimination of the extra chromosome during meiosis and of gametes containing n+1 chromosomes as the main causes for the difference in transmission rate between the trisomic

types of L. pedunculatus, and confine the mechanism to selection against the zygotes containing the extra chromosome. It has been shown that in Datura (Buchholz and Blakeslee, 1922; Blakeslee and Avery, 1938) and Lycopersicon (Lesley, 1928; Rick and Barton, 1954), elimination of the 2n+1 zygotes occurs at various stages of development, from the young embryos to mature plants. In L. pedunculatus, the presence of inviable seeds in the crosses of trisomics with diploids and in selfing trisomics indicates that some of the 2n+1 zygotes might be eliminated through their inability to develop good seeds. That this elimination of 2n+1 zygotes at the embryo stage might be the main cause which determines the extent of transmission of the extra chromosome is suggested by the positive correlation between the transmission rate and the number of viable seeds per pod in three of the four trisomic types studied.

The trisomic type Narrow does not show any correlation between transmission rate and the number of viable seeds per pod since it set fewer seeds than all the other trisomic types, however, it transmitted the extra chromosome more frequently than the trisomics Broad and Pointed. This paradox may be explained if it is considered that the developmental process of gamete formation was disturbed by the presence of an extra

chromosome in Narrow and that only a limited number of male and female gametes were formed in the sex organs of this trisomic type. The observation that Narrow contained fewer pollen grains in its anthers than any of the other trisomic types seems to substantiate this explanation. Therefore, the problem presented by Narrow does not contradict the hypothesis that inability of the 2n+1 zygotes to develop viable seeds is the main cause for the difference in transmission rate between the trisomic types of L. pedunculatus.

Indirect support for this hypothesis is presented by the finding that the rate of transmission of the four trisomic types is neither correlated with the percentage of seed germination nor with the percentage of seedlings surviving after germination. It seems not unreasonable to infer that embryo development is a critical stage for the 2n+1 zygotes. Once they successfully pass this stage, the 2n and 2n+1 seeds can germinate equally well, and it has already been pointed out that the 2n+1 seedlings and mature plants are not weaker than the normal diploid plants. The ability for the 2n+1 zygotes to pass this critical stage would depend upon the degree of genic unbalance created by the extra chromosome, the longer the extra chromosome, the greater the unbalance, and the less chance for the 2n+1 embryos to develop

viable seeds. This relationship accounts for the negative correlation between the length of the extra chromosome and the rate of transmission of the trisomics of L. pedunculatus.

The reason why the embryo stage is so critical in zygotic selection is now known. It is speculated that an interaction between embryo and endosperm might be involved. In the crosses of trisomics with diploids, the 2n+1 embryos would live in the triploid endosperms containing one or two extra chromosomes depending on the direction of the crosses. These aneuploid endosperms may constitute an unfavourable condition for the development of the 2n+1 embryos.

Since the extra chromosome of the four trisomic types of L. pedunculatus can be transmitted through both female and male gametes, one would expect some tetrasomics in the selfed progenies of trisomics. Since no tetrasomics were found in the present investigation, an explanation for their absence might also be the result of zygotic selection at the embryo stage. Consequently, zygotes containing two similar extra chromosomes might be unable to develop viable seeds.

Because telosomes are shorter than normal chromosomes, it may be assumed that they would create less

unbalance in the 2n+1 zygotes. Based on the hypothesis of zygotic selection at the embryo stage, one could postulate that there would be more viable seeds in pods when the telosomic trisomics were crossed to the diploid and also when they were selfed, and consequently, that the extra telosomes would be transmitted more frequently than the extra normal chromosomes. The data obtained from the two telosomic trisomics, 00004 and 10458, are in agreement with all these expectations, therefore, they present further evidence for the hypothesis that inability to develop viable seed is the main cause which determines the extent of transmission of the extra chromosome.

