A CYTOGENETIC STUDY OF INTERSPECIFIC DIPLOID HYBRIDS AND AMPHIDIPLOIDS IN THE GENUS LOTUS

by

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CLAIM TO ORIGINAL RESEARCH

This is to certify that the study reported in this thesis constitutes the original work of the author and is his contribution to cytogenetic knowledge.

ABSTRACT

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A study was carried out on a) 117 interspecific diploid hybrids obtained from 16 different crosses between 7 Lotus species, b) 15 autotetraploids of these diploid species, c) 40 amphidiploids from 12 different interspecific hybrids, and d) 15 different tetraploid hybrids obtained by crossing the amphidiploids and the natural tetraploid L. corniculatus L., in order to provide data on crossability, meiotic behavior of the chromosomes, and morphological and fertility relationships of the species. Whereas only varying degrees of success were achieved in producing diploid hybrids, crosses were more easily obtained between the amphidiploids and L. corniculatus. Comparative morphological studies between the induced tetraploids and L. corniculatus revealed that the autotetraploids of L. japonicus and L. alpinus and the amphidiploid (L. japonicus x L. alpinus) had a greater number of characteristics in common with L. corniculatus which indicated their closer relationship to this latter species. Meiotic and fertility studies provided valuable data on chromosome differentiation and species relationships. It is concluded that the transfer of desirable germplasm from the diploid taxa to L. corniculatus is possible and that L. corniculatus is an allotetraploid derived from L. japonicus and L. alpinus.

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INTRODUCTION

Cytological analyses of species, hybrids, and polyploids, have been of great value in determining the relationships and the origin of many species of plants. Interspecific hybridization, polyploidy, and structural changes of chromosomes, are some of the factors that have been involved in the evolution of plant species. In many genera, a study of chromosome behaviour in experimentally controlled plants, has shown, which of these factors, or combination of factors, have been responsible for species differentiation. The experimental production of polyploids, achieved through the application of colchicine has made it possible to examine how polyploidy has operated in the past to produce new cultivars and species of plants. Studies designed to investigate relationships between species, hybrids, and their polyploid derivatives are both of theoretical importance and of practical value.

Theoretically, it is possible to examine the pairing relationships of the chromosomes at meiosis in interspecific hybrids, and in so doing come to a decision concerning the natural relationships of species. In addition, the chromosome numbers of the interspecific hybrids can be doubled in an attempt to artificially synthesize a naturally existing tetraploid, or other polyploid species, as the case may be. A classical example of the contribution that can be made from this type of study is the investigation carried out by Clausen, Keck and Hiesey (1945) between species of the tribe Madiinae. They postulated certain evolutionary pathways and then by means of inter-

specific hybridization and chromosome doubling, were able to prove their hypotheses by experimentally synthesizing plants already in existence. Another example of the usefulness of these studies to scientific knowledge, is provided by Upcott (1939). She obtained valuable data on the various factors controlling hybrid sterility and on the effects of amphidiploidy upon Darwinian fitness.

The practical importance of studies concerned with hybridization and the induction of polyploidy is reflected in the improvements that have been made in many of our cultivated plants. The introduction of new germplasm for breeding purposes is a basic procedure for crop improvement and increasing crop yields, and there are few cultivated plants that are not subjected to hybridization whether interspecific or intraspecific. Hybridization has been particularly useful in producing various types of garden and greenhouse ornamentals such as tulips, roses, orchids, hyacinths and pansies. To a lesser extent, hybridization has played a role in the development of such useful fruits as peaches, cherries, plums and grapes. In the cereal grains, where the main interest is in the transfer of particular traits from one cultivar to another, intraspecific hybridization has been used most successfully.

Polyploidy has also played a major role in the natural evolution of some of our important crop plants such as wheat, oats, cotton, tobacco, coffee, sugar cane, and alfalfa. Carefully planned and executed cytogenetic studies have revealed the progenitor species for the majority of these polyploid plants. In addition to polyploids resulting from species hybridization, there are a number of examples

of induced autopolyploid crop plants. These include cultivated species such as rye, sugar beet, clover, grapes, watermelons and some ornamentals such as snapdragons and marigolds. These autopolyploids have proved valuable because of the intrinsically superior qualities conveyed by polyploidy alone. Artificially synthesized alloploids or amphidiploids, on the other hand, have been less successful as a means of establishing new cultivars. The only outstanding example is Triticale which seems most promising for the development of a new amphidiploid crop.

However, both amphidiploids and autotetraploids have proved to be invaluable for the improvement of established crops, since they are particularly useful as vehicles for the transfer of genes across barriers of hybrid sterility. A well known example is the transfer of the necrotic type of resistance to mosaic disease from Nicotiana glutinosa to N. tabacum by means of the amphidiploid N. digluta. Also in some genera, the transfer of desirable genes from one species to another is often difficult or impossible because of the differences in basic chromosome numbers. In such cases, the transfer can be effected more easily by producing an autotetraploid of the species with the lower number of chromosomes. Sears (1956) used this technique to transfer rust resistance from Aegilops umbellulata to cultivated wheat.

Another potential use of induced polyploidy is the partial stabilization of desirable hybrid characteristics found in the F_1 progenies. Sterile hybrids between distantly related species can be rendered fertile by amphidiploidy, and thus, valuable characters can be kept constant by this means.

These various examples have been chosen to demonstrate how cytogenetic studies of species hybrids and their polyploid derivatives have contributed to the development of theoretical knowledge and its application to the improvement of our crops. The success of these studies also serves as a source of stimulation for the improvement of many minor or potential crops.

Lotus corniculatus L., or Birdsfoot Trefoil as it is commonly called, is a highly successful leguminous forage crop in eastern North America and Canada. Since its potentialities as a forage crop were first recognized in the early 1930's, there has been a steady increase in its production especially within the last decade or so. There are several reasons for the increase in the use of this species as a forage crop:

1) It produces high quality forage under conditions of continuous grazing.

2) It is adapted to poor drainage conditions and secondary soils, where red clover and alfalfa are not successful.

3) It is relatively resistant to heat and drought.

4) It is winter-hardy

5) It has high palatability and good nutritive value.

6) It is of particular interest since it is a perennial and has never been reported to cause bloat in cattle.

Certain problems, however, have prevented more extensive use of this crop. These major factors are, uneven ripening of seed pods, seed pod dehiscence, lack of seedling vigor, and susceptibility to root rot.

There are approximately 200 species in the genus Lotus and L. corniculatus, the most widely cultivated species, is a tetraploid; two other taxa of economic interest are L. tenuis Waldst. et Kit. and L. pedunculatus Cav. (L. uliginosus Schkuhr) both of which are diploids. Interspecific hybridization studies aimed at resolving some of the problems mentioned above, have been attempted between a limited number of induced autotetraploids and L. corniculatus (Tome and Johnson, 1945; Keim, 1952; Mears, 1955; Erbe, 1955, and Bent, 1958) but so far with only limited success. Polyploidy is a complicating factor in studies where genes control the expression of characteristics useful for breeding purposes, and therefore, the selection of desirable recombinants from populations at the tetraploid level is a slow and difficult process.

The evolutionary history of L. corniculatus is also an unresolved problem. If the progenitor species of this tetraploid cultivated plant could be found, then, these, and other closely related species, would serve as valuable sources of potential germplasm for further breeding work. The genetics of diploids in comparison to tetraploids is less complicated and improvement for specific desirable traits could first be tried at the diploid level before finally incorporating at the tetraploid level.

In order to obtain pertinent cytogenetic information on L. corniculatus, a program of interspecific hybridization between species closely related to L. corniculatus has been initiated in this laboratory. The study reported in this dissertation, forms part of the continuing study of the cytogenetics of Lotus. This investigation was undertaken in an

attempt to achieve the following objectives:

1) The production of a number of diploid interspecific hybrids which could be studied morphologically and cytologically, in order to provide additional cytogenetic information on some of the closely related diploid species.

2) The induction of autotetraploids and amphidiploids which could be used in conjunction with L. corniculatus in a comparative morphological and cytological study. The information gained from this aspect of the investigation should help to clarify the evolutionary relationships of this cultivated tetraploid species.

3) Finally, to determine the feasibility of transferring germplasm from the diploid species to the tetraploid L. corniculatus by means of the synthetic amphidiploids, and to examine the meiotic chromosome behaviour in the progenies obtained from crosses of the induced amphidiploids with L. corniculatus. The knowledge acquired from these observations should clarify the relationships between the diploid species and the cultivated tetraploid, L. corniculatus.

It was considered that the information gained from this research project would provide valuable cytogenetic knowledge which would make it possible to establish effective breeding procedures in a Lotus improvement program.

LITERATURE REVIEW

The genus Lotus of the family Leguminosae has been of scientific interest as early as the fifteenth century. Taxonomists at that time introduced the name Lotus into the botanical literature, and since then, it has held a generic position in the tribe Loteae of the Leguminosae family. Many years later in the early 1930's, when it was discovered that certain members of the genus had potential as forage plants, agronomists became interested and initiated scientific research aimed at producing a good forage type. In most of these investigations emphasis has been placed on intraspecific breeding programs, selection experiments, and field trials of one type or another. Comparatively limited effort has been directed to studies relating to interspecific hybridization and the biosystematics of this group. However, following the introduction and classification of a number of species in some taxonomic treatments of the genus, a certain amount of research has been conducted in this direction, primarily within the last decade. While the results of these studies are not conclusive and final, they have led to a better understanding of the genus as a whole. In this review, literature pertaining mainly to interspecific hybridization and cytological studies shall be presented.

Geographical distribution and classification of Lotus species

The genus includes a heterogeneous group of annual and perennial species which have a world-wide distribution. There are approximately 200 species which are endemic to parts of Asia, Africa, Europe,

Australia and North America. There are two principal geographic centers of speciation (Meusel and Jäger cited in Grant, 1965). The primary center is found around the Mediterranean region from where species have spread southwards around the Sahara desert and eastwards to Asia throughout the more temperate areas. About sixty species are found in the secondary center of speciation in Western North America radiating out from California to British Columbia eastwards to Manitoba and Arkansas and to the southeastern United States.

The taxa vary greatly in form and are adapted to a wide range of ecological habitats which extend from saline conditions at sea level through progressively higher elevations to an alpine habitat. Some species are also found growing in dry desert conditions (Ottley, 1957).

Classification of the genus is not well defined nor clearly understood. This is due primarily to the fact that investigators have employed different morphological and cytological criteria to delimit the different taxa. As a result, classification of the genus into subgenera, and the question of inclusion or exclusion of different species, are still not completely resolved. Zandstra and Grant (1967) and de Nettancourt (1963) have described various attempts made at classifying the genus by different workers. Callen's (1959) classification of the genus, based on the morphology of the style, has been the last one proposed and it bears some correlation with the chromosome numbers in the different groups. Callen divided the genus into four subgenera:

Subgenus I. Pedrosia (Lowe) Brand. Style erect forked or toothed.

Subgenus II. Edentolotus Brand. Style erect simple.

Subgenus III. Deflectostylus Callen. Style deflected.

Subgenus IV. Tetragonolobus Callen. Style erect club-shaped terminally or subterminally.

The species may be divided into two distinct groups with basic chromosome numbers of $n = 6$ and $n = 7$ (Senn, 1938; Darlington and Wylie, 1956; Löve and Löve, 1961; Grant, Bullen and de Nettancourt, 1962). All species with a basic chromosome number of $n = 6$ are found either in the subgenus Edentolotus or the subgenus Deflectostylus (Callen, Bubar and Grant, 1959). Lotus corniculatus L. and its closely related species which are grouped in the subgenus Edentolotus, all have a basic chromosome number of $n = 6$.

Hybridization in Lotus

Natural Hybridization

The breakdown of isolating mechanisms appears to be rare in this genus since there seems to be no real evidence that natural hybridization is a common feature within this group of plants. Many of the species are self-fertile and difficulties encountered in making artificial crosses between certain species support the view that natural hybridization is very limited in this genus. Stebbins (1959) did not observe any hybrids between closely related species of Lotus which grew side by side in California; he suggested that hybrid swarms do not occur in the family Leguminosae.

However, there are reports of a conflicting nature suggesting that

a certain amount of natural hybridization is a possibility. In 1944, Ottley published a monograph on a number of American species of Lotus. She observed in certain cases, where two species overlapped, the plants studied from these regions were extremely difficult to classify and she concluded that natural interspecific hybridization had occurred. According to Larsen (1954), three species, L. corniculatus, L. tenuis and L. pedunculatus could be differentiated with relative ease in Northern Europe; in Southern Europe no clearcut boundaries could be found between these taxa. Gillett (1958) also observed considerable variability in these three species growing in the Mediterranean region. The great polymorphism found in these populations, according to these authors, is suggestive of a certain amount of natural crossing. These species are all obligate outbreeders and chances are an occasional interspecific fertilization could take place. In this connection, it should be noted also that artificial crosses have been obtained between L. corniculatus and L. tenuis, and L. corniculatus and L. pedunculatus, by several workers (Grant, 1965).

Other reports substantiating the case for the occurrence of natural hybridization are those of Mattick and Seaney, both cited by de Nettancourt (1963). Mattick found a plant of L. corniculatus with 26 chromosomes which he suggested may well have been the F_1 hybrid of a cross between L. corniculatus ($2n = 24$) and a 28-chromosomed species. Seaney also noted several plants of L. hispidus Desf. ($2n = 24$) with somatic chromosome numbers of 25 and 26.

While evidence supporting natural hybridization within the

genus is not clearcut and really substantial, the absence of hybrid populations is not necessarily evidence that natural hybridization does not take place. It is possible that hybrids are eliminated either during the embryonic or the seedling stage. On the other hand, artificial hybridization has been accomplished with relative ease for certain species. Some of the taxa are obligate outbreeders and the great morphological similarities between some species would indicate that natural hybridization of a limited nature is a possibility.

Experimental Hybridization

The importance of introducing favorable germplasm from wild species into the cultivated species L. corniculatus, has been recognized by many workers and several studies have been undertaken to explore the possibilities for experimental interspecific hybridization within the genus. Grant et al. (1962) and de Nettancourt (1963) summarized the interspecific hybridization studies done up to that time. Grant (1965) also published a comprehensive list of the interspecific crosses that have been reported by various workers. Attempts at interspecific hybridization in the literature have involved the following types of crosses:

(i) Crosses between tetraploid and diploid species.

(ii) Crosses between tetraploid species.

(iii) Crosses between diploid species.

The results which have been reported concerning these categories will now be considered in detail.

(i) Crosses between tetraploid and diploid species

Eliott (1946) reported successful interspecific crosses between L. corniculatus and L. tenuis (2n = 12). He made 140 pollinations and obtained 7 fully developed seeds, the viability of which remains doubtful, since no information regarding this aspect has been made available by Eliott. McKee (1949) also stated that in field and greenhouse tests he obtained viable seeds from crosses between L. corniculatus and L. tenuis (2n = 12), and L. corniculatus and L. divaricatus Boiss. (2n = 12). He considered that these were true crosses and indicated that progenies were being grown in the field to confirm this. However, no further report on this material has been found. Mears (1955) failed to obtain hybrids when she attempted reciprocal crosses between the diploid species L. tenuis and L. pedunculatus. The first authentic hybrid between a tetraploid and a diploid was reported by Bent (1958, 1962). He used an embryo-culture technique and succeeded in getting two hybrids from the crosses L. pedunculatus (2n) x L. corniculatus. These two hybrids which more closely resembled L. corniculatus than L. pedunculatus were triploids and these produced a small amount of pollen which was stainable. Bent failed to obtain successful backcrosses to the parental species although both hybrids showed appreciable seed set when used either as the male or the female parent. In addition, Bent attempted to cross reciprocally L. corniculatus to L. japonicus (Regel) Larsen (2n) and L. tenuis (2n) but he only succeeded in getting some embryos which were abnormal and did not grow.

Studies aimed at the possibilities of incorporating resistance to

seed pod dehiscence into L. corniculatus also have resulted in a number of attempts to cross certain diploid species with the cultivated tetraploid (Gershon, 1961; Phillips and Keim, 1968). Gershon made reciprocal crosses between L. corniculatus and L. edulis L. ($2n = 14$), L. wrightii (Gray) Green ($2n = 14$), L. tetragonolobus L. ($2n = 14$), L. weilleri Marie ($2n = 14$), L. coimbrensis Willd. ($2n = 12$) and L. arabicus L. ($2n = 14$). He was unsuccessful in these crosses although in the one cross involving L. coimbrensis and L. corniculatus he reported that some empty pods were formed. Phillips and Keim (1968) likewise did not succeed in obtaining seed in crosses between L. tetragonolobus ($2n = 12$) and L. corniculatus; however, their experimental cross between a pubescent plant of L. coimbrensis ($2n = 12$) and a glabrous plant of L. corniculatus was successful and resulted in the production of 13 offspring, all of which were found to have a diploid chromosome number of 24, low pollen fertility, and a phenotypic appearance resembling L. corniculatus but with pubescence typical of the male parent L. coimbrensis. At first, the hybrid nature of these plants was questioned since there was the possibility of contamination by pollen from a pubescent clone of L. corniculatus. However, this was later clarified by a study of chromosome pairing relationships in the hybrids which indicated that they were indeed hybrids that must have resulted from unreduced gametes of L. coimbrensis.

(ii) Crosses between tetraploid species

It can be ascertained from the foregoing account of tetraploid x

diploid crosses that only very limited success to-date has been achieved. A logical approach to double the diploid chromosome number and use the induced autotetraploids in hybridization tests has been followed by a number of workers. Tome and Johnson (1945) in accordance with this procedure, artificially induced tetraploids of L. tenuis and attempted crosses with L. corniculatus; they observed pod set but the seeds formed were empty and inviable. Keim (1952) was more successful employing embryo-culture techniques and obtained hybrid plants using these same species; he also succeeded in producing some hybrids directly from seeds. Later, Mears (1955), Erbe (1955) and Bent (1958) also obtained interspecific hybrids between L. corniculatus and tetraploid L. tenuis. Mears successfully carried out the reciprocal cross and observed that about 25 per cent of the hybrid seeds germinated when L. tenuis was used as the pistillate parent as compared to about 1 per cent in the case of the reciprocal cross. She grew to maturity 33 such hybrids and studied their fertility; in greenhouse tests, there was an average of 17 seeds per pod whereas the average seed set in the field was 20.7 seeds per pod. This differed markedly from Keim's (1952) report of low seed set in such a hybrid, and Mears considered that Keim's results possibly were due to the advanced generation 4x plants used in his study. Mears also made other tetraploid crosses; in the cross L. corniculatus x 4x L. pedunculatus, no pods developed following pollination, but pod formation was observed in crosses between 4x L. pedunculatus and 4x L. tenuis. None of the embryos in the latter cross developed when grown in culture medium.

In 1958, Bent conducted an interspecific hybridization study which included producing a number of reciprocal crosses at the tetraploid level. He was the first person to report success in crossing 4x *L. pedunculatus* and *L. corniculatus*; he produced nine mature hybrids from this cross by recourse to embryo culture. While many of these hybrids showed growth comparable to the parental species with an overall appearance closely resembling *L. corniculatus*, pubescence of sepals and HCN reaction were similar to that of *L. pedunculatus*. Fertility of these hybrids was variable, for example, two of the hybrids when used as female parents were high seed setters, but were low when used as male parents; many of the flowers were male sterile or nearly so. A few years following Bent's investigations, Fish (1961) reported successful crosses involving *L. corniculatus* x *L. japonicus* (4x) and *L. corniculatus* x *L. filicaulis* Dur. (4x) which previously had not been used in any hybridization program. In the former cross, the hybrids were intermediate to both parents for morphological characters; in the latter, the single hybrid closely resembled the female parent *L. filicaulis*, except for the florets which were intermediate in size and coloration. Pollen stainability was also high (80-85 per cent) in the *L. corniculatus* x *L. filicaulis* F_1 progeny.

(iii) Crosses between diploid species

It has long been realized that crossing diploid species closely related to *L. corniculatus* would lead to a better understanding of the relationships of the diploid species (Dawson, 1941; MacDonald, 1946;

Grant et al., 1962). This knowledge has been considered important since certain agronomic characters could first be improved at the diploid level where the genetics is less complicated; improved characters could then be transferred to the tetraploid species for ultimate use (Wernsman, 1963).

Dawson (1941) made reciprocal crosses between L. tenuis and L. pedunculatus but failed to obtain any hybrid seeds from a total of 425 flowers pollinated. In a study of self-fertility relationships, Macdonald (1946) also was unsuccessful in obtaining pod set when L. tenuis and L. pedunculatus were placed in adjacent areas in a bee-proof greenhouse during the winter months, or in bee-proof cages in the field or when bees were used as pollinating agents. McKee (1949) conducted fertilization studies in the genus Lotus and attempted hybridization between L. tenuis and L. filicaulis, and L. tenuis and L. divaricatus. He reported obtaining some hybrids in the latter cross; however, the hybrid nature of these plants was not clearly ascertained. In a study of interspecific crosses in Trifolium and Lotus, Keim (1952) crossed the diploid species L. pedunculatus and L. tenuis. He pollinated 234 flowers of which 158 formed pods and yielded 83 plump seeds; but unfortunately, he apparently did not grow these seeds to determine their genetic nature as he did not publish any further information on these. A few years later, Mears (1955) made reciprocal crosses between L. tenuis and L. pedunculatus and she obtained data which were at variance with Keim's. She succeeded in obtaining pod set only and found that early abortion of the embryo took place soon after

development was initiated. Mears suggested that an isolating mechanism of some unknown nature was operating during embryonic development of the hybrid. Bent (1958) was able to overcome this barrier by the use of an embryo-culture technique. He transferred 106 embryos to culture medium and observed growth initiation in 22 of these; however, only one developed normally into a mature plant. This diploid interspecific hybrid was intermediate to its parents in morphological features but reacted positively to a hydrogen cyanide test like its male parent L. tenuis. Although sterility in this cross was believed to be caused by anther atrophy, attempts to backcross this hybrid to either parent failed when it was used as the pistillate parent. Colchicine treatments carried out in attempts to double the chromosome number were also unsuccessful. In addition to this cross, Bent also made other reciprocal crosses between L. japonicus, L. tenuis and L. pedunculatus but failed to produce any hybrids. Phillips (1963) was unsuccessful in crossing L. japonicus with L. coimbrensis; he did get two pods to develop but these contained only shrunken seeds. Seaney (in Grant et al., 1962) is reported to have obtained a sterile hybrid between L. coimbrensis ($2n = 12$) and L. ornithopodioides L. ($2n = 14$). This plant had a somatic chromosome number of 13 and was difficult to propagate by means of cuttings; attempts to double the chromosome number were made but the results have not been reported.

In this laboratory, improvements in techniques of embryo culture and methods of emasculation and pollination, have made it possible to

produce a number of interspecific diploid hybrids (Grant et al., 1962; de Nettancourt, 1963; de Nettancourt and Grant, 1963, 1964a; Grant, 1965). Grant and his co-workers (1962) by means of embryo culture, produced 39 F_1 hybrids from nine different crosses; the number of hybrids obtained in each cross and the average per cent stainable pollen are as follows:

Cross	No. of F_1 hybrids	Average % stainable pollen
L. japonicus x L. alpinus Schleich.	6	27.37
L. japonicus x L. schoelleri Schweinf.	5	12.44
L. schoelleri x L. japonicus	1	6.46
L. japonicus x L. krylovii Schischk. and Serg.	12	31.43
L. krylovii x L. japonicus	5	14.67
L. krylovii x L. schoelleri	1	2.66
L. schoelleri x L. krylovii	2	2.12
L. japonicus x L. filicaulis	4	33.28
L. krylovii x L. filicaulis	3	3.43

It was found that ascending growth habit, red stem coloration, striping on the flower bud, reddish brown keel tip color, pod and seed stippling, and positive reaction for the presence of HCN in the leaves exhibited dominance in the hybrids. Also, measurements of florets length, standard width, ovary length and leaflet index were observed to be intermediate in the hybrids to those of their respective parents; however, the length of the central leaflet and style

length in some hybrids exceeded those found in either parent and these were interpreted as expressions of hybrid vigor. All these hybrids exhibited varying degree of pollen abortion and reduction in fertility; pollen sterility ranged from 67 to 97.5 per cent. de Nettancourt and Grant (1963, 1964a) also described additional diploid interspecific hybrids in Lotus; the number of hybrids obtained in each cross and their average per cent stainable pollen are as follows:

Cross	No. of F_1 hybrids	Average % stainable pollen
L. japonicus x L. schoelleri	4	12.44
L. schoelleri x L. japonicus	1	6.46
L. krylovii x L. japonicus	5	14.67
L. schoelleri x L. krylovii	2	2.12
L. krylovii x L. schoelleri	2	2.66
L. krylovii x L. filicaulis	3	3.43
L. tenuis x L. filicaulis	1	43.65

All these hybrids except those obtained from the L. japonicus x L. schoelleri cross were produced by means of embryo culture. Hybrids obtained in three reciprocal crosses showed no gross morphological differences but significant differences were observed between reciprocal hybrids for the quantitative characters of leaflet and floret size. These variations were particularly noticeable when leaflet lengths were compared in reciprocal hybrids of the cross L. japonicus x L. schoelleri and ovary lengths and style lengths in the reciprocal cross of L. japonicus x L. krylovii. It has been suggested

that perhaps a cytoplasmic factor might be operating here. Pollen fertility ranged from 2.12 per cent in L. schoelleri x L. krylovii to 43.65 per cent in L. tenuis x L. filicaulis; the average seed set per pod was also very low in all these hybrids and varied from 0.78 in L. schoelleri x L. japonicus to 2.74 in L. tenuis x L. filicaulis. The general vigor and profuse branching of the hybrids and the increase in the central leaflet length and the floret length over their respective parents were explained in terms of heterotic effects.

Cytological investigations in Lotus

Karyotypic studies

Studies of the somatic chromosomes of some of the Lotus species used in this investigation have been published by a number of authors: L. corniculatus by Tschechow and Kartaschowa (1932), Larsen (1954), Mears (1955), Ujhelyi (1960), Larsen and Zertova (1963), Gilot (1965), Przywara and Schmager (1967) and Zandstra and Grant (1967); L. tenuis by Tschechow and Kartaschowa (1932), Mears (1955), Przywara and Schmager (1967), and Zandstra and Grant (1967); L. krylovii by Larsen (1958), and Zandstra and Grant (1967); L. alpinus by Favarger (1953), Larsen (1954), and Grant et al., (1962). Many of these reports gave the chromosome numbers and provided drawings of the somatic chromosomes of these species but the only idiograms published were those of Zandstra and Grant (1967) for L. krylovii and L. tenuis. According to these authors, the total complement length (TCL) of L. krylovii varied from 27.46 μ to 41.14 μ and had an average of 32.68 μ. The length of the individual chromosomes ranged from 3.6 μ for the longest

to 1.97 μ for the shortest; no satellite chromosomes were found and only one pair of chromosomes was metacentric, the rest being submetacentric. For L. tenuis, the length of the total complement ranged from 22.04 μ to 37.06 μ with an average TCL of 28.78 μ. The longest chromosome had an average length of 3.72 μ and the shortest 1.56 μ; all the chromosomes were submetacentric having fairly similar arm ratios and were without satellites. Zandstra and Grant indicated that most of the chromosomes of L. corniculatus were submetacentric with a few approaching metacentricity. Also, this species was found to be devoid of satellite chromosomes. Mears (1955) did comparative studies between L. tenuis and L. corniculatus and reported no significant differences in the karyotypes of L. corniculatus and that of the induced autotetraploid of L. tenuis. These findings differed somewhat from those of Przywara and Schmager (1967) who studied karyotypes of plants of L. tenuis and L. corniculatus that originated from natural habitats in Poland. These workers classified the chromosomes of diploid L. tenuis into four groups as follows:

Group A — three pairs with median centromeres.

Group B — one pair with submedian centromeres.

Group C — one pair with subterminal centromeres.

Group D — one pair with median centromeres and satellites.

In the karyotype of L. corniculatus, they observed two pairs of chromosomes corresponding with each pair of chromosomes of the groups A, B and C of L. tenuis but only one pair with satellites was found. They inferred from these studies that the complement of L. corniculatus

was not a duplicate of the L. tenuis chromosomes.

Tschechow and Kartaschowa (1932) indicated that there were two pairs of long submetacentric chromosomes and ten pairs of short chromosomes in somatic cells of L. corniculatus var. alpestris Lamotte (2n = 24), and one pair of long submetacentric chromosomes, one pair metacentric and four pairs of short chromosomes in L. filicaulis (2n = 12). In 1953, Favarger concluded from his studies that the karyotype of L. alpinus was very similar to that of L. tenuis. Grant and his co-workers (1962) also reported similarity of chromosome size and morphology in five diploid species. They examined the mitotic chromosomes of L. japonicus, L. alpinus, L. krylovii, L. schoelleri and L. filicaulis which they utilized in their interspecific hybridization studies and stated that the chromosomes of all these species appeared to be of the same range and type. At metaphase the chromosomes were very small (1-3 μ) and did not have secondary constrictions; in each of the species the chromosome complement consisted of one long pair, one medium pair and four short pairs.

Meiotic studies

In 1941, Dawson observed tetrasomic segregation for HCN in L. corniculatus and this discovery prompted him to investigate the frequency of quadrivalents at meiosis in this species. He stated that owing to the small size of the chromosomes, it was not possible to get numerical data on this point; however, from the limited number of cells he studied he concluded that bivalents were usually formed

and that quadrivalents appeared to be rare. Wernsman, Keim and Davis (1964) made similar observations when they conducted meiotic studies in L. corniculatus. They found that diakinesis was characterized by the occurrence of twelve bivalents although quadrivalents with an average of one in every four cells and occasional trivalents and univalents were also seen.

Lotus tenuis and L. pedunculatus, two other species which are considered to be of agronomic value, were also subjected to meiotic studies by several workers (Wernsman et al., 1964; Gershon, 1961; Chen, 1967). Wernsman and his colleagues also compared chromosome behaviour in a first generation of autotetraploid L. tenuis with clones of induced autotetraploids of this species that were several generations removed following the induction of autoploidy. They reported that in the first generation autotetraploids approximately equal number of chromosomes were found as bivalents (II's) and quadrivalents (IV's), the mean number per cell being 5.90 II's and 2.85 IV's; in the later generation clones there was an increase in the frequency of bivalents and a decrease in quadrivalents. They inferred from these observations that following random mating of the autotetraploids, there could be selection for a gene, or genes, influencing chromosome association, and this could conceivably result in a decrease of the frequency of quadrivalents.

Gershon (1961) studied meiosis in a second generation colchicine induced autotetraploids of L. pedunculatus. Of the 57 cells he examined at diplotene, diakinesis and metaphase I, he stated that two

cells had 6 IV's each, 13 had a frequency of 3 IV's + 6 II's per cell, 21 had a frequency of 2 IV's + 8 II's per cell, three had a frequency of 2 IV's + 2 III's + 4 II's + 2 I's per cell, 16 had a frequency of 1 IV's + 10 II's per cell and two cells had 12 II's each. Chen (1967) also investigated meiotic chromosome behaviour in an auto-tetraploid L. pedunculatus and indicated that an examination of 15 pollen mother cells (PMC) at diakinesis and prometaphase showed three cells with 5 IV's, three cells with 2 IV's, six cells with 1 IV and three cells with 0 IV's. The mean number of quadrivalents per cell was 1.80, whereas the frequencies of trivalents, bivalents and univalents per cell were 0.13, 7.53 and 1.33, respectively. He pointed out that the large and medium sized chromosomes tended to form quadrivalents more frequently and that the medium and small chromosomes usually formed bivalents. Lagging chromosomes were also quite common at telophase I and II.

A limited number of investigations have also been carried out on tetraploid interspecific hybrids obtained by crossing L. tenuis, or L. pedunculatus, to L. corniculatus. Gershoy (in de Nettancourt, 1963) conducted a meiotic study of the hybrid L. tenuis x L. corniculatus and made the following report:

> "Meiotic studies indicate, in various genotypes, the presence of a small number of dividing univalents in anaphase I and anaphase II. Neither multivalent formation nor dicentric bridges have been observed, in undisturbed spindles, in smears of P.M.C.; thus homogametic pairing is assumed."

Gershoy did not find any consistent correlations between meiotic

irregularities, per cent polyspory, per cent stainable pollen, and seed set per pod.

The results of Wernsman et al., (1964) for the most part seem to agree with Gershoy's observations. They found that the hybrids from this cross (L. tenuis x L. corniculatus) were fertile and at meiosis were characterized by twelve bivalent pairs with an occasional quadrivalent. They also suggested that homogametic pairing was taking place and that the few quadrivalents observed might be due to partial homology between the L. tenuis and the L. corniculatus chromosomes. Wernsman and his colleagues then proceeded to investigate this chromosome homology by making backcrosses between the interspecific hybrids and their parents. Meiocytes in the progenies obtained by using L. corniculatus as the recurrent parent had an average number of bivalents of 11.57 per cell and 0.09 quadrivalents per cell. When L. tenuis was the recurrent parent, the mean number of bivalents and quadrivalents per cell was 10.34 and 0.70, respectively. There results suggested to Wernsman and his associates that there was a high degree of homology between the chromosomes of L. tenuis and L. corniculatus.

Six tetraploid interspecific hybrids obtained by crossing L. corniculatus and L. pedunculatus were studied by Gershon (1961). These hybrids differed in the amount of viable pollen they produced and meiotic studies were undertaken to investigate a possible correlation between pollen production and meiotic chromosome behaviour. In four of these hybrids, 100 meiocytes per hybrid were analysed at

various stages of meiosis; approximately ten per cent of the cells showed both lagging bivalents and univalents at anaphase I and telophase I; no multivalents were detected and most of the cells exhibited normal bivalent formation. Loose bivalents which were probably due to a low frequency of chiasmata, were apparent at the end of terminalization when short chromosomes were seen to be attached end to end. Meiosis was almost normal in the other two hybrids as bivalent formation was observed in 159 out of the 162 cells examined and only three cells exhibited multivalent formation. One of these contained 1 IV and 10 II's, a second cell had 2 III's and 9 II's, and the third cell contained 2 IV's, 2 III's 4 II's and 2 I's. Gershon concluded that the difference in pollen viability was not the result of irregularities in meiosis. He suggested that a certain amount of chromosome homology existed between L. pedunculatus and L. corniculatus.

Gershon (1961) also studied chromosome association in the triploid interspecific hybrid between 2n L. pedunculatus and L. corniculatus, and stated that meiosis in this triploid was very irregular. Because of the incomplete pairing, or lack of pairing, of the chromosomes, he found the stages of meiotic divisions difficult to define. In the quartet stage, 69 of the 72 cells analysed, had at least one micronucleus and many of the microspores were small indicating that they might be composed of a smaller number of chromosomes.

In 1968, Phillips and Keim reported meiotic studies in hybrids

obtained by crossing L. corniculatus (♀) with the diploid species L. coimbrensis (♂). These F_1 hybrids had 24 chromosomes and were believed to be the result of unreduced male gametes or the formation of restitution nuclei following meiotic reduction. They found that the pollen mother cells at diakinesis contained mostly bivalents with occasional univalents. However, when these hybrids were crossed to L. corniculatus and meiosis was studied in the backcross progenies, a high per cent of univalents were seen. In the opinion of the authors, the presence of these univalents was evidence for the hybrid nature of the original offspring, that is, the F_1 hybrids from the L. corniculatus x L. coimbrensis cross. They also suggested that some homology may exist between the chromosomes of L. coimbrensis and L. corniculatus.

Analyses of meiotic chromosome behavior in diploid species and interspecific diploid hybrids have been reported by de Nettancourt (1963), Chen (1967), Grant (1963), Grant et al. (1962) and de Nettancourt and Grant (1963, 1964a, 1964b). In these investigations, observations were largely confined to the chromosome associations at metaphase I and the presence of bridges and lagging chromosomes during anaphase of the first meiotic division.

The three diploid species L. japonicus, L. filicaulis and L. tenuis studied by de Nettancourt (1963) showed regular bivalent formation; three of the thirty-two cells of L. japonicus and one of the thirty-nine cells of L. filicaulis had chromosome associations of 5 II's + 2 I's each whereas 6 II's per cell were observed in the remaining cells; bridges were also seen at anaphase I and anaphase II

in L. filicaulis. Lotus tenuis was characterized by a regular meiosis of six bivalents per cell. Chen (1967) also stated that meiosis was regular in L. pedunculatus. He examined 84 microsporocytes at diakinesis and prometaphase I and indicated that six bivalents were usually seen, although in a few cases, five bivalents and two univalents were present. He recorded the frequencies of bivalents and univalents per cell as 5.93 and 0.14, respectively.

In 1962, for the first time, meiotic studies were reported on four diploid interspecific hybrids, namely, L. japonicus x L. alpinus, L. japonicus x L. filicaulis, L. japonicus x L. krylovii, and L. japonicus x L. schoelleri, by Grant and his co-workers. This was immediately followed by studies on L. schoelleri x L. japonicus, L. schoelleri x L. krylovii, L. krylovii x L. filicaulis, and L. tenuis x L. filicaulis (de Nettancourt and Grant, 1963, 1964a). In each of these diploid hybrids, normal association of six bivalents was found in at least 68 per cent of the cells examined. One cross L. japonicus x L. alpinus, had as many as 94.5 per cent of the cells with six bivalents. However, the high degree of bivalent pairing observed in these hybrids was nevertheless accompanied by some irregularities. One or more pairs of univalents were seen, and Grant (1963) interpreted this to be the result of desynapsis of the weak type as defined by Prakken. These authors pointed out that the longest pair of chromosomes of the complement never separated as univalents but that the smaller chromosomes appeared always to be involved. This precocious division of the bivalents which was believed to be the result

of segmental and genetic differences between the parental species, was particularly noticeable in crosses between L. japonicus and L. schoelleri, L. krylovii and L. filicaulis, L. japonicus and L. alpinus, and L. schoelleri and L. krylovii. Multivalents were also recorded in microsporocytes of three diploid hybrids. Lotus japonicus x L. krylovii had three cells with chromosome associations of 1 I + 4 II's + 1 III and six cells with associations of 4 II's + 1 IV; L. krylovii x L. filicaulis had four cells each with 4 II's + 1 IV and L. tenuis x L. filicaulis had two cells recorded with 1 I + 4 II's + 1 III and one cell with the chromosomes distributed as 4 II's + 1 IV. Another cytological abnormality observed in a hybrid plant of the cross L. japonicus x L. alpinus, was the presence of inter-microsporocyte bridges connecting more than seventeen per cent of the pollen mother cells in various stages of meiosis. This phenomenon was attributed to asynchronous pre-meiotic somatic division in which karyokinesis and cytokinesis were out of phase resulting in incomplete cell formation and chromatin bridges between the pollen mother cells (de Nettancourt and Grant, 1964b). Irregularities at anaphase I consisted chiefly of lagging chromosomes, many of which remained in the cytoplasm at telophase I and failed to be incorporated within the restitution nuclei. Also, chromosome bridges were observed in six different hybrids; these included L. japonicus x L. alpinus, L. japonicus x L. filicaulis, L. japonicus x L. krylovii, L. schoelleri x L. krylovii, L. krylovii x L. filicaulis, and L. tenuis x L. filicaulis. Generally, the frequencies of visible cytological anomalies were low and it was difficult to

reconcile the overall high pollen sterility in these hybrids with the cytological abnormalities.

The evolutionary history of Lotus corniculatus

The origin of L. corniculatus has been considered by several investigators. Dawson (1941) suggested that the natural tetraploid species, L. corniculatus, originated as an autotetraploid of L. tenuis or its prototype. He reached this conclusion after making the following observations: (a) L. tenuis possesses a somatic chromosome number of 12, whereas L. corniculatus has a somatic chromosome number of 24. (b) Meiotic studies in L. corniculatus indicate that bivalents are formed most of the time, and quadrivalents only occasionally. (c) L. tenuis has a number of morphological characteristics similar to those of L. corniculatus. These include flower shape, flower number per head, calyx tooth in the bud, fruit, seeds, and succulent runners. (d) Cyanogenic and acyanogenic plants of both L. corniculatus and L. tenuis are found in the same wild population. (e) Cyanogenesis in L. corniculatus is determined by a dominant gene and shows tetrasomic inheritance.

Tome and Johnson (1945) in a breeding program investigated the possible phylogenetic relationships between L. tenuis and L. corniculatus. They found conspicuous morphological differences between the diploid L. tenuis and tetraploid L. corniculatus; the most noticeable characters which distinguished the two species were the leaves, stipules, and floral parts; these authors stated that

the greatest dissimilarity between the species was exhibited in the stipule length-width ratio and to a lesser extent in the pollen shape and size. By means of colchicine treatment they artificially doubled the number of chromosomes of L. tenuis and found that the induced autotetraploid did not resemble L. corniculatus in general appearance; differences in the shape of the leaves and stipules were very apparent. Moreover, they did not succeed in obtaining viable seeds from the crosses between L. corniculatus and the induced autotetraploid L. tenuis.

Stebbins (1950) taking these various observations into account, suggested that L. corniculatus could be a segmental allotetraploid. His arguments were that L. corniculatus formed almost entirely bivalents at meiosis and at the same time showed tetrasomic inheritance for some genetic characters; this behaviour according to Stebbins is typical of segmental allopolyploids.

A few years later, Favarger (1953) speculated that the recently discovered diploid species, L. alpinus, could well be the ancestor of L. corniculatus; he also pointed out that the reverse situation could be true, namely, that L. alpinus could have had a parthenogenetic origin. Larsen (1954) supported Favarger's view; he considered that L. alpinus and L. corniculatus resemble each other more closely than do any of the other species of the Lotus corniculatus group, and that the latter species was derived through autopolyploidy from L. alpinus. He did make one reservation however, by stating that the possibility for L. corniculatus to have arisen by amphidiploidy could not be disregarded.

Wernsman, Keim and Davis (1964) presented data on chromosomal pairing relationships in 1) L. corniculatus and L. tenuis (4x), 2) their interspecific hybrids and 3) backcross progenies obtained by crossing these interspecific hybrids to each parent. Their results indicated that the chromosomes of L. tenuis and L. corniculatus possess a high degree of homology which supported the hypothesis of an autotetraploid origin of L. corniculatus from the diploid L. tenuis. Harney and Grant (1964) from a chromatographic study of the phenolic properties possessed by these species, considered that the biochemical data gave greater support for an allotetraploid origin of L. corniculatus. More recently, Przywara and Schmager (1967), on the basis of karyological, morphological and anatomical studies in L. tenuis and L. corniculatus, rejected the former species as being implicated in the origin of the latter. They favored Favarger's hypothesis and suggested that L. alpinus could be safely treated as the ancestral form of L. corniculatus. In 1967, Grant and Zandstra conducted a thin-layer chromatographic study of fluorescent compounds present in a number of Lotus species. They showed that the chromatograms of L. tenuis (2n) and L. krylovii (2n) were very similar and that the chromatogram of L. corniculatus differed only slightly from those of these two diploid species. Grant and Zandstra therefore suggested that L. krylovii should be included in studies aimed at investigating the progenitor or progenitors of the tetraploid L. corniculatus.

Conclusion

It can be seen from the foregoing review that there is still a

considerable lack of basic cytogenetic knowledge in the genus Lotus. There are about 200 species in the genus which constitutes a vast amount of potential germplasm for incorporation into the cultivated tetraploid species. However, cytogenetic interest in this group of plants has only developed in the last few decades and this perhaps explains the paucity of published cytogenetic information. Since L. corniculatus is a very promising forage crop in eastern North America and Canada, and is rapidly increasing in production, there is a real need for establishing a greater knowledge of information concerning Lotus species especially those in the L. corniculatus group.

From the various studies on interspecific hybridization in Lotus, it is difficult to assess the degree to which natural crossing occurs. Some investigators believed it to be absent altogether, whereas others have presented indirect evidence which would support the occurrence of natural interspecific hybridization to a limited extent. Experimental hybridization, on the other hand, is achieved with relative difficulty. Embryo-culture techniques, which have been used to successfully produce interspecific hybrids, have revealed that in many cases the reproductive barriers which isolate species of Lotus are probably operative at the postzygotic stages. Also, it does not appear that embryo culture is an absolute prerequisite to the success of a complete hybridization program.

The limited cytological studies indicate that there is little variability in chromosome morphology throughout the complex of the

species closely related to L. corniculatus. From the meiotic investigations, it is clear that a certain amount of chromosome homology exists between the chromosomes of L. tenuis, L. pedunculatus, L. coimbrensis, and L. corniculatus. Chromosome pairing relationships in diploid interspecific hybrids obtained by hybridizing species closely related to L. corniculatus has also revealed close chromosome homology between these species.

There has been considerable controversy centered around the progenitors of L. corniculatus and several putative species have been suggested. The status of these putative diploid ancestors, however, has not been investigated to establish conclusively their phylogenetic relationships with L. corniculatus. The evolutionary history of this tetraploid cultivated species thus appears still to be a matter of speculation and further studies are needed to resolve this problem.

It may be readily realized from this brief literature review that there is considerable need for further cytogenetic studies in this important genus in which several species are of economic importance.

MATERIALS AND METHODS

I. *Lotus* species used in the study.

The species used in this study, their accession numbers and their sources are given in Table 1.

Brief comments concerning each species are as follows:

L. alpinus Schleich. (syn.: *L. corniculatus* var. *alpinus* Ser.), $2n = 12$.

This perennial species was discovered by Favarger (1953) growing in the western Alps at an altitude of 2,000 meters. The very dark reddish brown color of the keel tip of the flower is dominant to yellow keel tip, and this serves as an excellent marker when this plant is used as the male parent in hybridization experiments. Also, this species has been implicated as a putative ancestor of *L. corniculatus* (Favarger, 1953; Larsen, 1954; Przywara and Schmager, 1967).

L. japonicus (Regel) Larsen, (syn.: *L. corniculatus* var. *japonicus* Regel), $2n = 12$.

This taxon which is native to Korea and Japan is an annual. It is self-fertile and flowers profusely; the flowers are relatively large and recover well after emasculation. *L. japonicus* resembles *L. alpinus* in stem color, floret size and color, and in the presence of HCN (de Nettancourt, 1963).

L. tenuis Waldst. et Kit. (syn.: *L. corniculatus* var. *tenuifolius* L., *L. corniculatus* ssp. *tenuifolius* (L.) Hartm.), $2n = 12$.

Lotus tenuis is a self-sterile perennial of European and North

African ancestry; this species has become naturalized in the United States (Isely, 1951; Tidestrom, 1925) and also in the provinces of Ontario and British Columbia in Canada (Zandstra and Grant, 1967). Many authors up to 1954, considered L. tenuis as a variety, or sub-species, of L. corniculatus despite morphological dissimilarities between these two taxa; in 1954, Larsen on the basis of cytotaxonomic evidence re-instated L. tenuis to specific rank. Also, a number of investigators believe that L. corniculatus originated as an autotetraploid of L. tenuis or its prototype (Dawson, 1941; Wernsman et al., 1964).

L. filicaulis Dur. (syn.: L. corniculatus var. filicaulis (Dur.) Brand), $2n = 12$.

This is a self-fertile species that seems to be restricted to North Africa; its center of origin is believed to be Algeria (Jahandiez and Maire, 1911). Fish (1961) pointed out certain morphological similarities between L. filicaulis and L. tenuis; they both have long narrow leaves, slender stems, small flowers, and pods and seeds with the same characteristics.

L. schoelleri Schweinf. (syn.: L. corniculatus var. eremanthus Chiov.), $2n = 12$.

Gillett (1958) reported the presence of this species in Sudan, Eritrea, Ethiopia, Kenya, and Tanganyka. He stated that L. schoelleri is morphologically similar to L. tenuis except for its wider leaflets which resemble those of L. corniculatus. Its resemblance to L. filicaulis,

TABLE 1

List of species studied, their accession numbers and sources.