V. Production of Unrelated Trisomic Types from Primary Trisomics

Blakeslee and Avery (1938) found that all the 12 primary trisomics of Datura could produce some unrelated trisomic types in their offspring and that the frequency of unrelated trisomics was higher than that for spontaneously occurring trisomics from normal diploids. Consequently, they suggested that the presence of the extra chromosome in a trisomic might interfere with the meiotic divisions of that plant which would be responsible for the production of unrelated types in trisomics of

Datura. As an alternative, Burnham (1962) postulated that a change in physiology in trisomic plants might cause a higher rate of chromosome non-disjunction. In the trisomics of L. pedunculatus, the production of unrelated trisomics in the offspring of Narrow and Small may be explained on the basis of the meiotic irregularities of these trisomic types. It was observed that when three univalents were present in the microsporocytes of Narrow and Small, two univalents were occasionally larger than the third one. Judging from their size in meiosis, the two larger univalents are presumably the unpaired homologues of chromosome 3 or 4 and the small univalent is chromosome 5 or 6 for which Narrow or Small is trisomic, respectively. Non-disjunction of the two larger univalents would result in the formation of gametes containing a larger extra chromosome from the trisomic type Narrow or Small. When these gametes were fertilized by normal gametes, unrelated trisomic types would be produced. The phenomenon here is similar to "univalent shift" as reported by Person (1956) in monosomics of wheat. The mechanism as to how the presence of an extra chromosome could cause dissociation of other chromosome pairs in meiosis is not known.

VI. The Telocentric Chromosomes in L. pedunculatus

The univalents in the triploids and trisomic plants of L. pedunculatus divide frequently in the first division of meiosis. In almost all the microsporocytes examined, the daughter univalents were similar in size indicating an equational division of the centromere. The observation that the daughter univalents were unequal in size in a single microsporocyte of the triploid HR-2 suggests that misdivision (transverse division) of the centromere of the univalent may occur occasionally. Misdivision of univalents would account for the occasional occurrence of telosomic trisomics in the progenies of the triploids and primary trisomics of L. pedunculatus.

Identification of the telocentric chromosomes at somatic metaphase for the three telosomic trisomics was difficult. Judging from their length, they could be any one of the short arms of chromosomes 3 to 6. Although the short arm of chromosome 4 is distinct in having a tiny satellite, in cells of some preparations, this satellite was not visible and also the satellite could be easily confused with the terminal centromere of the telosomes. Morphological studies of these telosomic trisomics did not provide any information as to the

telosomes they possess because these plants closely resembled their diploid siblings in many aspects. The telosomic trisomics 10458 and 11947 were derived from the progenies of diploid ♀ x Broad ♂ and of Pointed ♀ x diploid ♂, respectively, therefore their extra telosomes might have originated from misdivision at the centromere of their respective primary chromosomes, that is, 3 and 4, during meiosis. From this information it may be inferred that the extra telosomes of 10458 and 11947 are possibly the short arms of chromosomes 3 and 4, respectively. However, a study of the meiotic behaviour of the chromosomes for 10458 does not support this inference. It was found that the two trivalents, which were the only two in all the microsporocytes examined for this telosomic trisomic, were composed of a telosome and two normal chromosomes belonging to the small group (presumably chromosome 5 or 6). In this case, if meiotic pairing reveals homology, the extra telosome of 10458 ought to be the short arm of chromosome 5 or 6. On the other hand, if the trivalents resulted from non-homologous pairing between the short arm of chromosome 3 and chromosome 5 or 6, then the mechanism of this unusual type of association has to be sought. It is speculated that the terminal centromere of the telosome might have some connection with the non-homologous chromosome association.

VII. The Stability of Telocentric Chromosomes

Darlington (1939) expressed the opinion that telocentric chromosomes were unstable; once they arose through misdivision of centromeres they were either converted into isochromosomes or were lost. Rhoades (1940) presented evidence to show that one telocentric chromosome of maize was unstable both by giving rise to an isochromosome through misdivision of its centromere in meiosis and by undergoing structural changes in mitosis leading to loss. He considered that this somatic and meiotic instability was due to the terminal position of the centromere which also might account for the rare occurrence of telocentric chromosomes in nature.

The data obtained from the present investigation as well as those reported previously by other investigators do not support the view that all telocentric chromosomes are unstable. It has been recently shown in several organisms that the centromere has a complex structure composed of centromeric chromomeres connected by fibrils to the chromatids (Tjio and Levan, 1950; Lima-de-Faria, 1949, 1956, 1958). Marks (1957) suggests that misdivision may occur in different portions of the complex centromere resulting in telosomes which differ in the completeness of their centromeric regions.

Steinitz-Sears (1966) found three classes of telocentric chromosomes in wheat differing in their degree of somatic stability. She suggested that the relative instabilities of the telocentric chromosomes may be attributed to the degree of completeness of the centromere region. If this is the case, a telocentric chromosome possessing a complete centromere would be as stable as the meta- and submeta-centric chromosomes. Marks (loc. cit.) listed a number of such examples of stable telocentric chromosomes in plants and animals.