Species	Acc. No.	Source
L. alpinus Schleich.	B-77	Institut de Botanique, Université de Neuchâtel. Collector: C. Favarger. Origin: Western Alps. Received as L. corniculatus var. alpinus Ser.
L. japonicus (Regel) Larsen	B-129	Kyoto University. Collector: Isawo Hirayoshi. Origin: Riverbank near Gifu, Japan.
L. filicaulis Dur.	B-37	Dr. P. Henson, U. S. Department of Agriculture, Beltsville, Maryland. P.I. No. 51864. Origin: Botanic Garden, Madrid, Spain.
L. schoelleri Schweinf.	B-166	Grassland Research Station, Kitale, Kenya. Origin: Kenya, Africa. Received as L. corniculatus var. eremanthus Chiov.
L. krylovii Schischk. et Serg.	B-86	Hortus Botanicus Universitatis, Uppsala, Sweden. Received as L. corniculatus var. heterophyllarius Pet.-Stib.
L. tenuis Waldst. et Kit.	B-109	Australia. C.P.I. No. 23788. Origin: Turkey.
L. tenuis (autotetraploid)	B-340	Dr. A. Gershoy, University of Vermont, Burlington, Vermont.
L. corniculatus var. minor Baker	B-303	The Royal Botanic Garden, Edinburgh. Collector: B. L. Burtt. Origin: Grassy ground on bank of Kabul river, Peshawar, West Pakistan.

(Table 1 Cont'd.)

TABLE 1 (Cont'd)

Species	Acc. No.	Source
L. corniculatus L.	B-554	Plant Introduction Center, Izmir, Turkey. Origin: Samsun, Turkey. Izmir No. 1465-64.
L. pedunculatus Cav.	B-193	Service de la Recherche Agronomique et de l'Expérimentation Agricole, Rabat, Morocco. Received as L. uliginosus var. decumbens Poir. and originally accessioned as B-110 in this laboratory.
L. palustris Willd.	B-115	Government Agricultural Experiment Station, Naweh-Yaar, Israel. Collector: S. Gallilea.
L. borbasii Ujhelyi	B-253	Darmotske Kopce, steep hillside, south slope, Hegyfarok, Slowakia South, Czechoslovakia. Collector: A. Zertova.

L. tenuis and L. krylovii in growth habit, stem color, floret size and in the presence of HCN was also noted by de Nettancourt (1963).

L. krylovii Schischk. et Serg. (syn.: L. frondosus Freyn; L. corniculatus var. heterophyllarius Pet.-Stib.), 2n = 12.

This is a self-fertile Eurasian taxon which exhibits similar phenotypes for growth habit, stem color, floret size, floret striping and pod mottling as L. filicaulis and L. tenuis (de Nettancourt, 1963). On the basis of chromatographic studies of the phenolic properties possessed by this species, Grant and Zandstra (1967) considered it to be closely related to L. tenuis and L. corniculatus.

L. corniculatus var. minor Baker, 2n = 12.

This taxon to the author's knowledge, has not been used in any previous studies, and no cytogenetic information on it is available in the literature. It is believed to have a geographic distribution in West Pakistan (Hooker, 1879).

L. corniculatus L., 2n = 24.

The center of origin of this cultivated tetraploid species is believed to be Southern Europe. It is widely distributed and has been established in Europe, Asia, New Zealand, Australia and North America (Isely, 1951). The seed lot used was chosen at random from a world-wide collection available in this laboratory.

L. tenuis (autotetraploid), 2n = 24.

Tetraploid L. tenuis was available at the beginning of this

investigation; this autotetraploid is estimated to have advanced two to three generations following the induction of autoploidy.

In addition to the above mentioned species which were studied extensively, three other diploid species of European origin were also included in the hybridization program; these include L. pedunculatus Cav., L. palustris Willd., and L. borbasii Ujhelyi.

II. Production of interspecific diploid hybrids.

Hybridization techniques and the embryo-culture procedures followed, were essentially the same as those described by Grant et al. (1962). Flowers of diploid parental species were emasculated by means of an air suction method, after which the whole plant was sprayed with 10 parts per million of 2-4-5 trichlorophenoxy propionic acid (TCPPN) which prevented the emasculated flowers from dropping off. Plants were then placed in a growth chamber at $24^{o}C \pm 2^{o}C$, with the relative humidity maintained at 80-90% and a photoperiod of 16 hours. Two or three days later, when the flowers opened, a small flat wooden stick (a tongue depressor) to one end of which was glued a small piece of fine sand paper, was used for transferring pollen from the male parent; the sand paper ruptured the stigmatic membrane and so effective pollination was ensured. In a number of cases, where embryo culture was a pre-requisite, embryos were dissected out of the ovules 15-18 days following pollination and transferred to culture medium. The bottles containing these newly excised embryos were placed in a dark section of an incubator equipped with fluorescent lamps and maintained at

$25^{o}C \pm 2^{o}C$. After 2-3 days in darkness, the embryos were gradually exposed to increasing light intensities. When the cultured embryos had grown to mature seedlings, they were transferred to sterilized soil in small pots and placed in a growth chamber. Once these seedlings had become established, they were removed and maintained in a greenhouse or coldframe. It was estimated that 15 plants per cross were necessary for the various observations and investigations planned in this study. The appropriate numbers of cuttings from most of the hybrid plants were therefore propagated.

III. Colchicine technique for the induction of amphidiploids and autotetraploids.

Seeds obtained from crosses and from F_1 hybrids were scarified and germinated on moist filter paper in Petri dishes. The Petri dishes containing the seed were placed in the dark for the first few days to promote abnormal elongation of the hypocotyls after germination; the elongated hypocotyls facilitated handling of the seedlings at the time of transplanting into pots of sterilized soil and subsequent treatment with colchicine. A small ball of cotton soaked in 0.2% colchicine solution was applied to the apical meristem of the young seedling before the emergence of the first leaves. The cotton was kept continuously soaked for 8 hours by placing a large drop of the colchicine solution on the cotton every two to three hours. At the end of this period the cotton ball was removed and the seedling rinsed with water. The colchicine treatment was carried out in a growth chamber where the seedlings remained for a minimum

of three weeks. Surviving plants were grown to maturity and screened for polyploidy. Slower growth, fleshier and larger leaves, late flowering and larger flowers were the main criteria used to visibly detect any putative tetraploid plants and tetraploid sectors. Cuttings made from suspected tetraploid plants and tetraploid branches were treated with a rooting powder (Seradix) and planted in vermiculite which was kept moist. Mitotic root tip chromosome counts were made on all the material to ensure that chromosome doubling had been produced by the colchicine treatment and the tetraploid seedlings were then transferred to soil in pots. Cuttings were propagated from the tetraploid plants so that 15 plants from each amphidiploid were available for the various observations and investigations.

IV. Production of crosses between the synthetic amphidiploids and L. corniculatus.

The same hybridization technique used for the production of interspecific diploid hybrids was followed in this case; however, there was no need to use embryo culture since successful crosses between the amphidiploids and L. corniculatus were comparatively easy to obtain.

V. Morphological studies.

Morphological investigations were carried out on the diploid parental species, interspecific hybrids which had not been produced in this laboratory previously, induced autotetraploids, and synthetic amphidiploids. These plants were all grown in the field during the

summer of 1969. Data were obtained for the following characters: growth habit, central leaflet size, floret size, bud color, floret color, floret striping, keel tip color, number of florets per inflorescence, and HCN reaction.

The growth habit was recorded at the time of anthesis and plants were classified as decumbent, procumbent, ascending, or semi-erect. Decumbent plants had their stems lying more or less prostrate immediately surrounding the crown with branch tips ascending, whereas, procumbent plants had their stems lying prostrate on the ground; plants were described as ascending when their stems arched upwards and as semi-erect when the branches were more or less erect. Leaflet size and floral parts were measured to the nearest millimeter. Thirty leaves, two from each of fifteen plants, were collected from the upper parts of the plants when the first flowers appeared and the length and width of each central leaflet were recorded. Fifteen flowers, one from each plant were selected and measured for (a) the length and width of the floral bract, (b) the length and width of the standard, (c) the lengths of the calyx and the calyx tube and (d) the lengths of the style and the ovary. From these measurements, the following statistics were calculated:

(1) leaf index : width of the central leaflet/length of the central leaflet;

(2) floral bract index: width of the floral bract/length of the floral bract;

(3) standard index : width of the standard/length of the standard;

(4) calyx index : total length of the calyx/length of the calyx tube;

(5) style/ovary index: length of style/length of ovary.

The means and standard errors of the means were calculated for each phenotypic trait and each index. A t-test was employed to check the significance of the differences for all the phenotypic traits and all the indices. An IBM 650 data processing machine was used for statistical analyses. For observations on hydrogen cyanide reaction, an average of twenty plants from each category (diploid parental species, interspecific diploid hybrids, autotetraploids and amphi-diploids) were tested. The detailed procedure as reported by Dawson (1941) was followed.

VI. Pollen viability studies.

Estimates of pollen viability were obtained by scoring ten microscopic fields at magnification of 400 X from each of two preparations per plant. Ten plants from each of the categories mentioned above, were used. Determination of the pollen viability was carried out according to the method outlined by Marks (1954). An undehisced anther was macerated in a conveniently sized drop of acetocarmine glycerol jelly which gives an even distribution of both empty and plump pollen grains. A cover slip was applied and the counting of empty and full grains was done from one edge of the cover glass to the other in order to obtain representative results. Grains that were shrivelled and shrunken and which did not take up the stain because of lack of cytoplasm, were scored as inviable; only normal

sized pollen grains that were plump and took up the stain were counted as viable.

VII. Cytological studies.

Root tips for the determination of chromosome numbers were taken from cuttings grown in moist vermiculite and from plants grown in a growth chamber and cold frame. They were pretreated for two hours in 0.002M 8-hydroxyquinoline solution and then fixed in a mixture of one part of concentrated acetic acid and three parts of 95% ethyl alcohol for twenty-four hours. This was followed by hydrolysis in 1N HCl solution at 60°C for eight minutes and staining in leuco-basic fuchsin (Feulgen) for two to four hours in the dark. The stained root tips were treated with four per cent pectinase solution for one to two hours and squashed in 45% acetic acid.

For the study of meiosis in pollen mother cells (PMC's), flower buds were collected from diploid and tetraploid plants grown in the field (summer 1969) and from L. corniculatus x amphidiploid crosses grown in the greenhouse (autumn 1969). The temperature in the greenhouse was maintained at 24°C - 27°C during the day and 18°C - 19°C at night. Buds were fixed in a 6:3:2 mixture of 100% methanol, chloroform and propionic acid for six to twenty four hours. They were then washed in 80% methanol and stored for short periods in 100% methanol in the refrigerator until needed. A modified Snow's (1963) methanolic hydrochloric acid-carmine solution was used for staining; the stored materials were placed in the stain for a period of two to four weeks and anthers were squashed in 45% acetic acid for the examination of

the meiocytes.

In certain cases where poor staining was observed, heating the flower buds in the staining solution at 60°C for 3-4 hours in a paraffin oven quite often made an improvement. Chromosomal configurations were studied in the PMC's at diakinesis, prometaphase and metaphase; only those cells were recorded in which the pairing relationship of every chromosome was clear. Observations of chromosome behavior at anaphase I and II and telophase I and II were also noted. Meiosis was studied in all the plants that were flowering at the time flower buds were collected.

Slides for mitosis and meiosis were examined from temporary mounts. These remained in good condition for several days when kept in a refrigerator on a moist filter paper within a Petri dish. A phase contrast microscope was used for cytological examination and photomicrographs were taken from the temporary mounts on Adox KB14 film using a Zeiss photomicroscope.

RESULTS

I. Interspecific hybridization

In an attempt to produce interspecific diploid hybrids, 44 different cross combinations between ten diploid taxa were made. A total of about 3000 flowers were emasculated and pollinated and an estimated number of about 800 embryos were cultured. The results obtained in this hybridization program are shown in Table 2. Sixteen of the 44 cross combinations were successful and 117 diploid interspecific hybrids were produced. The accession numbers of these hybrids and the number of plants obtained in each cross combination are indicated in Table 3. Nine new hybrid combinations were produced. These include L. japonicus x L. corniculatus var. minor, L. alpinus x L. krylovii, L. alpinus x L. filicaulis, L. alpinus x L. schoelleri, L. krylovii x L. corniculatus var. minor, L. krylovii x L. tenuis, L. filicaulis x L. schoelleri, L. corniculatus var. minor x L. alpinus, and L. corniculatus var. minor x L. filicaulis.

Four of these 16 successful cross combinations L. alpinus x L. japonicus, L. alpinus x L. schoelleri, L. filicaulis x L. schoelleri and L. krylovii x L. tenuis were obtained by recourse to embryo-culture. Pods were dissected 13 to 15 days after pollination and embryos in an advanced state of development were cultured. The hybrids of the remaining 12 crosses, L. japonicus x L. krylovii, L. japonicus x L. filicaulis, L. japonicus x L. schoelleri, L. japonicus x L. corniculatus var. minor, L. japonicus x L. alpinus, L. alpinus x L. krylovii, L. alpinus x L. filicaulis, L. krylovii x L. schoelleri,

L. krylovii x L. filicaulis, L. krylovii x L. corniculatus var. minor, L. corniculatus var. minor x L. alpinus and L. corniculatus var. minor x L. filicaulis were grown directly from seeds. Many of these hybrid combinations were relatively easy to produce as indicated by the comparatively small number of flowers that were emasculated and pollinated (Table 2). In all these successful crosses, except L. krylovii x L. tenuis, both parents were self-compatible; in the case of L. krylovii x L. tenuis the female L. krylovii was self-compatible. Also it can be seen from Table 2 that certain species (e.g. L. japonicus, L. krylovii, L. alpinus and L. corniculatus var. minor) were more satisfactory as pistillate parents than as pollen parents. When used as the female parent, (1) L. japonicus crossed easily with L. alpinus, L. krylovii, L. schoelleri, L. filicaulis and L. corniculatus var. minor; (2) L. krylovii crossed easily with L. schoelleri, L. filicaulis, and L. corniculatus var. minor, and (3) L. alpinus hybridized easily with L. filicaulis, L. krylovii and L. corniculatus var. minor. Also the hybrids L. corniculatus var. minor x L. alpinus and L. corniculatus var. minor x L. filicaulis were produced relatively easily compared with the reciprocal combinations which were extremely difficult to produce. Both L. filicaulis and L. schoelleri were somewhat sensitive to emasculation and did not serve as satisfactory female parents in these crossing experiments.

Failure in obtaining hybrids was particularly noticeable in crosses involving L. pedunculatus, L. borbasii and L. palustris. The latter two species were very sensitive to emasculation and

pollination and no pod set was obtained when flowers of these species were pollinated. In a number of crosses (L. alpinus x L. pedunculatus, L. tenuis x L. krylovii, L. krylovii x L. pedunculatus, L. schoelleri x L. krylovii, L. schoelleri x L. filicaulis, L. corniculatus var. minor x L. palustris, and L. schoelleri x L. borbasii), relatively few flowers were pollinated; in these cases either no pod enlargement was seen or the developed pod withered and fell off very early--2 to 4 days following pollination. Generally, in the unsuccessful crosses, when pod set occurred in response to pollination, the pods withered after 8 to 10 days enlargement. Observations in two hybrid combinations, L. japonicus x L. tenuis and L. alpinus x L. tenuis were, however, somewhat different. In these two crosses, a high percentage of pod set was observed; these pods appeared to develop quite normally but when they were left undisturbed until the time when pods would normally mature, only withered and slightly enlarged ovules were found. All the unsuccessful cross combinations yielded ovules of varying sizes that contained embryos which were retarded in development. In some cases, embryo development had progressed only to the stage of an undifferentiated cell mass; at other times, embryos grew to a spherical or more rarely, a heart-shaped body. Copious endosperm was invariably present in ovules dissected from pods which were 8 to 10 days old. Ovules from older pods (for example, in the crosses L. japonicus x L. tenuis and L. alpinus x L. tenuis) were devoid of endosperm.

TABLE 2. Crosses made between diploid species of Lotus

Crosses	No. of flowers emasculated and pollinated	No. of successful crosses
L. japonicus X L. alpinus	114	8
L. alpinus X L. japonicus	46	2
L. pedunculatus X L. alpinus	163	0
L. alpinus X L. pedunculatus	6	0
L. tenuis X L. alpinus	228	0
L. alpinus X L. tenuis	266	0
L. pedunculatus X L. tenuis	146	0
L. tenuis X L. pedunculatus	127	0
L. krylovii X L. alpinus	29	0
L. alpinus X L. krylovii	56	30
L. japonicus X L. pedunculatus	141	0
L. pedunculatus X L. japonicus	240	0
L. tenuis X L. krylovii	19	0
L. krylovii X L. tenuis	179	1
L. krylovii X L. pedunculatus	6	0
L. pedunculatus X L. krylovii	41	0
L. krylovii X L. schoelleri	19	7
L. schoelleri X L. krylovii	11	0
L. filicaulis X L. schoelleri	7	1
L. schoelleri X L. filicaulis	7	1
L. schoelleri X L. corniculatus var. minor	54	0
L. corniculatus var. minor X L. schoelleri	7	0
L. japonicus X L. tenuis	184	0
L. tenuis X L. japonicus	36	0
L. japonicus X L. krylovii	23	3
L. japonicus X L. filicaulis	14	8
L. japonicus X L. schoelleri	10	4
L. japonicus X L. borbasii	74	0

Table 2 Cont'd

TABLE 2 (Cont'd)

Crosses	No. of flowers emasculated and pollinated	No. of successful crosses
L. japonicus X L. palustris	16	0
L. japonicus X L. corniculatus var. minor	33	10
L. pedunculatus X L. filicaulis	23	0
L. alpinus X L. filicaulis	12	5
L. alpinus X L. schoelleri	29	3
L. alpinus X L. borbasii	111	0
L. krylovii X L. filicaulis	17	7
L. krylovii X L. corniculatus var. minor	68	7
L. krylovii X L. borbasii	181	0
L. krylovii X L. palustris	43	0
L. corniculatus var. minor X L. alpinus	49	21
L. corniculatus var. minor X L. filicaulis	6	3
L. corniculatus var. minor X L. borbasii	166	0
L. corniculatus var. minor X L. tenuis	35	0
L. corniculatus var. minor X L. palustris	8	0
L. schoelleri X L. borbasii	17	0
L. schoelleri X L. tenuis	66	0

TABLE 3. Interspecific diploid hybrids produced

Crosses	Cross number	No. of plants obtained
L. japonicus X *L. krylovii*	JK-1	9
	JK-4	6
L. japonicus X *L. filicaulis*	JF-4	6
	JF-7	2
L. japonicus X *L. schoelleri*	JS-1	1
	JS-2	1
	JS-3	1
	JS-4	1
L. japonicus X *L. corniculatus* var. *minor*	JM-1	12
	JM-6	3
L. japonicus X *L. alpinus*	JA-1	1
	JA-2	1
	JA-4	1
	JA-5	15
L. alpinus X *L. japonicus*	AJ-1	1
	AJ-2	1
L. alpinus X *L. krylovii*	AK-13	2
	AK-16	7
	AK-17	4
	AK-18	5
	AK-20	7
L. alpinus X *L. filicaulis*	AF-1	1
	AF-4	1
	AF-5	6
L. alpinus X *L. schoelleri*	AS-1	2
L. krylovii X *L. schoelleri*	KS-1	1
	KS-4	1

Table 3 Cont'd

TABLE 3 (Cont'd)

Crosses	Cross number	No. of plants obtained
L. krylovii X L. filicaulis	KF-1	3
	KF-2	1
L. krylovii X L. corniculatus var. minor	KM-1	1
	KM-2	1
	KM-3	1
	KM-5	1
L. krylovii X L. tenuis	KT-1	1
L. filicaulis X L. schoelleri	FS-1	2
L. corniculatus var. minor X L. alpinus	MA-11	9
	MA-12	9
L. corniculatus var. minor X L. filicaulis	MF-2	2
	MF-3	12

As a result of this hybridization study, it is possible to categorize Lotus crosses into three groups: one group includes all those crosses in which hybrids can be grown directly from seeds; another class is made up of those crosses that can be obtained by the existing embryo-culture techniques, and a third category includes those crosses which are extremely difficult or impossible to produce.

II. Induction of autotetraploids and amphidiploids

A total of 208 seedlings of L. alpinus, L. japonicus, L. filicaulis, L. schoelleri, L. krylovii and L. corniculatus var. minor were subjected to colchicine treatment in order to induce autotetraploids of these species. The results of the treatments are shown in Table 4. One hundred and eighteen seedlings survived the treatment and from these, 15 autotetraploids were subsequently obtained. These autotetraploids were characterized by slower growth, fleshier leaves, larger flowers and late flowering (3-4 weeks later) than their diploid counterparts.

For the production of amphidiploids, 313 seedlings of 14 different interspecific diploid hybrids were treated with colchicine solution. The surviving 119 plants were scored for polyploidy and 40 amphidiploids from 12 different interspecific hybrids were secured (Table 5). These amphidiploids like the induced autotetraploids exhibited a slower growth, fleshier leaves, larger flowers and later flowering when they were compared with untreated diploid hybrids. Three amphi-

TABLE 4. Production of autotetraploids by colchicine treatment

Diploid species	No. of seedlings treated	Survival	No. of autotetraploids obtained	Accession numbers of induced autotetraploids
L. alpinus	40	25	2	T-77-1; T-77-2
L. japonicus	40	26	2	T-129-1; T-129-2
L. filicaulis	18	16	4	T-37-1; T-37-2; T-37-3; T-37-4
L. schoelleri	58	25	2	T-166-1; T-166-2
L. krylovii	22	12	2	T-86-1; T-86-2
L. corniculatus var. *minor*	30	14	2	T-303-1; T-303-2

TABLE 5. Production of amphidiploids by colchicine treatment

Interspecific diploid hybrids	No. of seedlings treated	Survival	No. of amphi-diploids obtained	Accession numbers of induced amphidiploids
L. japonicus X krylovii	12	4	1	A/JK-1
L. japonicus X schoelleri	12	12	4	A/JS-1; A/JS-2; A/JS-3; A/JS-4
L. japonicus X corniculatus var. minor	20	4	3	A/JM-2-1; A/JM-2-2; A/JM-4-1
L. japonicus X alpinus	23	5	3	A/JA-1; A/JA-2; A/JA-10
L. alpinus X krylovii	16	9	2	A/AK-2; AK-7
L. alpinus X filicaulis	75	20	7	A/AF-1; A/AF-2; A/AF-3; A/AF-4; A/AF-5; A/AF-6; A/AF-7
L. alpinus X schoelleri	35	13	3	A/AS-1; A/AS-2; A/AS-3
L. krylovii X schoelleri	15	5	2	A/KS-1; A/KS-7
L. krylovii x filicaulis	9	7	2	A/KF-2-5; A/KF-2-7
L. krylovii X corniculatus var. minor	25	3	0	-

Table 5 Cont'd

TABLE 5 (Cont'd)

Interspecific diploid hybrids	No. of seedlings treated	Survival	No. of amphi-diploids obtained	Accession numbers of induced amphidiploids
L. filicaulis X schoelleri	25	15	6	A/FS-1; A/FS-2 A/FS-3; A/FS-4; A/FS-5; A/FS-6
L. corniculatus var. minor X alpinus	13	3	2	A/MA-3-1; A/MA-3-2
L. corniculatus var. minor X filicaulis	27	14	5	A/MF-2-1; A/MF-2-2; A/MF-2-3; A/MF-2-4; A/MF-2-5; A/MF-2-6

diploids (L. alpinus x L. schoelleri)*, (L. alpinus x L. filicaulis) and (L. filicaulis x L. schoelleri) were obtained by treating F_1 seeds. The interspecific hybrids L. alpinus x L. schoelleri and L. filicaulis x L. schoelleri were produced by embryo-culture technique and the limited hybrid seeds obtained from the cross L. alpinus x L. filicaulis were reserved for growing diploid hybrids. The remaining amphidiploids (L. japonicus x L. krylovii), (L. japonicus x L. schoelleri), (L. japonicus x L. corniculatus var. minor), (L. alpinus x L. krylovii), (L. japonicus x L. alpinus), (L. krylovii x L. schoelleri), (L. krylovii x L. filicaulis), (L. corniculatus var. minor x L. alpinus) and (L. corniculatus var. minor x L. filicaulis) were produced by treating hybrid seeds obtained following hand pollination.

No amphidiploids were obtained from three interspecific hybrids, L. krylovii x L. corniculatus var. minor, L. japonicus x L. filicaulis and L. krylovii x L. tenuis. Only a small amount of hybrid seeds from the crosses L. krylovii x L. corniculatus var. minor and L. japonicus x L. filicaulis were available for colchicine treatment; the hybrid L. krylovii x L. tenuis was produced by means of embryo culture. F_1 seeds from these three crosses were not available at the time the colchicine treatments were carried out.

III. Crossing relationships between the synthetic amphidiploids and Lotus corniculatus

The results of crosses attempted between the synthetic amphi-

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* Amphidiploids are being designated by placing the parental combinations in brackets to distinguish them from F_1 hybrids (without brackets).

diploids and L. corniculatus are presented in Table 6. Twenty-two different cross combinations were attempted and a total of 236 plants were obtained from 15 different combinations. The successful crosses, their accession numbers and the number of plants grown to maturity for each cross are given in Table 7. Embryo culture was not a prerequisite for making these crosses. Twelve hybrid combinations were attempted using the synthetic amphidiploids as female parents and 11 of these were successful. These include (L. alpinus x L. schoelleri) x L. corniculatus, (L. alpinus x L. filicaulis) x L. corniculatus, (L. japonicus x L. alpinus) x L. corniculatus, (L. corniculatus var. minor x L. alpinus) x L. corniculatus, (L. corniculatus var. minor x L. filicaulis) x L. corniculatus, (L. japonicus x L. corniculatus var. minor) x L. corniculatus, (L. filicaulis x L. schoelleri) x L. corniculatus, (L. japonicus x L. schoelleri) x L. corniculatus, (L. japonicus x L. krylovii) x L. corniculatus, (L. krylovii x L. filicaulis) x L. corniculatus and (L. krylovii x L. schoelleri) x L. corniculatus. No successful cross was obtained between (L. alpinus x L. krylovii) x L. corniculatus. The highest percentages of successful crosses (85.15%) were realized in the (L. japonicus x L. alpinus) x L. corniculatus combination followed by 58.62% and 27.65% for (L. corniculatus var. minor x L. alpinus) x L. corniculatus and (L. alpinus x L. filicaulis) x L. corniculatus crosses, respectively. The percentage of successful crosses ranged from 85.15 for (L. japonicus x L. alpinus) x L. corniculatus to 3.57 for the L. corniculatus x (L. japonicus x L. krylovii) cross. It is

interesting to note that in these crosses mentioned above, the number of successful crosses was relatively higher when either L. japonicus or L. alpinus constituted one of the parents of the original interspecific hybrid that was doubled. It can be seen from Table 6, that, except for (L. corniculatus var. minor x L. filicaulis) x L. corniculatus with 25 per cent successful crosses, all the other crosses with values greater than 20 per cent involved either L. japonicus or L. alpinus or both of these species.

Considerable difficulty was experienced when L. corniculatus was utilized as the female parent in this crossing program. Early withering and shedding of the pods (3-5 days following pollination) was a reliable indication that L. corniculatus would not serve as the seed parent. Ten crosses were attempted with L. corniculatus as the pistillate parent (Table 6), and of these only L. corniculatus x (L. japonicus x L. alpinus), L. corniculatus x (L. japonicus x L. schoelleri), L. corniculatus x (L. japonicus x L. krylovii) and L. corniculatus x (L. krylovii x L. filicaulis) were successful. Except for L. corniculatus x (L. japonicus x L. schoelleri), the percentages of successful crosses recorded for the other three combinations were low when these values were compared with those obtained in the reciprocal crosses. For example, 12.5 per cent success was noted for the cross L. corniculatus x (L. japonicus x L. alpinus), whereas 85.15 per cent was obtained for the reciprocal cross; for L. corniculatus x (L. japonicus x L. krylovii) 3.57 per cent was obtained, whereas 20.00 per cent was recorded for its

TABLE 6. Crosses attempted between the synthetic amphidiploids and L. corniculatus

Cross combinations	No. of flowers emasculated and pollinated	Successful crosses	
		No.	%
(L. alpinus X L. schoelleri) X L. corniculatus	27	6	22.22
L. corniculatus X (L. alpinus X L. schoelleri)	15	0	0.00
(L. alpinus X L. filicaulis) X L. corniculatus	47	13	27.65
(L. alpinus X L. krylovii) X L. corniculatus	30	0	0.00
(L. japonicus X L. alpinus) X L. corniculatus	27	23	85.15
L. corniculatus X (L. japonicus X L. alpinus)	32	4	12.50
(L. corniculatus var. minor X L. alpinus) X L. corniculatus	29	17	58.62
L. corniculatus X (L. corniculatus var. minor X L. alpinus)	21	0	0.00
(L. corniculatus var. minor X L. filicaulis) X L. corniculatus	68	17	25.00
L. corniculatus X (L. corniculatus var. minor X L. filicaulis)	11	0	0.00
(L. japonicus X L. corniculatus var. minor) X L. corniculatus	13	3	23.07
L. corniculatus X (L. japonicus X L. corniculatus var. minor)	17	0	0.00

Table 6 Cont'd

TABLE 6 (Cont'd)

Cross combinations	No. of flowers emasculated and pollinated	Successful crosses	
		No.	%
(*L. filicaulis* X *L. schoelleri*) X *L. corniculatus*	32	2	6.25
L. corniculatus X (*L. filicaulis* X *L. schoelleri*)	6	0	0.00
(*L. japonicus* X *L. schoelleri*) X *L. corniculatus*	54	4	7.40
L. corniculatus X (*L. japonicus* X *L. schoelleri*)	43	3	6.97
(*L. japonicus* X *L. krylovii*) X *L. corniculatus*	75	15	20.00
L. corniculatus X (*L. japonicus* X *L. krylovii*)	56	2	3.57
(*L. krylovii* X *L. filicaulis*) X *L. corniculatus*	49	9	18.36
L. corniculatus X (*L. krylovii* X *L. filicaulis*)	15	1	6.66
(*L. krylovii* X *L. schoelleri*) X *L. corniculatus*	111	9	8.10
L. corniculatus X (*L. krylovii* X *L. schoelleri*)	23	0	0.00

TABLE 7. Successful crosses between the synthetic amphidiploids and *L. corniculatus*

Successful crosses		Accession number	No. of plants obtained
(*L. alpinus* X *L. schoelleri*) X *L. corniculatus*	A/AS-2 X C	AS-C-1	2
(*L. alpinus* X *L. filicaulis*) X *L. corniculatus*	A/AF-2 X C	AF-C-1	3
	A/AF-3 X C	AF-C-2	6
	A/AF-4 X C	AF-C-3	4
	A/AF-7 X C	AF-C-4	4
(*L. japonicus* X *L. alpinus*) X *L. corniculatus*	A/JA-2 X C	JA-C-1	15
	A/JA-10 X C	JA-C-2	21
L. corniculatus X (*L. japonicus* X *L. alpinus*)	C X A/JA-10	C-JA-1	9
(*L. corniculatus* var. *minor* X *L. alpinus*) X *L. corniculatus*	A/MA-3-1 X C	MA-C-1	22
	A/MA-3-2 X C	MA-C-2	21
(*L. corniculatus* var. *minor* X *L. filicaulis*) X *L. corniculatus*	A/MF-2-2 X C	MF-C-1	14
	A/MF-2-3 X C	MF-C-2	17
	A/MF-2-6 X C	MF-C-3	13
(*L. japonicus* X *L. corniculatus* var. *minor*) X *L. corniculatus*	A/JM-2-2 X C	JM-C-1	6
	A/JM-4-1 X C	JM-C-2	3
(*L. filicaulis* X *L. schoelleri*) X *L. corniculatus*	A/FS-2 X C	FS-C-1	5
(*L. japonicus* X *L. schoelleri*) X *L. corniculatus*	A/JS-3-7 X C	JS-C-1	3
	A/JS-3-6 X C	JS-C-2	3
L. corniculatus X (*L. japonicus* X *L. schoelleri*)	C X A/JS-3-7	C-JS-1	2
(*L. japonicus* X *L. krylovii*) X *L. corniculatus*	A/JK-1 X C	JK-C-1	13
L. corniculatus X (*L. japonicus* X *L. krylovii*)	C X A/JK-1	C-JK-1	4

Table 7 Cont'd

TABLE 7 (Cont'd)

Cross combinations		Accession number	No. of plants obtained
(*L. krylovii* X *L. filicaulis*) X *L. corniculatus*	A/KF-2-5 X C	KF-C-1	19
	A/KF-2-7 X C	KF-C-2	18
L. corniculatus X (*L. krylovii* X *L. filicaulis*)	C X A/KF-2-7	C-KF-1	1
(*L. krylovii* X *L. schoelleri*) X *L. corniculatus*	A/KS-1 X C	KS-C-1	10
	A/KS-7 X C	KS-C-2	2

reciprocal combination; in the L. corniculatus x (L. krylovii x L. filicaulis) and (L. krylovii x L. filicaulis) x L. corniculatus crosses, the percentages of successful crosses were 6.66 and 18.36, respectively. Successful crosses between L. corniculatus and (L. japonicus x L. schoelleri) were approximately 7 per cent, regardless in which direction the cross was performed. In those crosses in which L. corniculatus was used as the seed parent, the greatest success was achieved in the combination L. corniculatus x (L. japonicus x L. alpinus).

The crossability data derived from these crossing experiments indicate a close relationship between the synthetic amphidiploid (L. japonicus x L. alpinus) and L. corniculatus.

IV. Morphological studies

Morphology of Lotus corniculatus var. minor

Fifteen phenotypic traits were studied in order to compare L. corniculatus var. minor with the six other diploid species used in this study. The results of the t-tests on the morphological data are set out in Table 8. An examination of these results indicates that this taxon has distinct morphological attributes. L. corniculatus var. minor differs from L. japonicus in 12 of the quantitative traits studied, from L. alpinus and L. tenuis in 10, from L. schoelleri in 9 and from L. krylovii and L. filicaulis in 8 traits. However, a certain amount of morphological resemblance to each of these species is reflected in a few to several of the phenotypic characteristics

TABLE 8. A comparison of phenotypic traits between L. corniculatus var. minor and six other diploid species employing a t-test

Phenotypic traits	L. corniculatus var. minor versus					
	L. japonicus	L. alpinus	L. krylovii	L. filicaulis	L. schoelleri	L. tenuis
Central leaflet length	6.98**	10.57**	5.04**	5.33**	6.67**	0.42
Central leaflet width	12.16**	2.94**	0.63	8.79**	12.92**	6.11**
Central leaflet index	21.38**	12.67**	6.44**	6.79**	18.99**	6.82**
Floral bract length	1.32	0.32	0.77	4.80**	0.40	0.67
Floral bract width	10.07**	3.90**	3.55**	1.51	7.09**	0.00
Floral bract index	12.63**	4.29**	5.17**	4.19**	7.35**	1.67
Standard length	16.55**	4.26**	0.38	4.41**	1.40	14.20**
Standard width	11.00**	9.20**	2.64*	5.20**	5.18**	10.57**
Standard index	1.00	4.55**	2.94**	3.40**	7.31**	3.08**
Ovary length	7.08*	1.22	0.45	0.70	6.63**	2.89**
Style length	20.03**	1.00	0.84	1.41	1.00	19.80**
Style/ovary index	4.55**	1.81	0.80	1.65	7.58**	3.64**
Calyx length	4.77**	8.47**	3.14**	1.77	0.32	8.47**
Calyx tube length	1.03	1.62	2.38*	1.37	1.37	2.03
Calyx index	2.84**	2.59*	0.02	0.11	1.65	2.46*

* Significant ($P < .05$)

** Highly significant ($P < .01$)

investigated. For example, L. corniculatus var. minor bears a close morphological relationship to L. filicaulis, which it resembles in floral bract width, ovary length, style length, style/ovary index, calyx, calyx tube length and calyx index. On the basis of its flower color, floret size, floret striping, stem color, and low central leaflet index, L. corniculatus var. minor would appear to belong to a group comprising L. krylovii, L. filicaulis, L. schoelleri, and L. tenuis. It can, however, be easily separated from these taxa by its decumbent growth habit and somewhat restricted branching and overall size. L. corniculatus var. minor does not flower profusely as do the other species; it produces solitary flowers and is also characterized by the production of a relatively large number of pods regardless whether these plants are grown in a growth chamber, greenhouse, or in the field.

Morphology of the interspecific hybrids

Morphological studies were conducted and a comparison was made between nine F_1 hybrids and their respective parental species. The hybrid L. filicaulis x L. schoelleri was not included in these studies since only a limited number of plants was available at the time when phenotypic observations were made. In general, the interspecific hybrids resembled more closely one or the other parent as far as some morphological characters were concerned, but were intermediate for other characters (Tables 9, 10, 11). Many of the hybrids exhibited considerable heterosis which was reflected in the vigorous

growth, profuse branching and flowering, and in certain other morphological traits in which measurements exceeded those of either parent. The following is a brief description of these interspecific diploid hybrids based on visual observations of qualitative characters and on measurements of quantitative ones.

Lotus alpinus x L. japonicus

This hybrid exhibited a growth habit similar to both its parents and showed vigorous growth and branching. The color expressions of the flower buds and flowers were similar to those of both parents and the number of florets varied from 1-4. This hybrid reacted positively to the HCN reaction as did both its parents (Table 9). Heterosis was manifested in seven of the morphological characters studied namely, central leaflet length, central leaflet width, floral bract length, floral bract width, standard width, calyx tube length and ovary length (Tables 10, 11). As for central leaflet index, floral bract index, standard length, standard index, calyx length, calyx index, style length and style/ovary index, measurements for these characters were intermediate to those of their parents. In general, this hybrid, L. alpinus x L. japonicus, resembled L. japonicus more closely than L. alpinus.

Lotus japonicus x L. corniculatus var. minor

The 12 hybrids obtained in this cross were intermediate in growth habit to either parent. The color characteristics of the flowers were very similar to those of the male parent; however, the

HCN reaction was positive like that of the female parent (Table 9). The hybrids appeared to resemble L. japonicus more closely than L. corniculatus var. minor. This was confirmed by measurements made on morphological traits (Tables 10, 11). Except for the length of the floral bract which was greater than those of either parent, the hybrids were intermediate in all the characters studied.

Lotus corniculatus var. minor x L. alpinus

The hybrids of this cross combination had flower buds and flowers similar in color to those of the female parent. Except for this character, they exhibited many features of the male parent, L. alpinus. The growth habit was decumbent, and the keel tip of the flowers was dark reddish-brown (Table 9). Further resemblance to the male parent was exhibited in the central leaflet width, central leaflet index, floral bract length and width, standard length and width, standard index, and the lengths of the calyx tube, ovary and style. The hybrids were polymorphic for the presence of HCN. They were quite vigorous and showed heterosis in central leaflet width, and in the lengths of the floral bract, standard, ovary and style (Tables 10, 11).

Lotus alpinus x L. schoelleri

Plants derived from the cross L. alpinus x L. schoelleri exhibited ascending growth habit like that of the male parent, L. schoelleri. However, their general outward appearance more closely resembled L. alpinus. The flowers were deep yellow with prominent red streaking

and had reddish-brown keel tips (Table 9). A study of the quantitative traits showed that the floral bract, standard length and width, calyx tube length, style length and ovary length were very similar to those of L. alpinus (Tables 10, 11). The hybrids were characterized by vigorous growth and considerable branching. Heterosis was evident in the standard length and width and in the lengths of the style and ovary. HCN reaction was positive as was the female parent, L. alpinus.

Lotus alpinus x L. filicaulis

These hybrids had a growth habit intermediate to that of both parents. Apart from the color expression of the flower buds, they showed little resemblance to the male parent, L. filicaulis. The flowers were deep yellow with dark reddish-brown keel tips, similar to those of L. alpinus (Table 9). Also the statistical tests indicated their morphological similarity to the seed parent in floral bract width, standard length and width and ovary length. A certain amount of heterotic effects was reflected in the lengths of the standard, style, and ovary (Table 11). HCN reaction was positive, the same as the female parent.

Lotus corniculatus var. minor x L. filicaulis

Hybrids obtained from the cross L. corniculatus var. minor x L. filicaulis exhibited a growth habit which was intermediate to that of both parents, namely, decumbent. Visually, it was difficult to differentiate the flowers of this cross, from those of the

parental species since the color characteristics of the flower buds and the flowers on the whole, were not appreciably different from those of either parent (Table 9). In general appearance, the hybrids resembled L. filicaulis more closely than L. corniculatus var. minor; this was confirmed by measurements of floral bract length and width, and the standard width and calyx tube length which statistically were similar to the corresponding traits for L. filicaulis. Even in the phenotypic traits which were intermediate, such as the lengths of the central leaflet, the standard, the ovary and the style, the differences between the means for these characters of the hybrids and those of L. filicaulis were smaller than the differences between the means for those of the hybrids and those of L. corniculatus var. minor (Tables 10, 11). These hybrids gave a negative HCN reaction like both parents.

Lotus alpinus x L. krylovii

Plants with this hybrid combination had a decumbent growth habit and deep yellow flowers with dark reddish-brown keel tips like those of L. alpinus. The flower bud color, however, was rather similar to that of the pollen parent L. krylovii (Table 9). The hybrids were intermediate to either parents in a number of other characters. These include floral bract width, floral bract index, standard width, standard index, calyx length, calyx tube length, calyx index, style length and style/ovary index. Vigorous growth and profuse branching typified these plants and this increased vigor was further manifested in heterosis

as indicated by data from measurements on the central leaflet (length and width), floral bract length, standard length, and ovary length. Although in general appearance, these hybrids seemed to be intermediate between the two parental species, statistical tests indicated that there was a closer resemblance to L. alpinus (Tables 10, 11). The plants were polymorphic for the presence of HCN.

Lotus krylovii x L. corniculatus var. minor

The decumbent growth habit of these hybrids was intermediate to the procumbent type of L. corniculatus var. minor and the ascending type of L. krylovii. The color expression of the flower buds and the flowers of these hybrids and their parents were very similar and therefore difficult to separate from one another (Table 9). The hybrids, however, were intermediate in general appearance to both parents and this was confirmed by the metrical characters studied (Tables 10, 11). Increased growth of the hybrids, as compared with the parental species, was indicated by data from measurements made on the central leaflet width, floral bract length, standard length and width and the lengths of the calyx and the ovary. Statistically, the hybrids resembled the female parent, L. krylovii in central leaflet length, central leaflet index, floral bract width and calyx length, whereas they resembled the male parent, L. corniculatus var. minor, in floral bract index and standard index. The plants gave a negative HCN reaction like both parents.

Lotus krylovii x L. tenuis

The single hybrid obtained in this cross had an ascending growth habit like both its parents, L. krylovii and L. tenuis. It bore flower buds and flowers that were similar to those of its male parent. The flower buds were yellow with stripes whereas the flowers were deep yellow and exhibited prominent red striping (Table 9). In quantitative characters, the hybrid was intermediate to either parent in length and width of the central leaflet, the length and width of the standard, the standard index, calyx index, the lengths of the style and the ovary and in the style/ovary index. An increase in measurements to those of either parent was noted for central leaflet index, the length and width of the bract, the bract index, the calyx index and the calyx tube length (Tables 10, 11). The hybrid appeared to resemble L. tenuis more closely than L. krylovii; this was confirmed by statistical tests which indicated morphological similarity to L. tenuis in six traits namely, central leaflet width, floral bract length, the length and the width of the standard, standard index and the length of the style. A positive HCN reaction was observed for this hybrid.

Comparative morphological studies of the induced autotetraploids and Lotus corniculatus

The autotetraploids produced by means of colchicine treatment were characterized by slower growth, fleshier and larger leaves that were dark green, thicker stems, larger flowers and later flowering,

TABLE 9. Some morphological characteristics of the diploid species and their F_1 hybrids

Species and hybrids (2x)	Growth habit	Florets per umbel	Bud color	Flower color	Red streaking on flower	Keel tip color	HCN reaction
L. japonicus	decumbent	1 - 4	yellow, faint pink stripes	deep yellow	very faint	yellow	+
L. alpinus	decumbent	1 - 3	yellow, faint pink stripes	deep yellow	medium prominent	dark reddish brown	+
L. alpinus X japonicus	decumbent	1 - 4	yellow	deep yellow	medium	dark reddish brown	+
L. corniculatus var. minor	procumbent	1	yellow, pink stripes	very pale yellow	medium prominent	yellow	-
L. japonicus X corniculatus var. minor	procumbent-decumbent	1 - 2	yellow, orange-red stripes	pale yellow	medium prominent	yellow	+
L. corniculatus var. minor X alpinus	decumbent	1 - 3	yellow, pink stripes	pale yellow	prominent	dark reddish brown	+, -
L. schoelleri	ascending	1 - 3	yellow, red stripes	pale yellow	prominent	yellow	-
L. alpinus X schoelleri	ascending	1 - 3	yellow, red stripes	deep yellow	prominent	dark reddish brown	+
L. filicaulis	ascending	1 - 3	yellow, red stripes	pale yellow	prominent	yellow	-
L. alpinus X filicaulis	ascending-decumbent	1 - 3	yellow, orange-red stripes	deep yellow	prominent	dark reddish brown	+

Table 9 Cont'd

TABLE 9 (Cont'd)

Species and hybrids (2x)	Growth habit	Florets per umbel	Bud color	Flower color	Red streaking on flower	Keel tip color	HCN reaction
L. corniculatus var. minor X filicaulis	decumbent	1 - 3	yellow, red stripes	pale yellow	prominent	yellow	-
L. krylovii	ascending	1 - 3	yellow, red stripes	pale yellow	prominent	yellow	+, -
L. alpinus X krylovii	decumbent	1 - 3	yellow, orange-red stripes	deep yellow	prominent	dark reddish brown	+, -
L. krylovii X corniculatus var. minor	decumbent	1 - 2	yellow, pink stripes	pale yellow	prominent	yellow	-
L. tenuis	ascending	2 - 6	yellow, red stripes	deep yellow	medium prominent	yellow	+
L. krylovii X tenuis	ascending	2 - 4	yellow, red stripes	deep yellow	medium prominent	yellow	+

TABLE 10. Data on measurements of the central leaflet and floral bract for the parental species and hybrids. The mean values and standard errors are shown.

Species and hybrids	Central leaflet			Floral bract		
	length (mm)	width (mm)	index	length (mm)	width (mm)	index
L. japonicus	9.5 ± 0.2	5.4 ± 0.2	0.57 ± 0.01	7.1 ± 0.3	3.6 ± 0.2	0.52 ± 0.01
L. alpinus	8.1 ± 0.2	3.5 ± 0.1	0.43 ± 0.01	6.6 ± 0.2	2.5 ± 0.1	0.37 ± 0.01
L. japonicus X alpinus	11.4 ± 0.3	5.3 ± 0.2	0.46 ± 0.01	9.8 ± 0.3	3.5 ± 0.1	0.36 ± 0.01
L. alpinus X japonicus	11.6 ± 0.4[b]	5.5 ± 0.2[f]	0.48 ± 0.01[b]	10.9 ± 0.5[b]	4.3 ± 0.2[b]	0.40 ± 0.01[m]
L. corniculatus var. minor	11.8 ± 0.3	3.0 ± 0.1	0.26 ± 0.01	6.5 ± 0.3	1.9 ± 0.1	0.30 ± 0.01
L. japonicus X corniculatus var. minor	10.7 ± 0.3[b]	4.3 ± 0.1[b]	0.41 ± 0.01[b]	9.2 ± 0.4[b]	3.2 ± 0.2[m]	0.34 ± 0.01[b]
L. corniculatus var. minor X alpinus	10.0 ± 0.3[b]	3.8 ± 0.1[f]	0.38 ± 0.01[b]	8.6 ± 0.4[b]	2.5 ± 0.2[f]	0.29 ± 0.01[m]
L. schoelleri	9.6 ± 0.2	5.0 ± 0.1	0.52 ± 0.01	6.6 ± 0.2	3.2 ± 0.2	0.48 ± 0.02
L. alpinus X schoelleri	8.2 ± 0.2[m]	3.7 ± 0.1[m]	0.45 ± 0.01[m]	6.1 ± 0.3	2.4 ± 0.1[m]	0.40 ± 0.02[m]
L. filicaulis	13.6 ± 0.2	4.5 ± 0.1	0.33 ± 0.01	8.8 ± 0.3	2.1 ± 0.1	0.24 ± 0.01

Table 10 Cont'd

TABLE 10 (Cont'd)

Species and hybrids	Central leaflet			Floral bract		
	length (mm)	width (mm)	index	length (mm)	width (mm)	index
L. alpinus X *filicaulis*	10.7 ± 0.2^{b}	4.1 ± 0.1^{b}	0.38 ± 0.01^{b}	7.4 ± 0.1^{b}	2.5 ± 0.5^{m}	0.33 ± 0.01^{b}
L. corniculatus var. *minor* X *filicaulis*	10.9 ± 0.3^{b}	3.3 ± 0.1^{m}	0.30 ± 0.01^{b}	8.3 ± 0.5^{f}	2.3 ± 0.1^{f}	0.28 ± 0.01^{m}
L. krylovii	9.6 ± 0.3	3.1 ± 0.1	0.33 ± 0.01	6.1 ± 0.3	2.3 ± 0.1	0.38 ± 0.01
L. alpinus X *krylovii*	12.0 ± 0.2^{b}	5.4 ± 0.1^{b}	0.45 ± 0.01^{m}	6.3 ± 0.2	2.3 ± 0.1	0.36 ± 0.01
L. krylovii X *corniculatus* var. *minor*	10.0 ± 0.2^{m}	3.3 ± 0.1	0.33 ± 0.01^{m}	6.8 ± 0.3	2.3 ± 0.1^{m}	0.33 ± 0.02^{f}
L. tenuis	12.0 ± 0.3	4.3 ± 0.2	0.36 ± 0.01	6.8 ± 0.4	1.9 ± 0.1	0.27 ± 0.01
L. krylovii X *tenuis*	8.8 ± 0.1^{b}	4.1 ± 0.1^{f}	0.47 ± 0.01^{b}	7.8 ± 0.4^{f}	4.0 ± 0.1^{b}	0.54 ± 0.03^{b}

^{b} significantly different from both parents
^{f} significantly different from female parent
^{m} significantly different from male parent

Significance calculated at 5% level of probability

TABLE 11. Data on measurements of the standard, ovary, style and calyx for the parental species and hybrids. The mean values and standard errors are shown.

Species and hybrids	Standard			Calyx			ovary length (mm)	style length (mm)	style/ ovary index
	length (mm)	width (mm)	index	total length (mm)	tube length (mm)	index			
L. japonicus	12.4 ±0.2	8.8 ±0.1	0.71 ±0.01	7.1 ±0.2	3.5 ±0.1	0.49 ±0.01	6.9 ±0.1	5.8 ±0.1	0.84 ±0.01
L. alpinus	9.7 ±0.2	8.1 ±0.0	0.83 ±0.02	5.0 ±0.0	3.1 ±0.1	0.61 ±0.01	5.8 ±0.1	4.0 ±0.0	0.70 ±0.01
L. japonicus X alpinus	11.9 ±0.1	9.7 ±0.1	0.81 ±0.01	6.8 ±0.1	3.3 ±0.1	0.48 ±0.01	7.0 ±0.1	5.0 ±0.0	0.72 ±0.01
L. alpinus X japonicus	12.2^{f} ±0.2	9.4^{b} ±0.1	0.77^{b} ±0.01	6.8^{f} ±0.2	3.6^{f} ±0.1	0.53^{b} ±0.01	7.3^{b} ±0.1	5.0^{b} ±0.1	0.68^{m} ±0.02
L. corniculatus var. minor	8.7 ±0.1	6.4 ±0.2	0.73 ±0.02	6.0 ±0.1	3.3 ±0.1	0.55 ±0.02	5.5 ±0.2	4.0 ±0.0	0.74 ±0.02
L. japonicus X corniculatus var. minor	11.7^{b} ±0.2	8.3^{m} ±0.2	0.71 ±0.01	7.0^{m} ±0.1	3.2 ±0.1	0.46^{m} ±0.01	6.9^{m} ±0.2	5.2^{b} ±0.1	0.76^{f} ±0.02
L. corniculatus var. minor X alpinus	9.8^{f} ±0.1	8.1^{f} ±0.2	0.83^{f} ±0.01	6.0^{m} ±0.1	3.1 ±0.1	0.53^{m} ±0.01	6.3^{b} ±0.1	4.9^{b} ±0.1	0.78^{m} ±0.02
L. schoelleri	8.4 ±0.2	7.5 ±0.1	0.89 ±0.01	6.0 ±0.0	3.1 ±0.1	0.52 ±0.01	4.3 ±0.1	4.0 ±0.0	0.94 ±0.02
L. alpinus X schoelleri	10.0^{m} ±0.1	8.7^{b} ±0.1	0.87 ±0.01	6.0^{f} ±0.1	3.1 ±0.1	0.51^{f} ±0.01	5.9^{m} ±0.0	4.1 ±0.0	0.69^{m} ±0.01

Table 11 Cont'd

TABLE 11 (Cont'd)

Species and hybrids	Standard			Calyx			ovary length (mm)	style length (mm)	style/ ovary index
	length (mm)	width (mm)	index	total length (mm)	tube length (mm)	index			
L. filicaulis	9.5 ±0.1	7.6 ±0.2	0.80 ±0.01	5.7 ±0.1	3.1 ±0.1	0.55 ±0.02	5.7 ±0.1	4.0 ±0.0	0.70 ±0.01
L. alpinus X filicaulis	10.1^{m} ±0.1	8.1^{m} ±0.1	0.81 ±0.01	5.4^{f} ±0.1	3.0 ±0.0	0.57 ±0.02	5.9^{m} ±0.0	4.3^{b} ±0.1	0.72^{b} ±0.02
L. corniculatus var. minor X filicaulis	10.0^{b} ±0.0	7.9^{f} ±0.1	0.79^{f} ±0.01	6.7^{b} ±0.1	2.9^{f} ±0.0	0.44^{b} ±0.01	5.9^{b} ±0.0	4.2^{b} ±0.1	0.71 ±0.01
L. krylovii	8.6 ±0.1	5.8 ±0.1	0.68 ±0.01	5.4 ±0.1	3.0 ±0.1	0.55 ±0.02	5.4 ±0.1	4.1 ±0.1	0.76 ±0.02
L. alpinus X krylovii	10.0^{m} ±0.1	7.7^{b} ±0.1	0.77^{b} ±0.01	5.1^{m} ±0.0	3.0 ±0.0	0.59^{m} ±0.00	5.9^{m} ±0.1	4.0 ±0.0	0.68^{m} ±0.01
L. krylovii X corniculatus var. minor	9.1^{b} ±0.0	6.9^{b} ±0.1	0.76^{f} ±0.01	6.4^{b} ±0.1	3.0^{m} ±0.1	0.47^{b} ±0.01	5.6 ±0.1	4.0 ±0.0	0.71 ±0.02
L. tenuis	11.1 ±0.1	8.8 ±0.1	0.79 ±0.01	5.0 ±0.0	3.0 ±0.0	0.61 ±0.01	6.1 ±0.1	5.0 ±0.0	0.82 ±0.01
L. krylovii X tenuis	11.0^{f} ±0.1	8.7^{f} ±0.1	0.79^{f} ±0.01	6.7^{b} ±0.1	3.2^{b} ±0.1	0.48^{b} ±0.01	6.3^{b} ±0.1	5.0^{f} ±0.1	0.79 ±0.02

^{b} significantly different from both parents
^{f} significantly different from female parent
^{m} significantly different from male parent

Significance calculated at 5% level of probability

when they were compared with their corresponding diploids under similar environmental conditions. Under field conditions, they showed vigorous growth and considerable branching; however, the degree of branching was not as great as that manifested by the diploids. Visual observations were made on a number of qualitative characters between the induced autotetraploids and L. corniculatus (Table 12). Brief comments on these observations are as follows.

Growth habit

Whereas the growth habit of L. corniculatus was semi-erect, that exhibited by the autotetraploids was either decumbent or ascending. Decumbent growth habit was seen in the autotetraploids L. alpinus, L. krylovii, and L. corniculatus var. minor. In the remaining tetraploids, L. japonicus, L. tenuis, L. filicaulis and L. schoelleri, the ascending type of growth habit was observed.

Number of florets per umbel

Lotus tenuis had 2-6 florets per umbel like L. corniculatus. The other tetraploids had either 1-3 florets per umbel (L. filicaulis, L. schoelleri, L. krylovii and L. corniculatus var. minor) or 2-4 florets per umbel as in the case of L. alpinus and L. japonicus.

Bud color

All the autotetraploids and L. corniculatus produced flower buds which were yellow with characteristic red stripes.

Flower color

Except for L. filicaulis, L. schoelleri, L. krylovii and L. corniculatus var. minor which had pale yellow flowers, the remaining autotetraploids, L. alpinus, L. japonicus and L. tenuis produced deep yellow flowers with prominent red streaking characteristic of L. corniculatus. The keel tip color of the flowers of L. alpinus, however, was dark reddish-brown in contrast to the yellow keel tip found in the other taxa.

Leaf shape

Observations were made on 10 leaves randomly selected from each of the autotetraploids and from L. corniculatus in order to compare their general leaf. A photograph of a representative leaf from each polyploid is shown in Plate II. It can be seen that the general morphology of the leaves of these autotetraploids was distinctly different from that of L. corniculatus.

HCN reaction

A positive HCN reaction was recorded for leaves of all plants of L. alpinus, L. japonicus, L. tenuis, L. krylovii and L. corniculatus. The other polyploids gave a negative reaction.

To further investigate possible morphological resemblances between the autotetraploids and L. corniculatus, 15 quantitative phenotypic traits were measured. The means and standard errors for

TABLE 12. Some morphological characteristics of the induced autotetraploids and *L. corniculatus*

Autotetraploids and *L. corniculatus*	Growth habit	Florets per umbel	Bud color	Flower color	Red streaking on flower	Keel tip color	HCN reaction
L. alpinus	decumbent	2 - 4	yellow, red stripes	deep yellow	prominent	dark reddish brown	+
L. japonicus	ascending	2 - 4	yellow, red stripes	deep yellow	prominent	yellow	+
L. tenuis	ascending	2 - 6	yellow, red stripes	deep yellow	prominent	yellow	+
L. filicaulis	ascending	1 - 3	yellow, red stripes	pale yellow	prominent	yellow	-
L. schoelleri	ascending	1 - 3	yellow, red stripes	pale yellow	prominent	yellow	-
L. krylovii	decumbent	1 - 3	yellow, red stripes	pale yellow	medium prominent	yellow	+
L. corniculatus var. *minor*	decumbent	1 - 3	yellow, red stripes	very pale yellow	prominent	yellow	-
L. corniculatus	semi-erect	2 - 6	yellow, red stripes	deep yellow	prominent	yellow	+

each of these characters were calculated and the results are presented in Tables 13 and 14. From these tables, it may be seen that very small differences were found in the mean values for certain phenotypic traits in L. corniculatus and some of the autotetraploids. A t-test was therefore employed to check the significance of these differences and the results are presented in Table 15. Inspection of this Table shows that each autotetraploid differed significantly from L. corniculatus in 8 to 14 phenotypic traits; L. filicaulis resembled L. corniculatus in standard index and L. schoelleri resembled it in calyx index. In the case of L. krylovii and L. corniculatus var. minor, the standard index and calyx index were very similar to the cultivated autotetraploid. Lotus tenuis and L. japonicus, each exhibited morphological likeness to L. corniculatus in 4 phenotypic characters. These morphological features in L. tenuis similar to L. corniculatus were central leaflet length, standard index, ovary length and calyx index; in L. japonicus, they were central leaflet width, standard length, ovary length and style length. It should be noted that although both L. japonicus and L. tenuis showed significant differences in 11 of the 15 traits studied, in the case of L. tenuis all these traits were significantly different at the 2 per cent level of probability whereas, in L. japonicus, three of these traits were significant at the 5 per cent level and not at the 2 per cent level. This means that L. japonicus has a closer morphological affinity to L. corniculatus than L. tenuis. With regards to L. alpinus, the statistical tests indicate that this autotetraploid

had the closest resemblance to the cultivated tetraploid. This was manifested by its morphological similarity in 7 of the 15 characters investigated. These include central leaflet width, floral bract width, standard length and width, standard index, ovary length and calyx index.

The results presented in Table 15, show that none of the autotetraploids can be considered a synthetic form of the cultivated species. This is further demonstrated by the shapes of the polygons illustrating the morphological relationships of these plants (Plate I). All of the polygons of these autotetraploids differed from the one depicting L. corniculatus.

The statistical tests indicate that the probable phyletic relationship of L. corniculatus to the induced autotetraploids listed in descending degree of affinity is L. alpinus, L. japonicus, L. tenuis, L. krylovii, L. corniculatus var. minor (or the reverse in the case of these two species, that is, L. corniculatus var. minor, L. krylovii), L. filicaulis and L. schoelleri.

Comparative morphological studies of synthetic amphidiploids and L. corniculatus

Twelve amphidiploids were obtained by doubling the chromosome complement of the interspecific diploid hybrids L. alpinus x L. schoelleri, L. alpinus x L. filicaulis, L. alpinus x L. krylovii, L. japonicus x L. alpinus, L. corniculatus var. minor x L. alpinus, L. corniculatus var. minor x L. filicaulis, L. japonicus x L. corniculatus var. minor, L. filicaulis

TABLE 13. Data on measurements of the central leaflet and floral bract for the autotetraploids and *L. corniculatus*. The mean values and standard errors are shown.

Autotetraploids and *L. corniculatus*	Central leaflet			Floral bract		
	length (mm)	width (mm)	index	length (mm)	width (mm)	index
L. alpinus	11.7 ± 0.2	6.0 ± 0.2	0.52 ± 0.01	7.6 ± 0.3	3.5 ± 0.2	0.46 ± 0.02
L. japonicus	11.7 ± 0.2	6.3 ± 0.2	0.55 ± 0.01	6.4 ± 0.2	2.9 ± 0.1	0.46 ± 0.02
L. tenuis	15.1 ± 0.3	5.2 ± 0.2	0.35 ± 0.01	9.9 ± 0.2	2.1 ± 0.1	0.21 ± 0.01
L. filicaulis	10.2 ± 0.2	3.8 ± 0.1	0.37 ± 0.01	6.6 ± 0.4	1.9 ± 0.1	0.29 ± 0.01
L. schoelleri	9.2 ± 0.2	5.1 ± 0.1	0.55 ± 0.02	5.4 ± 0.3	3.1 ± 0.1	0.58 ± 0.03
L. krylovii	10.8 ± 0.2	3.8 ± 0.2	0.35 ± 0.01	5.9 ± 0.4	1.8 ± 0.2	0.30 ± 0.01
L. corniculatus var. *minor*	13.4 ± 0.4	4.0 ± 0.1	0.31 ± 0.01	6.9 ± 0.3	1.6 ± 0.1	0.23 ± 0.01
L. corniculatus	15.0 ± 0.3	6.5 ± 0.2	0.43 ± 0.01	11.1 ± 0.3	3.8 ± 0.2	0.34 ± 0.02

TABLE 14. Data on measurements of the standard, ovary, style, and calyx for the autotetraploids and *L. corniculatus*. The mean values and standard errors are shown.

Autotetraploids and *L. corniculatus*	Standard			Calyx			ovary length (mm)	style length (mm)	style/ ovary index
	length (mm)	width (mm)	index	total length (mm)	tube length (mm)	index			
L. alpinus	12.8 ±0.3	10.0 ±0.2	0.78 ±0.01	6.0 ±0.1	3.0 ±0.0	0.50 ±0.01	7.2 ±0.2	5.5 ±0.2	0.76 ±0.02
L. japonicus	13.3 ±0.1	10.5 ±0.2	0.79 ±0.01	5.9 ±0.3	3.1 ±0.1	0.54 ±0.02	7.1 ±0.1	5.8 ±0.1	0.82 ±0.01
L. tenuis	11.5 ±0.1	8.9 ±0.1	0.77 ±0.01	6.1 ±0.1	3.2 ±0.1	0.52 ±0.01	6.8 ±0.1	5.0 ±0.0	0.74 ±0.01
L. filicaulis	10.0 ±0.1	7.6 ±0.1	0.76 ±0.01	5.3 ±0.1	2.9 ±0.1	0.55 ±0.01	5.9 ±0.1	4.2 ±0.1	0.71 ±0.02
L. schoelleri	8.2 ±0.2	7.5 ±0.1	0.91 ±0.01	6.0 ±0.1	3.0 ±0.1	0.51 ±0.01	4.5 ±0.1	3.5 ±0.1	0.79 ±0.02
L. krylovii	8.8 ±0.1	6.7 ±0.1	0.77 ±0.01	5.3 ±0.1	2.7 ±0.1	0.51 ±0.01	5.7 ±0.1	4.2 ±0.1	0.74 ±0.03
L. corniculatus var. *minor*	9.6 ±0.2	7.6 ±0.2	0.79 ±0.01	6.8 ±0.1	3.2 ±0.1	0.47 ±0.01	5.7 ±0.1	4.5 ±0.1	0.78 ±0.01
L. corniculatus	12.9 ±0.1	9.8 ±0.1	0.76 ±0.01	7.5 ±0.2	3.7 ±0.1	0.50 ±0.01	6.8 ±0.1	6.0 ±0.1	0.88 ±0.02

TABLE 15. A comparison of phenotypic traits between L. corniculatus and the autotetraploids employing a t-test

Phenotypic traits	L. corniculatus vs.						
	L. alpinus	L. japonicus	L. tenuis	L. filicaulis	L. schoelleri	L. krylovii	L. corniculatus var. minor
Central leaflet length	8.60**	8.43**	0.14	12.78**	15.44**	10.34**	3.04**
Central leaflet width	1.95	0.61	5.32**	13.36**	5.92**	11.06**	11.30**
Central leaflet index	5.37**	6.93**	5.86**	5.96**	6.98**	6.09**	8.69**
Floral bract length	7.62**	11.65**	3.02**	9.29**	13.93**	10.80**	10.34**
Floral bract width	1.22	3.34**	6.61**	7.60**	2.89**	7.20**	8.83**
Floral bract index	4.29**	5.19**	6.67**	3.01**	7.81**	2.16**	5.57**
Standard length	0.39	2.03	7.37**	17.45**	23.76**	24.93**	14.31**
Standard width	0.63	3.52**	5.46**	11.01**	12.24**	16.41**	10.59**
Standard index	1.46	2.49*	1.10	0.38	8.79**	0.83	1.96
Ovary length	1.85	1.47	0.47	6.72**	14.00**	6.64**	7.30**
Style length	2.29*	1.19	8.37**	11.23**	16.43**	12.44**	10.21**
Style/ovary index	3.86**	2.22*	5.32**	6.05**	2.66*	3.93**	3.91**
Calyx length	6.72**	4.84**	5.84**	9.21**	5.92**	9.01**	2.91*
Calyx tube length	6.55**	4.64**	3.99**	6.69*	5.46**	7.98**	3.76**
Calyx index	0.74	2.28*	1.75	3.83**	1.00	1.02	1.76

* significant ($P < 0.05$)
** highly significant ($P < 0.01$)

Plate I

Polygonal representations of five morphological indices for the autotetraploids and *Lotus corniculatus*

T 303 *L. corniculatus* var. *minor*

T 166 *L. schoelleri*

T 86 *L. krylovii*

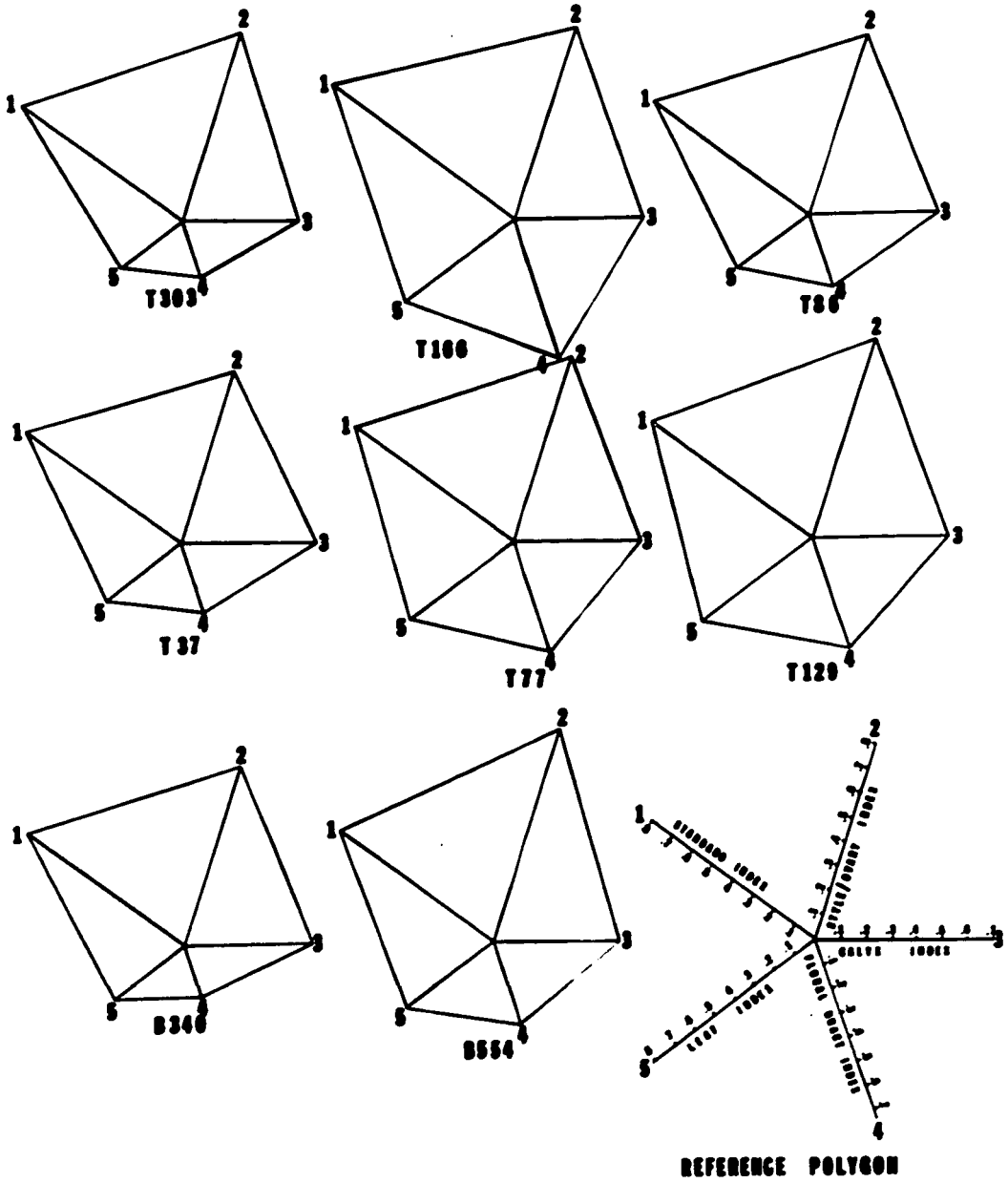
T 37 *L. filicaulis*

T 129 *L. japonicus*

T 77 *L. alpinus*

B 340 *L. tenuis*

B 554 *L. corniculatus*


T303
T166
T86
T37
T77
T129
B340
B554
REFERENCE POLYGON

x L. schoelleri, L. japonicus x L. schoelleri, L. japonicus x L. krylovii, L. krylovii x L. filicaulis and L. krylovii x L. schoelleri. These induced tetraploids were manifestly intermediate between their diploid progenitor species and were quite distinct from one another. They were also slower in development and flowered later (3-4 weeks) than the corresponding diploid interspecific hybrids under similar conditions. Such induced amphiploids possessed thicker stems, fleshier and darker green leaves which were larger. The florets were also larger than those of the diploids, and were borne on more robust pedicels. Under field conditions, all the amphidiploids except (L. alpinus x L. filicaulis) and (L. filicaulis x L. schoelleri) showed vigorous growth and considerable branching. Visual observations were made on a number of qualitative traits in these amphidiploids and the cultivated tetraploid L. corniculatus (Table 16). Brief comments on each of these morphological attributes are given below:

Growth habit

A decumbent growth habit was observed for all the amphidiploids except in (L. alpinus x L. schoelleri), (L. krylovii x L. filicaulis) and (L. krylovii x L. schoelleri) which had an ascending type of growth habit. In contrast, L. corniculatus was semi-erect in its growth habit.

Number of florets per umbel

Except for (L. japonicus x L. alpinus) and (L. japonicus x L. krylovii) which had 1-4 florets per umbel, all the amphidiploids had

1-3 florets. In L. corniculatus, a range of 2-6 florets per umbel was usually observed.

Bud color

Three amphidiploids, (L. alpinus x L. krylovii), (L. japonicus x L. alpinus) and (L. krylovii x L. filicaulis) produced flower buds which were yellow with orange-red stripes. The flower buds of the remaining amphidiploids were yellow with characteristic red stripes, the same as those of L. corniculatus.

Flower color

Pale yellow flowers with prominent red streaking were recorded for (L. alpinus x L. schoelleri), (L. corniculatus var. minor x L. alpinus), (L. corniculatus var. minor x L. filicaulis), (L. japonicus x L. corniculatus var. minor), (L. japonicus x L. schoelleri), (L. japonicus x L. krylovii), (L. krylovii x L. filicaulis) and (L. krylovii x L. schoelleri). The remaining four amphidiploids and L. corniculatus bore deep yellow flowers with prominent red streaking. Except for (L. alpinus x L. krylovii), (L. japonicus x L. alpinus) and (L. corniculatus var. minor x L. alpinus), the keel tip of the flowers of all the amphidiploids and L. corniculatus were yellow.

Leaf shape

Ten leaves randomly selected from each amphidiploid and from L. corniculatus were compared for similarity in general morphology. A photograph of a representative leaf from each amphidiploid is shown

in Plate II. It can be seen that the leaf of (L. japonicus x L. alpinus) exhibited the closest resemblance to that of L. corniculatus. A slight difference in the general shape of the leaves of these two polyploids was found in the shape of the central leaflet which was somewhat wider in the amphidiploid (L. japonicus x L. alpinus).

HCN reaction

A negative test for the presence of HCN was obtained for (L. alpinus x L. schoelleri), (L. corniculatus var. minor x L. alpinus), (L. corniculatus var. minor x L. filicaulis), and (L. filicaulis x L. schoelleri). The remaining amphidiploids and L. corniculatus gave a positive test for the presence of HCN.

To further investigate possible morphological resemblances between the amphidiploid and L. corniculatus, 15 quantitative traits were studied. The means and standard errors were calculated for each of the characters listed in Tables 17, 18. From the calculated means of the central leaflet index, the floral bract index, the standard index, the calyx index and the style/ovary index, polygons were drawn for each amphidiploid and for L. corniculatus. These are shown in Plate III. It can be seen that each of the amphidiploids exhibited a certain degree of resemblance to the cultivated species. It may be seen also from Tables 17 and 18 that very small differences were found in the mean values for certain phenotypic traits in L. corniculatus and some of the amphidiploids. A t-test was therefore used to check the significances of the differences and these results

TABLE 16. Some morphological characteristics of the amphidiploids

Amphidiploids (4x)	Growth habit	Florets per umbel	Bud color	Flower color	Red streaking on flower	Keel tip color	HCN reaction
(L. alpinus X schoelleri)	ascending	1 - 3	yellow, red stripes	pale yellow	prominent	yellow; dark reddish brown	-
(L. alpinus X filicaulis)	decumbent	1 - 3	yellow, red stripes	deep yellow	prominent	yellow	+
(L. alpinus X krylovii)	decumbent	1 - 3	yellow, orange-red stripes	deep yellow	prominent	dark reddish brown	+
(L. japonicus X alpinus)	decumbent	1 - 4	yellow, orange-red stripes	deep yellow	prominent	dark reddish brown	+
(L. corniculatus var. minor X alpinus)	decumbent	1 - 3	yellow, red stripes	pale yellow	prominent	dark reddish brown	-
(L. corniculatus var. minor X filicaulis)	decumbent	1 - 3	yellow, red stripes	pale yellow	prominent	yellow	-
(L. japonicus X corniculatus var. minor)	procumbent	1 - 3	yellow, red stripes	pale yellow	prominent	yellow	+
(L. filicaulis X schoelleri)	decumbent	1 - 3	yellow, red stripes	deep yellow	prominent	yellow	-
(L. japonicus X schoelleri)	decumbent	1 - 3	yellow, red stripes	pale yellow	prominent	yellow	+

Table 16 Cont'd

TABLE 16 (Cont'd)

Amphidiploids (4x)	Growth habit	Florets per umbel	Bud color	Flower color	Red streaking on flower	Keel tip color	HCN reaction
(L. japonicus X krylovii)	decumbent	1 - 4	yellow, red stripes	pale yellow	prominent	yellow	+
(L. krylovii X filicaulis)	ascending	1 - 3	yellow, orange-red stripes	pale yellow	prominent	yellow	+
(L. krylovii X schoelleri)	ascending	1 - 3	yellow, red stripes	pale yellow	prominent	yellow	+
L. corniculatus	semi-erect	2 - 6	yellow, red stripes	deep yellow	prominent	yellow	+

are presented in Table 19. Inspection of Table 19 shows these amphidiploids resembled L. corniculatus in only 1 to 6 of the phenotypic traits studied. These traits, designated as "morphologically similar characters", and the amphidiploid with which they are associated are as follows:

Amphidiploids	Morphologically similar characters
(L. alpinus x L. schoelleri)	calyx index
(L. krylovii x L. filicaulis)	floral bract index
(L. krylovii x L. schoelleri)	floral bract width
(L. corniculatus var. minor x L. filicaulis)	floral bract index, style/ovary index
(L. filicaulis x L. schoelleri)	central leaflet index, floral bract index, and calyx index
(L. japonicus x L. krylovii)	central leaflet index, floral bract width and ovary length
(L. alpinus x L. filicaulis)	central leaflet index, floral bract index and calyx ratio
(L. japonicus x L. corniculatus var. minor)	floral bract index, standard index, ovary length and calyx length
(L. japonicus x L. schoelleri)	central leaf width, floral bract width, ovary length, calyx length and calyx tube length
(L. alpinus x L. krylovii)	central leaflet length, standard width, calyx length, calyx tube length and calyx index
(L. corniculatus var. minor x L. alpinus)	central leaflet index, floral bract length, floral bract width, floral bract index, and calyx length
(L. japonicus x L. alpinus)	central leaflet width, floral bract width, standard length, style length, calyx tube length and calyx index

TABLE 17. Data on measurements of the central leaflet and floral bract for the amphidiploids and L. corniculatus. The mean values and standard errors are shown.

Amphidiploids (4x)	Central leaflet			Floral bract		
	length (mm)	width (mm)	index	length (mm)	width (mm)	index
(L. alpinus X schoelleri)	8.6 ± 0.2	5.0 ± 0.1	0.59 ± 0.02	6.3 ± 0.3	2.7 ± 0.1	0.43 ± 0.01
(L. alpinus X filicaulis)	10.5 ± 0.2	4.5 ± 0.1	0.43 ± 0.01	8.0 ± 0.4	2.6 ± 0.2	0.33 ± 0.01
(L. alpinus X krylovii)	14.3 ± 0.2	7.5 ± 0.2	0.52 ± 0.01	9.7 ± 0.3	4.5 ± 0.2	0.47 ± 0.01
(L. japonicus X alpinus)	12.8 ± 0.2	6.9 ± 0.2	0.54 ± 0.01	9.0 ± 0.3	3.9 ± 0.1	0.43 ± 0.01
(L. corniculatus var. minor X alpinus)	13.9 ± 0.3	5.1 ± 0.1	0.37 ± 0.01	9.5 ± 0.8	3.2 ± 0.3	0.33 ± 0.01
(L. corniculatus var. minor X filicaulis)	13.4 ± 0.3	3.9 ± 0.1	0.29 ± 0.01	8.2 ± 0.5	2.6 ± 0.2	0.32 ± 0.02
(L. japonicus X corniculatus var. minor)	12.6 ± 0.3	5.0 ± 0.2	0.39 ± 0.01	8.8 ± 0.5	3.0 ± 0.2	0.34 ± 0.02
(L. filicaulis X schoelleri)	10.6 ± 0.2	4.4 ± 0.1	0.42 ± 0.01	6.8 ± 0.3	2.3 ± 0.1	0.35 ± 0.02
(L. japonicus X schoelleri)	12.8 ± 0.2	6.8 ± 0.2	0.53 ± 0.01	7.0 ± 0.2	3.3 ± 0.2	0.48 ± 0.03

Table 17 Cont'd

TABLE 17 (Cont'd)

Amphidiploids (4x)	Central leaflet			Floral bract		
	length (mm)	width (mm)	index	length (mm)	width (mm)	index
(*L. japonicus* X *krylovii*)	14.0 ± 0.2	5.8 ± 0.2	0.42 ± 0.01	8.2 ± 0.6	3.4 ± 0.3	0.42 ± 0.02
(*L. krylovii* X *filicaulis*)	13.3 ± 0.3	4.7 ± 0.1	0.35 ± 0.01	8.9 ± 0.8	2.6 ± 0.2	0.30 ± 0.02
(*L. krylovii* X *schoelleri*)	12.7 ± 0.3	5.1 ± 0.2	0.40 ± 0.01	7.6 ± 0.6	3.1 ± 0.3	0.41 ± 0.02
L. corniculatus	15.0 ± 0.3	6.5 ± 0.2	0.43 ± 0.01	11.1 ± 0.3	3.8 ± 0.2	0.34 ± 0.02

TABLE 18. Data on measurements of the standard, ovary, style and calyx for the amphidiploids and *L. corniculatus*. The mean values and standard errors are shown.

Amphidiploids (4x)	Standard			Calyx			ovary length (mm)	style length (mm)	style/ ovary index
	length (mm)	width (mm)	index	total length (mm)	tube length (mm)	index			
(*L. alpinus* X *schoelleri*)	10.0 ±0.2	8.8 ±0.2	0.88 ±0.02	6.0 ±0.2	3.2 ±0.1	0.53 ±0.01	6.3 ±0.2	4.4 ±0.1	0.71 ±0.02
(*L. alpinus* X *filicaulis*)	10.3 ±0.2	8.9 ±0.3	0.87 ±0.03	5.7 ±0.1	2.9 ±0.1	0.51 ±0.01	6.3 ±0.2	4.6 ±0.1	0.74 ±0.02
(*L. alpinus* X *krylovii*)	11.7 ±0.1	9.9 ±0.2	0.85 ±0.01	7.7 ±0.2	3.9 ±0.1	0.51 ±0.01	7.3 ±0.1	5.4 ±0.1	0.74 ±0.02
(*L. japonicus* X *alpinus*)	12.6 ±0.1	10.8 ±0.1	0.86 ±0.01	7.0 ±0.1	3.4 ±0.1	0.49 ±0.01	7.7 ±0.1	5.7 ±0.1	0.74 ±0.01
(*L. corniculatus* var. *minor* X *alpinus*)	10.5 ±0.1	8.6 ±0.1	0.81 ±0.01	7.4 ±0.1	3.3 ±0.1	0.45 ±0.01	7.2 ±0.2	5.0 ±0.0	0.70 ±0.02
(*L. corniculatus* var. *minor* X *filicaulis*)	10.4 ±0.1	8.4 ±0.2	0.81 ±0.01	6.7 ±0.2	3.1 ±0.0	0.46 ±0.01	6.6 ±0.1	5.0 ±0.0	0.76 ±0.01
(*L. japonicus* X *corniculatus* var. *minor*)	11.3 ±0.2	8.8 ±0.2	0.78 ±0.01	7.1 ±0.2	3.2 ±0.1	0.45 ±0.01	6.7 ±0.1	5.5 ±0.1	0.82 ±0.01
(*L. filicaulis* X *schoelleri*)	9.9 ±0.1	8.3 ±0.2	0.84 ±0.02	5.7 ±0.1	2.9 ±0.0	0.52 ±0.01	5.9 ±0.1	4.2 ±0.1	0.71 ±0.02

Table 18 Cont'd

TABLE 18 (Cont'd)

Amphidiploids (4x)	Standard			Calyx			ovary length (mm)	style length (mm)	style/ ovary index
	length (mm)	width (mm)	index	total length (mm)	tube length (mm)	index			
(L. japonicus X schoelleri)	11.2 ±0.1	9.2 ±0.2	0.82 ±0.01	7.6 ±0.2	3.4 ±0.1	0.45 ±0.01	6.7 ±0.1	4.8 ±0.1	0.73 ±0.01
(L. japonicus X krylovii)	10.5 ±0.2	8.8 ±0.2	0.84 ±0.01	6.8 ±0.2	3.1 ±0.0	0.45 ±0.01	6.6 ±0.1	4.7 ±0.1	0.72 ±0.02
(L. krylovii X filicaulis)	10.4 ±0.1	8.3 ±0.2	0.79 ±0.01	6.6 ±0.1	3.0 ±0.0	0.46 ±0.01	6.4 ±0.2	4.6 ±0.1	0.73 ±0.02
(L. krylovii X schoelleri)	9.4 ±0.2	7.8 ±0.1	0.83 ±0.01	6.7 ±0.1	3.1 ±0.1	0.46 ±0.01	5.9 ±0.2	4.2 ±0.1	0.71 ±0.02
L. corniculatus	12.9 ±0.1	9.8 ±0.1	0.76 ±0.01	7.5 ±0.2	3.7 ±0.1	0.50 ±0.01	6.8 ±0.1	6.0 ±0.1	0.88 ±0.20

Plate II

General morphology of the leaves of the induced tetraploids (A-G), amphidiploids (H and J-T) and Lotus corniculatus (I).

A L. alpinus

B L. tenuis

C L. japonicus

D L. corniculatus var. minor

E L. filicaulis

F L. krylovii

G L. schoelleri

H (L. alpinus x L. schoelleri)

I L. corniculatus

J (L. japonicus x L. alpinus)

K (L. alpinus x L. krylovii)

L (L japonicus x L. schoelleri)

M (L. japonicus x L. corniculatus var. minor)

N (L. krylovii x L. schoelleri)

O (L. japonicus x L. krylovii)

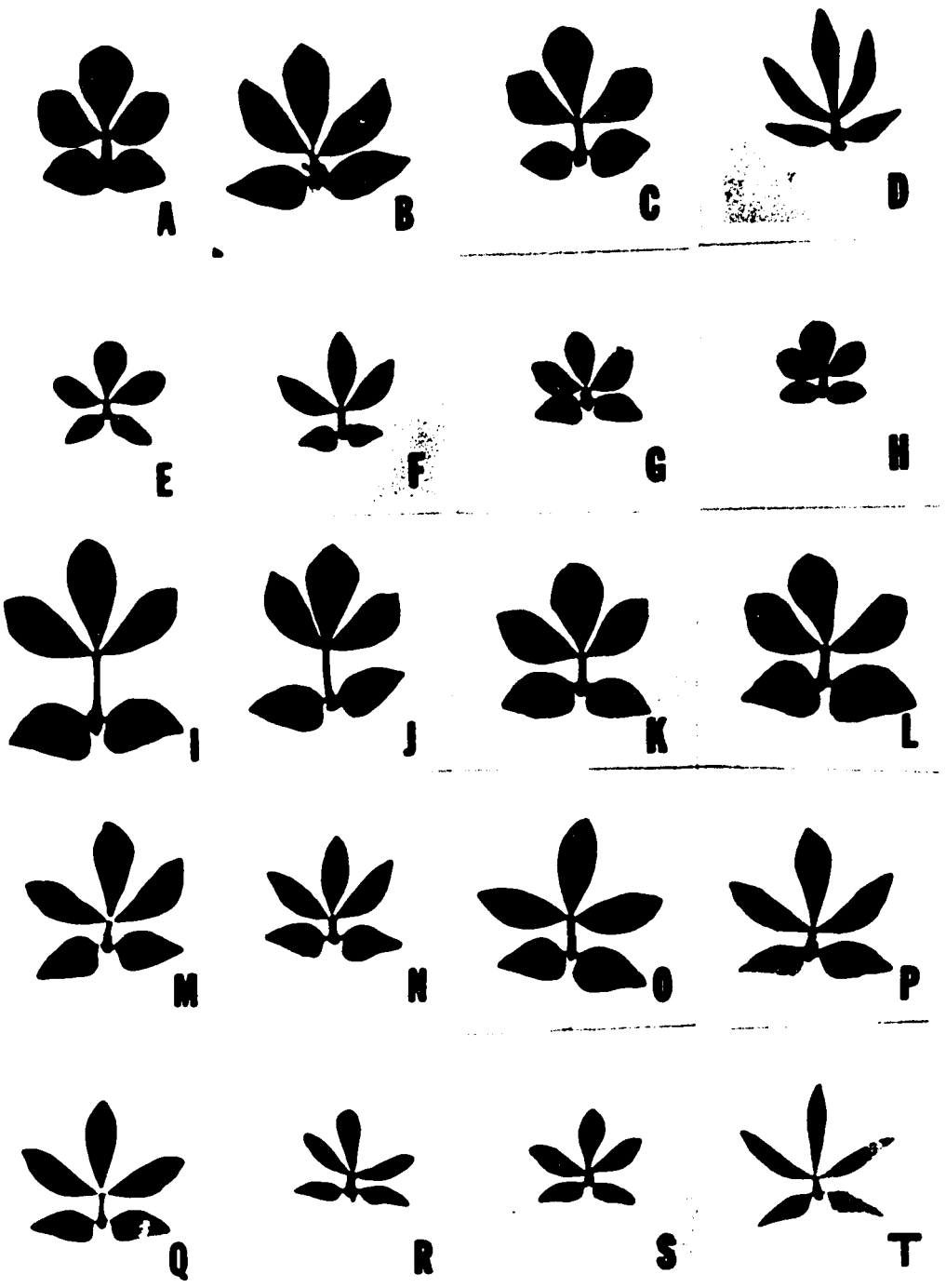
P (L. corniculatus var. minor x L. alpinus)

Q (L. krylovii x L. filicaulis)

R (L. alpinus x L. filicaulis)

S (L. filicaulis x L. schoelleri)

T (L. corniculatus var. minor x L. filicaulis)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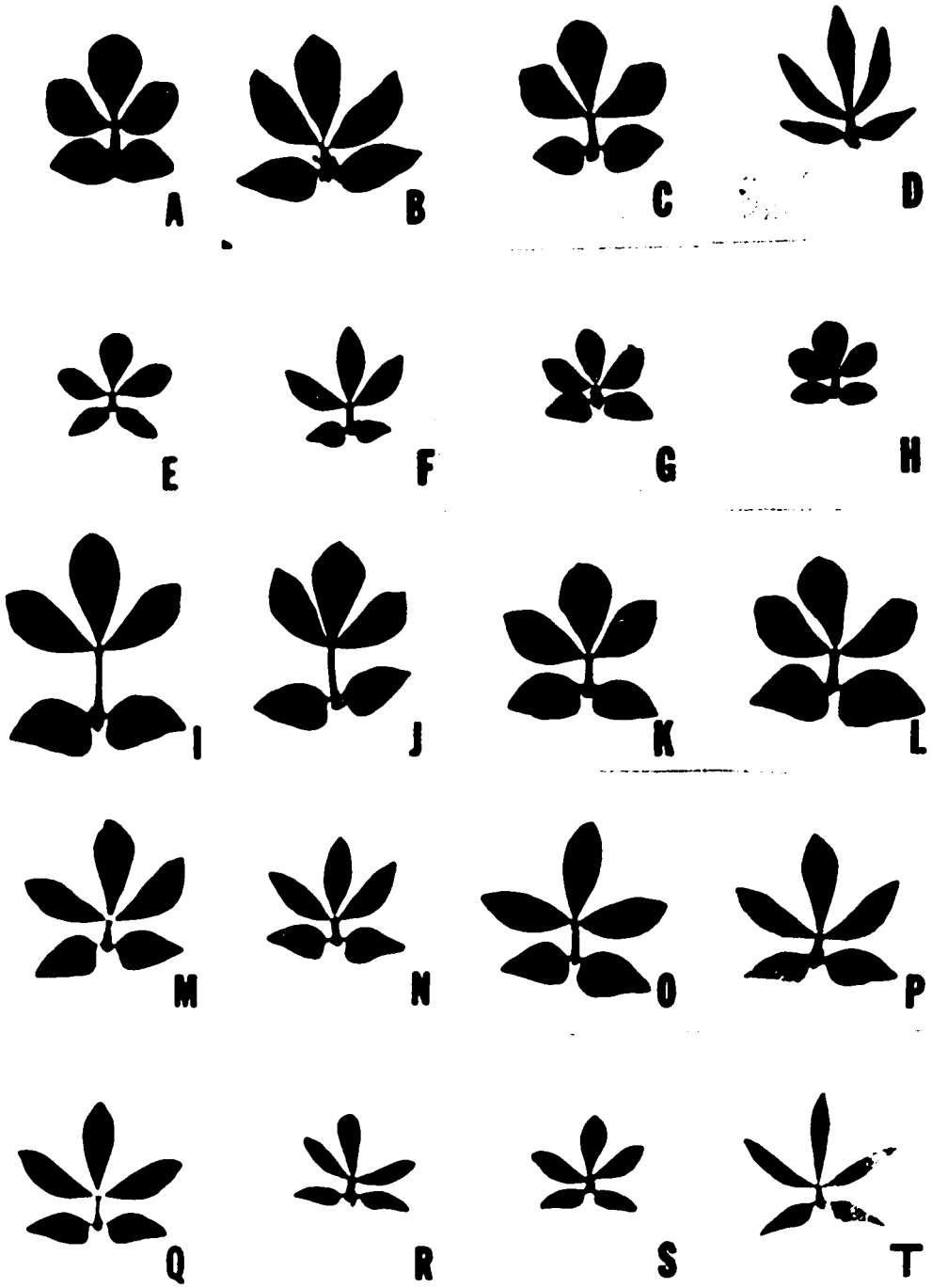
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Plate III

Polygonal representations of five morphological indices for the amphidiploids and *Lotus corniculatus*

A/JM (*L. japonicus* x *L. corniculatus* var. *minor*)

A/MF (*L. corniculatus* var. *minor* x *L. filicaulis*)

A/MA (*L. corniculatus* var. *minor* x *L. alpinus*)

A/AF (*L. alpinus* x *L. filicaulis*)

A/FS (*L. filicaulis* x *L. schoelleri*)

A/JK (*L. japonicus* x *L. krylovii*)

A/KF (*L. krylovii* x *L. filicaulis*)

A/AK (*L. alpinus* x *L. krylovii*)

A/JS (*L. japonicus* x *L. schoelleri*)

A/AS (*L. alpinus* x *L. schoelleri*)

A/KS (*L. krylovii* x *L. schoelleri*)

A/JA (*L. japonicus* x *L. alpinus*)

B554 *L. corniculatus*

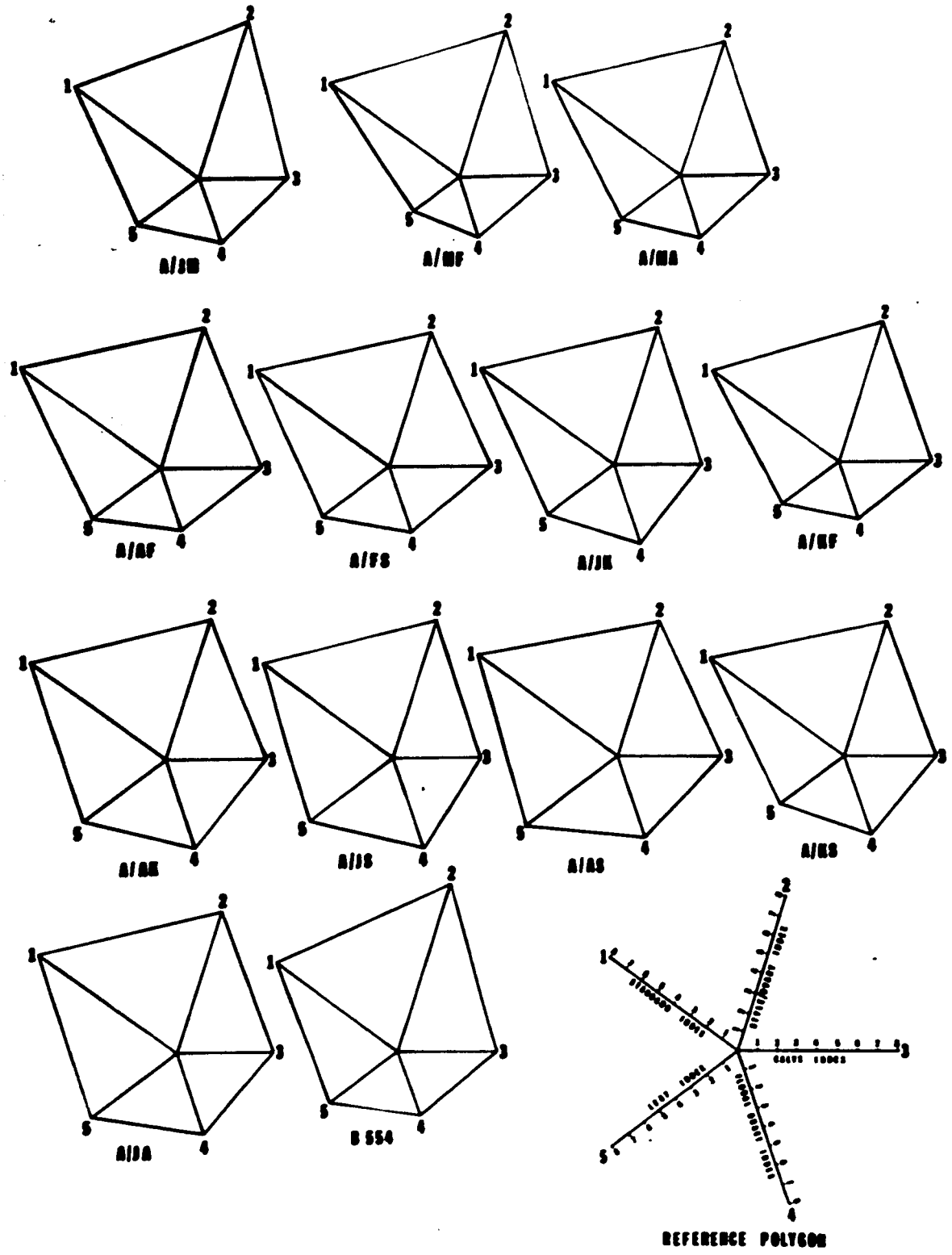

1
2
3
4
5
A/SB
A/BF
A/BA
A/BC
A/FS
A/SE
A/EF
A/BE
A/JS
A/AS
A/ES
A/DA
B 554
REFERENCE POLYGON

TABLE 19. A comparison of phenotypic traits between L. corniculatus and the amphidiploids employing a t-test

Phenotypic traits	L. corniculatus versus					
	(L. alpinus X L. schoelleri)	(L. alpinus X L. filicaulis)	(L. alpinus X L. krylovii)	(L. japonicus X L. alpinus)	(L. corniculatus var. minor X L. alpinus)	(L. corniculatus var. minor X L. filicaulis)
Central leaflet length	15.90**	11.57**	1.78	5.53**	2.31*	3.55**
Central leaflet width	6.60**	8.38**	3.72**	1.74	6.26**	10.99**
Central leaflet index	8.54**	0.13	6.98**	7.42**	6.22**	11.00**
Floral bract length	11.46**	5.84**	3.10**	4.48**	1.88	4.80**
Floral bract width	4.42**	4.21**	2.46**	0.12	1.74	4.00**
Floral bract index	4.17**	0.52	6.29**	4.60**	0.51	1.00
Standard length	11.60**	13.36**	7.10**	1.89	12.99**	14.98**
Standard width	3.97**	3.03**	0.63	5.77**	6.19**	6.32**
Standard index	6.32**	3.86**	5.39**	7.52**	3.74**	3.40**
Ovary length	2.48*	3.05**	2.82**	5.97**	1.90	1.45
Style length	10.22**	9.10**	4.46**	1.87	8.37**	8.37**
Style/ovary index	5.45**	4.40**	4.93**	5.18**	6.15**	4.27**
Calyx length	5.28**	7.20**	1.01	2.14*	0.40	3.04**
Calyx tube length	4.00**	6.69*	1.67	1.86	2.97**	5.45**
Calyx index	1.96	0.96	0.77	0.17	3.73**	2.39*

Table 19 Cont'd

TABLE 19 (Cont'd)

Phenotypic traits	*L. corniculatus* versus					
	(*L. japonicus* X *L. corniculatus* var. *minor*)	(*L. filicaulis* X *L. schoelleri*)	(*L. japonicus* X *L. schoelleri*)	(*L. japonicus* X *L. krylovii*)	(*L. krylovii* X *L. filicaulis*)	(*L. krylovii* X *L. schoelleri*)
Central leaflet length	5.23**	11.49**	5.78**	2.42*	3.94**	5.32**
Central leaflet width	5.62**	10.30**	1.00	2.64*	7.84**	5.58**
Central leaflet index	2.98**	1.71	7.11**	1.07	6.87**	2.12*
Floral bract length	3.71**	10.13**	10.32**	4.37**	2.53**	5.01*
Floral bract width	2.39*	5.41**	1.81	1.12	3.75**	1.71
Floral bract index	0.02	0.07	4.37**	3.06**	1.78	2.18*
Standard length	7.75**	15.50**	10.17**	10.66**	14.36**	17.54**
Standard width	4.03**	6.21**	2.94**	4.13**	6.75**	10.06**
Standard index	1.07	4.11**	3.57**	6.13**	2.21*	4.63**
Ovary length	0.60	6.95**	1.26	1.45	2.08*	4.68**
Style length	2.96**	12.08**	8.64*	6.73**	9.63**	12.44**
Style/ovary index	2.33*	5.69**	5.74*	4.82**	5.21**	5.25**
Calyx length	1.42	7.20**	0.60	2.22*	3.38**	3.15**
Calyx tube length	4.00**	6.60**	1.86	5.45*	5.95**	5.03**
Calyx index	3.21**	1.62	3.18**	3.20**	2.68*	2.65*

* significant ($P < 0.05$)
** highly significant ($P < 0.01$)

It can be seen that the amphidiploid (L. japonicus x L. alpinus) displayed the closest morphological affinity to L. corniculatus. This resemblance was reflected to a certain degree in all the plant organs examined, namely, the leaf, the floral bract, the standard, the gynoecium, and the calyx. In addition, this induced polyploid exhibited a very intense positive reaction for the presence of HCN characteristic of L. corniculatus.