Elimination of the extra telocentric chromosome in somatic tissues has been taken as evidence of instability of the terminal centromere (Rhoades, 1940; Steinitz-Sears, 1966). It should be noted that chromosome elimination has also been found in the primary trisomic type Compact of Nicotiana sylvestris (Goodspeed and Avery, 1939) and in Curled of Spinacea oleracea (Ellis and Janick, 1959). In these trisomics, the extra normal chromosome was eliminated in some cell lines resulting in a chimera. In determining the chromosome number of one plant of the trisomic type Broad, 00088, of L. pedunculatus, the author found five root tips containing 13 chromosomes and four root tips containing 12 chromosomes. The aerial portion of this plant seemed relatively unaffected as it retained the trisomic

phenotype. From these examples it is apparent that somatic instability is not a phenomenon which occurs only in plants containing telocentric chromosomes. The extra normal chromosome in primary trisomics may be eliminated possibly as a result of genic unbalance which interferes with normal mitotic behaviour. It is, therefore, postulated that elimination of the telocentric chromosomes having a complete centromere may not be due to instability of the terminal centromere but due to genic unbalance created by duplication or deficiency of portions of chromosomes.

In maize, Rhoades (1940) found that 86 out of 19,242 offspring were secondary trisomics when one specific telosomic trisomic was used as the male parent in crosses with the diploid, and that the frequency of secondary trisomics was 27 out of 17,175 when the telosomic trisomic was used as the female parent. The two telosomic trisomics of L. pedunculatus did not produce any secondary trisomics among a total of 349 offspring. On account of the small population used in the present study, no conclusion can be made as to whether the two telocentric chromosomes were stable or not. However, when it is considered that the four primary trisomic types produced two telosomic trisomics through misdivision of the centromere in a total of 1,076 offspring, it would

appear that the two telocentric chromosomes in L. pedunculatus are not more unstable than the normal chromosomes.

SUMMARY

This thesis describes the morphological, cytological and genetical characteristics of trisomics which have been obtained for the first time in the genus Lotus. A detailed procedure of their mode of origin is given which involves hybridization of different cytodemes of the species Lotus pedunculatus Cav. A morphological and cytological study of the various cytodemes was made for comparative purposes.

1. Lotus pedunculatus is a diploid species with 12 somatic chromosomes. Crossing the diploid with a colchicine induced tetraploid resulted in one triploid and with one plant of the trisomic type Narrow resulted in another triploid.

2. The diploid was fertile, whereas the triploid and the tetraploid were highly sterile as indicated by pollen stainability and seed-set. The diploid, triploid and tetraploid plants differed only in quantitative characters; the two triploids were intermediate between the diploid and the tetraploid in almost all the plant parts.

3. Meiotic behaviour of the diploid was regular. The homologous chromosomes usually formed bivalents in diakinesis and prometaphase I, and no lagging chromosomes were observed at either telophase I or II. The frequency of trivalents in the two triploids was 4.57 and 3.85 per cell. In the tetraploid, most of the chromosomes were associated as bivalents and only 1.80 quadrivalents per cell were formed. Lagging chromosomes were frequently observed at telophase I and II in the microsporocytes of the triploid and tetraploid plants.

The meiotic chromosomes of L. pedunculatus could be arranged into four classes according to size: one large, one medium large, two medium and two small. The larger chromosomes tended to form multivalents more frequently than the smaller ones in the triploid and tetraploid plants.

4. A total of 202 mature plants were raised from backcrossing the triploids to the original diploid and from selfing the triploids. Among these plants, 126 (62.38%) were normal diploids, 72 (36.14%) were simple trisomics, two (0.99%) were double trisomics, and one (0.49%) was a telosomic trisomic. Evidence was presented to show that the relatively greater proportion of individuals with lower chromosome numbers in the offspring

of the triploids was due to elimination of extra chromosomes during meiosis and to sporophytic inviability. Although selection against the gametes containing extra chromosomes has not been demonstrated, it is considered that such a factor may play a role in distorting the distribution of chromosome numbers in the progenies of the triploids.

5. The 73 simple trisomic plants acquired from the progenies of the two triploids were classified into five distinct morphological groups. Each group differed from other groups and from the diploids in a number of qualitative and quantitative characters. A t test showed that some of the quantitative differences were significant statistically. It was considered that these differences resulted from change in genic balance of the plants which was brought about by adding different single extra chromosomes to the normal complement. The five morphological groups represent five simple trisomic types, and they were named Round, Broad, Pointed, Narrow and Small according to their distinguishing leaf characters.