V. Meiotic studies

In Lotus a wide range of meiotic stages was found in the anthers within the same flower. Microspore mother cells were often observed in every stage of meiosis from early pachynema, diakinesis, and metaphase I. Therefore it was necessary to examine a large number of preparations in order to obtain cell plates that were suitable for study. Difficulty in obtaining good chromosome separation and the extreme spiralization of most of the chromosomes at late diakinesis and metaphase I made it impossible to study chiasma frequency. Similarly, observations on chromosome disjunction at anaphase I and II and telophase I and II were limited to the occurrence of lagging chromosomes and bridge formation.

Meiosis in the diploid parental species

Chromosome behavior at diakinesis and metaphase I in the microsporocytes of the diploid species were analysed and the data are presented in Tables 20 and 21. It may be seen from these tables

that meiosis was characterized by a high degree of bivalent pairing as over 98 per cent of the chromosomes were associated as bivalents in each of the seven diploids. Six bivalents per cell were generally present (Plate IV, Figures 1, 2, 4, 7, 8 and 9). The percentage of cells with this type of chromosome association ranged from 89.93 for L. corniculatus var. minor to 98.44 for L. krylovii. The chromosomes were united by a terminal chiasma or less often by two terminal chiasmata, one at either end; this resulted in rod and less frequently in ring formation. No interstitial chiasmata were observed. The number of cells with 5 II's + 2 I's were variable between the species and ranged from 1.56 per cent in L. krylovii to 10.07 per cent in L. corniculatus var. minor. Six cells with 4 II's + 4 I's were recorded in L. filicaulis (Plate IV, Figure 6), and one cell each, also, with this type of chromosome association was detected in L. tenuis and L. filicaulis. Anaphase I and II and telophase I and II appeared to be regular since a high percentage of the cells examined, did not have lagging chromosomes (Table 22). Since micronuclei were rarely seen it would appear that most of the lagging chromosomes were able to reach their respective poles in time to be included in the telophase nuclei. Anaphase I bridges were recorded in 10 of the 419 cells in L. japonicus and in 3 of the 214 of L. krylovii. No accompanying fragments were seen and it appears likely that these bridges resulted from the failure of chiasma to terminalize. Micro-photographs of lagging chromosomes and anaphase bridges are given in

TABLE 20. Diakinesis and/or Metaphase I chromosome associations in the parental species

Parental species	Accession no.	Total no. of cells examined	6 II's		5 II's + 2 I		4 II's + 4 I	
			No. of cells	%	No. of cells	%	No. of cells	%
L. alpinus	B77	544	491	90.26	53	9.74	-	-
L. japonicus	B129	392	369	94.13	23	5.87	-	-
L. tenuis	B109	368	352	95.65	15	4.08	1	0.27
L. filicaulis	B37	530	477	90.00	47	8.87	6	1.13
L. schoelleri	B166	255	245	96.08	9	3.53	1	0.39
L. krylovii	B86	320	315	98.44	5	1.56	-	-
L. corniculatus var. minor	B303	139	125	89.93	14	10.07	-	-

TABLE 21. Diakinesis and/or Metaphase I chromosome behavior in the parental species

Parental species	Accession no.	Total no. of cells examined	Means and ranges per cell of		Per cent chromosomes as	
			I	II's	I	II's
L. alpinus	B77	544	0.19 (0 - 2)	5.90 (5 - 6)	1.62	98.38
L. japonicus	B129	392	0.12 (0 - 2)	5.94 (5 - 6)	0.98	99.02
L. tenuis	B109	368	0.09 (0 - 4)	5.95 (4 - 6)	0.77	99.23
L. filicaulis	B37	530	0.22 (0 - 4)	5.89 (4 - 6)	1.86	98.14
L. schoelleri	B166	255	0.09 (0 - 4)	5.96 (4 - 6)	0.72	99.28
L. krylovii	B86	320	0.03 (0 - 2)	5.98 (5 - 6)	0.26	99.74
L. corniculatus var. minor	B303	139	0.20 (0 - 2)	5.90 (5 - 6)	1.68	98.32

TABLE 22. Meiotic chromosome behavior at AI--TI and AII--TII in the parental species

Parental species	Accession no.	Anaphase I - Telophase I					Anaphase II - Telophase II				
		Total no. of cells	normal	%	laggards	%	Total no. of cells	normal	%	laggards	%
L. alpinus	B77	49	43	87.76	6	12.24	117	113	97.58	4	3.42
L. japonicus	B129	419*	400	95.47	9	2.15	281	280	99.64	1	0.36
L. tenuis	B109	324	314	96.91	10	3.08	113	110	97.35	3	2.65
L. filicaulis	B37	354	326	92.09	28	7.91	148	144	97.30	4	2.70
L. schoelleri	B166	215	211	98.14	4	1.86	129	127	98.45	2	1.55
L. krylovii	B86	214**	207	96.73	4	1.87	-	-	-	-	-
L. corniculatus var. minor	B303	234	226	96.58	8	3.42	101	97	96.04	4	4.96

* bridges seen in 10 cells
** bridges seen in 3 cells

Plate IV

Meiosis in the diploid species

Figure 1 MI in L. alpinus showing 6 II's. x ca. 1370

Figure 2 MI in L. alpinus showing 5 II's + 2 I's (left) and 6 II's (right). x ca. 700

Figure 3 Prometaphase I in L. japonicus showing 5 II's + 2 I's. x ca. 1400

Figure 4 Six bivalents at prometaphase I in L. tenuis. x ca. 1370

Figure 5 Prometaphase I in L. tenuis showing 6 II's. x ca. 1850

Figure 6 MI in L. filicaulis showing (a) 4 II's + 4 I's and (b) 6 II's. x ca. 970

Figure 7 Prometaphase I in L. schoelleri showing 6 II's. x ca. 1050

Figure 8 Prometaphase in L. krylovii showing three cells each with 6 II's. x ca. 760

Figure 9 Diakinesis in L. corniculatus var. minor showing 6 II's. x ca. 1330

Figure 1

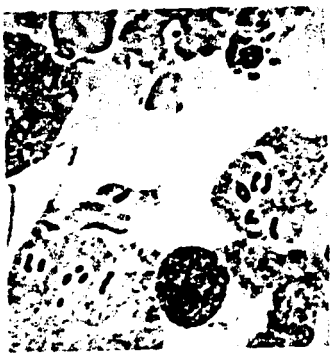


Figure 2

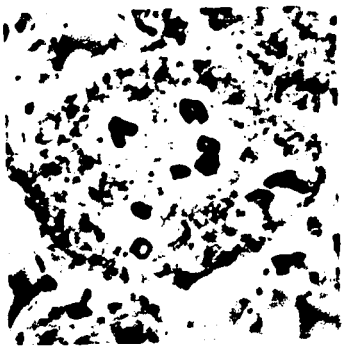
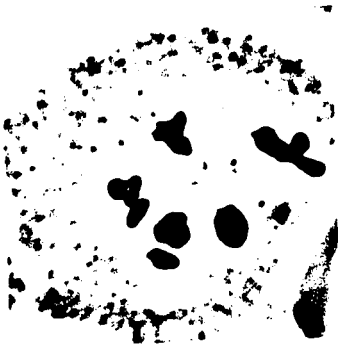


Figure 3

Figure 4



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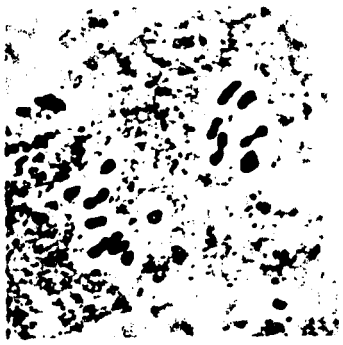

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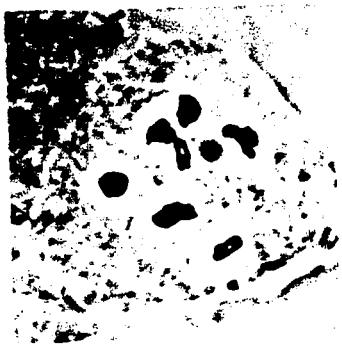

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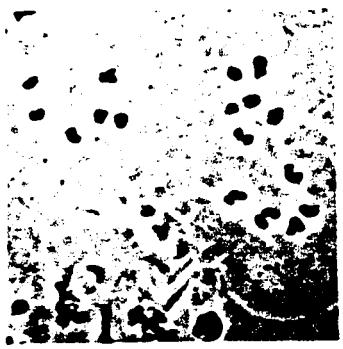


Figure 8

Figure 9

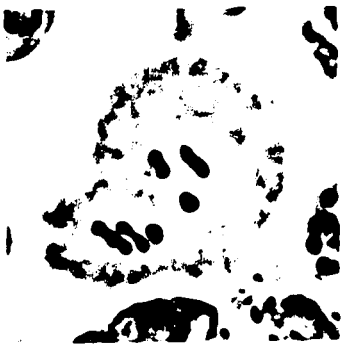
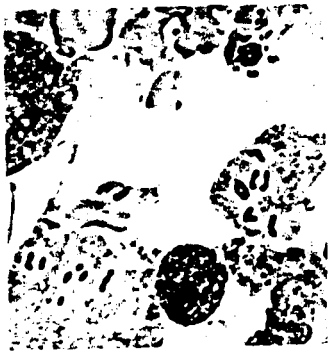


Figure 1



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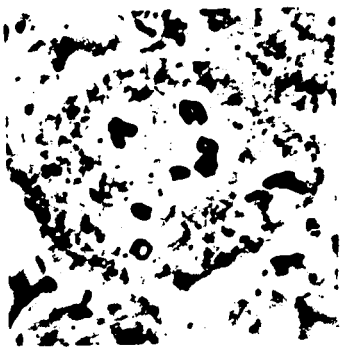
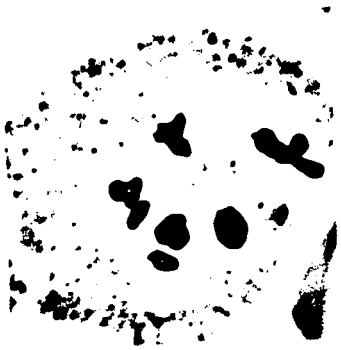


Figure 3

Figure 4



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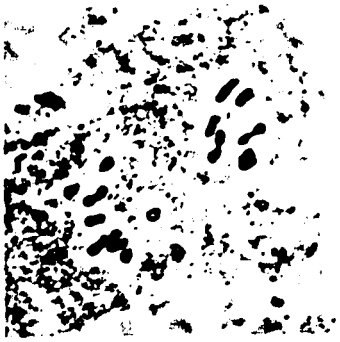

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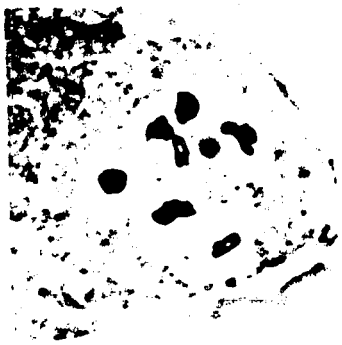


Figure 7

Figure 8

Figure 9

Plate V

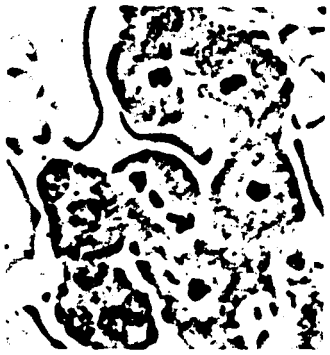


Figure 1
AI in L. japonicus showing lagging chromosome.
x ca. 740

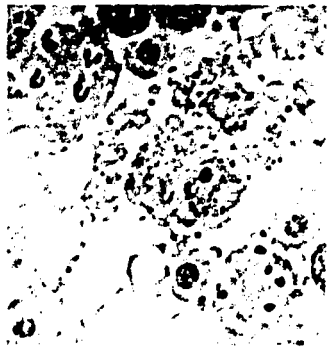


Figure 2
AII in L. alpinus showing (a) normal cell (upper left) and lagging chromosome (lower right).
x ca. 500

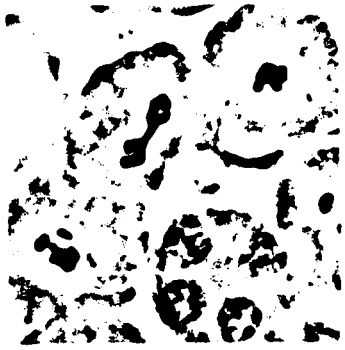


Figure 3
AI in L. japonicus showing bridge.
x ca. 1070

Figure 4
AI in L. krylovii showing bridge.
x ca. 1330

Plate V

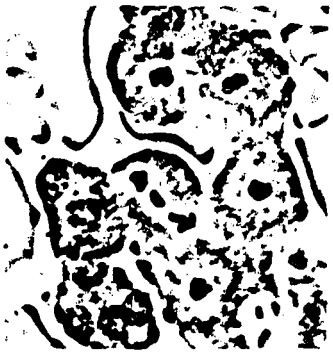


Figure 1
AI in L. japonicus showing lagging chromosome.
x ca. 740

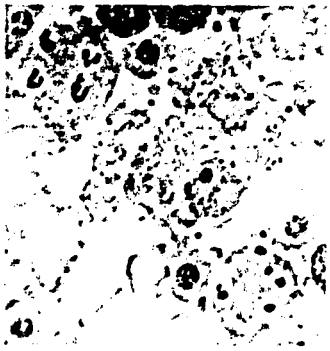


Figure 2
AII in L. alpinus showing (a) normal cell (upper left) and lagging chromosome (lower right).
x ca. 500

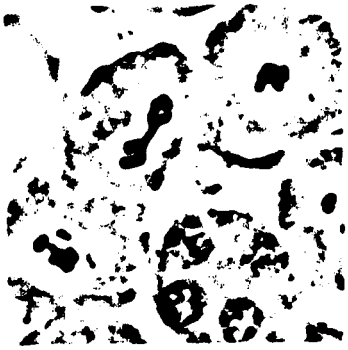


Figure 3
AI in L. japonicus showing bridge.
x ca. 1070

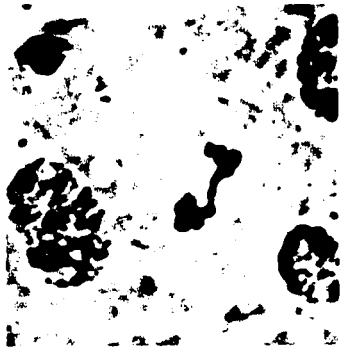


Figure 4
AI in L. krylovii showing bridge.
x ca. 1330

Plate V, Figures 1 to 4.

In general, the meiotic behavior of the diploid species was quite regular. The homologous chromosomes usually formed bivalents as seen in diakinesis and metaphase I. A small percentage of lagging chromosomes were observed at anaphase I and II and telophase I and II.

Meiosis in the interspecific diploid hybrids

Meiosis was studied in 16 hybrid combinations. A minimum of two genotypes were investigated for each cross combination, except L. krylovii x L. tenuis for which only one genotype was obtained. Meiocytes of 51 genotypes were analysed at diakinesis, metaphase I, anaphase I and II and telophase I and II. The data obtained in these studies are summarized in Tables 23, 24 and 25. A high degree of bivalent pairing was exhibited by all the hybrids; it can be seen in Table 23 which gives the percent of chromosomes associated as bivalents (II's) in each cross that L. japonicus x L. alpinus had the highest percentage bivalents (96.13%), whereas, L. alpinus x L. schoelleri exhibited the lowest value (89.49%). In the remaining cross combinations, percentages ranging from 90.35 (L. krylovii x L. filicaulis) to 93.87 (L. krylovii x L. corniculatus var. minor) were recorded. For each genotype studied, bivalent pairing of the 12 chromosomes of the complement was observed in at least 51 per cent of the cells examined (Table 24). When the percentages of cells with 6 II's were averaged for each cross combination, the

value was never below 57 per cent. The highest percentage cells with normal pairing (80.21) was observed in hybrid plants derived from the cross between L. japonicus x L. alpinus. Hybrid combinations with low percentages of such cells were L. alpinus x L. schoelleri with 57.78 per cent and L. krylovii x L. filicaulis with 58.30 per cent. In the remaining crosses, meiocytes with 6 II's ranged from 60.19 per cent, L. japonicus x L. filicaulis, to 67.95 per cent, L. japonicus x L. schoelleri. The bivalents were generally rod shaped and the chiasmata were usually terminal. An occasional ring-shaped bivalent resulting from two terminal chiasmata was also detected. Pollen mother cells of the hybrids showing normal pairing of the chromosomes are shown in Plates VII, VIII and IX. Loose bivalents in which thread-like connections joined some of the chromosomes were observed at both diakinesis and metaphase I in all the hybrids (Plate VIII, Figures 6, 10 and 11). Relatively few such bivalents were noticed in hybrids derived from the crosses L. alpinus x L. schoelleri and L. krylovii x L. corniculatus var. minor; in plants from the crosses L. alpinus x L. krylovii loose bivalents were somewhat more frequent and these appeared to constitute between 5-8 per cent of the total bivalents recorded.

In each hybrid, a number of cell plates were observed to be abnormal. As a rule, the irregularities were caused by a tendency of some of the smaller chromosomes to appear as univalents at diakinesis and metaphase I. Some of these univalents also appeared

to have resulted from the precocious separation of bivalents at metaphase I; the separated univalents were often found to be lying opposite each other on either side of the equatorial plate. It may be seen in Table 24, that in some cells more than 50 per cent of the chromosomes were observed as univalents, e.g. in the cross L. alpinus x L. filicaulis. A range of 0-4 univalents per cell was recorded for many of the hybrids and as many as 6 and 8 of these entities were observed in L. krylovii x L. corniculatus var. minor, L. krylovii x L. tenuis, L. corniculatus var. minor x L. filicaulis, and L. alpinus x L. filicaulis. The frequency of univalents varied between different genotypes of a cross and from hybrid to hybrid. It can be seen from Table 24, that there was considerable variability with regards to the frequency of unpaired chromosomes in the genotypes of L. alpinus x L. krylovii. Inspection of Table 23 also shows that L. japonicus x L. filicaulis had an average of 1.00 univalent per cell and 8.33 per cent of its chromosomes occurred as univalents. In L. japonicus x L. alpinus, the values of only 0.30 and 2.53 were recorded as the mean and percentage of univalent chromosomes, respectively. In the remaining hybrids the mean number of univalents per cell ranged from 0.54 to 0.95 and the per cent chromosomes as univalent ranged from 4.52 to 7.92.

Multivalent associations occurring as trivalents and quadrivalents were also observed in hybrids obtained from the crosses L. japonicus x L. krylovii, L. japonicus x L. alpinus, L. alpinus x L. japonicus,

L. alpinus x L. schoelleri, L. krylovii x L. filicaulis, L. corniculatus var. minor x L. alpinus and L. alpinus x L. filicaulis. Quadrivalents in the form of closed rings, or a chain of 4 chromosomes, were frequently seen at diakinesis and prometaphase. The rare quadrivalents seen at metaphase I appeared mainly as chains of 4 chromosomes or as N-shaped configurations. Trivalents occurred less frequently than quadrivalents and were generally present as a chain of 3 chromosomes mainly at metaphase I. The decrease in the multivalent frequency at metaphase I as compared to that at early diakinesis may indicate the falling apart of some of the multivalents at late diakinesis and early metaphase I. Also, the trivalents observed, may have resulted from a precocious segregation of a member of a chain of 4 chromosomes. Photomicrographs of multivalent configurations are shown in Plate IX, Figures 1 to 12. The largest number of PMC's exhibiting multivalent configurations was found in the hybrid L. alpinus x L. schoelleri (Table 24). In this cross combination, 4.49 per cent of the cells were recorded with 4 II's + 1 III + 1 IV, 0.53 per cent had 3 II's + 3 I's + 1 III, 7.92 per cent had 4 II's + 1 IV and 0.79 per cent showed association of 3 II's + 2 I's + 1 IV. The total percentage of cells with multivalent association for the hybrids L. japonicus x L. alpinus, L. alpinus x L. japonicus, L. alpinus x L. schoelleri, L. krylovii x L. filicaulis, L. corniculatus var. minor x L. alpinus, L. alpinus x L. filicaulis and L. japonicus x L. krylovii were 4.40, 5.49, 13.73, 7.34, 5.15,

1.05 and 11.29, respectively. In the cross L. alpinus x L. filicaulis only trivalents were observed in the 570 meiocytes analysed and a mean of 0.03 was noted for this type of chromosome association. Table 23 gives the mean, range, and the per cent of chromosomes associated as multivalents for the crosses in which they were found.

In one genotype (AF-5-2) of the cross L. alpinus x L. filicaulis an extra chromosome considered to be a B-chromosome was seen in over 90 per cent of the cells at diakinesis and metaphase I. This extra chromosome when highly contracted, was somewhat spherical in appearance (Plate VI, Figures 8 and 9) and did not pair with any of the other chromosomes of the normal set; it invariably appeared as a univalent. An examination of the meiocytes of 4 other genotypes of this cross (L. alpinus x L. filicaulis) was made, but no supernumerary chromosome was detected in any of these plants.

Observations of meiocytes at post-metaphase I stages were made and the results are set out in Table 25. It can be seen that at least 86 per cent of the anaphase I cells and 82 per cent of the anaphase II cells were devoid of bridges and lagging chromosomes. The range of cells tabulated as normal at anaphase I was 86.93 per cent for L. corniculatus var. minor x L. alpinus, to 93.55 per cent in L. alpinus x L. schoelleri; the range at AII was 82.29 per cent for L. alpinus x L. japonicus to 97.24 per cent in L. filicaulis x L. schoelleri.

Irregularities recorded after metaphase I were lagging chromosomes,

asynchronous separation of chromosomes, bridges, and bridges with associated fragments. The number of lagging chromosomes at anaphase I and II varied from 0 to 3 for many of the hybrids. A few cells of L. alpinus x L. japonicus were observed with as many as 5 laggards. The laggards appeared to be some of the smaller chromosomes which had separated during the early stages of meiosis and which presumably had failed to travel to the pole at the beginning of anaphase I. The lagging chromosomes either divided on the spindle or became included intact in one or another of the groups of chromosomes at the poles. The variable number of univalents on the spindle may be due therefore to some of the lagging chromosomes reaching the pole divided or undivided. In a number of meiocytes, medium to large sized chromosomes remained at the equatorial plate at first anaphase (Plate XI, Figures 1 and 2).

The B-chromosome observed in the genotype AF-5-2 of the cross L. alpinus x L. filicaulis often moved undivided to one pole at anaphase I; at other times, it was observed to be dividing midway between the two poles after the A-chromosomes had already reached their respective poles (Plate XI, Figure 5).

At anaphase I, hybrids of L. japonicus x L. filicaulis had the greatest number of cells (12.85%) with lagging chromosomes and L. alpinus x L. krylovii plants had the lowest percentage (5.96). At anaphase II, the highest number (16.67%) was recorded for L. alpinus x L. japonicus and the lowest number (2.76%) was observed in hybrids

of L. filicaulis x L. schoelleri. Generally the percentage of cells with laggards was somewhat higher at anaphase I and telophase I than at anaphase II and telophase II.

In some cases, the telophase I chromosome complement consisted of more or less than the normal chromosome number as a result of unequal distribution of the chromosomes at anaphase I. Bridges were observed at anaphase I--telophase I and anaphase II--telophase II in L. japonicus x L. krylovii, L. japonicus x L. corniculatus var. minor, L. japonicus x L. alpinus, L. alpinus x L. japonicus, L. krylovii x L. tenuis and L. corniculatus var. minor x L. alpinus. In L. japonicus x L. schoelleri and L. krylovii x L. filicaulis, cells with bridges were seen only at anaphase I (Table 25). The highest number of bridges was recorded in hybrids L. corniculatus var. minor x L. alpinus (5.19%) for anaphase I and in L. japonicus x L. krylovii crosses (6.92%) for anaphase II. Percentages of anaphase I plates with bridges ranged from 0.37 to 5.19 and anaphase II cells with bridges varied from 0.60 per cent to 6.92 per cent. The frequency of these bridges was somewhat higher at anaphase I; anaphase II bridges were usually broken towards the end of this stage. An examination of the data presented in Table 25, also indicates that in L. japonicus x L. krylovii, L. japonicus x L. alpinus, L. alpinus x L. japonicus, L. krylovii x L. tenuis and L. corniculatus var. minor x L. alpinus acentric fragments were also present in some of the cells in which bridges occurred. These fragments were seen both at anaphase

TABLE 23. Diakinesis and/or Metaphase I chromosome behavior in the interspecific diploid hybrids

Hybrid	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
L. japonicus X krylovii	177	0.54 (0 - 4)	5.50 (4 - 6)	-	0.11 (0 - 1)	4.52	91.71	-	3.77
L. japonicus X filicaulis	324	1.00 (0 - 4)	5.50 (4 - 6)	-	-	8.33	91.67	-	-
L. japonicus X schoelleri	418	0.74 (0 - 4)	5.63 (4 - 6)	-	-	6.14	93.86	-	-
L. japonicus X corniculatus var. minor	322	0.75 (0 - 4)	5.63 (4 - 6)	-	-	6.21	93.79	-	-
L. japonicus X alpinus	250	0.30 (0 - 4)	5.77 (4 - 6)	0.02 (0 - 1)	0.03 (0 - 1)	2.53	96.13	0.40	0.93
L. alpinus X japonicus	255	0.83 (0 - 4)	5.49 (3 - 6)	0.03 (0 - 1)	0.02 (0 - 1)	6.93	91.50	0.78	0.78
L. alpinus X krylovii	606	0.77 (0 - 4)	5.62 (4 - 6)	-	-	6.41	93.59	-	-
L. alpinus X filicaulis	570	0.95 (0 - 8)	5.51 (2 - 6)	0.01 (0 - 1)	-	7.92	91.81	0.26	-
L. alpinus X schoelleri	379	0.73 (0 - 6)	5.37 (2 - 6)	0.06 (0 - 1)	0.09 (0 - 1)	6.05	89.49	1.39	3.08

Table 23 Cont'd

TABLE 23 (Cont'd)

Hybrid	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
L. krylovii X schoelleri	221	0.94 (0 - 4)	5.53 (4 - 6)	-	-	7.84	92.16	-	-
L. krylovii X filicaulis	259	0.87 (0 - 4)	5.42 (3 - 6)	-	0.07 (0 - 1)	7.27	90.35	-	2.45
L. krylovii X corniculatus var. minor	163	0.74 (0 - 6)	5.63 (3 - 6)	-	-	6.13	93.87	-	-
L. krylovii X tenuis	320	0.79 (0 - 6)	5.60 (3 - 6)	-	-	6.61	93.39	-	-
L. filicaulis X schoelleri	558	0.75 (0 - 4)	5.32 (4 - 6)	-	-	6.57	93.43	-	-
L. corniculatus var. minor X alpinus	486	0.73 (0 - 4)	5.53 (3 - 6)	-	0.05 (0 - 1)	6.10	92.18	-	1.71
L. corniculatus var. minor X filicaulis	444	0.89 (0 - 6)	5.55 (3 - 6)	-	-	7.43	92.57	-	-

TABLE 24. Diakinesis and/or Metaphase I chromosome associations in the interspecific diploid hybrids

Hybrid	Plant number	No. of cells examined	6 II's		5 II's + 2 I's		4 II's + 4 I's		4 II's + 1 III+1 I		3 II's + 3 I's+1 III		4 II's + 1 IV		3 II's + 2 I's+1 IV	
			No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%
L. japonicus X krylovii	JK-1-4	80	46	57.50	22	27.50	1	1.25	-	-	-	-	9	11.25	2	2.50
	JK-4-2	40	28	70.00	8	20.00	-	-	-	-	-	-	3	7.50	1	2.50
	JK-4-3	57	40	70.18	12	21.06	-	-	-	-	-	-	4	7.01	1	1.75
	Total	177	114	64.41	42	23.73	1	0.56	-	-	-	-	16	9.04	4	2.25
L. japonicus X filicaulis	JF-4-4	160	89	55.63	55	34.37	16	10.00	-	-	-	-	-	-	-	-
	JF-7-1	164	106	64.63	41	25.00	17	10.37	-	-	-	-	-	-	-	-
	Total	324	195	60.19	96	29.63	33	10.18	-	-	-	-	-	-	-	-
L. japonicus X schoelleri	JS-1-1	110	82	74.55	27	24.55	1	0.90	-	-	-	-	-	-	-	-
	JS-2-1	80	54	67.50	23	28.75	3	3.75	-	-	-	-	-	-	-	-
	JS-3-1	108	78	72.22	25	23.15	5	4.63	-	-	-	-	-	-	-	-
	JS-4-1	120	70	58.33	39	32.50	11	9.17	-	-	-	-	-	-	-	-
	Total	418	284	67.95	114	27.27	20	4.78	-	-	-	-	-	-	-	-
L. japonicus X corniculatus var. minor	JM-1-1	62	40	64.52	17	27.42	5	8.06	-	-	-	-	-	-	-	-
	JM-1-2	80	53	66.25	24	30.00	3	3.75	-	-	-	-	-	-	-	-
	JM-1-3	69	45	65.22	21	30.43	3	4.35	-	-	-	-	-	-	-	-

Table 24 Cont'd

TABLE 24 (Cont'd)

Hybrid	Plant number	No. of cells examined	6 II's		5 II's + 2 I's		4 II's + 4 I's		4 II's + 1 III+1 I		3 II's + 3 I's+1 III		4 II's + 1 IV		3 II's + 2 I's+1 IV	
			No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%
L. japonicus X corniculatus var. minor (cont'd)	JM-6-1	32	19	59.38	12	37.50	1	3.12	-	-	-	-	-	-	-	-
	JM-1-10	79	58	73.42	20	25.32	1	1.26	-	-	-	-	-	-	-	-
	Total	322	215	66.77	94	29.19	13	4.04	-	-	-	-	-	-	-	-
L. japonicus X alpinus	JA-1-1	150	119	79.33	25	16.67	-	-	3	2.00	-	-	3	2.00	-	-
	JA-5-3	100	84	84.00	11	11.00	-	-	1	1.00	-	-	4	4.00	-	-
	Total	250	203	81.20	36	14.40	-	-	4	1.60	-	-	7	2.80	-	-
L. alpinus X japonicus	AJ-1-1	125	77	61.60	30	24.00	6	4.80	3	2.40	3	2.40	4	3.20	2	1.60
	AJ-2-1	130	85	65.39	33	25.38	10	7.69	-	-	2	1.54	-	-	-	-
	Total	255	162	63.53	63	24.71	16	6.27	3	1.18	5	1.96	4	1.57	2	0.78
L. alpinus X krylovii	AK-13-2	120	80	66.67	34	28.33	6	5.00	-	-	-	-	-	-	-	-
	AK-16-2	40	27	67.50	12	30.00	1	2.50	-	-	-	-	-	-	-	-
	AK-17-4	58	34	58.62	21	36.20	3	5.18	-	-	-	-	-	-	-	-
	AK-18-1	154	114	74.03	34	22.07	6	3.90	-	-	-	-	-	-	-	-
	AK-20-7	234	157	67.09	54	23.08	23	9.83	-	-	-	-	-	-	-	-
	Total	606	412	67.98	155	25.58	39	6.44	-	-	-	-	-	-	-	-

Table 24 Cont'd

TABLE 24 (Cont'd)

Hybrid	Plant number	No. of cells examined	6 II's		5 II's + 2 I's		4 II's + 4 I's		4 II's + 1 III+1 I		3 II's + 3 I's+1 III		4 II's + 1 IV		3 II's + 2 I's+1 IV	
			No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%
L. alpinus X filicaulis	AF-1-3	115[a]	63	54.78	35	30.43	13	11.30	1	0.87	-	-	-	-	-	-
	AF-4-3	142[b]	99	69.72	30	21.13	8	5.63	4	2.82	-	-	-	-	-	-
	AF-5-1	81[c]	53	65.43	22	27.16	3	3.70	1	1.23	-	-	-	-	-	-
	AF-5-2	82[d]	52	63.41	21	25.61	7	8.54	-	-	-	-	-	-	-	-
	AF-5-3	150	92	61.33	44	29.33	14	9.34	-	-	-	-	-	-	-	-
	Total	570	359	62.98	152	26.67	45	7.89	6	1.05	-	-	-	-	-	-
L. alpinus X schoelleri	AS-1-2	213*	129	60.56	41	19.25	3	1.41	14	6.57	1	0.47	22	10.33	1	0.47
	AS-1-4	166[d]	90	54.22	51	30.72	9	5.42	3	1.81	1	0.61	8	4.82	2	1.20
	Total	379	219	57.78	92	24.27	12	3.17	17	4.49	2	0.53	30	7.92	3	0.79
L. krylovii X schoelleri	KS-1-4	57	42	73.68	14	24.56	1	3.77	-	-	-	-	-	-	-	-
	KS-4-1	164	102	62.20	36	21.95	26	15.85	-	-	-	-	-	-	-	-
	Total	221	144	65.15	50	22.62	27	12.12	-	-	-	-	-	-	-	-
L. krylovii X filicaulis	KF-1-5	110	65	59.10	29	26.36	5	4.55	-	-	-	-	7	6.36	4	3.63
	KF-1-7	70	40	57.14	22	31.43	7	10.00	-	-	-	-	1	1.43	-	-
	KF-2-1	79	46	58.23	20	25.32	6	7.59	-	-	-	-	5	6.33	2	2.53
	Total	259	151	58.30	71	27.41	18	6.95	-	-	-	-	13	5.02	6	2.32

Table 24 Cont'd

TABLE 24 (Cont'd)

Hybrid	Plant number	No. of cells examined	6 II's		5 II's + 2 I's		4 II's + 4 I's		4 II's + 1 III+1 I		3 II's + 3 I's+1 III		4 II's + 1 IV		3 II's + 2 I's+1 IV	
			No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%
L. krylovii X corniculatus var. minor	KM-1-2	53	38	71.70	13	24.53	2	3.77	-	-	-	-	-	-	-	-
	KM-2-5	60[b]	42	70.00	10	16.66	7	11.67	-	-	-	-	-	-	-	-
	KM-3-1	50	34	68.00	16	32.00	-	-	-	-	-	-	-	-	-	-
	Total	163	114	69.92	39	23.93	9	5.52	-	-	-	-	-	-	-	-
L. krylovii X tenuis	KT-1-1	320[e]	217	67.81	83	25.94	16	5.00	-	-	-	-	-	-	-	-
L. filicaulis X schoelleri	FS-1-1	320	214	66.88	90	28.12	16	5.00	-	-	-	-	-	-	-	-
	FS-1-3	238	157	65.97	64	26.89	17	7.14	-	-	-	-	-	-	-	-
	Total	558	371	66.49	154	27.60	33	5.91	-	-	-	-	-	-	-	-
L. corniculatus var. minor X alpinus	MA-11-1	77	50	64.94	14	18.18	3	3.90	-	-	-	-	8	10.39	2	2.60
	MA-11-3	125	78	62.40	39	31.20	6	4.80	-	-	-	-	2	1.60	-	-
	MA-11-4	81	42	51.85	34	41.98	2	2.47	-	-	-	-	-	-	3	3.70
	MA-12-7	80	54	67.50	14	17.50	5	6.25	-	-	-	-	4	5.00	3	3.75
	MA-12-8	123	88	71.54	27	21.95	5	4.07	-	-	-	-	3	2.44	-	-
	Total	486	312	64.20	128	26.34	21	4.32	-	-	-	-	17	3.50	8	1.65

Table 24 Cont'd

TABLE 24 (Cont'd)

Hybrid	Plant number	No. of cells examined	6 II's		5 II's + 2 I's		4 II's + 4 I's		4 II's + 1 III+1 I		3 II's + 3 I's+1 III		4 II's + 1 IV		3 II's + 2 I's+1 IV	
			No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%	No. of cells	%
L. corniculatus var. minor X filicaulis	MF-2-1	80	54	67.50	23	28.75	3	3.75	-	-	-	-	-	-	-	-
	MF-2-2	83[b]	46	55.42	25	30.12	11	13.25	-	-	-	-	-	-	-	-
	MF-3-1	82	58	70.73	21	25.61	3	3.66	-	-	-	-	-	-	-	-
	MF-3-2	104	59	56.73	37	35.58	8	7.69	-	-	-	-	-	-	-	-
	MF-3-3	95	60	63.16	31	32.63	4	4.21	-	-	-	-	-	-	-	-
	Total	444	277	62.39	137	30.86	29	6.53	-	-	-	-	-	-	-	-

[a] 3 cells each with 6 I's + 3 II's
[b] 1 cell with 6 I's + 3 II's
[c] 2 cells each with 8 I's + 2 II's
[d] 2 cells each with 6 I's + 3 II's
[e] 4 cells each with 6 I's + 3 II's
* Also 2 cells each with 1 I + 2 II's + 1 III + 1 IV

TABLE 25. Meiotic chromosome behavior at AI--TI and AII--TII in the interspecific diploid hybrids

Genotype	Anaphase I--Telophase I						Anaphase II--Telophase II					
	Normal		Bridges		Laggards		Normal		Bridges		Laggards	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
					L. japonicus X *L. krylovii*							
JK-1-4	130	82.80	9	5.73	18	11.47	51	77.27	5	7.58	10	15.15
JK-4-2	115	95.04	1	0.83	5	4.13	30	88.24	1	2.94	3	8.82
JK-4-3	28	93.33	-	-	2	6.66	26	86.67	3	10.00	1	3.33
Total	273	88.64	10[a]	3.25	25	8.11	107	82.31	9[a]	6.92	14	10.77
					L. japonicus X *L. filicaulis*							
JF-4-4	205	86.86	-	-	31	13.14	55	84.62	-	-	10	15.38
JF-7-1	107	87.70	-	-	15	12.30	80	90.91	-	-	8	9.09
Total	312	87.15	-	-	46	12.85	135	88.24	-	-	18	11.76
					L. japonicus X *L. schoelleri*							
JS-1-1	170	92.39	-	-	14	7.61	90	93.75	-	-	6	6.25
JS-2-1	84	84.00	2	2.00	14	14.00	39	90.70	-	-	4	9.30
JS-3-1	163	93.68	-	-	11	6.32	195	85.90	-	-	32	14.10
JS-4-1	160	93.57	2	1.17	9	5.26	51	87.93	-	-	7	12.07
Total	577	91.73	4	0.64	48	7.63	375	88.44	-	-	49	11.56

Table 25 Cont'd

TABLE 25 (Cont'd)

Genotype	Anaphase I--Telophase I						Anaphase II--Telophase II					
	Normal		Bridges		Laggards		Normal		Bridges		Laggards	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
							L. japonicus X *L. corniculatus* var. *minor*					
JM-1-1	120	93.02	-	-	9	6.98	60	93.75	-	-	4	6.25
JM-1-2	82	84.54	4	4.12	11	11.34	59	79.73	-	-	15	20.27
JM-1-3	70	94.60	1	1.35	3	4.05	70	93.33	2	2.67	3	4.00
JM-6-1	109	81.96	1	0.75	23	17.29	-	-	-	-	-	-
JM-1-10	105	87.50	12[b]	10.00	3	2.50	112	89.60	3	2.40	10	8.00
Total	486	87.89	18[b]	3.25	49	8.86	301	89.05	5[c]	1.48	32	9.47
							L. japonicus X *L. alpinus*					
JA-1-1	123	91.11	5	3.70	7	5.19	159	93.53	1	0.59	10	5.88
JA-5-3	69	89.61	1	1.30	7	9.09	96	88.07	9	8.26	4	3.67
Total	192	90.57	6[d]	2.83	14	6.60	255	91.40	10[e]	3.58	14	5.02
							L. alpinus X *L. japonicus*					
AJ-1-1	230	89.84	1	0.39	25	9.77	55	76.39	1	1.39	16	22.22
AJ-2-1	85	94.44	-	-	5	5.56	24	100.00	-	-	-	-
Total	315	91.04	1[f]	0.29	30	8.67	79	82.29	1[f]	1.04	16	16.67

Table 25 Cont'd

TABLE 25 (Cont'd)

Genotype	Anaphase I--Telophase I						Anaphase II--Telophase II					
	Normal		Bridges		Laggards		Normal		Bridges		Laggards	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
					L. alpinus X *L. krylovii*							
AK-13-2	37	92.50	-	-	3	7.50	55	91.67	-	-	5	8.33
AK-16-2	48	92.31	-	-	4	7.69	160	86.49	-	-	25	13.51
AK-17-4	75	93.75	-	-	5	6.25	72	91.14	-	-	7	8.86
AK-18-1	75	93.75	-	-	5	6.25	63	95.45	-	-	3	4.55
AK-20-7	175	95.11	-	-	9	4.89	-	-	-	-	-	-
Total	410	94.04	-	-	26	5.96	350	89.74	-	-	40	10.26
					L. alpinus X *L. filicaulis*							
AF-1-3	138	90.79	-	-	14	9.21	-	-	-	-	-	-
AF-4-3	201	94.81	-	-	11	5.19	55	88.71	-	-	7	11.29
AF-5-1	60	98.36	-	-	1	1.64	-	-	-	-	-	-
AF-5-2	135	81.82	-	-	30	18.18	-	-	-	-	-	-
AF-5-3	135	95.07	-	-	7	4.93	42	95.45	-	-	2	4.55
Total	669	91.39	-	-	63	8.61	97	91.51	-	-	9	8.49

Table 25 Cont'd

TABLE 25 (Cont'd)

Genotype	Anaphase I--Telophase I						Anaphase II--Telophase II					
	Normal		Bridges		Laggards		Normal		Bridges		Laggards	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
					L. alpinus X *L. schoelleri*							
AS-1-2	340	92.64	-	-	27	7.36	105	98.13	-	-	2	1.87
AS-1-4	168	95.45	-	-	8	4.55	120	95.24	-	-	6	4.76
Total	508	93.55	-	-	35	6.45	225	96.57	-	-	8	3.43
					L. krylovii X *L. schoelleri*							
KS-1-4	55	96.49	-	-	2	3.51	120	90.91	-	-	12	9.09
KS-4-1	152	91.02	-	-	15	8.98	119	94.44	-	-	7	5.56
Total	207	92.41	-	-	17	7.59	239	92.64	-	-	19	7.36
					L. krylovii X *L. filicaulis*							
KF-1-5	114	97.44	-	-	3	2.56	95	84.07	7	6.19	11	9.73
KF-1-7	115	90.55	-	-	12	9.45	90	92.78	1	1.03	6	6.19
KF-2-1	103	79.23	9	6.92	18	13.85	65	97.01	-	-	2	2.99
Total	332	88.77	9	2.41	33	8.82	250	90.25	8	2.89	19	6.81

Table 25 Cont'd

TABLE 25 (Cont'd)

Genotype	Anaphase I--Telophase I						Anaphase II--Telophase II					
	Normal		Bridges		Laggards		Normal		Bridges		Laggards	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
					L. krylovii X *L. corniculatus* var. *minor*							
KM-1-2	99	93.40	-	-	7	6.60	43	95.56	-	-	2	4.44
KM-2-5	171	93.44	-	-	12	6.56	31	96.88	-	-	1	3.12
KM-3-1	140	93.33	-	-	10	6.67	90	93.75	-	-	6	6.25
Total	410	93.39	-	-	29	6.61	164	94.80	-	-	9	5.20
					L. krylovii X *L. tenuis*							
KT-1-1	243	91.01	1[f]	0.37	23	8.61	315	94.88	2[g]	0.60	15	4.52
					L. filicaulis X *L. schoelleri*							
FS-1-1	260	90.28	-	-	28	9.72	61	98.39	-	-	1	1.61
FS-1-3	255	96.23	-	-	10	3.77	150	96.77	-	-	5	3.33
Total	515	93.13	-	-	38	6.87	211	97.24	-	-	6	2.76

Table 25 Cont'd

TABLE 25 (Cont'd)

Genotype	Anaphase I--Telophase I						Anaphase II--Telophase II					
	Normal		Bridges		Laggards		Normal		Bridges		Laggards	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
					L. corniculatus var. *minor* X *L. alpinus*							
MA-11-1	137	88.39	13	8.39	5	3.22	35	85.37	2	4.88	4	9.76
MA-11-3	105	87.50	4	3.33	11	9.17	42	85.71	2	4.08	5	10.20
MA-11-4	82	90.11	2	2.20	7	7.69	110	86.61	5	3.94	12	9.45
MA-12-7	98	91.59	3	2.80	6	5.61	30	90.91	3	9.09	-	-
MA-12-8	97	78.23	9	7.26	18	14.51	105	93.75	5	4.46	2	1.79
Total	519	86.93	31[h]	5.19	47	7.87	322	88.95	17[i]	4.70	23	6.35
					L. corniculatus var. *minor* X *L. filicaulis*							
MF-2-1	78	95.12	-	-	4	4.88	21	75.00	-	-	7	25.00
MF-2-2	110	81.48	-	-	25	18.52	60	88.24	-	-	8	11.76
MF-3-1	87	94.57	-	-	5	5.43	30	88.24	-	-	4	11.76
MF-3-2	160	93.57	-	-	11	6.43	57	86.36	-	-	9	13.64
MF-3-3	112	91.80	-	-	10	8.20	64	90.14	-	-	7	9.86
Total	547	90.86	-	-	55	9.14	232	86.89	-	-	35	13.11

[a] bridges and fragments in 2 of these cells
[b] bridges and fragments in 4 of these cells
[c] bridges and fragments in 1 of these cells
[d] 4 of these cells also have fragments
[e] 3 of these cells also have fragments
[f] with fragments
[g] both cells with fragments
[h] fragments seen in one of these cells
[i] fragments seen in two of these cells

Plate VI

Meiosis in the interspecific diploid hybrids

Figures 1, 2, and 3 Prometaphase in L. alpinus x L. schoelleri.

Fig. 1 6 II's. x ca. 1700

Fig. 2 6 II's. x ca. 1550

Fig. 3 4 II's + 1 loose II + 2 I's. x ca. 960

Figures 4, 5, and 6 MI in L. alpinus x L. krylovii.

Fig. 4 3 II's + 6 I's. x ca. 1400

Fig. 5 4 II's + 2 loose II's (left); 5 II's + 2 I's (center); 6 II's (right). x ca. 840

Fig. 6 5 II's + 2 I's. x ca. 1330

Figure 7 Prometaphase in L. alpinus x L. krylovii showing 5 II's + 2 I's. x ca. 1600

Figures 8 and 9 B-chromosomes (indicated by arrow) in MI of L. alpinus x L. filicaulis.

Fig. 8 5 II's + 2 I's B-chromosome (upper left); 6 II's + B-chromosome (lower right). x ca. 1000

Fig. 9 4 II's + 4 I's B-chromosome. x ca. 1800

Figure 10 Prometaphase in L. alpinus x L. filicaulis showing 6 II's. x ca. 850

Figure 1

Figure 2

Figure 3

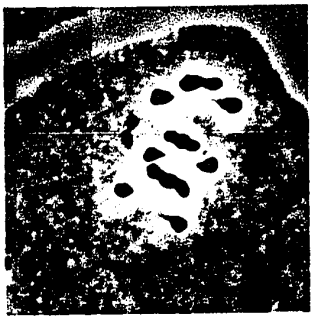


Figure 4

Figure 5

Figure 6

Figure 7

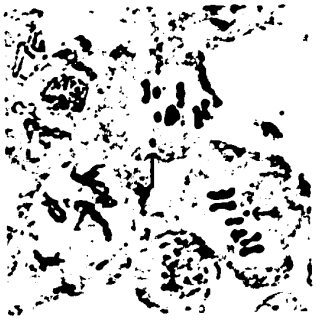


Figure 8

Figure 9

Figure 10

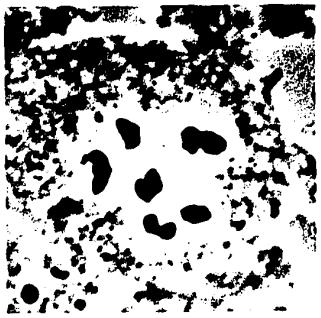


Figure 1

Figure 2

Figure 3

Figure 4

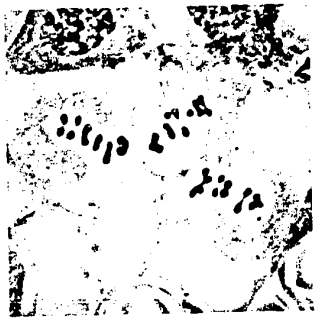


Figure 5

Figure 6

Figure 7

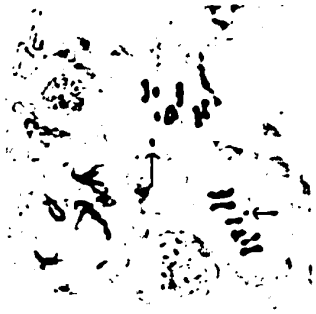


Figure 8

Figure 9

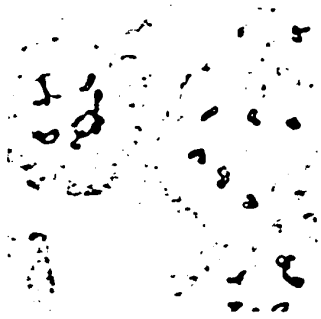


Figure 10

Plate VII

Meiosis in the interspecific diploid hybrids

Figure 1 — Prometaphase in *L. japonicus* x *L. alpinus* showing 6 II's. x ca. 2150

Figure 2 — MI in *L. japonicus* x *L. krylovii* showing 6 II's. x ca. 2000

Figures 3, 4 — MI in *L. krylovii* x *L. filicaulis*.

Fig. 3 — 6 II's. x ca. 1150

Fig. 4 — 3 II's + 6 I's. x ca. 1400

Figure 5 — Prometaphase in *L. japonicus* x *L. schoelleri* showing 6 II's. x ca. 1350

Figure 6 — MI in *L. japonicus* x *L. schoelleri* showing 3 II's + 6 I's. x ca. 1200

Figures 7, 8 — MI in *L. japonicus* x *L. filicaulis*

Fig. 7 — 6 II's. x ca. 850

Fig. 8 — 5 II's + 2 I's. x ca. 1100

Figures 9, 10 — Prometaphase I in *L. krylovii* x *L. schoelleri*.

Fig. 9 — 4 II's + 4 I's. x ca. 1100

Fig. 10 — 5 II's + 2 I's (upper left); 6 II's. x ca. 1400

Figure 1

Figure 2

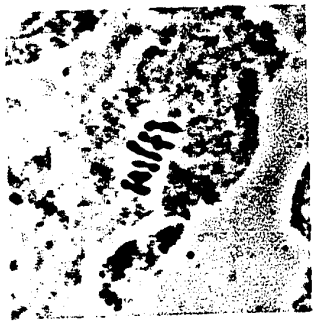


Figure 3

Figure 4

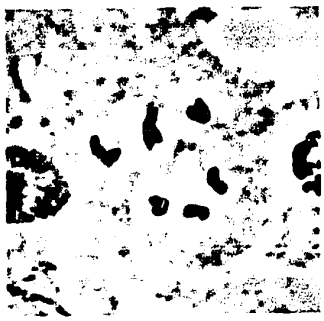


Figure 5

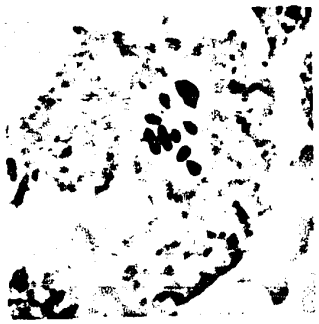


Figure 6

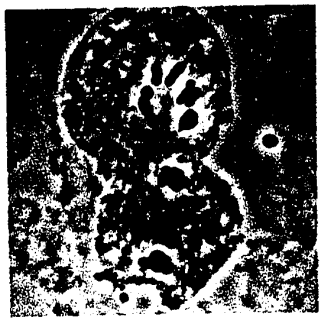


Figure 7

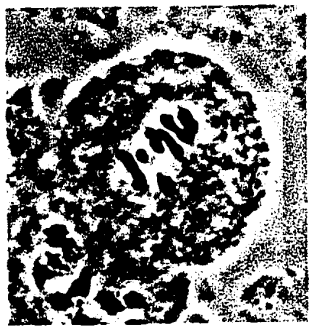


Figure 8

Figure 9

Figure 10

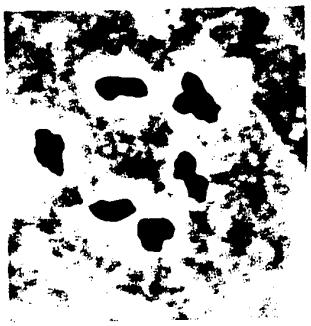


Figure 1

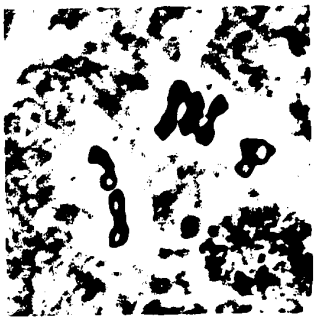


Figure 2

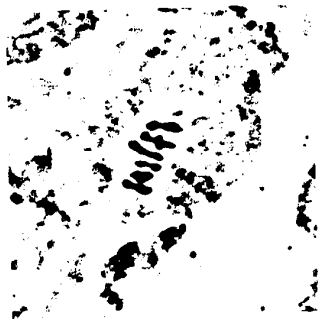


Figure 3

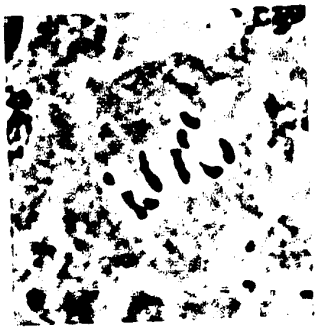


Figure 4

Figure 5

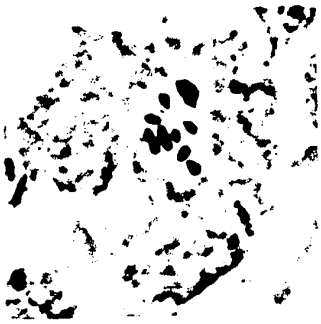


Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Plate VIII

Meiosis in the interspecific diploid hybrids

Figure 1 — *L. japonicus* x *L. corniculatus* var. *minor*. Prometaphase I. 5 II's + 2 I's. x ca. 1350

Figures 2, 3 — Diakinesis in *L. japonicus* x *L. corniculatus* var. *minor*.

Fig. 2 — 5 II's + 1 loose II. x ca. 1370

Fig. 3 — 4 II's + 2 loose II's. x ca. 1330

Figure 4 — MI in *L. corniculatus* var. *minor* x *L. filicaulis* showing 2 II's + 3 loose II's + 2 I's. x ca. 1100

Figure 5 — MI in *L. krylovii* x *L. corniculatus* var. *minor*. 6 II's. x ca. 1100

Figure 6 — MI in *L. krylovii* x *L. corniculatus* var. *minor*. 2 II's + 3 loose II's + 2 I's. x ca. 1100

Figures 7, 8 — MI in *L. filicaulis* x *L. schoelleri*.

Fig. 7 — 6 II's. x ca. 1400

Fig. 8 — 5 II's + 2 I's (right). x ca. 1000

Figure 9 — Early anaphase I in *L. filicaulis* x *L. schoelleri* showing late disjunction of one chromosome. x ca. 1330

Figure 10 — Prometaphase I in *L. filicaulis* x *L. schoelleri*. 4 II's + 2 loose II's. x ca. 1330

Figure 11 — Prometaphase I in *L. corniculatus* var. *minor* x *L. alpinus* showing 4 II's + 2 loose II's. x ca. 1400

Figure 12 — MI in *L. corniculatus* var. *minor* x *L. alpinus*. 4 II's + 4 I's. x ca. 1400

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

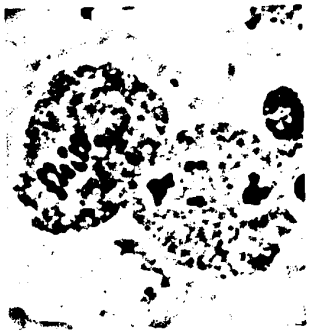


Figure 8

Figure 9

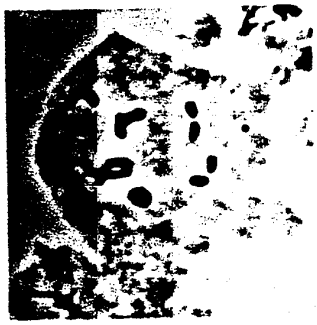


Figure 10

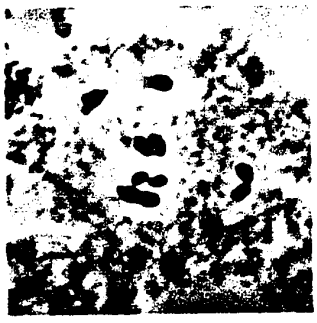


Figure 11

Figure 12

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

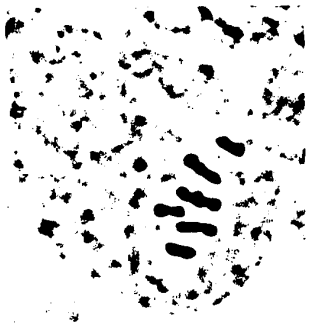


Figure 7

Figure 8

Figure 9

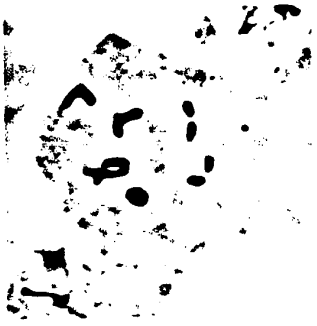


Figure 10

Figure 11

Figure 12

Plate IX

Meiosis in the interspecific diploid hybrids

Figures 1, 2, 3, 4 — Prometaphase I in *L. alpinus* x *L. schoelleri*.

Fig. 1 — 4 II's + 1 IV. x ca. 1150

Fig. 2 — 4 II's + 1 IV. x ca. 1400

Fig. 3 — 4 I's + 2 II's + 1 IV. x ca. 950

Fig. 4 — 4 II's + 1 IV. x ca. 1200

Figure 5 — MI in *L. japonicus* x *L. krylovii* showing 2 I's + 3 II's + 1 IV. x ca. 2100

Figure 6 — Diakinesis in *L. japonicus* x *L. krylovii*. 4 II's + 1 IV. x ca. 1370

Figures 7, 8 — Prometaphase I in *L. corniculatus* var. *minor* x *L. alpinus*

Fig. 7 — 4 II's + 1 IV. x ca. 1600

Fig. 8 — 4 II's + 1 IV. x ca. 1850

Figure 9 — Prometaphase I in *L. krylovii* x *L. filicaulis*. 4 II's + 1 IV. x ca. 1425

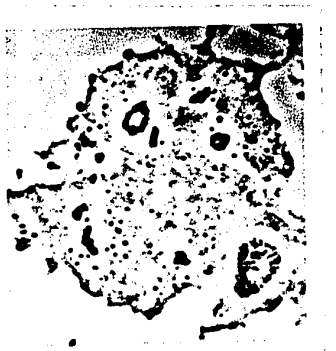
Figure 10 — Diakinesis in *L. krylovii* x *L. filicaulis*. 2 II's + 2 loose II's + 1 IV. x ca. 1400

Figure 11 — Diakinesis in *L. japonicus* x *L. alpinus*. 4 II's + 1 IV. x ca. 1950

Figure 12 — MI in *L. alpinus* x *L. filicaulis* showing 1 I + 4 II's + 1 III. x ca. 1525

Figure 1

Figure 2



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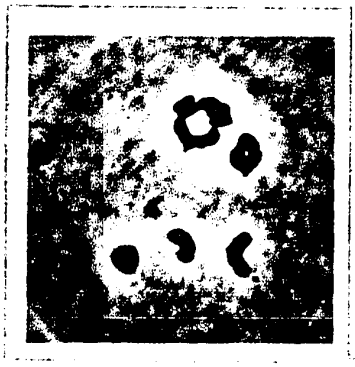

Figure 4

Figure 5

Figure 6

Figure 7

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Figure 9

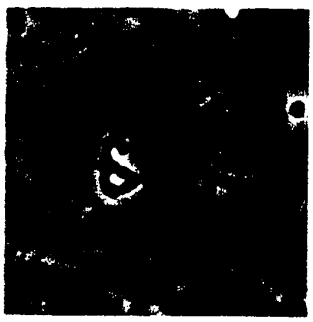


Figure 10

Figure 11

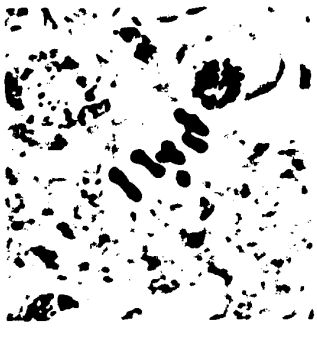


Figure 12

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

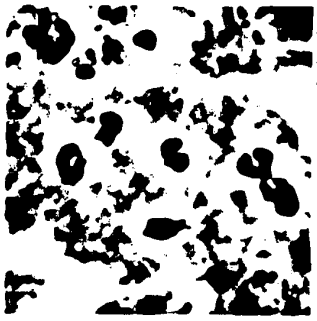
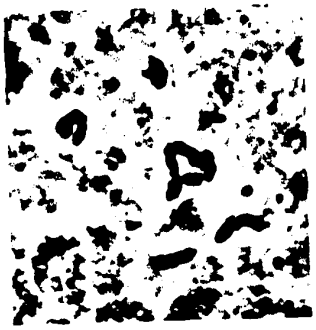


Figure 7

Figure 8



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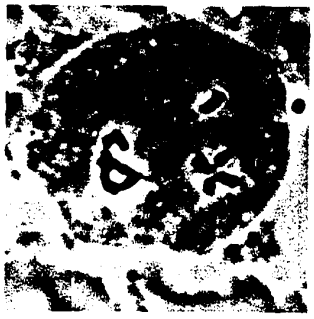


Figure 10

Figure 11

Figure 12

Plate X

Meiosis in the interspecific diploid hybrids

Figures 1-6 AI and TI bridges arising as a result of either stickiness of chromosomes or belated separation of chromosomes.

Fig. 1 *L. corniculatus* var. *minor* x *L. alpinus*. x ca. 2000

Fig. 2 *L. japonicus* x *L. corniculatus* var. *minor*. x ca. 1700

Fig. 3 *L. japonicus* x *L. corniculatus* var. *minor*. x ca. 1300

Fig. 4 *L. alpinus* x *L. schoelleri*. x ca. 665

Fig. 5 *L. krylovii* x *L. filicaulis*. x ca. 1450

Fig. 6 *L. japonicus* x *L. corniculatus* var. *minor*. x ca. 1370

Figures 7, 8 TI bridges arising as a result of failure of chiasma terminalization.

Fig. 7 *L. corniculatus* var. *minor* x *L. alpinus*. x ca. 1900

Fig. 8 *L. japonicus* x *L. alpinus*. x ca. 1330

Figure 9 AI in *L. japonicus* x *L. corniculatus* var. *minor* showing inversion bridge + 1 laggard. x ca. 1600

Figure 10 TI inversion bridge + fragment in *L. japonicus* x *L. krylovii*. x ca. 1370

Figure 11 TI in *L. japonicus* x *L. corniculatus* var. *minor* showing inversion bridge. x ca. 515

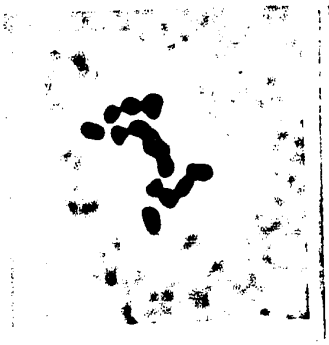


Figure 1

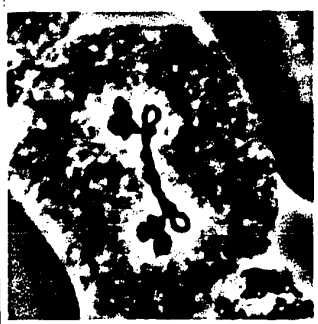


Figure 2

Figure 3

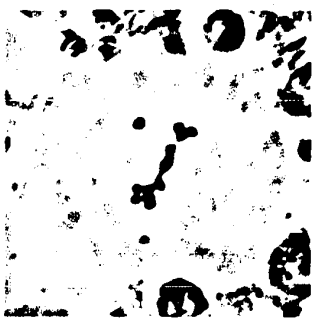


Figure 4

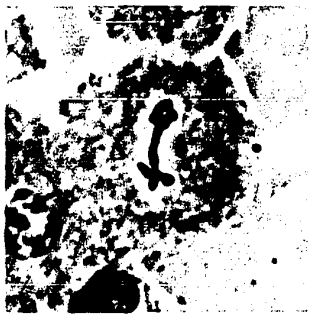


Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

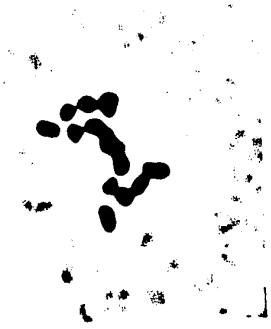


Figure 1

Figure 2

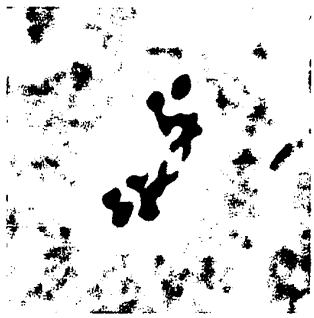


Figure 3

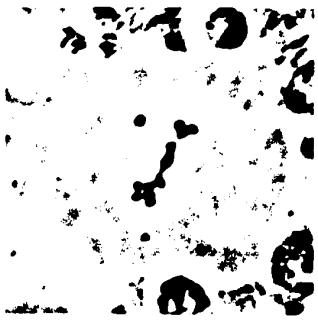


Figure 4

Figure 5

Figure 6

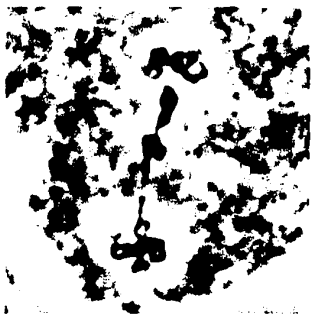


Figure 7

Figure 8

Figure 9

Figure 10

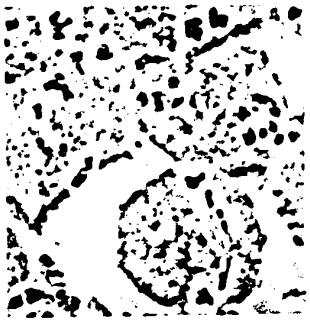


Figure 11

Plate XI

Meiosis in the interspecific diploid hybrids

Figures 1, 2, 3, 4 AI showing late separation of one bivalent.

Fig. 1 *L. japonicus* x *L. krylovii*. x ca. 1750

Fig. 2 *L. japonicus* x *L. alpinus*. x ca. 1330

Fig. 3 *L. alpinus* x *L. krylovii*. x ca. 1400

Fig. 4 *L. alpinus* x *L. filicaulis*, (note the B-chromosome). x ca. 1400

Figure 5 AI in *L. alpinus* x *L. filicaulis* showing the B-chromosome dividing at the equator. x ca. 1400

Figure 6 AI in *L. alpinus* x *L. krylovii* showing normal separation of chromosomes. x ca. 1430

Figure 7 Late AI in *L. alpinus* x *L. filicaulis* showing lagging chromosomes (left) and TI (right). x ca. 900

Figure 8 Early TI in *L. japonicus* x *L. schoelleri* showing lagging chromosomes. x ca. 1330

Figure 9 TI in *L. krylovii* x *L. corniculatus* var. *minor* showing 2 laggards. x ca. 1100

Figure 10 TII in *L. alpinus* x *L. krylovii* showing normal plate (left) and lagging chromosomes (right). x ca. 820

Figure 11 TII in *L. corniculatus* var. *minor* x *L. filicaulis* showing 2 lagging chromosomes. x ca. 1400

Figure 12 AII in *L. krylovii* x *L. filicaulis* showing normal separation (left) and 5 aggregations of chromosomes (right). x ca. 800

Figure 1

Figure 2

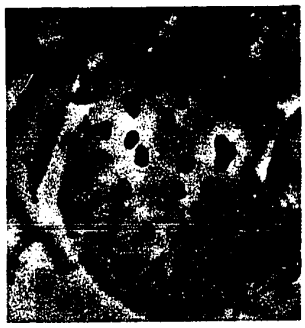


Figure 3

Figure 4

Figure 5

Figure 6

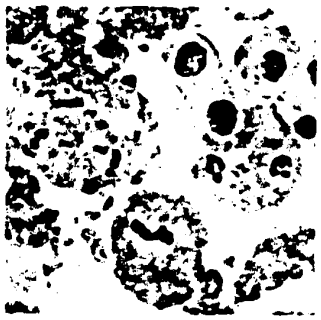


Figure 7

Figure 8

Figure 9

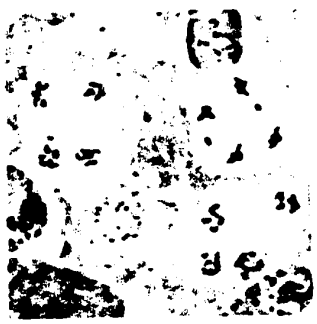


Figure 10

Figure 11

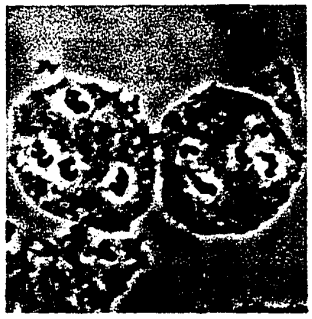


Figure 12

Figure 1

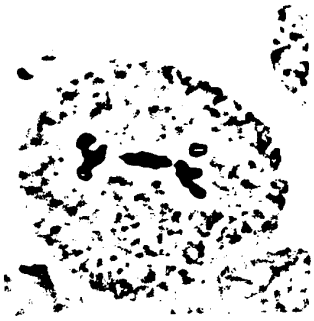


Figure 2

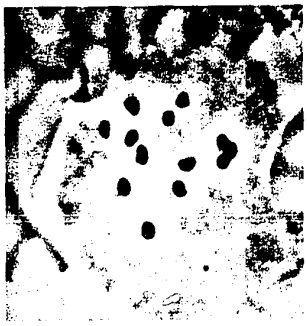


Figure 3

Figure 4

Figure 5

Figure 6

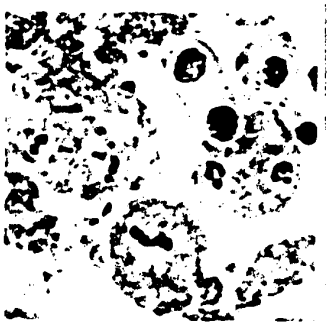


Figure 7

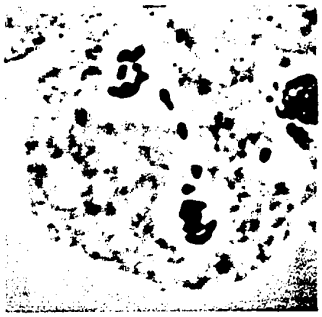


Figure 8

Figure 9

Figure 10

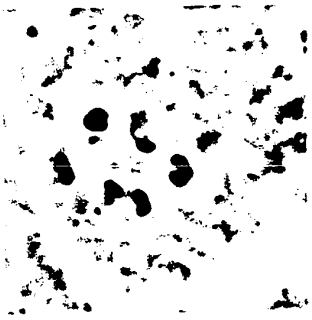


Figure 11

Figure 12

Plate XII

Meiosis in the interspecific diploid hybrids

Figure 1 Late TII showing bridge and lagging chromosome in *L. krylovii* x *L. tenuis*. x ca. 1600

Figure 2 TII in *L. japonicus* x *L. corniculatus* var. *minor* showing inversion bridge. x ca. 1070

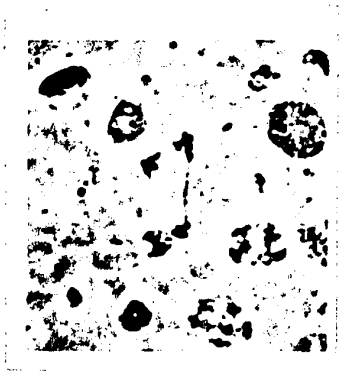
Figure 3 TII in *L. japonicus* x *L. corniculatus* var. *minor* showing (a) inversion bridge + acentric fragment (b) normal telophase. x ca. 666

Figure 4 TII in *L. corniculatus* var. *minor* x *L. alpinus* showing inversion bridge. x ca. 1400

Figure 5 TII bridge in *L. alpinus* x *L. krylovii*. x ca. 1400

Figure 6 Quartet stage in *L. krylovii* x *L. filicaulis* showing failure of bridge separation. x ca. 1700

Figure 1



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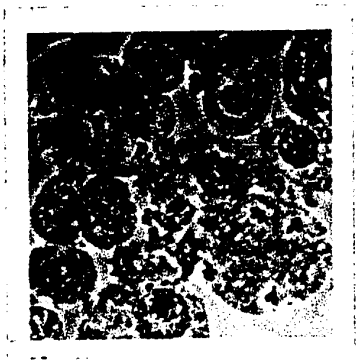


Figure 3

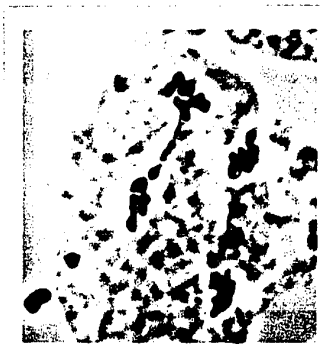


Figure 4

Figure 5

Figure 6

Figure 1

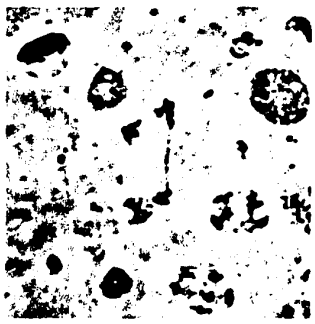


Figure 2

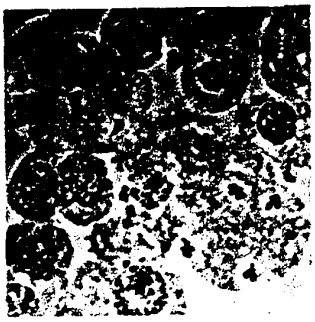


Figure 3

Figure 4

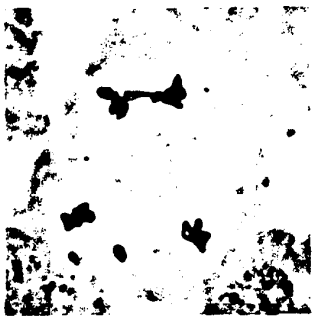


Figure 5

Figure 6

and telophase I and anaphase and telophase II. In L. japonicus x L. corniculatus var. minor, no acentric fragments were observed at the second division stages. No fragments were detected in bridges containing cells of L. japonicus x L. schoelleri and L. krylovii x L. filicaulis. These dicentric chromatid bridges and associated fragments indicate that these hybrids were heterozygous for inversions. The characteristic configurations seen in some of these anaphase plates indicate that chromosome stickiness, belated separation of chromosomes and failure of chiasma terminalization were other factors contributing to the presence of these bridges (see Plate X). For example, the chromosomes of certain bivalents had difficulty in separating and as a result they lagged behind their counterparts at anaphase. The configuration of such belated separation was quite different from inversion bridges (see Plate X, Figures 4 to 6). Also failure of chiasma terminalization caused the arms of affected chromosomes to become noticeably stretched into thin strands; in such bridges the point of attachment of the two chromosomes was usually distinct and served to identify these types of bridges (Plate X, Figures 7 and 8). Photomicrographs of the various types of chromosome anomalies encountered in meiocytes of the hybrids at anaphase I and II and telophase I and II are shown in Plates X, XI, and XII.

Meiosis in the autotetraploids

The data concerning chromosome behavior during meiosis in the

induced autotetraploids of L. alpinus, L. japonicus, L. tenuis, L. filicaulis, L. schoelleri, L. krylovii and L. corniculatus var. minor are presented in Tables 26 and 27. It can be seen that univalents, bivalents, trivalents and quadrivalents occurred in the meiocytes of all these autotetraploids. Except for L. tenuis (4x) which had a mean number of univalents per cell of 1.34, the frequency of unpaired chromosomes in the remaining autotetraploids did not vary to any great extent between these induced species and did not exceed a mean of 3.96 per cell (L. filicaulis, 4x). The frequency of sporocytes with univalents in the derived tetraploids was considerably higher than that in the corresponding diploids. The number of bivalents observed was unexpectedly high and there was no great variation in the frequency of bivalents in the seven different autotetraploids. L. japonicus (4x) had the highest number of bivalents per cell (a mean value of 9.11) and L. filicaulis (4x) exhibited the lowest mean value (8.37). The preponderance of rod bivalents indicate that a chiasma was formed in one chromosome arm most of the time. Multivalent configurations consisting of trivalents and quadrivalents were observed in all the autotetraploids. Trivalents were usually seen in the same cells as univalents. Apparently, one of the four homologous chromosomes occasionally failed to synapse with one of its three homologues. The trivalents appeared most frequently as a chain of three chromosomes; the frying-pan and the Y-shaped configurations were seldom observed. The frequency of quadrivalents was low in all the autotetraploids studied. It can be

seen in Table 26, that the mean number of quadrivalents per cell ranged from 0.57 (L. japonicus, 4x) to 1.10 (L. tenuis, 4x). These values corresponded to 9.55 and 18.35 per cent quadrivalents, respectively. Inspection of Table 26 also shows that there was variability with regards to the frequency of quadrivalents in the genotypes of L. japonicus (4x), L. filicaulis (4x), and L. schoelleri (4x). In L. japonicus (4x) one genotype (T-129-1) had 0.38 quadrivalents per cell whereas in the other (T-129-2) a mean number of 0.70 was recorded. In L. filicaulis (4x), the two genotypes T-37-2 and T-37-9 displayed 0.82 and 0.63 quadrivalents per cell, respectively. In the genotypes T-166-1 and T-166-2 of L. schoelleri (4x), 0.87 and 1.08 quadrivalents per cell, respectively, were detected. From the different types of quadrivalents observed, symmetrical types, that is, simple chains, rings and figures of 8, were found to be the most frequent (Plates XIII and XIV). A few zigzag and N-shaped configurations were recorded also. Generally, the large and medium chromosomes tended to form quadrivalents more often than the smaller chromosomes.

Meiotic irregularities manifested as lagging chromosomes and anaphase bridges were seen at both anaphase I and II and telophase I and II. Except for L. alpinus (4x) and L. japonicus (4x), the number of cells scored as normal was always greater at the first-division stages. Laggards ranging from 1-4 per cell, were detected both at anaphase I and II (Plate XV, Figures 1 to 3). The percentages of cells with these entities were generally greater at anaphase II than

at anaphase I. Two derived tetraploids, L. alpinus (4x) and L. japonicus (4x), however, had a greater number of cells with laggards at anaphase I with 33.00 and 32.14 per cent, respectively. At anaphase II, in L. japonicus (4x) 10.71 per cent of the cells and in L. alpinus (4x) 12.75 per cent of the cells contained lagging chromosomes. It is interesting to note that these two autotetraploids displayed the lowest number of cells with these "abnormal" chromosomes. The lagging chromosomes were either divided as monads or undivided as dyads. The number of meiocytes in the autotetraploids with these types of chromosomes at anaphase I and II were significantly greater than that observed in their corresponding diploids (Tables 22 and 27).

Anaphase bridges were occasionally seen in certain autotetraploids. In L. alpinus (4x) and L. schoelleri (4x) bridges were detected at anaphase I and II whereas in L. japonicus (4x) and L. tenuis (4x), these configurations were noticed at anaphase II only. The characteristic appearance of these anaphase bridges indicate that they could have arisen as a result of stickiness of chromosomes or failure of certain quadrivalents to separate at anaphase I. One to several micronuclei occurred in a percentage of quartet groups in all the autotetraploids. On the whole, there appeared to be a correlation between the number of anaphase II cells with laggards and the percentage of quartets with micronuclei. For example, L. filicaulis (4x) which exhibited 48.84 per cent of anaphase II cells with lagging chromosomes had a high frequency of quartets with micronuclei. L. alpinus (4x), on the other hand, which had 10.71 per cent of anaphase II cells with lagging chromosomes

TABLE 26. Diakinesis and/or Metaphase I chromosome behavior of the autotetraploids

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
				L. alpinus (4x)					
T-77-1	60	3.28 (0 - 8)	8.88 (3 - 11)	0.12 (0 - 2)	0.65 (0 - 3)	13.68	74.03	1.46	10.83
				L. japonicus (4x)					
T-129-1	36	3.11 (0 - 8)	9.44 (7 - 11)	0.10 (0 - 1)	0.38 (0 - 1)	12.96	78.70	1.39	6.94
T-129-2	46	2.93 (0 - 8)	8.85 (4 - 12)	0.20 (1 - 2)	0.70 (0 - 2)	12.23	73.73	2.45	11.59
Total	82	3.01 (0 - 8)	9.11 (4 - 12)	0.16 (1 - 2)	0.57 (0 - 2)	12.55	75.91	1.98	9.55
				L. tenuis (4x)					
B-340-1	79	1.65 (0 - 6)	8.92 (4 - 11)	0.10 (0 - 1)	1.05 (0 - 4)	6.86	74.37	1.27	17.51
B-340-2	40	0.75 (0 - 4)	9.08 (2 - 12)	0.10 (0 - 1)	1.20 (0 - 5)	3.13	75.63	1.25	20.00
Total	119	1.34 (0 - 6)	8.97 (2 - 12)	1.10 (0 - 1)	1.10 (0 - 5)	5.60	74.79	1.26	18.35

Table 26 Cont'd

TABLE 26 (Cont'd)

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
				L. filicaulis (4x)					
T-37-2	65	3.65 (0 - 10)	8.38 (3 - 12)	0.11 (0 - 1)	0.82 (0 - 3)	15.19	69.87	1.35	13.59
T-37-9	35	4.54 (0 - 10)	8.34 (5 - 12)	0.09 (0 - 1)	0.63 (0 - 2)	18.93	65.92	1.07	10.48
Total	100	3.96 (0 - 10)	8.37 (3 - 12)	0.10 (0 - 1)	0.75 (0 - 3)	16.50	69.75	1.25	12.50
				L. schoelleri (4x)					
T-166-1	30	3.50 (0 - 8)	8.37 (3 - 11)	0.10 (0 - 1)	0.87 (0 - 3)	14.58	69.72	1.25	14.44
T-166-2	50	2.32 (0 - 8)	8.56 (5 - 12)	0.08 (0 - 1)	1.08 (0 - 3)	9.67	71.33	1.00	18.00
Total	80	2.76 (0 - 8)	8.49 (3 - 12)	0.09 (0 - 1)	1.00 (0 - 3)	11.51	70.73	1.09	16.67

Table 26 Cont'd

TABLE 26 (Cont'd)

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
				L. krylovii (4x)					
T-86-1	50	3.04 (0 - 6)	8.84 (6 - 11)	0.16 (0 - 2)	0.70 (0 - 3)	12.67	73.67	2.00	11.67
T-86-2	20	2.55 (0 - 5)	9.30 (5 - 11)	0.15 (0 - 1)	0.60 (0 - 3)	10.63	77.50	1.88	10.00
Total	70	2.90 (0 - 6)	8.97 (5 - 11)	0.16 (0 - 2)	0.69 (0 - 3)	12.08	74.76	1.96	11.19
				L. corniculatus var. *minor* (4x)					
T-303-1	30	3.43 (0 - 8)	8.77 (5 - 11)	0.17 (0 - 1)	0.63 (0 - 3)	14.31	73.06	2.08	10.56

TABLE 27. Meiotic chromosome behavior at AI--TI and AII--TII in autotetraploids

Genotypes	Anaphase I--Telophase I							Anaphase II-Telophase II						
	No. of cells	Normal		Bridges		Laggards		No. of cells	Normal		Bridges		Laggards	
		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%
L. alpinus (4x)														
T-77-1	100	66	66.00	1	1.00	33	33.00	28	22	78.57	3	10.71	3	10.71
L. japonicus (4x)														
T-129-1	140	95	67.86	-	-	45	32.14	102	85	83.33	4	3.92	13	12.75
L. tenuis (4x)														
B-340-2	205	140	68.29	-	-	65	31.71	117	74	63.25	2	1.71	41	35.04
L. filicaulis (4x)														
T-37-2	75	53	70.67	-	-	22	29.33	43	22	51.16	-	-	21	48.84
L. schoelleri (4x)														
T-166-1	106	73	68.87	1	0.94	32	30.19	118	73	61.86	4	3.39	41	34.75
L. krylovii (4x)														
T-86-1	100	78	78.00	-	-	22	22.00	36	22	61.11	-	-	14	38.89
L. corniculatus var. *minor* (4x)														
T-303-1	109	88	80.73	-	-	21	19.27	59	46	77.97	-	-	13	22.03

was characterized by a low frequency of micronuclei in the quartets.

Microphotographs showing meiotic chromosome behavior in these induced autotetraploids are shown in Plates XIII, XIV and XV.

Meiosis in Lotus corniculatus

In tetraploid L. corniculatus (4x) meiosis was studied in 230 microspore cells. The results of the observations are summarized in Tables 28 and 29. Diakinesis and metaphase I plates in this cultivated species were characterized by a high degree of bivalent pairing; twelve bivalents and less frequently eleven bivalents plus two univalents were detected in many cells. A mean of 10.97 bivalents per cell was recorded. The high degree of bivalent association is also reflected in the high percentage of chromosomes (91.38) that formed bivalents. Both rod and ring bivalents were seen; however, the frequency of ring bivalents was considerably lower and they were invariably present at early diakinesis only. In addition to bivalents there were univalents, trivalents and quadrivalents also present; the mean values recorded for these were 1.03, 0.02 and 0.24, respectively. A noteworthy point is that only 4.06 per cent of the chromosomes were associated as quadrivalents and these generally appeared as closed rings or simple chains (Plate XIV, Figures 1 to 4), that is symmetrical types.

Lagging chromosomes were observed at anaphase I and II in 11.92 and 18.92 per cent of the cells, respectively (Table 29), but no bridges were observed in either division. Quartets with one or more

TABLE 28. Diakinesis and/or Metaphase I chromosome behavior of Lotus corniculatus

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
				L. corniculatus (4x)					
B-554-1	106	0.94 (0 - 4)	11.04 (9 - 12)	0.04 (0 - 1)	0.22 (0 - 1)	3.93	91.98	0.47	3.62
B-554-2	34	0.82 (0 - 4)	11.06 (9 - 12)	-	0.26 (0 - 1)	3.43	92.16	-	4.41
B-554-3	90	1.21 (0 - 4)	10.84 (8 - 12)	0.01 (0 - 1)	0.27 (0 - 2)	5.05	90.37	0.14	4.44
Total	230	1.03 (0 - 4)	10.97 (8 - 12)	0.02 (0 - 1)	0.24 (0 - 2)	4.29	91.38	0.27	4.06

TABLE 29. Meiotic chromosome behavior at AI--TI and AII--TII in L. corniculatus

Genotypes	Anaphase I--Telophase I*				Anaphase II--Telophase II*				
	No. of cells	Normal		Laggards		No. of cells	Normal		Laggards	
		No.	%	No.	%		No.	%	No.	%
				L. corniculatus (4x)						
B-554-1	144	124	86.11	20	13.89	214	185	86.45	29	13.55
B-554-2	115	105	91.30	10	8.70	105	85	80.95	20	19.05
B-554-3	110	96	87.27	14	12.73	125	90	72.00	35	28.00
Total	369	325	88.08	44	11.92	444	360	81.08	84	18.92

* No bridges were observed

Plate XIII

Meiosis in the induced autotetraploids

Figure 1 — MI in L. japonicus (4x) showing 4 I's + 8 II's + 1 IV. x ca. 1600

Figures 2-4 — MI in L. filicaulis (4x).

Fig. 2 — 2 I's + 7 II's + 2 IV's. x ca. 1350

Fig. 3 — 8 I's + 4 II's + 2 IV's. x ca. 1267

Fig. 4 — 2 I's + 9 II's + 1 IV. x ca. 1430

Figure 5 — Early AI in L. filicaulis (4x) showing asynchronous separation of chromosomes. x ca. 1500

Figures 6, 7 — Diakinesis in L. krylovii (4x).