6. The somatic chromosomes of L. pedunculatus could be distinguished from each other by length, by the presence and absence of a satellite and by arm ratio in

root tip cells. They were numbered from 1 to 6 in the order of decreasing length. The extra chromosomes of Round, Broad, Pointed, Narrow and Small were chromosomes 1, 3, 4, 5 and 6, respectively.

7. The two double trisomic plants were similar in morphology. In some aspects, they resembled the simple trisomic type Pointed. One of them which was studied cytologically possessed a deficient chromosome 4 and a normal chromosome 6 as the extra chromosomes.

8. Three telosomic trisomic plants were obtained in L. pedunculatus, one from triploids and two from simple trisomics. They occurred through misdivision of the centromere of univalents. These telosomic trisomics closely resembled their diploid siblings in external morphology. Cytological identification of the extra telosomes was not possible.

9. Meiotic behaviour of the chromosomes was studied for four of the five simple trisomic types. At diakinesis and prometaphase I, chromosome association of 6 II + 1 I was more frequent than the association of 1 III + 5 II. The frequency of cells with one trivalent varied within and between trisomic types. There was a positive correlation between chromosome length and trivalent formation. An explanation for this phenomenon

was that the homologous chromosomes are held together by chiasmata at diakinesis and metaphase I, and that the longer chromosomes have a greater chance of having such chiasmata than shorter chromosomes. Most of the trivalents were chain shape; frying-pan and V shape trivalents were observed only occasionally. The absence of ring shape trivalents and univalents and of pentavalents indicates that these simple trisomics are primaries and not secondaries or tertiaries.

At anaphase I and telophase I, the univalents either remained undivided at the equator or divided later than the bivalents. The frequency of lagging chromosomes at anaphase I and/or telophase I was not correlated with the frequency of trivalents (or univalents) at diakinesis and/or prometaphase I. No particular trend was apparent as to which extra chromosome, when present as an univalent, tended to divide more frequently than another chromosome of the complement. Lagging chromosomes were frequently observed at anaphase II and telophase II.

10. Meiotic behaviour of the telosomic trisomics was essentially similar to that of the primary simple trisomics. At diakinesis and prometaphase I, the extra telosome formed trivalents less frequently than the

primary trisomes. Lagging chromosomes were present at telophase I and II.

11. The average transmission rate for the four simple trisomic types was 9.07% through the female gametes and 8.67% through the male gametes. This result suggested that selection against the n+1 gametes might not occur in the trisomics of L. pedunculatus. Although there were variations in transmission rate within the same trisomic types, the shorter chromosomes tended to be transmitted more frequently than the longer chromosomes. Transmission in the four simple trisomic types was roughly correlated with the average number of viable seeds per pod, and there was no correlation of the transmission rate with the frequency of lagging chromosomes at telophase, pollen fertility, the percentage of seed germination and the percentage of seedlings surviving after germination. Therefore, it was suggested that embryo development constituted a critical stage for sporophyte selection. The ability of the 2n+1 zygotes to pass successfully through this stage would depend upon the degree of genic unbalance created by the extra chromosome, the longer the extra chromosome, the greater the unbalance and hence, the less chance for the 2n+1 zygotes to develop viable seeds. This hypothesis accounted for the negative correlation between the

length of the extra chromosome and the rate of transmission of the trisomics.

12. The two telosomic trisomics transmitted the extra chromosome more frequently than the primary trisomics. About 8% of the plants contained two extra telosomes in the selfed progenies. All the data obtained from the telosomic trisomics supported the hypothesis that the inability of the 2n+1 zygotes to develop viable seeds was the main cause which determined the extent of transmission of the extra chromosome.

13. The trisomic type Small produced 0.77% unrelated trisomics through the female gametes and the trisomic type Narrow produced 1.69% through the male gametes. The production of unrelated trisomics in the offspring of Small and Narrow was explained as the result of meiotic irregularities in these trisomic types.

14. The stability of the telocentric chromosomes in mitosis and meiosis was discussed. The author is of the opinion that the relative instabilities of the telocentric chromosomes may be attributed to the degree of completeness of the centromere region possessed by the telocentric chromosomes, and that a telocentric chromosome with a complete centromere should be as stable as a meta- or submeta-centric chromosome. Somatic elimination

of the telocentric chromosome having a complete centromere may not be due to the terminal position of the centromere but due to genic unbalance which interferes with normal mitotic behaviour.

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