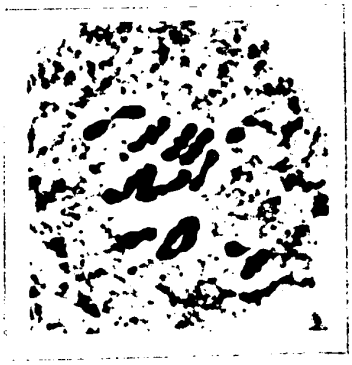
Fig. 6 — 1 I + 10 II's + 1 III. x ca. 1150

Fig. 7 — 5 II's + 2 III's + 2 IV's. x ca. 1500

Figure 8 — Prometaphase I in L. schoelleri (4x) 1 I + 8 II's + 1 III + 1 IV. x ca. 1383

Figure 9 — Diakinesis in L. schoelleri (4x) showing 2 I's + 7 II's + 1 IV. x ca. 1700

Figure 1



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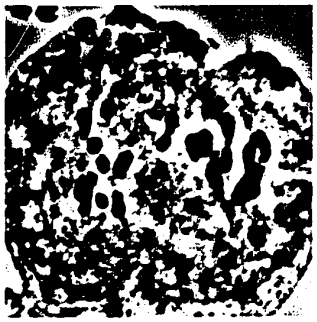
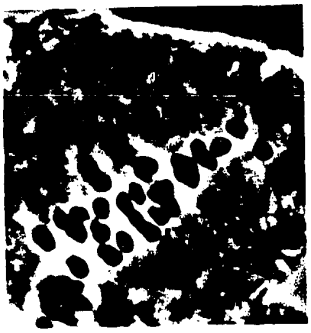


Figure 3

Figure 4



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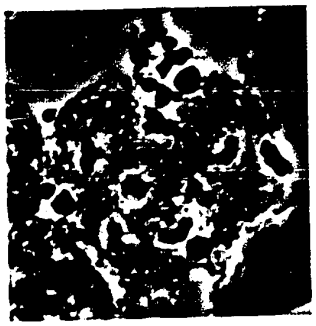

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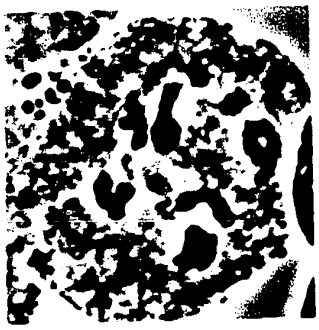


Figure 7

Figure 8

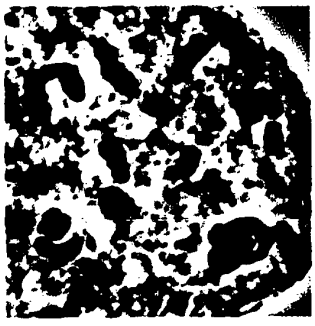
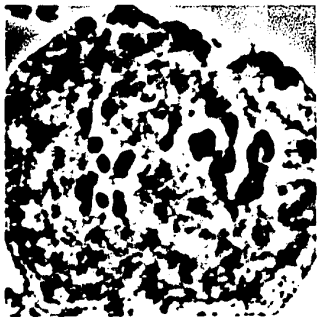


Figure 9

Figure 1

Figure 2



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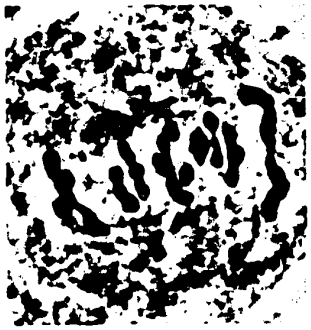
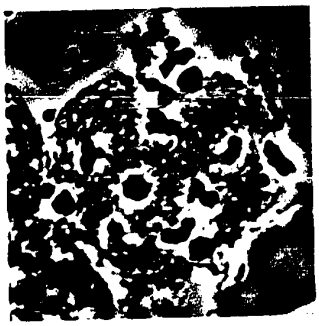


Figure 4

Figure 5



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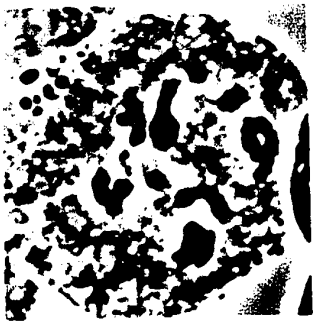


Figure 7

Figure 8

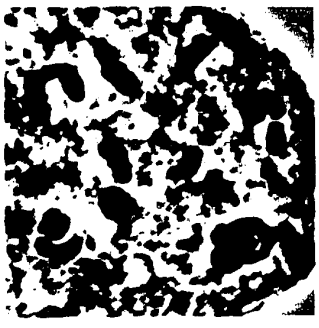


Figure 9

Plate XIV

Meiosis in L. corniculatus (4x) and in the autotetraploids

Figure 1 Prometaphase I in L. corniculatus showing 12 II's. x ca. 1150

Figures 2-5 Diakinesis in L. corniculatus

Fig. 2 2 I's + 1 III (upper cell). x ca. 755

Fig. 3 2 I's + 9 II's + 1 IV. x ca. 1600

Fig. 4 10 II's + 1 IV. x ca. 1550

Fig. 5 1 I + 10 II's + 1 III. x ca. 1200

Figures 6-8 MI in L. tenuis (4x)

Fig. 6 2 II's + 5 IV's. x ca. 2100

Fig. 7 6 II's + 3 IV's. x ca. 1800

Fig. 8 8 II's + 2 IV's. x ca. 1800

Figure 9 MI in L. alpinus (4x) showing 2 I's + 9 II's + 1 IV. x ca. 1375

Figure 10 Diakinesis in L. alpinus (4x) showing 4 I's + 8 II's + 1 IV. x ca. 1267

Figure 11 Prometaphase in L. japonicus (4x). 4 I's + 8 II's + 1 IV. x ca. 1500

Figure 12 MI in L. japonicus (4x) showing 8 II's + 8 I's. x ca. 2150

Figure 1

Figure 2

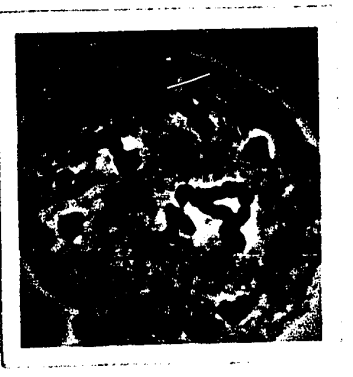


Figure 3

Figure 4

Figure 5

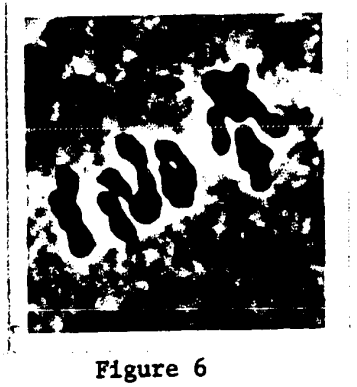


Figure 6

Figure 7

Figure 8

Figure 9

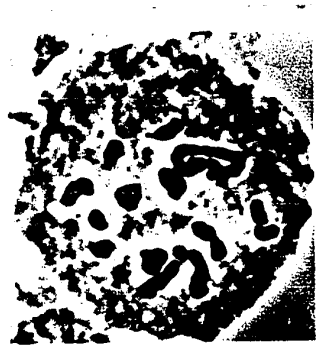


Figure 10

Figure 11

Figure 12

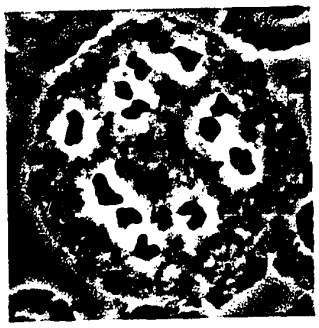
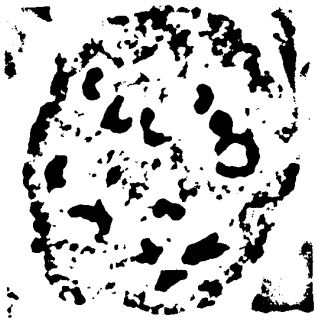


Figure 1

Figure 2

Figure 3



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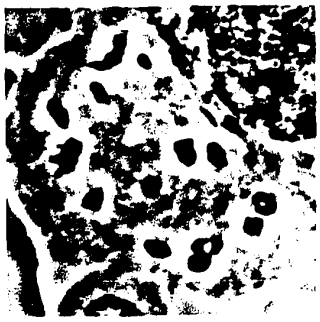


Figure 5

Figure 6

Figure 7

Figure 8

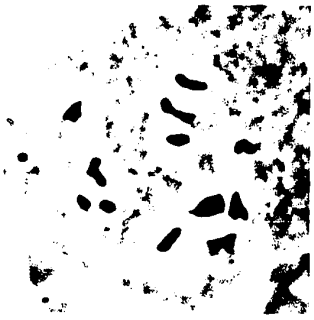


Figure 9

Figure 10

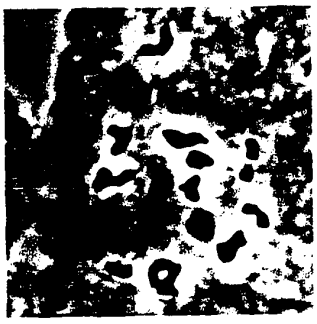


Figure 11

Figure 12

Plate XV

Meiosis in L. corniculatus (4x) and the autotetraploids

Figure 1 AI in L. tenuis (4x) showing one lagging chromosome. x ca. 1283

Figure 2 TI in L. alpinus (4x) showing late separation of chromosomes. x ca. 1375

Figure 3 TII in L. alpinus (4x) showing (a) normal plate (b) laggards. x ca. 1000

Figure 4 TI in L. tenuis (4x) showing (a) normal division (b) lagging chromosome not incorporated into the restitution nucleus (c) TII bridge. x ca. 406

Figures 5-8 TII bridges lagging chromosomes.

Fig. 5 L. japonicus (4x). x ca. 1350

Fig. 6 L. japonicus (4x). x ca. 875

Fig. 7 L. alpinus (4x). x ca. 1373

Fig. 8 L. schoelleri (4x). x ca. 863

Figure 9 Quartet stage in L. schoelleri (4x) showing 2 micronuclei. x ca. 1333

Figure 10 Quartet stage in L. filicaulis (4x) showing 8 micronuclei. x ca. 1650

Figure 11 Quartet stage in L. corniculatus (4x) showing (a) cell with normal 4 nuclei and (b) a cell with 5 nuclei. x ca 850

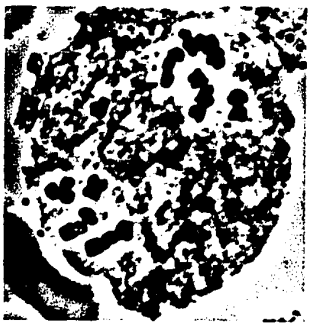


Figure 1

Figure 2

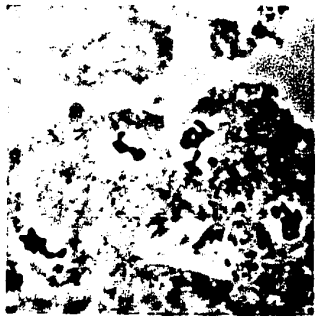


Figure 3

Figure 4

Figure 5

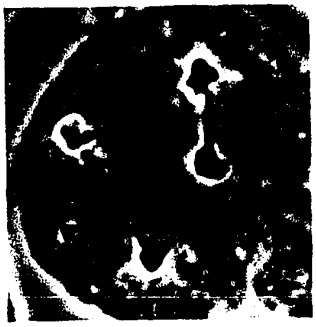


Figure 6

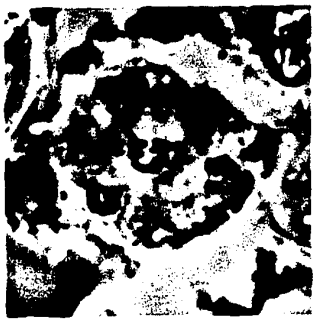
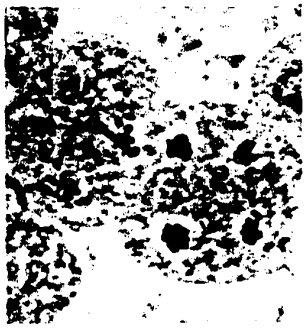


Figure 7



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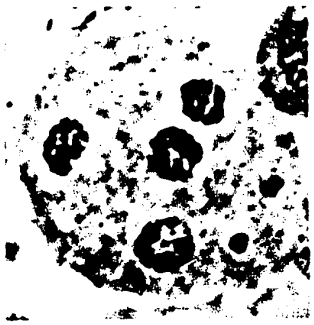


Figure 9

Figure 10

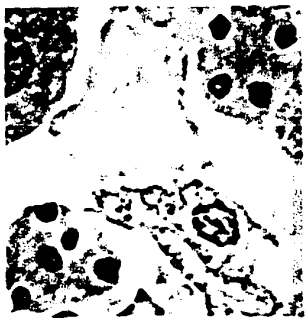


Figure 11

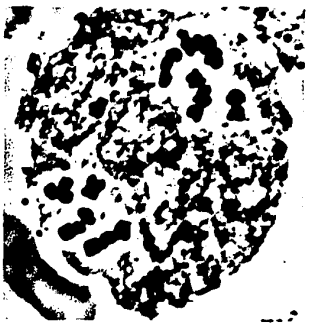


Figure 1

Figure 2

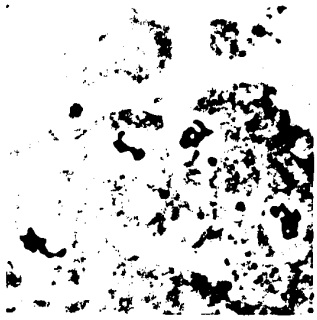


Figure 3

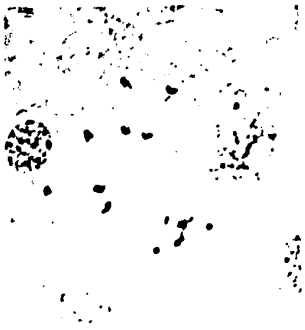


Figure 4

Figure 5

Figure 6

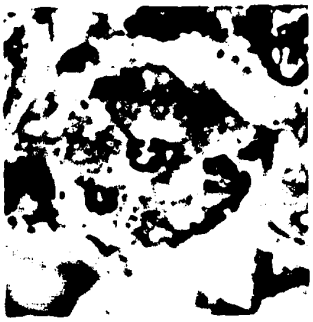


Figure 7

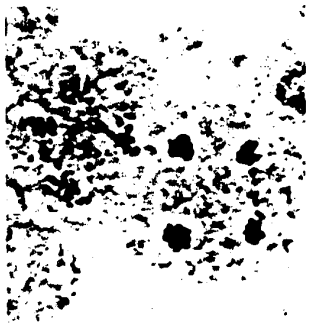


Figure 8

Figure 9

Figure 10

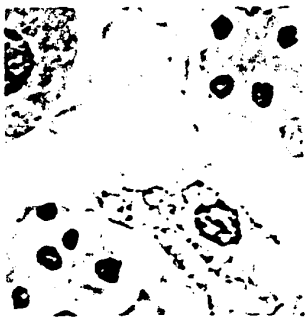


Figure 11

micronuclei occurred in about 12 per cent of the cells. The pairing behavior of the chromosomes of L. corniculatus are shown in Plate XIV, Figures 1 to 5.

Meiosis in the amphidiploids

An analysis of the meiotic chromosome behavior was carrried out on 22 genotypes representing 12 synthetic amphidiploids produced during the course of this study. The cytological data are presented in Tables 30 and 31. One of these amphidiploids (L. alpinus x L. krylovii), seemed primarily desynaptic and its meiocytes were characterized by a high occurrence of univalents (65.89 per cent) and relatively low frequency of bivalents (31.35 per cent) and quadrivalents (1.04 per cent). Photomicrographs of meiocytes of this amphidiploid are shown in Plate XVI, Figures 9 to 11. As many as 24 unpaired chromosomes were recorded in some pollen mother cells at diakinesis and metaphase I in this desynaptic plant (Table 30). Meiotic behavior in the remaining induced alloploids was more "normal" and univalents, bivalents, and quadrivalents were present in the PMC's examined. Univalents which occurred in some cases appeared to have resulted from the precocious separation of bivalents, the separated univalents lying opposite each other on either side of the equatorial plate. These were scored as separate univalent chromosomes. Occasionally as many as 12 unpaired chromosomes were observed, for example, in certain genotypes of (L. corniculatus var. minor x L. alpinus) and (L. japonicus x L. schoelleri). The frequency of univalents varied

slightly between the genotypes of an amphidiploid [for example in (L. japonicus x L. alpinus)], and from amphidiploid to amphidiploid. The mean number of univalents per cell ranged from 1.83 in (L. japonicus x L. alpinus) to 3.03 in (L. corniculatus var. minor x L. alpinus) (Table 30). A relatively high frequency of bivalents was detected in the sporocytes of all the amphidiploids [except the asynaptic (L. alpinus x L. krylovii)]. Although sometimes as few as five bivalents were seen in some cells [for example some genotypes of (L. alpinus x L. filicaulis) and (L. japonicus x L. schoelleri)], the percentage of chromosomes with this type of association was never below 78. Twelve bivalents, or eleven bivalents plus two univalents, were frequently recorded in many cells at diakinesis and metaphase I in all of the amphidiploids (Plate XVI, Figures 4 and 5). The mean number of bivalents per cell ranged from 9.40 in (L. alpinus x L. filicaulis) to 10.51 in (L. filicaulis x L. schoelleri). The bivalents were generally rod shaped; ring shaped configurations were rarely seen and the chiasmata were usually terminal. Loose bivalents constituted a very small percentage of the total bivalents and this type of pairing was detected somewhat more often at diakinesis. Multivalent configurations were also observed in all the amphidiploids. Except for (L. alpinus x L. schoelleri), (L. japonicus x L. corniculatus var. minor) and (L. krylovii x L. filicaulis) in which only quadrivalents were seen, the meiocytes of the other induced alloploids had both trivalents and quadrivalents present. The number of trivalents per cell was seldom greater than 2 and the percentage chromosomes associated as trivalents

TABLE 30. Diakinesis and/or Metaphase I chromosome behavior of the amphidiploids

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
				(*L. alpinus* X *L. schoelleri*) (4x)					
A/AS-2	119	2.07 (0 - 6)	10.38 (7 - 12)	-	0.29 (0 - 2)	8.61	86.48	-	4.90
				(*L. alpinus* X *L. filicaulis*) (4x)					
A/AF-2	39	3.03 (0 - 10)	9.26 (5 - 12)	0.21 (0 - 2)	0.46 (0 - 2)	12.61	77.14	2.56	7.69
A/AF-3	49	2.57 (0 - 10)	9.39 (6 - 12)	0.04 (0 - 1)	0.63 (0 - 3)	10.71	78.23	0.51	10.54
A/AF-4	30	2.53 (0 - 8)	9.60 (6 - 12)	0.13 (0 - 1)	0.47 (0 - 2)	10.56	80.00	1.67	7.78
Total	118	2.73 (0 - 10)	9.40 (5 - 12)	0.12 (0 - 2)	0.53 (0 - 3)	11.30	78.32	1.48	8.90
				(*L. alpinus* X *L. krylovii*) (4x)					
A/AK-7	80	15.81 (8 - 24)	3.76 (0 - 8)	0.14 (0 - 1)	0.06 (0 - 1)	65.89	31.35	1.72	1.04

Table 30 Cont'd

TABLE 30 (Cont'd)

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
				(*L. japonicus* X *L. alpinus*) (4x)					
A/JA-1	42	2.12 (0 - 6)	9.81 (7 - 12)	0.02 (0 - 1)	0.55 (0 - 2)	8.83	81.75	0.30	9.13
A/JA-2	32	1.34 (0 - 6)	9.72 (6 - 12)	0.03 (0 - 1)	0.78 (0 - 2)	5.60	80.99	0.39	13.02
A/JA-10	18	1.89 (0 - 4)	9.83 (8 - 12)	-	0.61 (0 - 2)	7.87	81.94	-	10.19
Total	92	1.83 (0 - 6)	9.78 (6 - 12)	0.02 (0 - 1)	0.64 (0 - 2)	7.52	81.52	0.27	10.69
				(*L. corniculatus* var. *minor* X *L. alpinus*) (4x)					
A/MA-3-1	50	3.14 (0 - 10)	9.68 (6 - 12)	0.02 (0 - 1)	0.36 (0 - 1)	13.08	80.67	1.25	6.00
A/MA-3-2	25	2.88 (0 - 12)	9.80 (6 - 12)	0.08 (0 - 1)	0.32 (0 - 1)	12.00	81.67	1.00	5.33
Total	75	3.03 (0 - 12)	9.72 (6 - 12)	0.04 (0 - 1)	0.35 (0 - 1)	12.72	81.00	0.50	5.78
				(*L. corniculatus* var. *minor* X *L. filicaulis*) (4x)					
A/MF-2-3	57	3.00 (0 - 8)	9.96 (7 - 12)	0.05 (0 - 1)	0.23 (0 - 2)	12.50	83.04	0.66	3.80

Table 30 Cont'd

TABLE 30 (Cont'd)

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
		(*L. japonicus* X *L. corniculatus* var. *minor*) (4x)							
A/JM-2-2	30	2.93 (0 - 10)	9.60 (7 - 12)	-	0.47 (0 - 1)	12.22	80.00	-	7.78
A/JM-4-1	40	2.50 (0 - 6)	9.95 (7 - 12)	-	0.40 (0 - 1)	10.42	82.92	-	6.67
Total	70	2.74 (0 - 10)	9.80 (7 - 12)	-	0.43 (0 - 1)	11.19	81.67	-	7.14
		(*L. filicaulis* X *L. schoelleri*) (4x)							
A/FS-2	100	1.58 (0 - 6)	10.51 (8 - 12)	-	0.35 (0 - 1)	6.58	87.58	-	5.83
		(*L. japonicus* X *L. schoelleri*) (4x)							
A/JS-3-4	50	2.36 (0 - 8)	9.80 (5 - 12)	0.04 (0 - 1)	0.48 (0 - 2)	9.83	81.67	0.50	8.00
A/JS-3-6	30	2.93 (0 - 12)	9.87 (6 - 12)	-	0.33 (0 - 1)	12.22	82.22	-	5.56
A/JS-3-7	43	2.28 (0 - 6)	9.70 (6 - 12)	-	0.58 (0 - 2)	9.50	80.81	-	9.69
Total	123	2.47 (0 - 12)	9.78 (5 - 12)	0.02 (0 - 1)	0.48 (0 - 2)	10.30	81.50	0.20	7.99

Table 30 Cont'd

TABLE 30 (Cont'd)

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
				(L. japonicus X L. krylovii) (4x)					
A/JK-1	60	2.58 (0 - 8)	9.67 (6 - 12)	0.05 (0 - 1)	0.48 (0 - 2)	10.76	80.56	0.63	8.06
				(L. krylovii X L. filicaulis) (4x)					
A/KF-2-7	64	3.19 (0 - 10)	9.97 (7 - 12)	-	0.22 (0 - 1)	13.28	83.07	-	3.65
A/KF-2-5	70	2.63 (0 - 6)	10.20 (8 - 12)	-	0.24 (0 - 1)	10.95	85.00	-	4.05
Total	134	2.90 (0 - 10)	10.10 (7 - 12)	-	0.23 (0 - 1)	12.06	84.08	-	3.86
				(L. krylovii X L. schoelleri) (4x)					
A/KS-1	23	2.52 (0 - 6)	10.22 (8 - 12)	-	0.26 (0 - 1)	10.51	85.14	-	4.35
A/KS-7	32	2.81 (0 - 8)	9.88 (8 - 12)	0.06 (0 - 1)	0.31 (0 - 1)	11.72	82.29	0.78	5.21
Total	55	2.69 (0 - 8)	10.02 (8 - 12)	0.04 (0 - 1)	0.29 (0 - 1)	11.21	83.48	0.45	4.85

was not greater than 1.72 [for example, in (L. alpinus x L. krylovii)]. Most of the trivalents formed chains, usually two chromosomes closely associated together with the third one only loosely paired with them (Plate XVII, Figure 4). Other configurations less frequently found were the Y-shaped and frying-pan types. Quadrivalents generally varied from 0 to 2 per cell. In a few meiocytes of the amphidiploid (L. japonicus x L. alpinus) as many as 3 quadrivalents were detected in a cell. Excluding the desynaptic (L. alpinus x L. krylovii) the mean number of quadrivalents per cell varied from 0.23 in (L. corniculatus var. minor x L. filicaulis) to 0.64 in (L. japonicus x L. alpinus). Such a variability in the frequency of quadrivalents was also exhibited between certain genotypes of an amphidiploid. For example, in the amphidiploid (L. alpinus x L. filicaulis) means from 0.46 to 0.63 quadrivalents per cell were noted for the genotypes studied; in (L. japonicus x L. alpinus), for A/JA-1, A/JA-2 and A/JA-10, means of 0.55, 0.78 and 0.61, respectively were recorded; in (L. japonicus x L. schoelleri), A/JS-3-4 had 0.48, A/JS-3-6, 0.33 and A/JS-3-7, 0.58 quadrivalents per cell. Various types of quadrivalents occurred such as zigzag, open ring, closed ring, frying pan, chains of 4 chromosomes and N-shaped configurations (Plates XVI, XVII and XVIII). Of these, the closed ring, the open ring and the chain types were most frequently seen.

The cytological behavior of meiotic chromosomes of these amphidiploids were also investigated during the post-metaphase I stages. No suitable preparations of anaphase II and telophase II stages were obtained for (L. alpinus x L. schoelleri) and (L. alpinus x L. krylovii).

In the latter amphidiploid, 84 cells were studied at anaphase I and only 9.2 per cent of these were recorded as normal. Lagging chromosomes ranging from 0 to 4 were seen in 14.29 per cent of the meiocytes and in the remaining 76.19 per cent of the cells, unequal distribution of the chromosomes to the poles was observed (Plate XIX, Figures 1 and 2). In the remaining amphidiploids between 63.18 (L. corniculatus var. minor x L. filicaulis) and 80.50 per cent of the cells (L. japonicus x L. corniculatus var. minor) were scored as normal at first division anaphase. At anaphase II, the percentage of cells devoid of bridges and lagging chromosomes varied from 63.24 for (L. alpinus x L. filicaulis) to 76.88 for (L. japonicus x L. corniculatus var. minor). The number of cells with lagging chromosomes varied between the different amphidiploids at both anaphase I and telophase I and anaphase II and telophase II, and did not exceed 31.59 per cent in (L. corniculatus var. minor x L. filicaulis), at anaphase I, and 35.14 per cent in (L. alpinus x L. filicaulis), at anaphase II. Generally between 1 and 5 laggards were observed.

Bridges, and bridges with associated fragments, were some of the other cytological anomalies observed in certain microsporocytes at telophase I and II (See Plates XX and XXI). Occasionally in a few cells two bridges were detected in the same division figure, for example, (L. japonicus x L. alpinus) (Plate XX, Figure 9). Compared with the lagging monads and dyads, the fragments accompanying the bridges were relatively small and rounded into spheres. In the amphidiploid (L. filicaulis x L. schoelleri) bridges but no fragments

were detected. A similar situation was found in anaphase I pollen mother cells of (L. corniculatus var. minor x L. filicaulis) and in (L. krylovii x L. schoelleri). In these two derived alloploids, however, accompanying fragments were discerned in cells with bridges at anaphase II. In (L. alpinus x L. filicaulis) and (L. krylovii x L. filicaulis), on the other hand, fragments were seen at anaphase I but not at anaphase II. At the quartet stage, cells were observed with 5, 6 and sometimes 7 nuclei. An average of about 35 per cent of the quartets, were estimated with micronuclei in all the amphidiploids except the desynaptic (L. alpinus x L. krylovii). In this latter plant, from a total of 133 cells, micronuclei were seen in 115; up to 9 such micronuclei were observed (Plate XXI, Figure 12).

Photomicrographs of meiotic chromosomes in these synthetic amphidiploids are shown in Plates XVI to XXI.

Meiosis in crosses between the synthetic amphidiploids and Lotus corniculatus

Meiosis was studied in pollen mother cells of 26 genotypes derived from 15 cross combinations obtained by crossing the synthetic amphidiploids and L. corniculatus. The data acquired in this investigation are presented in Tables 32 and 33. It may be seen from Table 32, that diakinesis and metaphase I plates of all 15 crosses were characterized by univalents and by chromosomes associated as bivalents, trivalents and quadrivalents. There was little variability in the frequency of univalents between the different hybrid combinations; the mean number

TABLE 31. Meiotic chromosome behavior at AI--TI and AII--TII in the amphidiploids

Genotypes	Anaphase I--Telophase I							Anaphase II--Telophase II						
	No. of cells	Normal		Bridges		Laggards		No. of cells	Normal		Bridges		Laggards	
		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%
						(L. alpinus X L. schoelleri) (4x)								
A/AS-2	130	91	70.00	7*	5.38	32	24.62	-	-	-	-	-	-	-
						(L. alpinus X L. filicaulis) (4x)								
A/AF-2	100	78	78.00	1	1.00	21	21.00	100	72	72.00	3	3.00	25	25.00
A/AF-3	105	69	65.71	-	-	36	34.29	85	45	52.94	-	-	40	47.06
A/AF-4	110	86	78.18	3	2.73	21	19.09	-	-	-	-	-	-	-
Total	315	233	73.97	4**	1.27	78	24.76	185	117	63.24	3	1.62	65	35.14
						(L. alpinus X L. krylovii) (4x)								
A/AK-7	84[a]	8	9.52	-	-	12	14.29	-	-	-	-	-	-	-
						(L. japonicus X L. alpinus) (4x)								
A/JA-1	140	76	54.29	23	16.43	41	29.29	96	58	60.42	22	22.92	16	16.66
A/JA-2	115	90	78.26	4	3.48	21	18.26	60	47	78.33	7	11.67	6	10.00
A/JA-10	120	98	81.67	2	1.67	20	16.66	120	77	64.17	3	2.50	40	33.33
Total	375	264	70.40	29[b]	7.73	82	21.87	276	182	65.94	32[c]	11.59	62	22.46

Table 31 Cont'd

TABLE 31 (Cont'd)

Genotypes	Anaphase I--Telophase I							Anaphase II--Telophase II						
	No. of cells	Normal		Bridges		Laggards		No. of cells	Normal		Bridges		Laggards	
		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%
						(L. corniculatus var. minor X L. alpinus) (4x)								
A/MA-3-1	100	74	74.00	6	6.00	20	20.00	105	68	64.76	10	9.52	27	25.71
A/MA-3-2	100	85	85.00	3	3.00	12	12.00	110	78	70.91	3	2.73	29	26.36
Total	200	159	79.50	9[d]	4.50	32	16.00	215	146	67.91	13[e]	6.05	56	26.04
						(L. corniculatus var. minor X L. filicaulis) (4x)								
A/MF-2-3	95	60	63.16	5	5.26	30	31.59	54	37	68.52	4[f]	7.41	13	24.07
						(L. japonicus X L. corniculatus var. minor) (4x)								
A/JM-2-2	100	87	87.00	-	-	13	13.00	70	55	78.57	1	1.43	14	20.00
A/JM-4-1	100	74	74.00	3	3.00	23	23.00	90	68	75.56	9	10.00	13	14.44
Total	200	161	80.50	3[g]	1.50	36	18.00	160	123	76.88	10[h]	6.25	27	16.87
						(L. filicaulis X L. schoelleri) (4x)								
A/FS-2	115	82	71.30	3	2.61	30	26.09	115	87	75.65	2	1.74	26	22.61

Table 31 Cont'd

TABLE 31 (Cont'd)

Genotypes	Anaphase I--Telophase I							Anaphase II--Telophase II						
	No. of cells	Normal		Bridges		Laggards		No. of cells	Normal		Bridges		Laggards	
		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%
						(L. japonicus X L. schoelleri) (4x)								
A/JS-3-4	70	51	72.86	3	4.29	16	22.86	-	-	-	-	-	-	-
A/JS-3-6	75	57	76.00	1	1.33	17	22.67	120	79	65.83	8[h]	6.67	33	27.50
Total	145	108	74.48	4[g]	2.76	33	22.76	-	-	-	-	-	-	-
						(L. japonicus X L. krylovii) (4x)								
A/JK-1	122	80	65.57	7[e]	5.74	35	28.69	126	95	75.40	19[d]	15.08	12	9.52
						(L. krylovii X L. filicaulis) (4x)								
A/KF-2-1	110	65	59.09	5	4.55	40	36.36	25	16	64.00	3	12.00	6	24.00
A/KF-2-5	102	86	84.31	1	0.98	15	14.71	85	66	77.65	3	3.53	16	18.83
Total	212	151	71.23	6[h]	2.83	55	25.94	110	82	74.55	6	5.45	22	20.00
						(L. krylovii X L. schoelleri) (4x)								
A/KS-1	101	60	59.41	1	0.99	40	39.60	120	88	73.33	3	2.50	29	24.17
A/KS-7	115	100	86.96	-	-	15	13.04	90	68	75.56	1	1.11	21	23.33
Total	216	160	74.07	1	0.46	55	25.46	210	156	74.29	4[g]	1.90	50	23.80

* fragments also seen in 6 of these cells
** fragments also seen in these cells
a abnormal disjunction of chromosomes seen in 64 of these cells
b fragments also seen in 17 of these cells
c fragments also seen in 5 of these cells
d fragments also seen in 2 of these cells
e fragments also seen in 4 of these cells
f fragments also seen in these 4 cells
g fragment also seen in 1 of these cells
h fragments also seen in 5 of these cells

Plate XVI

Meiosis in the amphidiploids

Figure 1 MI in (*L. corniculatus* var. *minor* x *L. alpinus*) showing 10 II's + 1 IV. x ca. 1130

Figures 2-5 MI in (*L. alpinus* x *L. schoelleri*)

Fig. 2 8 II's + 2 IV's. x ca. 1425

Fig. 3 2 I's + 9 II's + 1 IV. x ca. 1450

Fig. 4 4 I's + 8 II's. x ca. 975

Fig. 5 12 II's. x ca. 1700

Figure 6 MI in (*L. alpinus* x *L. filicaulis*) showing 2 I's + 8 II's + 2 IV's. x ca. 2500

Figures 7-8 Prometaphase I in (*L. alpinus* x *L. filicaulis*)

Fig. 7 2 I's + 9 II's + 1 IV. x ca. 1670

Fig. 8 4 I's + 8 II's + 1 IV. x ca. 1400

Figures 9-10 MI in (*L. alpinus* x *L. krylovii*)

Fig. 9 20 I's + 2 II's. x ca. 1800

Fig. 10 12 I's + 4 II's + 1 IV. x ca. 1600

Figure 11 Diakinesis in (*L. alpinus* x *L. krylovii*) showing 14 I's + 3 loose II's + 1 IV. x ca. 1550

Figure 12 MI in (*L. krylovii* x *L. schoelleri*) showing 4 I's + 10 II's. x ca. 1370

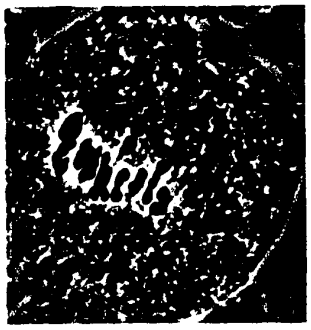
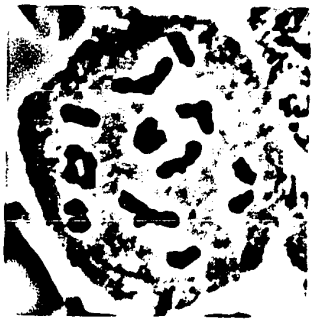


Figure 1

Figure 2



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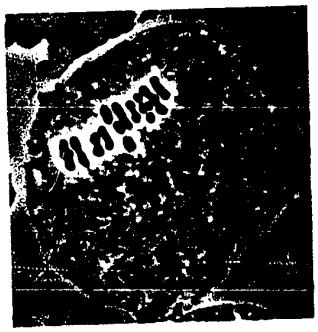

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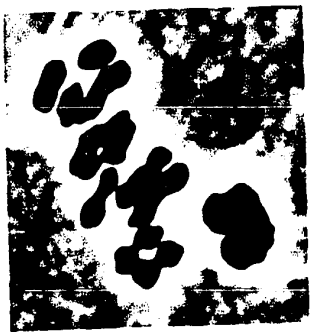

Figure 6

Figure 7

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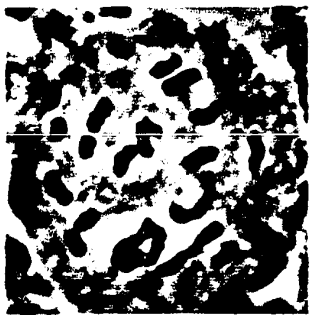

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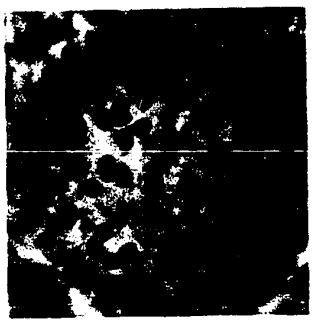

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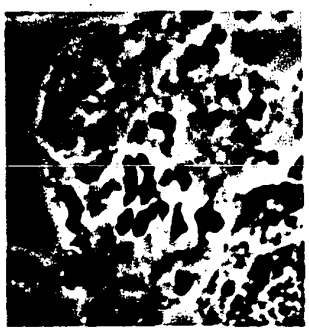

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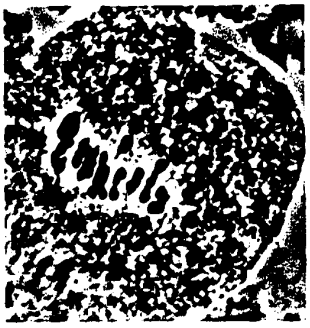

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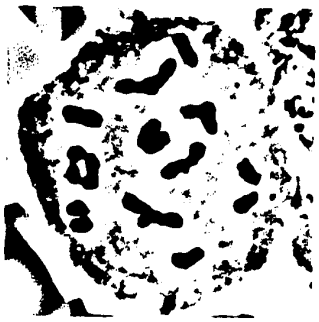

Figure 3

Figure 4

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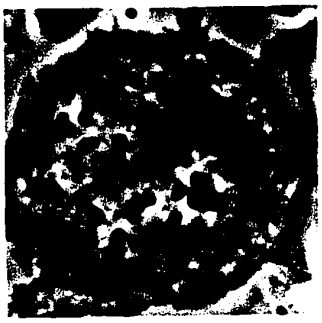

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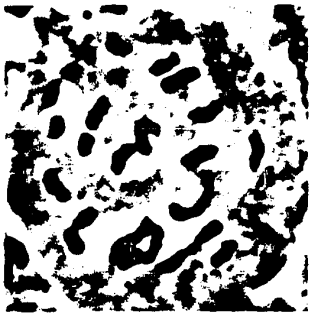
Figure 9



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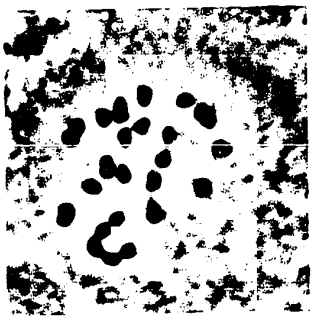


Figure 11

Figure 12

Plate XVII

Meiosis in the amphidiploids

Figures 1-4 Diakinesis in *L. japonicus* x *L. alpinus*

Fig. 1 2 I's + 7 II's + 2 IV's. x ca. 1575

Fig. 2 6 II's + 3 IV's. x ca. 1850

Fig. 3 4 I's + 10 II's. x ca. 1500

Fig. 4 3 I's + 7 II's + 1 III + 1 IV. x ca. 1500

Figure 5 Prometaphase I in *L. japonicus* x *L. krylovii* showing 2 I's + 9 II's + 1 IV. x ca. 1575

Figure 6 MI in *L. japonicus* x *L. krylovii* showing 4 I's + 10 II's. x ca. 1400

Figures 7-8 MI in *L. filicaulis* x *L. schoelleri*.

Fig. 7 6 I's + 9 II's. x ca. 1300

Fig. 8 2 I's + 9 II's + 1 IV. x ca. 1175

Figure 9 MI in *L. japonicus* x *L. krylovii* showing 6 I's + 7 II's + 1 IV. x ca. 1400

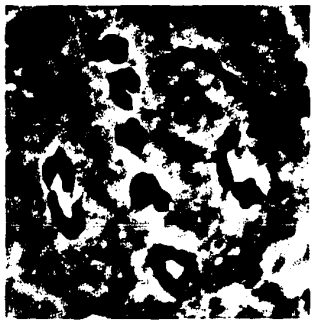
Figures 10-11 MI in *L. filicaulis* x *L. schoelleri*.

Fig. 10 2 I's + 9 II's + 1 IV. x ca. 1300

Fig. 11 4 I's + 10 II's. x ca. 1550

Figure 12 Prometaphase I in *L. corniculatus* var. *minor* x *L. filicaulis* showing 1 I + 10 II's + 1 III. x ca. 1100

Figure 1



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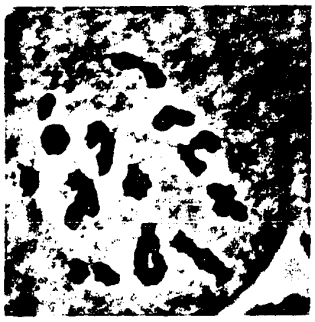

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Figure 4

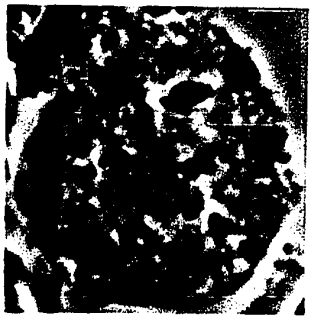


Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

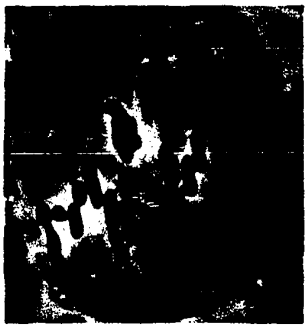
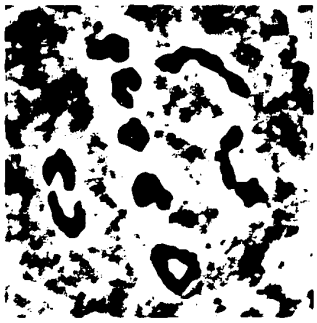


Figure 11

Figure 12

Figure 1



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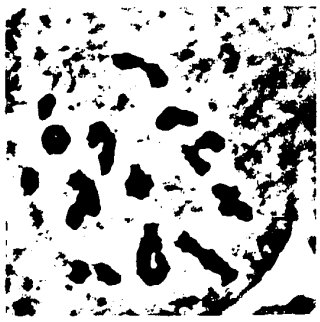


Figure 3

Figure 4

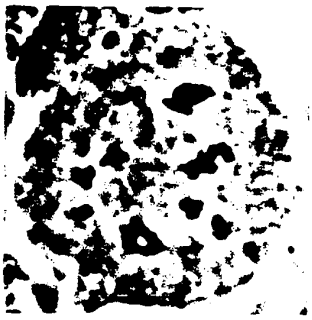
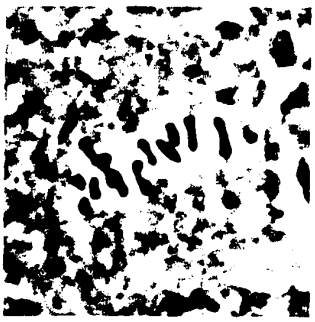


Figure 5

Figure 6



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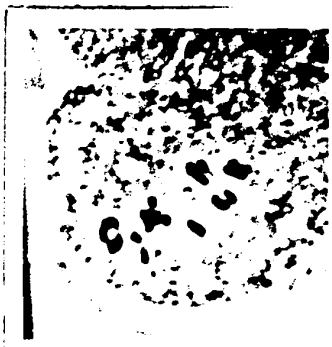

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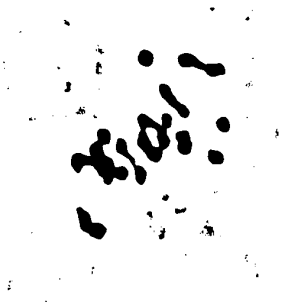


Figure 9

Figure 10

Figure 11

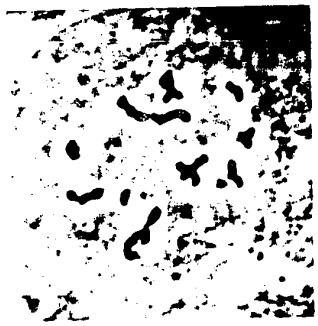


Figure 12

Plate XVIII

Meiosis in the amphidiploids

Figures 1-2 Diakinesis in (*L. japonicus* x *L. corniculatus* var. minor).

Fig. 1 12 II's. x ca. 1150

Fig. 2 7 II's + 2 III's + 1 IV. x ca. 1700

Figures 3-5 MI in (*L. krylovii* x *L. filicaulis*)

Fig. 3 6 I's + 9 II's. x ca. 1000

Fig. 4 8 II's + 2 IV's. x ca. 1700

Fig. 5 6 I's + 7 II's + 1 IV. x ca. 2150

Figure 6 Prometaphase I in (*L. krylovii* x *L. filicaulis*) showing 6 I's + 7 II's + 1 IV. x ca. 1300

Figures 7-8 Diakinesis in (*L. japonicus* x *L. schoelleri*)

Fig. 7 1 I + 8 II's + 1 III + 1 IV. x ca. 1400

Fig. 8 12 II's. x ca. 1350

Figures 9-10 MI in (*L. japonicus* x *L. schoelleri*).

Fig. 9 4 I's + 8 II's + 1 IV. x ca. 1400

Fig. 10 4 I's + 8 II's + 1 IV. x ca. 1600

Figures 11-12 MI in (*L. corniculatus* var. *minor* x *L. alpinus*).

Fig. 11 2 I's + 9 II's + 1 IV. x ca. 1800

Fig. 12 4 I's + 10 II's. x ca. 1125

Figure 1

Figure 2

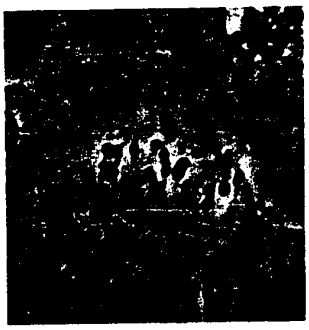
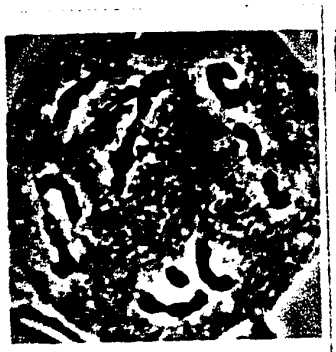


Figure 3

Figure 4

Figure 5

Figure 6



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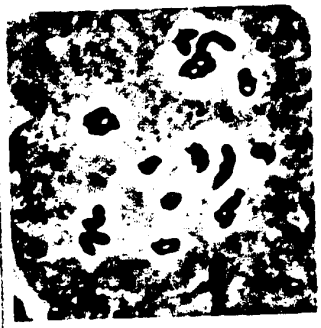

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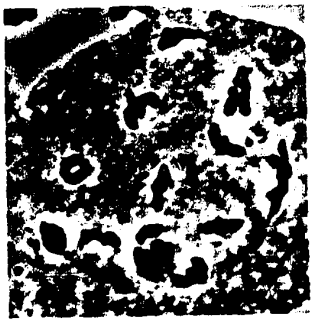


Figure 9

Figure 10

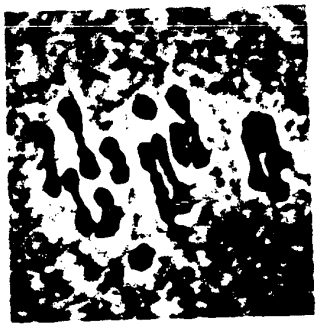


Figure 11

Figure 12

Figure 1

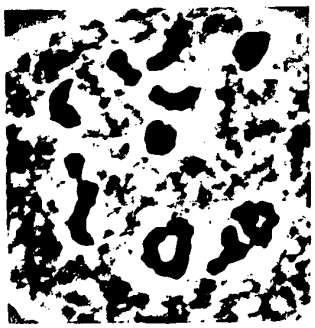
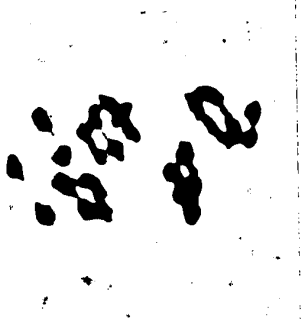


Figure 2

Figure 3



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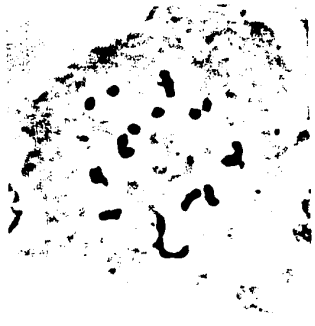

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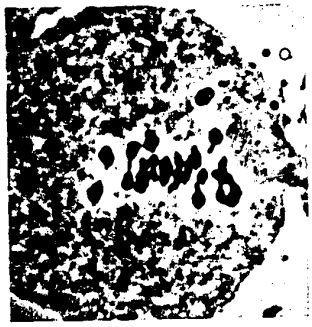

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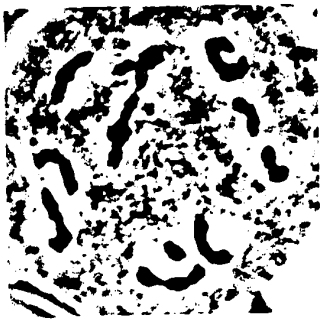

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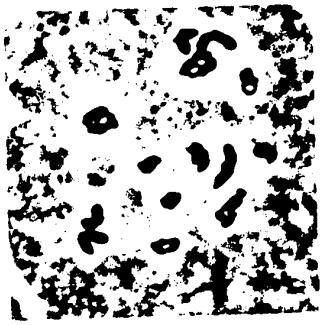

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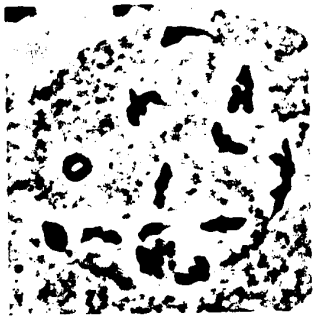
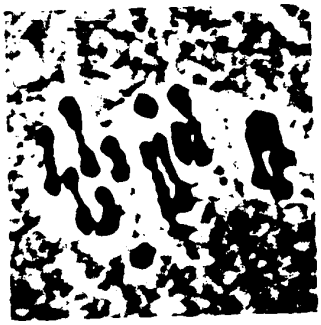


Figure 9

Figure 10



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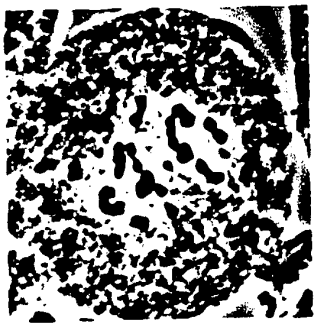


Figure 12

Plate XIX

Meiosis in the amphidiploids

Figures 1-2 AI in (L. alpinus x L. krylovii).

Fig. 1 Unequal separation of chromosomes showing 8 chromosomes at one pole and 16 at the other pole. x ca. 1700

Fig. 2 Unequal separation of chromosomes and lagging chromosomes. x ca. 780

Figure 3 Late AI in (L. filicaulis x L. schoelleri) showing 2 lagging chromosomes. x ca. 1130

Figure 4 AI in (L. japonicus x L. schoelleri) showing 12 chromosomes at each pole. x ca. 1350

Figures 5-6 TI in (L. krylovii x L. schoelleri).

Fig. 5 7 lagging chromosomes. x ca. 1300

Fig. 6 2 monads and 1 dyad. x ca. 1200

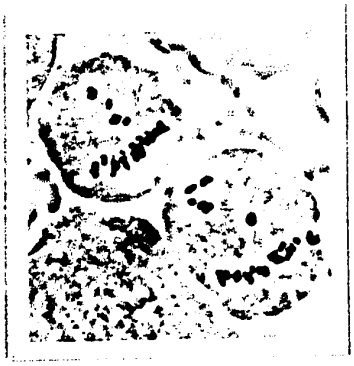
Figures 7-8 TII showing lagging chromosomes.

Fig. 7 (L. krylovii x L. schoelleri). x ca. 1370

Fig. 8 (L. alpinus x L. filicaulis). x ca. 1525

Figure 9 TII in (L. corniculatus var. minor x L. alpinus) showing a normal anaphase II (lower right) and lagging chromosomes (upper left). x ca. 900

Figure 1



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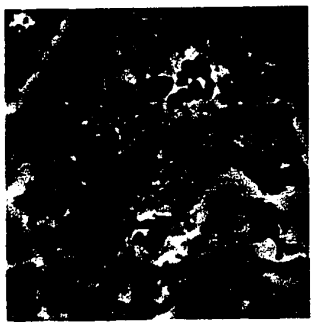

Figure 3

Figure 4

Figure 5

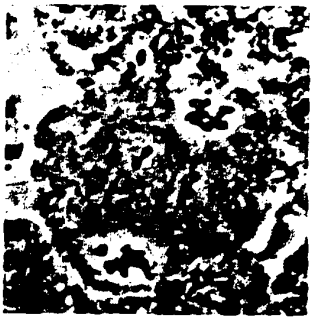

Figure 6

Figure 7

Figure 8

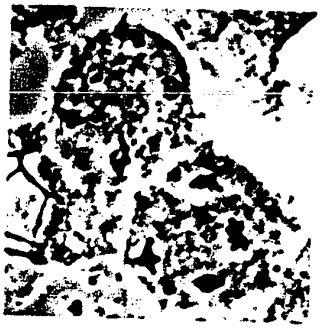

Figure 9

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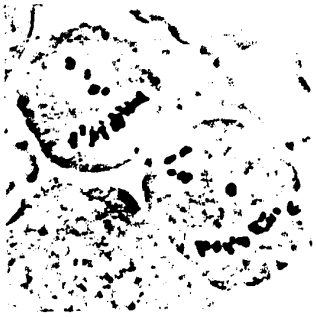

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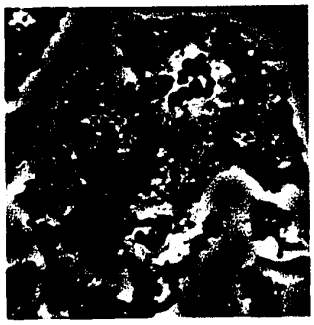


Figure 3

Figure 4

Figure 5

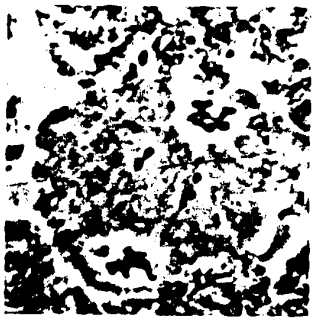


Figure 6

Figure 7

Figure 8

Figure 9

Plate XX

Meiosis in the amphidiploids

Figure 1 — TI in (*L. krylovii* x *L. filicaulis*) showing bridge and 3 lagging chromosomes. x ca. 1270

Figures 2-4 — TI inversion bridges and associated acentric fragments.

Fig. 2 — (*L. krylovii* x *L. filicaulis*). x ca. 375

Fig. 3 — (*L. japonicus* x *L. krylovii*). x ca. 600

Fig. 4 — (*L. alpinus* x *L. schoelleri*). x ca. 1550

Figure 5 — TI in (*L. alpinus* x *L. schoelleri*). Inversion bridge + fragment + 2 lagging chromosomes. x ca. 1300

Figure 6 — TI in (*L. alpinus* x *L. schoelleri*) showing (a) lagging chromosomes (left), inversion bridge + fragment + 1 laggard (right) and normal telophase I figure. x ca. 730

Figure 7 — TI in (*L. japonicus* x *L. schoelleri*) showing (a) inversion bridges without acentric fragments, (b) lagging chromosomes. x ca. 650

Figures 8-10 — TI in (*L. japonicus* x *L. alpinus*)

Fig. 8 — Inversion bridge + 3 laggards. x ca. 650

Fig. 9 — 2 inversion bridges + associated acentric fragments + 3 laggards. x ca. 650

Figure 11 — TI in (*L. alpinus* x *L. filicaulis*). (a) normal TI cell (b) bridge + fragment. x ca. 1050

Figure 12 — TII in (*L. corniculatus* var. *minor* x *L. alpinus*) Inversion bridge + fragment. x ca. 1300

Figure 1

Figure 2

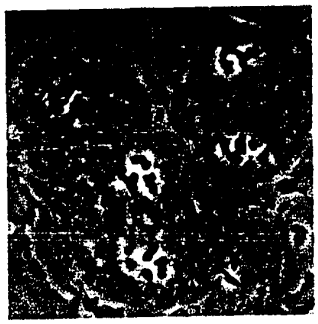


Figure 3

Figure 4

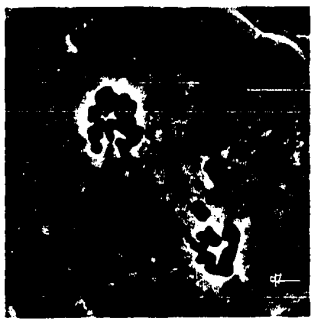


Figure 5

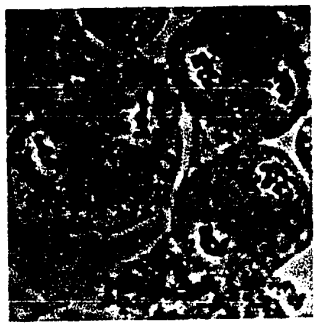


Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

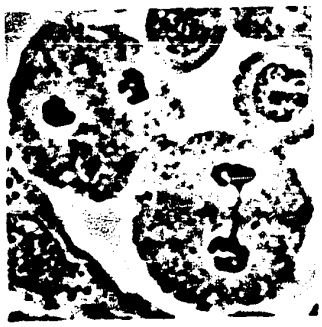


Figure 11

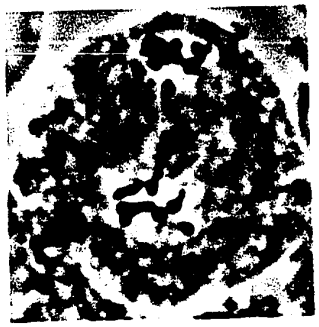


Figure 12

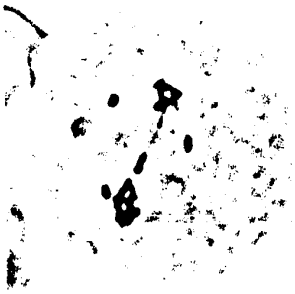


Figure 1

Figure 2

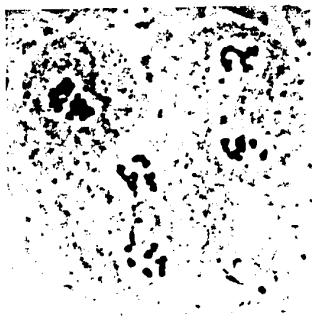


Figure 3

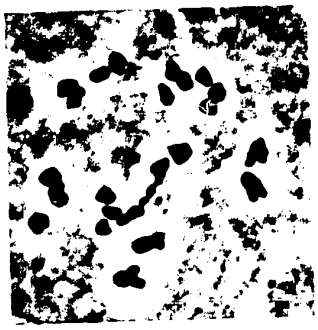


Figure 4

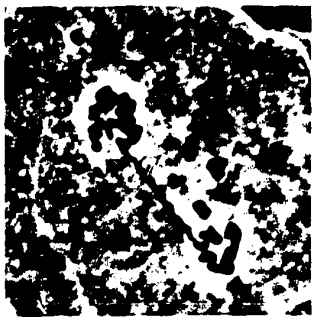


Figure 5

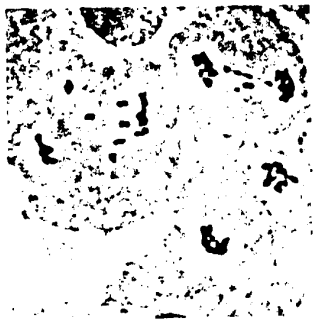


Figure 6

Figure 7

Figure 8

Figure 9

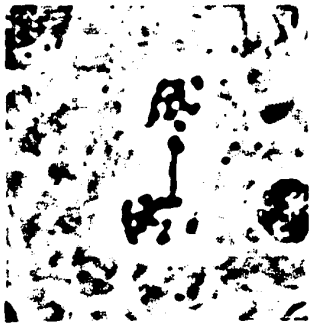


Figure 10

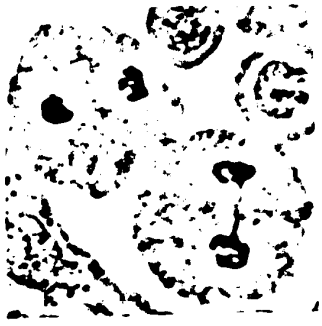


Figure 11

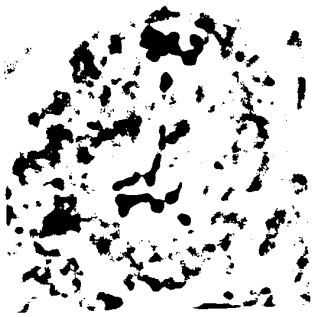


Figure 12

Plate XXI

Meiosis in the amphidiploids

Figure 1 — AII in (*L. japonicus* x *L. schoelleri*) showing bridge + laggard. x ca. 1400

Figures 2-3 — TII in (*L. filicaulis* x *L. schoelleri*).

Fig. 2 — Bridge + fragment. x ca. 1700

Fig. 3 — Normal division (left); and laggards (right). x ca. 900

Figure 4 — Bridge in (*L. japonicus* x *L. alpinus*). x ca. 1400

Figure 5 — TII bridge in (*L. corniculatus* var. *minor* x *L. alpinus*). x ca. 1375

Figure 6 — TII in (*L. corniculatus* var. *minor* x *L. alpinus*). (a) normal division (b) bridge + fragment. x ca. 830

Figure 7 — TII bridge + fragment in (*L. krylovii* x *L. schoelleri*). x ca. 1550

Figure 8 — TII in (*L. krylovii* x *L. schoelleri*) showing bridge + laggards. x ca. 1330

Figure 9 — Six aggregations of chromosomes in (*L. alpinus* x *L. krylovii*). x ca. 850

Figures 10-11 — Quartet stage.

Fig. 10 — (a) normal cell; (b) 2 micronuclei in (*L. alpinus* x *L. filicaulis*). x ca. 733

Fig. 11 — 3 micronuclei in (*L. filicaulis* x *L. schoelleri*). x ca. 1375

Figure 12 — Nine aggregations of chromosomes at TII in (*L. alpinus* x *L. krylovii*). x ca. 800

Figure 1

Figure 2

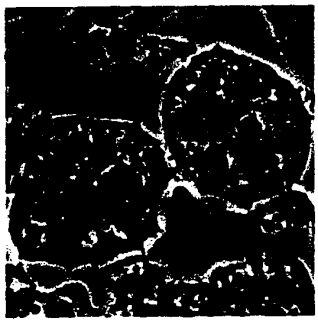


Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

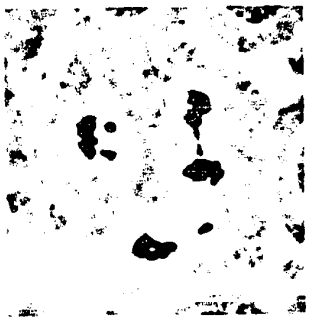


Figure 8

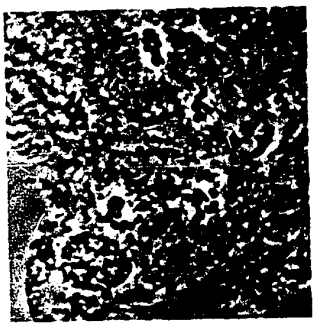


Figure 9

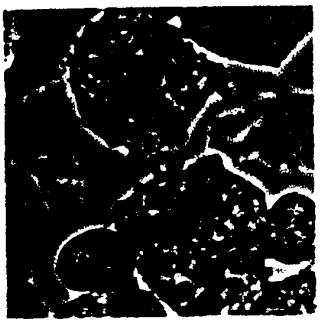


Figure 10

Figure 11

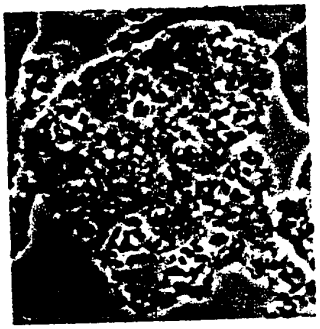


Figure 12

Figure 1

Figure 2

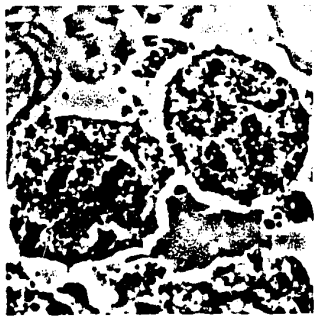


Figure 3

Figure 4

Figure 5

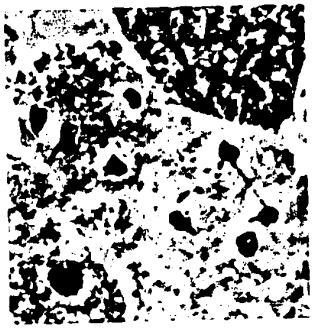


Figure 6

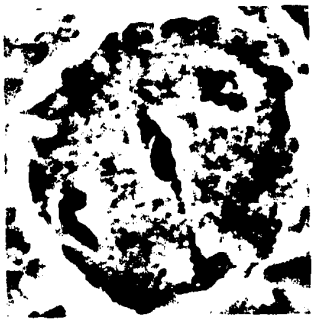


Figure 7

Figure 8

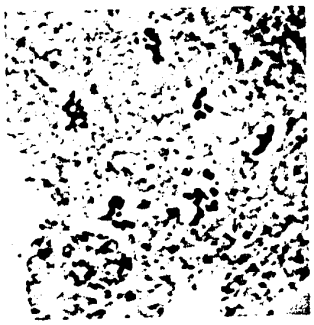


Figure 9

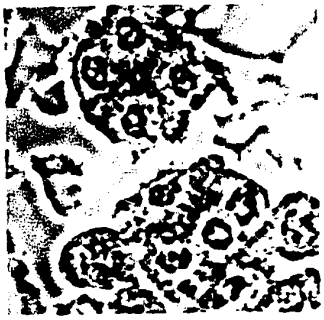


Figure 10

Figure 11

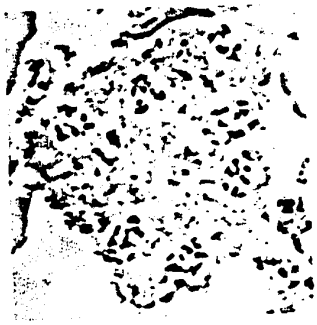


Figure 12

of univalents per cell ranged from 8.88 in (L. japonicus x L. alpinus) x L. corniculatus to 11.86 in (L. krylovii x L. schoelleri) x L. corniculatus. Except for the hybrid (L. japonicus x L. alpinus) x L. corniculatus, the frequency of univalents did not vary from genotype to genotype. In (L. japonicus x L. alpinus) x L. corniculatus, one genotype, JA-C-1-1 had 12.50 per cent univalents whereas, in an other genotype, JA-C-2-3, only 6.25 per cent were observed. It is interesting to note that the frequency of unpaired chromosomes in these hybrids was about the same as that recorded for the amphidiploids (see Table 30) and was within the range of univalents observed in the autotetraploids of the parental species (see Tables 26, 30 and 32).

The number of bivalents found in these hybrids was unexpectedly high (Table 32). The percentage bivalents ranged from 73.82 in L. corniculatus x (L. japonicus x L. alpinus) to 85.94 in (L. japonicus x L. krylovii) x L. corniculatus. Although sometimes only four bivalents were present in some microsporocytes, values below 80 per cent bivalents were observed only in two hybrid combinations. These were L. corniculatus x (L. japonicus x L. alpinus) with 73.82 per cent and (L. filicaulis x L. schoelleri) x L. corniculatus with 77.54 per cent. Twelve bivalents were frequently recorded in many microsporocytes of all the hybrid combinations (see Plate XXII, Figures 2 and 12).

Multivalent associations consisting of trivalents and quadrivalents were observed in all the 15 crosses. The number of trivalents detected varied from 1-3 per cell and was low compared with the frequency of quadrivalents. Mean trivalents per cell varied from 0.05 in (L. corniculatus

var. minor x L. filicaulis) x L. corniculatus to 0.26 in L. corniculatus x (L. japonicus x L. alpinus). The most frequent trivalent configuration was the chain type; a few Y-shaped types were also detected (Plate XXII, Figure 1). A noteworthy point is that trivalents occurred with greater frequency in these crosses than in the synthetic amphidiploids (see Tables 30 and 32).

Quadrivalent associations were observed in all of the crosses studied. Generally these configurations were detected with a frequency of 0-1 and 0-2 per cell in many of the hybrids; in L. corniculatus x (L. japonicus x L. alpinus) some meiocytes were found with as many as three quadrivalents. The frequency of these multivalent associations was more variable between hybrids than between the genotypes of a hybrid. The mean number of quadrivalents per cell for the crosses ranged from 0.25 for (L. krylovii x L. filicaulis) x L. corniculatus to 0.80 in L. corniculatus x (L. japonicus x L. alpinus). These values correspond to 4.17 and 13.33 per cent chromosomes associated as quadrivalents, respectively. Variability in the frequency of quadrivalents was found only in the genotypes of the crosses (L. japonicus x L. alpinus) x L. corniculatus and L. corniculatus x (L. japonicus x L. alpinus). In the former cross, the genotype JA-C-1-1 had 0.44 quadrivalents per cell whereas, in the other genotype JA-C-2-3, 0.62 quadrivalents were present; in the latter cross, a mean number of quadrivalents per cell of 0.97 and 0.68 were recorded for the genotypes C-JA-1-5 and C-JA-1-6, respectively. A noteworthy point is that the highest frequency of quadrivalents was also recorded in these reciprocal crosses. Another

interesting observation is the differences in the meiotic behavior exhibited by these reciprocal crosses. The cross (L. japonicus x L. alpinus) x L. corniculatus had chromosomes distributed as 8.88 per cent univalents, 80.31 per cent bivalents, 1.63 per cent trivalents and 9.18 per cent quadrivalents, whereas in the reciprocal cross combination the percentages of univalents, bivalents, trivalents and quadrivalents were 9.61, 73.82, 3.24 and 13.33, respectively. Such differences were not seen in the other reciprocal hybrids obtained by crossing 1) (L. japonicus x L. schoelleri) and L. corniculatus, 2) (L. japonicus x L. krylovii) and L. corniculatus and 3) (L. krylovii x L. filicaulis) and L. corniculatus.

Data obtained on the behavior of meiotic chromosomes at anaphase I and II and telophase I and II are presented in Table 33. At anaphase I the percentage of cells scored as normal was never less than 67.62, calculated for the cross (L. japonicus x L. schoelleri) x L. corniculatus. The highest number of normal cells was found in the cross (L. alpinus x L. filicaulis) x L. corniculatus (88.57 per cent). At anaphase II and telophase II, there was considerable variation between the crosses in the frequency of normal cells. A low value of 47.50 per cent was calculated in the (L. alpinus x L. schoelleri) x L. corniculatus cross and a high of 87.83 per cent for (L. japonicus x L. schoelleri) x L. corniculatus. Lagging chromosomes (monads and dyads) occurred with a frequency of 0-2, 0-3 and 0-4 per cell at both anaphase I and anaphase II. The number of cells with laggards was variable at both first and

second division anaphase stages; this variability, however, was more marked at anaphase II where the range was from 12.17 per cent for (L. japonicus x L. schoelleri) x L. corniculatus, to 52.50 per cent for (L. alpinus x L. schoelleri) x L. corniculatus. Bridges and bridges with accompanying fragments were seen in hybrids of L. corniculatus x (L. japonicus x L. alpinus), (L. corniculatus var. minor x L. filicaulis) x L. corniculatus, (L. japonicus x L. corniculatus var. minor) x L. corniculatus, (L. filicaulis x L. schoelleri) x L. corniculatus, and (L. krylovii x L. schoelleri) x L. corniculatus at telophase I and II. However, in (L. japonicus x L. alpinus) x L. corniculatus, (L. japonicus x L. krylovii) x L. corniculatus and (L. krylovii x L. filicaulis) x L. corniculatus, these irregularities were observed only at telophase II. Associated fragments which appeared as rounded spheres of chromatin were distinct in some of the cells containing bridges in L. corniculatus x (L. japonicus x L. alpinus), (L. corniculatus var. minor x L. filicaulis) x L. corniculatus (both telophase I and II) and (L. japonicus x L. corniculatus var. minor) x L. corniculatus and (L. japonicus x L. krylovii) x L. corniculatus (telophase II only). In all of the hybrids, micronuclei were observed in a small percentage of the cells in the quartet stage. Photomicrographs of meiocytes of these crosses are shown in Plates XXII, XXIII, XXIV and XXV.

TABLE 32. Diakinesis and/or Metaphase I chromosome behavior in crosses between the amphidiploids and *L. corniculatus*

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
		(*L. alpinus* X *L. schoelleri*) X *L. corniculatus* (4x)							
AS-C-1-1	85	2.53 (0 - 8)	10.06 (6 - 12)	0.06 (0 - 1)	0.29 (0 - 1)	10.54	83.82	0.74	4.90
		(*L. alpinus* X *L. filicaulis*) X *L. corniculatus* (4x)							
AF-C-1-1	42	2.38 (0 - 4)	10.09 (7 - 12)	0.09 (0 - 1)	0.28 (0 - 1)	9.92	84.12	1.19	4.76
AF-C-2-1	72	2.05 (0 - 6)	10.12 (6 - 12)	0.13 (0 - 2)	0.31 (0 - 2)	8.56	84.37	1.73	5.32
Total	114	2.18 (0 - 6)	10.11 (6 - 12)	0.12 (0 - 2)	0.31 (0 - 2)	9.06	84.28	1.54	5.12
		(*L. japonicus* X *L. alpinus*) X *L. corniculatus* (4x)							
JA-C-1-1	29	3.00 (0 - 8)	9.24 (6 - 12)	0.24 (0 - 2)	0.44 (0 - 2)	12.50	77.01	3.01	7.47
JA-C-2-3	40	1.50 (0 - 6)	9.92 (6 - 12)	0.05 (0 - 1)	0.62 (0 - 2)	6.25	82.71	0.63	10.41
Total	69	2.13 (0 - 8)	9.64 (6 - 12)	0.13 (0 - 2)	0.55 (0 - 2)	8.88	80.31	1.63	9.18

Table 32 Cont'd

TABLE 32 (Cont'd)

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
		L. corniculatus X (*L. japonicus* X *L. alpinus*) (4x)							
C-JA-1-5	35	2.11 (0 - 6)	8.74 (6 - 11)	0.17 (0 - 1)	0.97 (0 - 3)	8.80	72.86	2.14	16.20
C-JA-1-6	50	2.44 (0 - 7)	8.94 (4 - 12)	0.32 (0 - 3)	0.68 (0 - 3)	10.17	74.50	4.00	11.33
Total	85	2.31 (0 - 7)	8.86 (4 - 12)	0.26 (0 - 3)	0.80 (0 - 3)	9.61	73.82	3.24	13.33
		(*L. corniculatus* var. *minor* X *L. alpinus*) X *L. corniculatus* (4x)							
MA-C-2-1	33	2.24 (0 - 6)	10.24 (8 - 12)	0.06 (0 - 1)	0.27 (0 - 1)	9.34	85.35	0.76	4.55
MA-C-1-2	60	2.12 (0 - 8)	10.22 (6 - 12)	0.08 (0 - 1)	0.30 (1 - 2)	8.82	85.14	1.04	5.00
Total	93	2.16 (0 - 8)	10.23 (6 - 12)	0.08 (0 - 1)	0.29 (1 - 2)	9.01	85.22	0.94	4.84

Table 32 Cont'd

TABLE 32 (Cont'd)

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
(*L. corniculatus* var. *minor* X *L. filicaulis*) X *L. corniculatus* (4x)									
MF-C-1-1	35	2.11 (0 - 4)	10.29 (8 - 12)	0.06 (0 - 1)	0.29 (0 - 1)	8.81	85.71	0.71	4.76
MF-C-2-3	60	2.10 (0 - 8)	10.33 (6 - 12)	0.03 (0 - 1)	0.28 (0 - 1)	8.75	86.11	0.42	4.72
MF-C-3-1	30	2.67 (0 - 10)	9.90 (7 - 12)	0.07 (0 - 1)	0.33 (0 - 1)	11.11	82.50	0.83	5.56
Total	125	2.24 (0 - 10)	10.22 (6 - 12)	0.05 (0 - 1)	0.30 (0 - 1)	9.33	85.13	0.60	4.93
(*L. japonicus* X *L. corniculatus* var. *minor*) X *L. corniculatus* (4x)									
JM-C-2-3	50	2.12 (0 - 6)	9.96 (7 - 12)	0.12 (0 - 2)	0.40 (0 - 1)	8.83	83.00	1.50	6.67
JM-C-1-2	32	2.84 (0 - 9)	9.47 (6 - 12)	0.16 (0 - 1)	0.44 (0 - 2)	11.85	78.71	1.95	7.29
Total	82	2.40 (0 - 9)	9.77 (6 - 12)	0.13 (0 - 2)	0.41 (0 - 2)	10.01	81.40	1.68	6.91

Table 32 Cont'd

TABLE 32 (Cont'd)

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
		(*L. filicaulis* X *L. schoelleri*) X *L. corniculatus* (4x)							
FS-C-1-1	50	3.68 (0 - 9)	9.00 (5 - 11)	0.24 (0 - 2)	0.40 (0 - 1)	15.33	75.00	3.00	6.67
FS-C-1-3	32	2.72 (0 - 8)	9.78 (7 - 12)	0.16 (0 - 1)	0.31 (0 - 1)	11.33	81.51	1.95	5.21
Total	82	3.30 (0 - 9)	9.30 (5 - 12)	0.21 (0 - 2)	0.37 (0 - 1)	13.77	77.54	2.59	6.10
		(*L. japonicus* X *L. schoelleri*) X *L. corniculatus* (4x)							
JS-C-1-2	30	3.03 (0 - 8)	9.60 (5 - 12)	0.10 (0 - 2)	0.37 (0 - 1)	12.64	80.00	1.25	6.11
JS-C-2-3	40	1.93 (0 - 8)	10.00 (7 - 12)	0.13 (0 - 1)	0.43 (0 - 2)	8.02	83.33	1.56	7.08
Total	70	2.40 (0 - 8)	9.83 (7 - 12)	0.11 (0 - 2)	0.40 (0 - 2)	10.00	81.90	1.43	6.67
		L. corniculatus X (*L. japonicus* X *L. schoelleri*) (4x)							
C-JS-1-2	50	2.22 (0 - 6)	9.88 (7 - 12)	0.22 (0 - 2)	0.34 (0 - 1)	9.25	82.33	2.75	5.67

Table 32 Cont'd

TABLE 32 (Cont'd)

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
		(*L. japonicus* X *L. krylovii*) X *L. corniculatus* (4x)							
JK-C-1-7	64	1.94 (0 - 6)	10.31 (8 - 12)	0.06 (0 - 1)	0.31 (0 - 1)	8.07	85.94	0.78	5.21
		L. corniculatus X (*L. japonicus* X *L. krylovii*) (4x)							
C-JK-1-4	31	2.84 (0 - 8)	9.97 (6 - 12)	0.06 (0 - 2)	0.26 (0 - 1)	11.83	83.06	0.81	4.30
		(*L. krylovii* X *L. filicaulis*) X *L. corniculatus* (4x)							
KF-C-1-10	32	2.47 (0 - 8)	10.16 (8 - 12)	0.16 (0 - 1)	0.19 (0 - 1)	10.29	84.63	1.95	3.13
KF-C-2-9	52	2.19 (0 - 8)	10.21 (8 - 12)	0.08 (0 - 1)	0.29 (0 - 1)	9.13	85.10	0.96	4.81
Total	84	2.30 (0 - 8)	10.19 (8 - 12)	0.11 (0 - 1)	0.25 (0 - 1)	9.58	84.92	1.34	4.17

Table 32 Cont'd

TABLE 32 (Cont'd)

Genotypes	No. of cells examined	Means and ranges per cell of				Per cent chromosomes as			
		I	II	III	IV	I	II	III	IV
		L. corniculatus X (*L. krylovii* X *L. filicaulis*) (4x)							
C-KF-1-1	50	2.42 (0 - 6)	9.98 (5 - 12)	0.06 (0 - 1)	0.36 (0 - 2)	10.08	83.17	0.75	6.00
		(*L. krylovii* X *L. schoelleri*) X *L. corniculatus* (4x)							
KS-C-1-5	42	3.00 (0 - 6)	9.79 (7 - 12)	0.10 (0 - 1)	0.29 (0 - 1)	12.50	81.55	1.19	4.76
KS-C-2-2	17	2.47 (0 - 4)	10.29 (9 - 12)	- -	0.24 (0 - 1)	10.29	85.78	-	3.92
Total	59	2.85 (0 - 6)	9.93 (7 - 12)	0.07 (0 - 1)	0.27 (0 - 1)	11.86	82.77	0.85	4.52

TABLE 33. Meiotic chromosome behavior at AI--TI and AII--TII in crosses between the amphidiploids and L. corniculatus

Genotypes	Anaphase I--Telophase I							Anaphase II--Telophase II						
	No. of cells	Normal		Bridges		Laggards		No. of cells	Normal		Bridges		Laggards	
		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%
(L. alpinus X L. schoelleri) X L. corniculatus (4x)														
AS-C-1-1	98	82	83.67	-	-	16	16.33	80	38	47.50	-	-	42	52.50
(L. alpinus X L. filicaulis) X L. corniculatus (4x)														
AF-C-1-1	105	93	88.57	-	-	12	11.43	72	58	80.56	-	-	14	19.44
(L. japonicus X L. alpinus) X L. corniculatus (4x)														
JA-C-1-1	95	70	73.68	-	-	25	26.32	115	71	61.74	2	1.74	42	36.52
L. corniculatus X (L. japonicus X L. alpinus) (4x)														
C-JA-1-5	106	90	84.91	1*	0.94	15	14.15	125	97	77.60	4**	3.20	24	19.20
(L. corniculatus var. minor X L. alpinus) X L. corniculatus (4x)														
MA-C-2-1	100	81	81.00	-	-	19	19.00	119	92	77.31	-	-	27	22.69
(L. corniculatus var. minor X L. filicaulis) X L. corniculatus (4x)														
MF-C-1-1	105	86	81.90	7**	6.66	12	11.43	110	69	62.73	26**	23.64	15	13.64

Table 33 Cont'd

TABLE 33 (Cont'd)

Genotypes	Anaphase I--Telophase I							Anaphase II--Telophase II						
	No. of cells	Normal		Bridges		Laggards		No. of cells	Normal		Bridges		Laggards	
		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%
	(L. japonicus X L. corniculatus var. minor) X L. corniculatus (4x)													
JM-C-2-3	110	86	78.18	2	1.82	22	20.00	100	72	72.00	5***	5.00	23	23.00
	(L. filicaulis X L. schoelleri) X L. corniculatus (4x)													
FS-C-1-2	117	83	70.94	2	1.71	32	27.35	121	83	68.60	3	2.48	35	28.93
	(L. japonicus X L. schoelleri) X L. corniculatus (4x)													
JS-C-1-2	105	71	67.62	-	-	34	32.38	115	101	87.83	-	-	14	12.17
	L. corniculatus X (L. japonicus X L. schoelleri) (4x)													
C-JS-1-2	100	84	84.00	-	-	16	16.00	50	41	82.00	-	-	9	18.00
	(L. japonicus X L. krylovii) X L. corniculatus (4x)													
JK-C-1-7	75	63	84.00	-	-	12	16.00	121	90	74.38	2**	1.65	29	23.97
	L. corniculatus X (L. japonicus X L. krylovii (4x)													
C-JK-1-4	110	89	80.91	-	-	21	19.09	75	60	80.00	-	-	15	20.00

Table 33 Cont'd

TABLE 33 (Cont'd)

Genotypes	Anaphase I--Telophase I							Anaphase II--Telophase II						
	No. of cells	Normal		Bridges		Laggards		No. of cells	Normal		Bridges		Laggards	
		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%
(L. krylovii X L. filicaulis) X L. corniculatus (4x)														
KF-C-1-10	105	89	84.76	-	-	16	15.24	114	90	78.95	1	0.88	23	20.18
L. corniculatus X (L. krylovii X L. filicaulis) (4x)														
C-KF-1-1	120	99	82.50	-	-	21	17.50	70	60	85.71	-	-	10	14.29
(L. krylovii X L. schoelleri) X L. corniculatus (4x)														
KS-C-1-5	104	84	80.77	3	2.88	17	16.35	153	116	75.82	1	0.65	36	23.53

*
** fragments also seen
***fragment also seen in 1of these cells
fragments also seen in 3 of these cells

Plate XXII

Meiosis in the crosses between the amphidiploids x L. corniculatus

Figures 1-2 MI in (L. alpinus x L. schoelleri) x L. corniculatus.

Fig. 1 3 I's + 7 II's + 1 III + 1 IV. x ca. 1420

Fig. 2 12 II's. x ca. 1650

Figures 3-4 Diakinesis in (L. alpinus x L. filicaulis) x L. corniculatus.

Fig. 3 10 II's + 1 IV. x ca. 1700

Fig. 4 7 II's + 2 III's + 1 IV. x ca. 1400

Figures 5-6 MI in (L. alpinus x L. filicaulis) x L. corniculatus.

Fig. 5 4 I's + 8 II's + 1 IV. x ca. 2050

Fig. 6 2 I's + 11 II's. x ca. 1850

Figures 7-10 MI in (L. corniculatus var. minor x L. filicaulis) x L. corniculatus.

Fig. 7 10 II's + 1 IV. x ca. 2100

Fig. 8 10 II's + 1 IV. x ca. 1950

Fig. 9 10 II's + 1 IV. x ca. 1900

Fig. 10 2 I's + 9 II's + 1 IV. x ca. 1900

Figure 11 Diakinesis in (L. japonicus x L. schoelleri) x L. corniculatus showing 10 II's + 1 IV. x ca. 1650

Figure 12 MI in L. corniculatus x (L. japonicus x L. krylovii) showing 12 II's. x ca. 1650

Figure 1

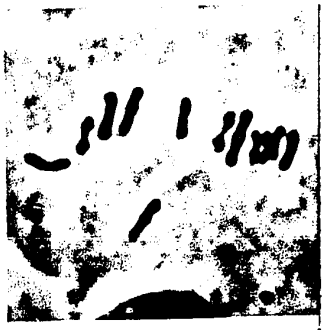
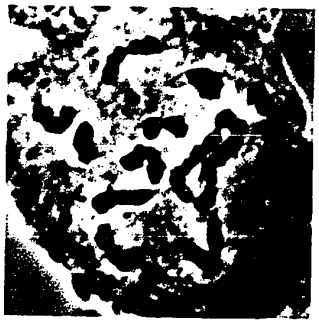


Figure 2



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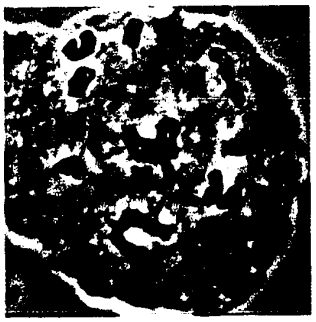


Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

Figure 12

Figure 1

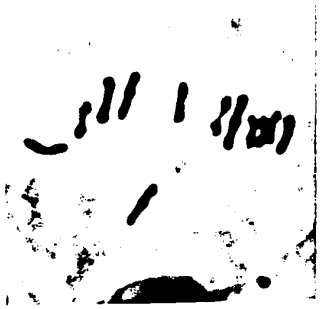


Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

Figure 12

Plate XXIII

Meiosis in the crosses between the amphidiploids and L. corniculatus

Figure 1 Diakinesis in L. corniculatus x (L. krylovii x L. filicaulis) showing 6 I's + 5 II's + 2 IV's. x ca. 1600

Figure 2 Prometaphase in L. corniculatus x (L. japonicus x L. schoelleri) showing 10 II's + 1 IV. x ca. 1600

Figures 3-4 MI in L. corniculatus x (L. japonicus x L. alpinus).

Fig. 3 4 I's + 6 II's + 2 IV's. x ca. 1330

Fig. 4 2 I's + 11 II's. x ca. 1550

Figures 5-7 Diakinesis in (L. japonicus x L. alpinus) x L. corniculatus.

Fig. 5 2 I's + 6 II's + 2 III's + 1 IV. x ca. 2125

Fig. 6 2 I's + 7 II's + an association of 8 chromosomes. x ca. 1575

Fig. 7 2 I's + 7 II's + 2 IV's. x ca. 2075

Figures 8-9 MI in (L. krylovii x L. schoelleri) x L. corniculatus.

Fig. 8 4 I's + 10 II's. x ca. 2200

Fig. 9 2 I's + 9 II's + 1 IV. x ca. 1700

Figures 10-11 MI in (L. krylovii x L. schoelleri) x L. corniculatus.

Fig. 10 1 I + 8 II's + 1 III + 1 IV. x ca. 1800

Fig. 11 12 II's. x ca. 1625

Figure 12 MI in (L. filicaulis x L. schoelleri) x L. corniculatus showing 2 I's + 9 II's + 1 IV. x ca. 1850

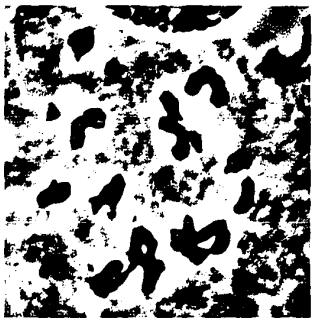
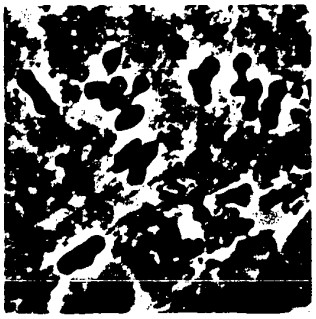


Figure 1

Figure 2

Figure 3



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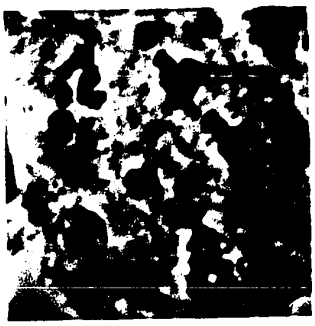


Figure 5

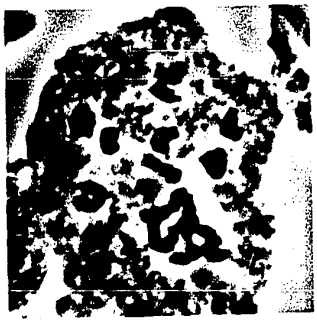


Figure 6

Figure 7

Figure 8

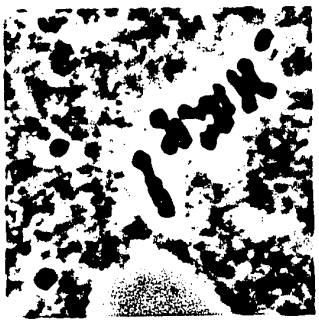


Figure 9

Figure 10

Figure 11

Figure 12

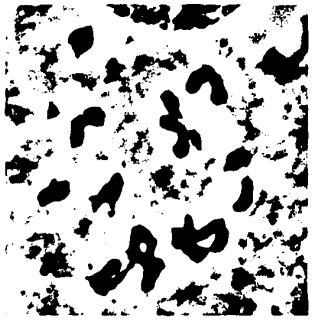


Figure 1

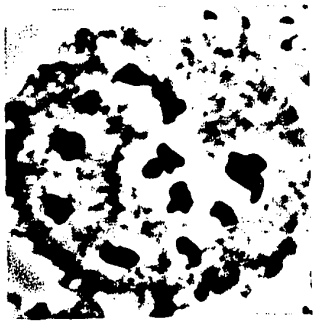


Figure 2

Figure 3

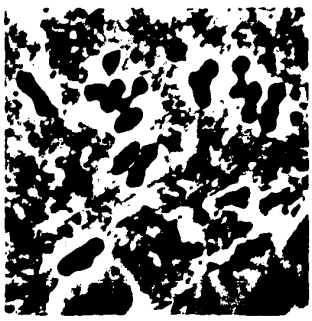
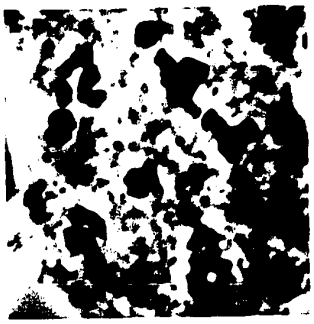


Figure 4



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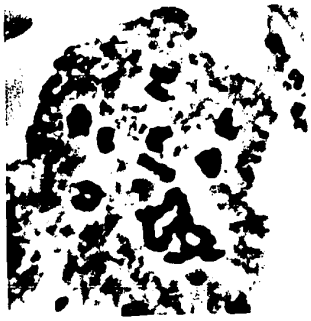


Figure 6

Figure 7

Figure 8

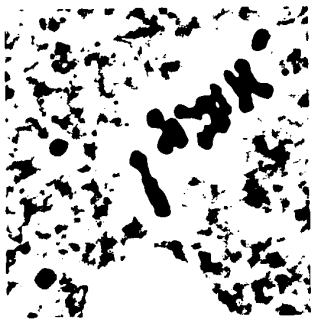


Figure 9

Figure 10

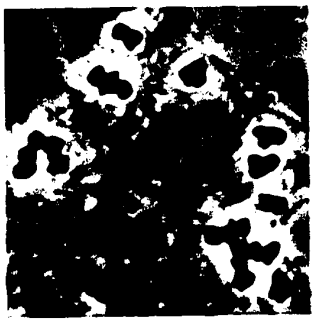


Figure 11

Figure 12

Plate XXIV

Meiosis in the crosses between the amphidiploids and L. corniculatus

Figures 1-3 MI in (L. filicaulis x L. schoelleri) x L. corniculatus.

Fig. 1 4 I's + 8 II's + 1 IV. x ca. 1330

Fig. 2 11 I's + 3 II's + 1 III + 1 IV. x ca. 1000

Fig. 3 4 I's + 8 II's + 1 IV. x ca. 1600

Figure 4 Diakinesis in (L. corniculatus var. minor x L. alpinus) x L. corniculatus showing 8 II's + 2 IV's. x ca. 1725

Figure 5 Prometaphase in (L. corniculatus var. minor x L. alpinus) x L. corniculatus showing 12 II's. x ca. 1750

Figure 6 AI in (L. filicaulis x L. schoelleri) x L. corniculatus showing failure of separation of a quadrivalent. x ca. 1000

Figure 7 AI in (L. filicaulis x L. schoelleri) x L. corniculatus showing delayed separation of one chromosome. x ca. 1650

Figure 8 AI in L. corniculatus x (L. japonicus x L. krylovii) showing delayed separation of a quadrivalent. x ca. 1900

Figure 9 AI in (L. krylovii x L. schoelleri) x L. corniculatus showing lagging chromosomes. x ca. 1350

Figure 10 AI in (L. filicaulis x L. schoelleri) x L. corniculatus showing delayed separation of a bivalent. x ca. 2350

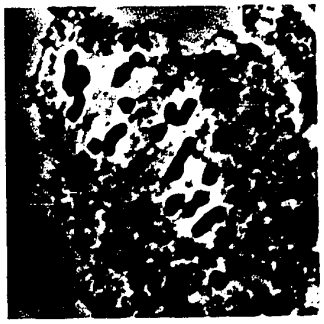


Figure 1

Figure 2

Figure 3

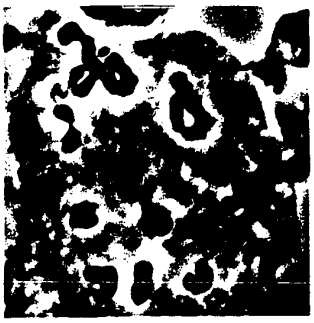


Figure 4

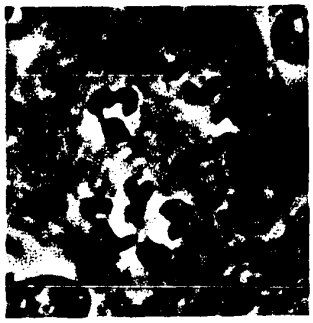
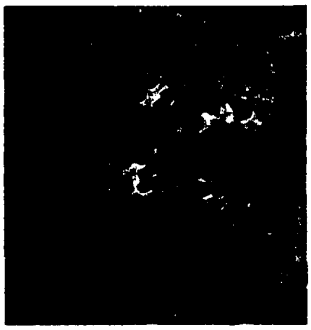


Figure 5

Figure 6



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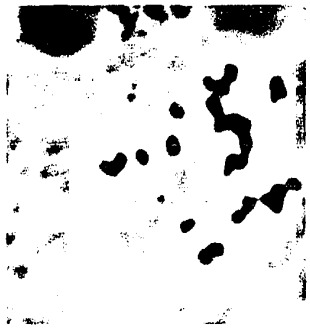

Figure 8

Figure 9

Figure 10

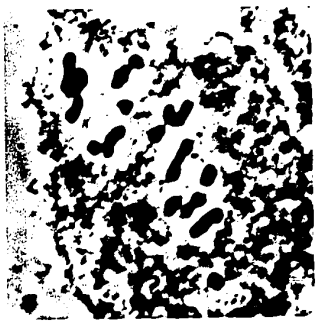

Figure 1

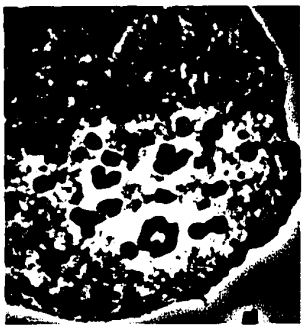

Figure 2

Figure 3

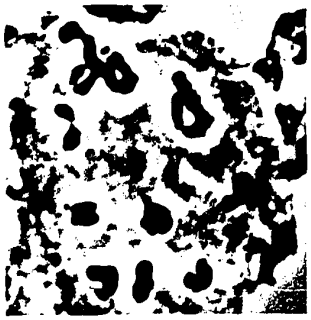

Figure 4

Figure 5

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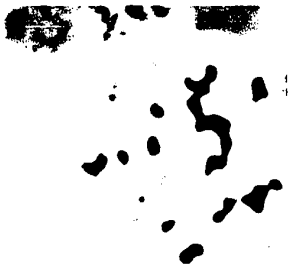


Figure 8

Figure 9

Figure 10

Plate XXV

Meiosis in the crosses between the amphidiploids and L. corniculatus

Figure 1 — TI in L. corniculatus x (L. japonicus x L. schoelleri) showing 2 laggards. x ca. 1550

Figure 2 — AI in (L. krylovii x L. filicaulis) x L. corniculatus showing lagging chromosomes. x ca. 1400

Figure 3 — AI in L. corniculatus x (L. japonicus x L. alpinus) showing inversion bridge + fragment + 1 lagging chromosome. x ca. 1700

Figures 4-5 — TII in (L. alpinus x L. schoelleri) x L. corniculatus.

Fig. 4 — Dividing laggards. x ca. 1180

Fig. 5 — (a) Normal plate (left) and (b) lagging chromosomes (upper right). x ca. 600

Figure 6 — TII in (L. corniculatus var. minor x L. filicaulis) x L. corniculatus showing laggards. x ca. 1600

Figure 7 — TI in (L. corniculatus var. minor x L. filicaulis) x L. corniculatus showing bridges + fragments. x ca. 950

Figure 8 — AII in L. corniculatus x (L. japonicus x L. alpinus) showing bridges and laggards. x ca. 1500

Figure 9 — TII in L. corniculatus x (L. japonicus x L. alpinus) showing bridge. x ca. 1000

Figures 10-11 — TII in (L. japonicus x L. krylovii) x L. corniculatus showing bridges and fragments.

Fig. 10 — x ca. 1280

Fig. 11 — x ca. 1030

Figure 12 — Late TII in L. corniculatus x (L. japonicus x L. alpinus) showing 7 aggregations of chromosomes. x ca. 1550

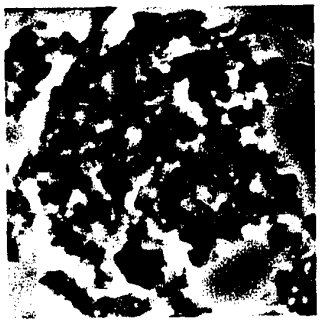
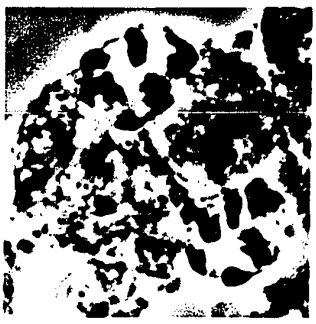


Figure 1

Figure 2



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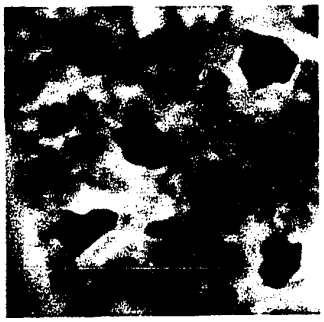


Figure 4

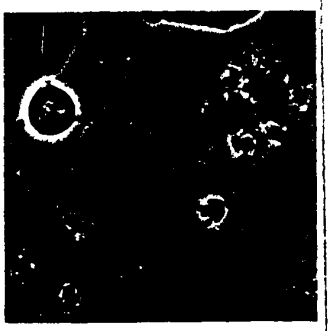
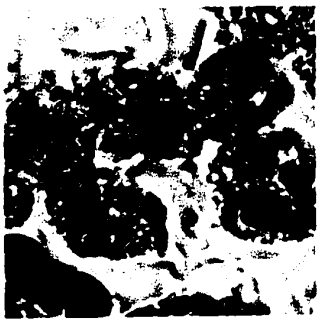


Figure 5

Figure 6



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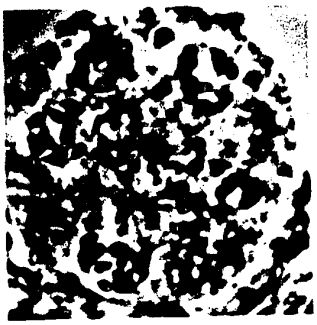

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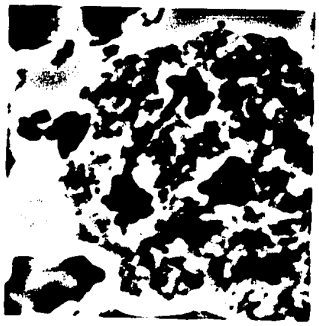

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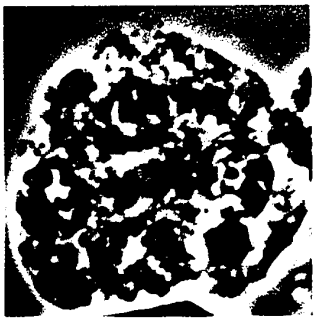

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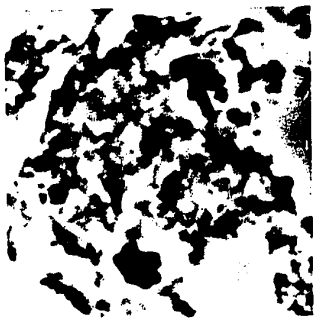


Figure 1

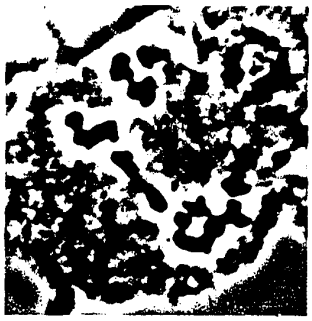
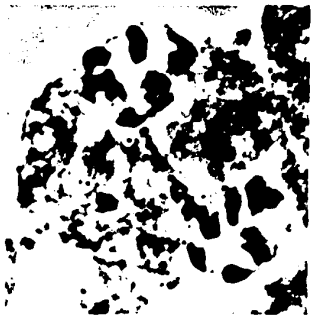


Figure 2



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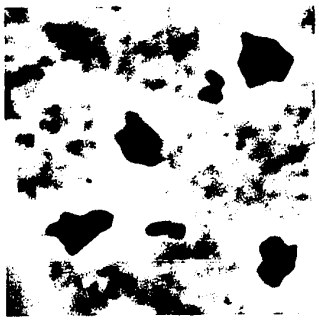
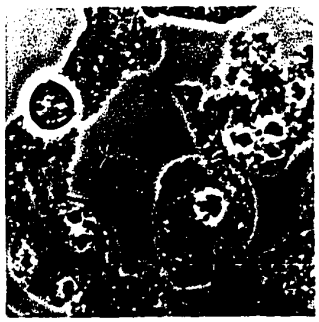


Figure 4



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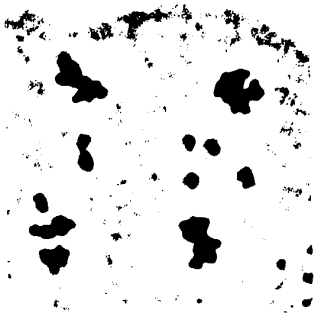

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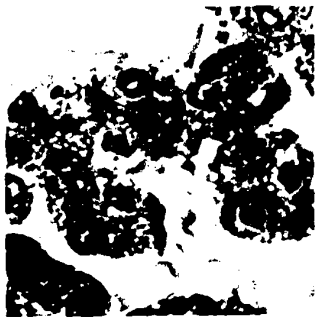

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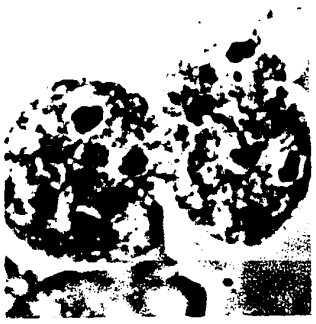

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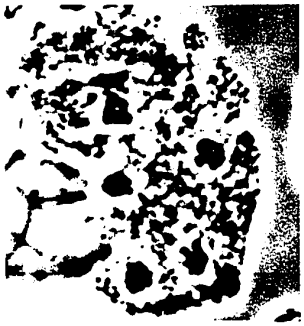

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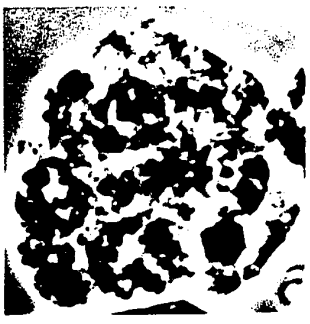


Figure 12

VI. Fertility studies

Fertility in the diploid parental species

Data obtained from observations made on pollen stainability and seed set in the diploid species, L. alpinus, L. tenuis, L. corniculatus var. minor, L. japonicus, L. schoelleri, L. filicaulis, and L. krylovii are set out in Table 34. The percentage stainable pollen was found to be low in many of the diploids and considerable variation was observed from species to species. The highest percentage of good pollen (96.43), as judged by its stainability in acetocarmine, was recorded in L. japonicus. In the remaining six species, a range of 92.93 (in L. corniculatus var. minor) to 67.19 per cent (in L. alpinus) was recorded.

The average seed set per pod for each of the seven diploid species was determined from plants growing in the field. Counts were based on 30 pods which were harvested at random; no hand pollination was performed. A high value of 24.80 seeds per pod was observed for L. corniculatus var. minor and the lowest value of 9.26 seeds per pod for L. alpinus. The seed set values for the remaining five species ranged from 19.23 to 21.40 seeds per pod.

Fertility in the interspecific diploid hybrids

Table 35 summarizes the pollen stainability and seed set data in the interspecific diploid hybrids. Pollen grains of various sizes and with varying degree of stainability were encountered in the hybrids but only those grains possessing a diameter in the size range of normal

pollen of the species were scored as normal and tabulated as stainable pollen. Occasionally, in some of the diploid hybrids "giant" pollen grains probably resulting from abnormal meiosis were also present. Their presence resulted in an overlapping of measurement within the range of pollen size in the induced tetraploids, but on the whole, the diploid pollen was smaller than the pollen of the corresponding tetraploids. It may be seen from Table 35 that the differences in the percentage stainable pollen between genotypes of the same cross were not very great. This was the case for all the cross combinations except two genotypes AF-1-3 and AF-4-3 of the cross L. alpinus x L. filicaulis, which exhibited 68.35 and 84.88 per cent stainable pollen, respectively. However, when the data on the average stainability of the pollen were considered for each cross combination, wide differences were seen between certain groups. Such differences can be seen from Plate XXVI which depicts the relative pollen fertility relationships in these hybrids. For example, L. alpinus x L. krylovii and L. alpinus x L. filicaulis with 86.11 and 76.25 per cent normal pollen respectively, can be grouped into one class, L. corniculatus var. minor x L. alpinus (50.29 per cent), L. krylovii x L. corniculatus var. minor (53.02 per cent) and L. japonicus x L. corniculatus var. minor (55.09 per cent) may constitute a second group, and the remaining crosses with percentage stainable pollen ranging from 32.28 per cent, L. corniculatus var. minor x L. filicaulis, to 16.63 per cent, L. filicaulis x L. schoelleri, can be placed in a third group.

From an examination of Tables 34 and 35, it can be seen that

TABLE 34. Pollen stainability and seed set in the diploid parental species

Species	Accession numbers	No. of pollen grains examined	No. of stainable pollen grains	Average stainable pollen (%)	Average seed set per pod
L. alpinus	B-77	3142	2111	67.19	9.26
L. japonicus	B-129	2663	2568	96.43	21.40
L. tenuis	B-109	2337	1926	82.41	21.17
L. filicaulis	B-37	2960	2288	77.30	19.23
L. schoelleri	B-166	2145	1596	74.41	20.03
L. krylovii	B-86	2470	2150	87.04	20.73
L. corniculatus var. minor	B-303	2604	2420	92.93	24.80

TABLE 35. Pollen stainability and seed set in the interspecific diploid hybrids

Hybrid	Genotypes	No. of pollen grains examined	No. of stainable pollen grains	Stainable pollen (%)	Average stainable pollen (%)	Average seed set per pod
L. alpinus X schoelleri	AS-1-4	866	198	22.86		
	AS-1-2	1180	269	22.80	22.83	2.90
L. alpinus X filicaulis	AF-1-3	613	419	68.35		
	AF-5	655	486	74.20		
	AF-4-3	344	292	84.88	74.25	6.70
L. alpinus X krylovii	AK	2103	1811	86.11	86.11	5.70
L. alpinus X japonicus	AJ-1	1108	208	18.77		
	AJ-2	1273	292	22.94	21.00	4.33
L. japonicus X alpinus	JA	2271	608	26.77	26.77	2.66
L. corniculatus var. minor X alpinus	MA-12	1389	740	53.26		
	MA-11	1236	580	46.93	50.29	9.03
L. corniculatus var. minor X filicaulis	MF-1	645	207	32.09		
	MF-2	760	226	29.74		
	MF-3	593	212	35.76	32.28	6.57

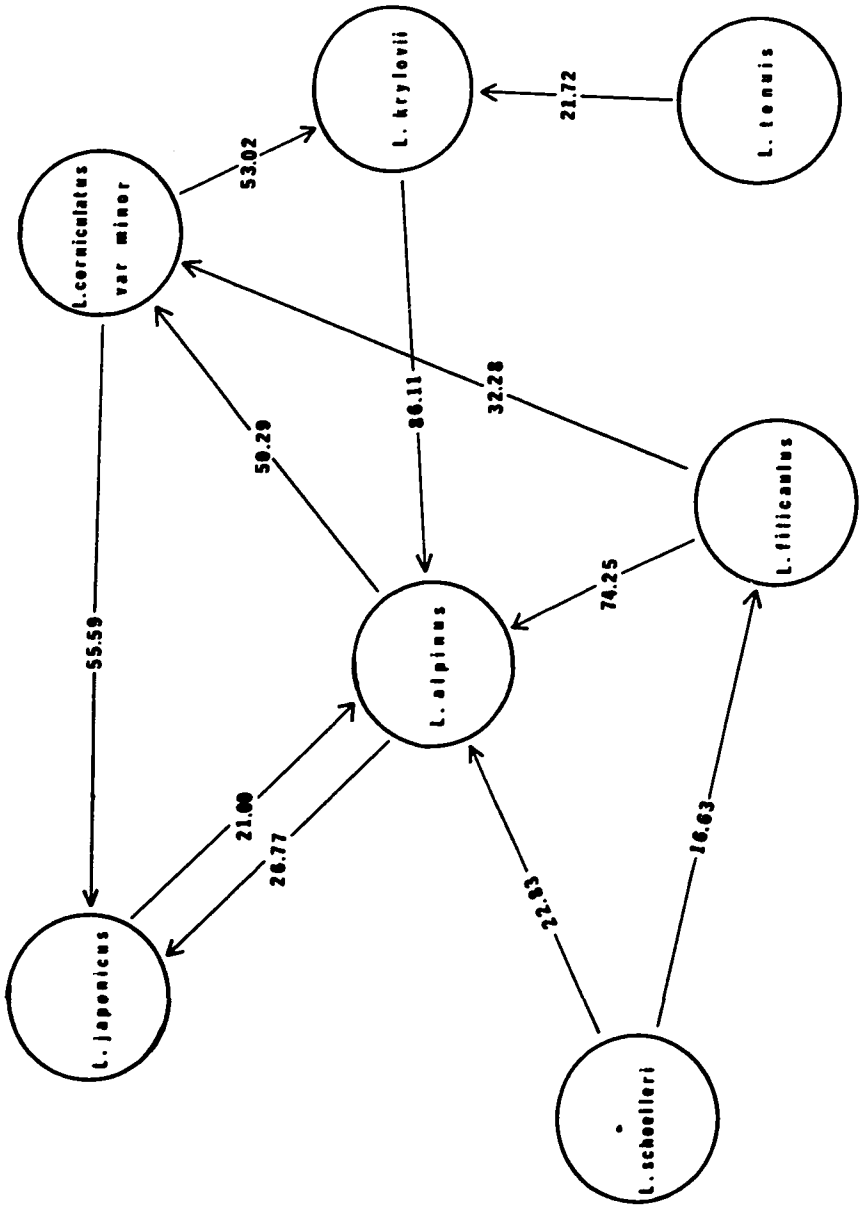
Table 35 Cont'd

TABLE 35 (Cont'd)

Hybrid	Genotypes	No. of pollen grains examined	No. of stainable pollen grains	Stainable pollen (%)	Average stainable pollen (%)	Average seed set per pod
L. krylovii X corniculatus var. minor	KM-1	521	266	51.06		
	KM-2	1003	527	52.54		
	KM-3	858	470	54.78	53.02	6.27
L. japonicus X corniculatus var. minor	JM-1	696	411	59.05		
	JM-2	1576	852	54.06	55.59	7.43
L. krylovii X tenuis	KT-1	1791	389	21.72	21.72	0.66
L. filicaulis X schoelleri	FS-1-1	1121	175	15.61		
	FS-1-3	1428	249	17.44	16.63	-*

* No information available

Plate XXVI



Diagrammatic representation of per cent stainable pollen in the hybrids between seven species of Lotus. The arrows point to the maternal parent in the cross.

there is a considerable difference between the percentage stainable pollen of any given hybrid and its parental species. In L. alpinus x L. krylovii and L. alpinus x L. filicaulis, however, the frequency of stainable pollen was close to that of the respective female parent.

Seed set per pod in the hybrids was evaluated under open pollinated conditions in the field. All the hybrid plants produced some seeds; however a very low figure of 0.66 seed per pod was recorded for L. krylovii x L. tenuis. All the crosses in which L. corniculatus var. minor served as a parent (for example, L. corniculatus var. minor x L. alpinus, L. corniculatus var. minor x L. filicaulis, L. krylovii x L. corniculatus var. minor, and L. japonicus x L. corniculatus var. minor) had relatively high seed set values. A comparatively high seed set value of 6.70 was also noted for L. alpinus x L. filicaulis. It is interesting to note that the average seed set per pod in these interspecific hybrids was considerably lower than the values recorded for the diploid parental species (Tables 34 and 35).

Fertility in the induced autotetraploids and Lotus corniculatus

Pollen fertility data for the autotetraploids and L. corniculatus are set out in Table 36. Deformed pollen grains and a wide variability in pollen grain size were commonly present in all the tetraploids studied. Some of the pollen grains were noticeably large and probably contained aneuploid gametes. Only pollen grains that were stained and within the tetraploid size range were considered normal and tabulated as stainable pollen. In comparison with their diploid

counterparts, these derived tetraploid plants exhibited a much lower amount of normal pollen. The amount of stainable pollen ranged from 73.21 per cent in L. japonicus (4x) to 44.57 per cent in L. alpinus (4x). No data on seed set are available since inclement weather interrupted the harvesting of pods from these plants. In L. corniculatus, 78.81 per cent of the pollen was stainable and a seed set value of 10.13 seeds per pod was recorded. Thus all the induced autotetraploids exhibited a lower per cent of stainable pollen than that recorded for L. corniculatus. A noteworthy observation, however, is that the per cent stainable pollen in autotetraploid L. japonicus (73.21 per cent) was only about 5 per cent below that recorded for L. corniculatus.

Fertility in the synthetic amphidiploids

The data on stainable pollen and seed set for the synthetic amphidiploids are presented in Table 37. It can be ascertained from this table, that there were wide differences in pollen stainability for the different genotypes in certain amphidiploids. The greatest variability in pollen stainability was seen in the genotypes of the amphidiploid (L. japonicus x L. alpinus) where the stainable pollen ranged from 30.11 per cent in the genotype A/JA-1 to 69.06 per cent in the genotype A/JA-10. A considerable variation in pollen stainability ranging from 48.76 per cent (A/AF-3) to 71.93 per cent (A/AF-4) was also found among the different genotypes of the amphidiploid (L. alpinus x L. filicaulis). The highest percentage of normal pollen (65.89) was

found for the amphidiploid (L. japonicus x L. corniculatus var. minor); this was followed by 59.04 per cent in the amphidiploid (L. alpinus x L. filicaulis). The lowest value of 11.15 per cent was noted in (L. alpinus x L. krylovii). The remaining amphidiploids may be grouped into three classes which showed considerable differences between one another in pollen stainability.

For example in one group comprising (L. corniculatus var. minor x L. alpinus), (L. japonicus x L. alpinus) and (L. corniculatus var. minor x L. filicaulis) pollen stainability ranged from 46.02 per cent to 52.24 per cent; in another group made up of (L. filicaulis x L. schoelleri), (L. japonicus x L. krylovii) and (L. krylovii x L. filicaulis), the range of good pollen varied from 36.16 per cent to 38.93 per cent; and a final group consisting of (L. alpinus x L. schoelleri), (L. japonicus x L. schoelleri) and (L. krylovii x L. schoelleri) exhibited a range of 22.83 to 25.03 per cent. It may be seen that the variability in the amount of good pollen within each group was small.

The average seed set per pod recorded for the amphidiploids was low; the highest figure of 5.50 seeds per pod was noted for the amphidiploid (L. krylovii x L. schoelleri) and the lowest (2.13 seeds per pod) was recorded for (L. japonicus x L. corniculatus var. minor). No seed set was observed for (L. alpinus x L. schoelleri) and (L. alpinus x L. krylovii).

When the pollen stainability figures for the amphidiploids (Table 37) were compared with those for the interspecific diploid

TABLE 36. Pollen stainability and seed set in the induced autotetraploids and *L. corniculatus*

Autotetraploid and *L. corniculatus*	Accession numbers	No. of pollen grains examined	No. of stainable pollen grains	Average stainable pollen (%)	Average seed set per pod
L. alpinus	T-77	1557	694	44.57	-
L. japonicus	T-129	1646	1242	73.21	-
L. tenuis	B-340	2144	1303	60.77	-
L. filicaulis	T-37	2619	1187	45.32	-
L. schoelleri	T-166	1873	892	47.62	-
L. krylovii	T-86	1480	717	48.45	-
L. corniculatus var. *minor*	T-303	1331	818	61.46	-
L. corniculatus	B-554	2549	2009	78.81	10.13

TABLE 37. Pollen stainability and seed set in the amphidiploids

Amphidiploid (4x)	Genotypes	No. of pollen grains examined	No. of stainable pollen grains	Stainable pollen (%)	Average stainable pollen (%)	Average seed set per pod
(L. alpinus X schoelleri)	A/AS-2	2309	524	22.83	22.83	-*
(L. alpinus X filicaulis)	A/AF-1	605	341	53.36		
	A/AF-2	511	309	60.47		
	A/AF-3	525	256	48.76		
	A/AF-4	488	351	71.93	59.04	3.77
(L. alpinus X krylovii)	A/AK-2	1283	145	11.30		
	A/AK-7	1150	99	8.60	11.15	-*
(L. japonicus alpinus)	A/JA-1	963	290	30.11		
	A/JA-2	801	486	60.67		
	A/JA-10	666	460	69.06	50.86	2.17
(L. corniculatus var. minor X alpinus)	A/MA-3-1	864	471	54.51		
	A/MA-3-2	896	339	37.84	46.02	2.30
(L. corniculatus var. minor X filicaulis)	A/MF-2-3	710	415	58.45		
	A/MF-2-4	367	117	46.80		
	A/MF-2-5	420	250	59.52	52.24	4.80

Table 37 Cont'd

TABLE 37 (Cont'd)

Amphidiploid (4x)	Genotypes	No. of pollen grains examined	No. of stainable pollen grains	Stainable pollen (%)	Average stainable pollen (%)	Average seed set per pod
(L. japonicus X corniculatus var. minor)	A/JM-4-1	865	571	66.01		
	A/JM-2-1	976	652	66.80		
	A/JM-2-2	648	417	64.35	65.89	2.13
(L. filicaulis X schoelleri)	A/FS-2	2406	870	36.16	36.16	4.27
(L. japonicus X schoelleri)	A/JS-3-4	601	111	18.47		
	A/JS-3-6	850	142	16.71		
	A/JS-3-7	686	261	38.05	24.05	2.87
(L. japonicus X krylovii)	A/JK-1		1068	38.93	38.93	
(L. krylovii X filicaulis)	A/KF-2-7	888	309	34.80		
	A/KF-2-5	1074	441	41.06	38.23	4.30
(L. krylovii X schoelleri)	A/KS-7	1040	259	24.90		
	A/KS-1	798	201	25.19	25.03	5.50

* No information available

hybrids (Table 35), all the amphidiploids except (L. alpinus x L. filicaulis), (L. alpinus x L. krylovii), and (L. corniculatus var. minor x L. alpinus) had higher values than those in the corresponding interspecific hybrids. A similar comparison for seed set data, however, indicated that higher values were obtained for the diploid hybrids. It is interesting to note that the pollen stainability figure (78.81 per cent) and seed set value (10.13 seeds per pod) in L. corniculatus were higher than those observed in all the synthetic amphidiploids.

Fertility in the crosses between the amphidiploids and Lotus corniculatus

The percentage of morphologically good and stainable pollen was determined for the hybrids obtained from crosses between the synthetic amphidiploids and L. corniculatus. The data recorded in this study are summarized in Table 38. It may be seen that the pollen fertility in all the hybrids was relatively high. There was little variability between the genotypes of a particular cross. All the hybrids except L. corniculatus x (L. japonicus x L. alpinus), L. corniculatus x (L. japonicus x L. schoelleri) and (L. krylovii x L. schoelleri) x L. corniculatus exhibited pollen stainability above 70 per cent. In the last three crosses, the percentages of stainable pollen were 58.69, 62.02 and 66.61 per cent, respectively. The range of fertile pollen in all the hybrids varied from 62.02 per cent for L. corniculatus x (L. japonicus x L. schoelleri) to 85.43 per cent for L. corniculatus x (L. krylovii x L. filicaulis).

TABLE 38. Pollen stainability in the crosses between the amphidiploids and L. corniculatus

Cross	Genotypes	No. of pollen grains examined	No. of stainable pollen grains	Stainable pollen (%)	Average stainable pollen (%)
(L. alpinus X L. schoelleri) X L. corniculatus	AS-C-1-1	1113	832	74.75	74.75
(L. alpinus X L. filicaulis) X L. corniculatus	AF-C-1-1	1008	744	73.80	
	AF-C-2-1	983	758	77.11	75.43
(L. japonicus X L. alpinus) X L. corniculatus	JA-C-1-1	988	744	75.30	
	JA-C-2-3	788	604	76.64	75.90
L. corniculatus X (L. japonicus X L. alpinus)	C-JA-1-5	1513	888	58.69	58.69
(L. corniculatus var. minor X L. alpinus) X L. corniculatus	MA-C-1-2	963	735	76.32	
	MA-C-2-1	914	754	82.49	79.32
(L. corniculatus var. minor X L. filicaulis) X L. corniculatus	MF-C-1-1	659	490	74.35	
	MF-C-2-3	592	451	76.49	
	MF-C-3-1	651	514	78.95	76.49
(L. japonicus X L. corniculatus var. minor) X L. corniculatus	JM-C-2-3	1115	880	78.92	
	JM-C-1-2	834	673	80.69	79.68

Table 38 Cont'd

TABLE 38 (Cont'd)

Cross	Genotypes	No. of pollen grains examined	No. of stainable pollen grains	Stainable pollen (%)	Average stainable pollen (%)
(*L. filicaulis* X *L. schoelleri*) X *L. corniculatus*	FS-C-1-1	1103	796	72.17	72.17
(*L. japonicus* X *L. schoelleri*) X *L. corniculatus*	JS-1-2	1011	745	73.68	
	JS-2-3	969	754	77.81	75.70
L. corniculatus X (*L. japonicus* X *L. schoelleri*)	C-JS-1-2	948	588	62.02	62.02
(*L. japonicus* X *L. krylovii*) X *L. corniculatus*	JK-C-1-7	1749	1296	74.09	74.09
L. corniculatus X (*L. japonicus* X *L. krylovii*)	C-JK-1-4	1666	1222	73.34	73.34
(*L. krylovii* X *L. filicaulis*) X *L. corniculatus*	KF-C-1-10	943	729	77.30	
	KF-2-9	1157	869	75.10	76.09
L. corniculatus X (*L. krylovii* X *L. filicaulis*)	C-KF-1-1	1167	997	85.43	85.43
(*L. krylovii* X *L. schoelleri*) X *L. corniculatus*	KS-C-1-5	951	621	65.29	
	KS-C-2-2	1014	688	67.85	66.61

Differences in pollen fertility were observed in certain reciprocal crosses. For example, the percentages of stainable pollen were: 1) 75.90 for (L. japonicus x L. alpinus) x L. corniculatus with 58.69 in the reciprocal combination, 2) 75.70 for (L. japonicus x L. schoelleri) x L. corniculatus with 62.02 for hybrids of the reciprocal cross and 3) 76.09 for (L. krylovii x L. filicaulis) x L. corniculatus with 85.43 in the reciprocal cross. Hybrids derived from crosses between (L. corniculatus var. minor x L. alpinus) and L. corniculatus, and (L. japonicus x L. corniculatus var. minor) and L. corniculatus, exhibited pollen stainability greater than those observed for both parents. In the remaining cross combinations, pollen fertility varied from 2.32 to 20.12 per cent below that observed in L. corniculatus (Tables 36 and 38). An examination of the pollen stainability data for the amphidiploids (Table 37) indicates that in all cases there was a marked increase in the pollen fertility in these hybrids as compared with their corresponding amphidiploid parents.

DISCUSSION

I. Interspecific hybridization

In this study varying degrees of success were achieved in effecting hybridization between the diploid species. The embryos of many cross combinations aborted during the early stages of development and many young embryos did not proliferate and grow when they were transferred to the culture medium. Similar observations were made by other workers who conducted interspecific hybridization studies in this genus (Keim, 1952; Mears, 1955; Bent, 1958; Grant et al., 1962). It would seem therefore, that in order to conduct a comprehensive hybridization program in Lotus at the diploid level, embryo-culture technique is virtually a necessity.

In the present study, the degeneration of the embryos were observed to take place at different times following pollination and this invariably occurred in the presence of copious endosperm within the ovules. Thus, the limiting factor was not somatoplastic sterility, that is, sterility resulting from starvation of the endosperm because of its inability to keep pace with the hypertrophied growth of the nucellar tissue. More likely, in the unsuccessful crosses, the failure of embryos to develop was due partly to genetic disturbances within the embryo, and, partly to the fact that the requirement of the embryos with respect to the culture medium may be more critical when such requirements are considered in relation to those embryos which were successfully cultured.

The rather high number of embryos exhibiting no proliferation in the culture medium, raises the question concerning the suitability of the modified Randolph-Cox culture medium (Grant et al., 1962) utilized in this study. In preliminary tests, the sucrose content was increased and various vitamins were also added but this further modification did not help. Although a higher percentage of the embryos appeared to remain alive for a longer period of time, no significant improvement in growth and development was seen. These observations would indicate that the most ideal embryo-culture procedures for the embryos of Lotus crosses have not been achieved. The voluminous literature which deals with the culture of immature embryos of many plant genera, on appropriate media indicates conclusively that methodology is all important; each study has shown that the use of materials such as vitamins, coconut milk, its fractionated derivatives and casein hydrolysate have made it possible to grow progressively younger embryos in culture medium. Perhaps a further modification of the medium by the inclusion of some of these materials may prove rewarding.

Certain workers (Keim, 1952; Mears, 1955) have indicated that if a sufficiently high number of cross pollinations was made, enough viable seeds would be produced to make unnecessary the use of embryo culture. In this connection, Grant et al. (1962) also stated that embryo culture is not necessary provided the investigator is prepared to make about 10,000 emasculations and pollinations. In this study, similar conclusions can be drawn for those crosses which

were successfully produced by the embryo-culture procedure. However, the author believes also that in many cases a modest number of pollinations is all that is necessary for the successful production of viable seeds. This was exemplified by the crosses L. japonicus x L. krylovii, L. japonicus x L. filicaulis, L. japonicus x L. corniculatus var. minor, L. japonicus x L. alpinus, L. alpinus x L. krylovii, L. alpinus x L. filicaulis, L. krylovii x L. schoelleri, L. krylovii x L. filicaulis, L. krylovii x L. corniculatus var. minor, L. corniculatus var. minor x L. alpinus, and L. corniculatus var. minor x L. filicaulis, all of which produced viable hybrid seeds that germinated. It is interesting to note that five of the above-mentioned crosses (L. japonicus x L. krylovii, L. japonicus x L. filicaulis, L. japonicus x L. alpinus, L. krylovii x L. filicaulis and L. krylovii x L. schoelleri) were obtained by Grant and his co-workers (Grant et al., 1962; de Nettancourt and Grant, 1964a); these authors inferred that the production of these hybrids would have been extremely difficult or impossible without the use of embryo-culture technique.

The success in effecting crosses in Lotus species depends very much on the proper choice of the pistillate parent. All the fertile combinations in this study involved female parents which displayed self-compatibility. Even in crosses in which both parents were self-compatible, one self-compatible parent generally was more successful than the other as a seed parent for a particular cross. This was exemplified by reciprocal crosses between L. krylovii and L. alpinus

and L. krylovii and L. schoelleri. In crosses involving two self-incompatible species, one parent generally performed better than the other parent in a particular cross and a high degree of pod set was obtained only when one of the two species was used as the pistillate parent. These observations are in agreement with those of other workers; Keim (1952), Bent (1962) and Grant et al. (1962) reported that successful hybridization of Lotus species depended on the choice of a self-compatible female parent. Mears (1955) also pointed out that in Lotus crosses involving two self-incompatible parents, the choice of the seed parent is important. The relationship between self-compatibility and successful hybridization in Lotus is probably explained by the findings of Bubar (1958). This investigator studied the relationship between ovule development and self-fertility and found that there is considerable variability in the rate of ovule development in self-sterile Lotus species; in contrast, he observed that the ovules of self-fertile species mature simultaneously soon after the flowers are open. The success in obtaining hybrids when a more self-compatible species is used as the seed parent may be due to the fact that there is a greater number of ovules to be fertilized after a single pollination.

Considering the relative ease with which a number of hybrid combinations have been produced, there is a strong indication that a certain amount of natural hybridization might have occurred during the early history of the genus. Data on chromosome pairing affinities (to be discussed later) further substantiate this postulate.

Dawson (1941) and MacDonald (1946) confined their hybridization studies to three species, L. corniculatus, L. tenuis and L. pedunculatus and concluded that in Lotus species there is little tendency towards hybridization. Bent (1962) and Grant et al. (1962) reached a similar conclusion after conducting interspecific hybridization and embryo-culture studies. On the other hand, supporting evidence for natural hybridization in Lotus is provided by the studies of Ottley (1944), Larsen (1954), Gillett (1958) and other workers (see Literature Review).

The rarity of natural hybrids may be due to a number of factors. For example, although the flowering periods for many of the species are about the same, they probably have different peaks of flowering periods. Some of the species are normally both cross- and self-fertile and they are pollinated by insects. It is possible that insects would usually restrict their activities to one species at a time and this would further limit the production of natural interspecific hybrids. If cross-pollination did occur, viable seeds of the F_1 hybrids would only be formed part of the time. Therefore, the investigator would have to search very closely for natural hybrids, or find them by chance. In Lotus, lack of morphological characteristics of interspecific hybrids might have made it difficult to recognize them when they did occur. In this study, it has been shown that some of the hybrids exhibited a closer phenotypic resemblance to one of its two parents. Geographical separation also is another factor that could limit the production of natural hybrids of certain species. For example, L. japonicus which crosses relatively easily with some of the other

hybrids is restricted to Japan and Korea (Eastern Asia). Also L. corniculatus var. minor crosses easily with certain other species, and this taxon is found only in West Pakistan.

If the Lotus species which now have a world wide distribution, have a common center of origin, then at some time in their evolutionary history, a certain amount of hybridization could have occurred. Hence, it is more than likely that certain species originated as a result of interspecific hybridization.

II. Crossing relationships between the synthetic amphidiploids and Lotus corniculatus

Many of the diploid Lotus species which may be of interest from a practical breeding point of view cannot be easily crossed with L. corniculatus, the cultivated species. The results obtained in this study, however, have shown that the induced tetraploids of species hybrids could be crossed successfully with L. corniculatus without recourse to embryo culture. Furthermore, a greater degree of success was realized when the cultivated species was used as the male parent. The reason for this is not apparent. The success of a cross usually depends on the environmental conditions in which emasculation and pollination are performed as well as on the physiological make-up, and the genotypes of the plants selected for crossing. In this study, all the crosses were made under the same environmental conditions and all the L. corniculatus plants were obtained from a single clone. A possible explanation for the apparent suitability of the synthetic amphidiploids as female parents, is that they were self-

compatible. It has been indicated that crosses were more successful at the diploid level when self-compatible plants were used as the seed parent; the explanation presented for the diploid crosses to explain this phenomenon may be applicable here also.

In other hybridization studies, Mears (1955), Bent (1962), Beamish, Cooper and Hougas (1957) and Magoon, Cooper and Hougas (1958a) reported that the choice of the pistillate parent is an important consideration in obtaining successful crosses. In crosses between induced autotetraploids and L. corniculatus, Mears (1.c.) and Bent (1.c.) were successful in producing hybrids when L. corniculatus was used as the pistillate parent (see Literature Review). Beamish, Cooper and Hougas (1.c.) and Magoon, Cooper and Hougas (1.c.) also found that in the genus Solanum, when crosses were attempted between induced autotetraploids and synthetic amphidiploids and the cultivated species S. tuberosum, successful hybridization was achieved only when the latter species was utilized as the female parent.

From the crossing relationships of the various amphidiploids and L. corniculatus, it would appear that L. alpinus and L. japonicus are more closely related to L. corniculatus than the other species. This is apparent from the figures recorded for the percentage of successful crosses. Relatively higher values were obtained when either of these two species constituted one of the parents of the original interspecific hybrid that was doubled. The high degree of success realized in crosses between (L. japonicus x L. alpinus) and

L. corniculatus would indicate that there is very little incompatibility barrier between this amphidiploid and the cultivated species.

III. Morphological studies

Morphology of the parental species

Morphological characters were studied in detail in six of the seven species utilized in this study namely, L. japonicus, L. alpinus, L. krylovii, L. filicaulis, L. schoelleri and L. tenuis by de Nettancourt (1963). On the basis of certain morphological attributes, de Nettancourt arbitrarily grouped these taxa into two categories. One group consisted of L. japonicus and L. alpinus; the close morphological resemblance of these two species was manifested in their non-ascending growth habit, high leaflet index, large deep-yellow flowers, red stem and the presence of cyanogenetic glycoside. The remaining four species made up the other category and showed ascending growth habit, low leaflet index, pale small-yellow flowers, green stems, mottled pods, and speckled seeds.

In the present investigation, a comparative morphological study between L. corniculatus var. minor and the above-mentioned six species suggests, that L. corniculatus var. minor with its low leaflet index, pale small-yellow flowers, green stem, and absence of cyanogenetic glycoside, could also be included in the second category proposed by de Nettancourt. Statistical analysis of the data of 15 morphological traits studied, also showed that this taxon has distinct morphological attributes. On the basis of these observations, its geographical

distribution, crossing behavior, and chromosome number, L. corniculatus var. minor is hardly likely to be a sub-species of L. corniculatus. Perhaps it should be considered a separate species and should be accorded specific status.

Morphology of the interspecific hybrids

Observations made on certain qualitative characters showed that in addition to vigorous growth and profuse branching, many of the hybrids generally exhibited with slight variation in intensity, the phenotype of one of the two parents. This feature was exemplified by studies of floret striping, flower bud and flower color, keel tip color of the flower, and the presence of cyanogenetic glycoside. Some of the hybrids were intermediate between the parents for several of the metrical characters studied. However, the F_1 hybrids transgressed the limits of the parents in one or more traits. Such a tendency towards heterotic vigor was particularly noticeable in the crosses L. alpinus x L. japonicus, L. krylovii x L. corniculatus var. minor, L. krylovii x L. tenuis, L. corniculatus var. minor x L. alpinus, L. alpinus x L. krylovii and L. corniculatus var. minor x L. filicaulis. The first three of these crosses showed heterotic effects in seven traits, whereas, the last three exhibited transgression in six characters. In hybrids of the cross L. japonicus x L. corniculatus var. minor, heterosis was manifested only in the floral bract length.

Hybrid vigor was also observed in interspecific diploid hybrids of Lotus by other workers (Bent, 1962; Grant et al., 1962; de Nettancourt

and Grant, 1963, 1964a). Bent reported on morphological studies in L. pedunculatus x L. tenuis and noted that the width of the standard was greater in the hybrid than in the parental species; Grant et al. (1962) observed that in the hybrid L. japonicus x L. alpinus the mean style length in the hybrid exceeded that of either parent. Heterotic effects have been observed for the central leaflet length in L. tenuis x L. filicaulis (de Nettancourt and Grant, 1963). de Nettancourt and Grant (1964a) found also that in L. japonicus x L. krylovii, L. krylovii x L. japonicus, L. schoelleri x L. japonicus, L. schoelleri x L. krylovii and L. krylovii x L. schoelleri hybrid vigor was expressed in the central leaflet, whereas in L. schoelleri x L. japonicus and in the reciprocal crosses between L. japonicus and L. krylovii and L. schoelleri and L. krylovii, this phenomenon was exhibited by the floral length. The degree of heterosis reported by these authors was not as great as that observed in this study where the plants were grown in the field and there was no restriction on root development as compared to plants grown in pots.

These examples of transgression can be explained either by the chance combination of certain dominant genes in the interspecific crosses or by the general vigor of the F_1 hybrids. This general vigor of the interspecific hybrids may be attributed to the interaction of a changed nucleus with a relatively unaltered cytoplasm as suggested by Shull (1912). Harney and Grant (1963) conducted chromatographic studies on a number of Lotus interspecific hybrids

and reported the presence of a "hybrid substance" in certain hybrids, namely, L. alpinus x L. japonicus and L. japonicus x L. krylovii. These authors suggested that the occurrence of a "hybrid substance" in these species may be due to gene expression in a foreign cytoplasm. Such a nucleo-cytoplasmic interaction may be partly responsible for the hybrid vigor manifested by the interspecific diploid hybrids in this study.

Comparative morphological studies between the induced tetraploids and Lotus corniculatus

The induced autotetraploid and the synthetic amphidiploids were on the whole slow in development in comparison to the diploids. They exhibited larger and darker green leaves, larger flowers, and bigger pods when compared with the corresponding diploid species and species hybrids. The slow rate of development of these derived polyploids in all phase of growth may be attributed to a number of factors such as genetic interaction, reduced rate of cell division, and lower content of growth hormone, which could lead to a decrease in the rate of metabolic activities such as respiration, transpiration and photosynthesis. The darker green color of the tetraploid leaves may be due partly to the thickness of the leaf tissue in the polyploid state and partly in the higher chloroplast content. A proportionate increase in the number of chloroplasts in the cell and in the concentration of chlorophyll per unit area was noted in 2x, 4x and 8x forms of the hybrid Nicotiana alata x N. sandereae by Kostoff (cited

by Goodspeed and Bradley, 1942).

The increase in size of many morphological characters is perhaps due to enlargement of all plant organs which is in turn the product of increases in cell volume. Unbalanced physiology is likely to be responsible for the restricted development and poor vegetative vigor of the two amphidiploids (L. filicaulis x L. schoelleri) and (L. alpinus x L. schoelleri). The latter alloploid was produced from F_1 seeds and segregation of genetic factors could also lead to a decrease in size and vigor.

The results of comparative morphological studies between the induced autotetraploids and L. corniculatus indicate that L. alpinus and L. japonicus are more closely related to the cultivated species than are the other autotetraploids of L. tenuis, L. filicaulis, L. schoelleri, L. krylovii and L. corniculatus var. minor. It should be noted that whereas both L. japonicus and L. tenuis showed close resemblance to L. corniculatus in four of the 15 metrical traits studied, L. japonicus, in addition, exhibited a distant relationship in three additional characters namely, standard index, style/ovary index, and calyx index. This taxon is considered, therefore, to have a closer affinity to L. corniculatus. L. alpinus which resembles L. corniculatus in seven of the 15 quantitative characters, would appear to stand closest to the cultivated species in morphological relationships.

Comparative morphological studies between 4x L. tenuis and L.

corniculatus by Tome and Johnson (1945) and by Guttman (in Keim, 1952) indicated that although some morphological relationships exist between these two taxa, distinct differences were also very apparent; the results of the present study, therefore, confirm these earlier investigations.

Morphologically, the twelve synthetic amphidiploids showed varying degrees of resemblance to L. corniculatus. However, the five amphidiploids which manifested the greatest morphological affinity to the cultivated species (in four to six quantitative traits), had the genome of either L. japonicus, or L. alpinus, or the chromosome complements of both these species. Two amphidiploids which statistically showed the closest resemblance to L. corniculatus are (L. alpinus x L. krylovii) and (L. japonicus x L. alpinus). In the former alloploid, the krylovii genomes are believed to play an insignificant role in the relationship since the amphidiploids (L. krylovii x L. filicaulis) and (L. krylovii x L. schoelleri) which also had krylovii chromosome manifested slight resemblance to the cultivated species. The amphidiploid (L. japonicus x L. alpinus) showed the closest affinity to L. corniculatus and this resemblance was reflected in the overall morphology of this plant, as well as in characters such as the leaf, the floral bract, standard, the gynoecium, and the calyx. In addition this alloploid gave a very intense positive reaction for the presence of HCN, as did the cultivated species.

IV. Meiotic studies

Chromosome behavior during the course of meiosis in interspecific hybrids has assumed an important role in studies relating to evolutionary changes within a group of related species. The most suitable stage for the analysis of pairing behavior of meiotic chromosomes is pachynema. At this stage the chromosomes are not highly condensed and the homologues derived from the two parents are closely paired. It is thus possible to conduct point by point analysis of the structural differences between homologous or homoelogous chromosomes. In Lotus the lack of a suitable technique for the study of prophase stages has prevented a critical study of stages earlier than diakinesis. At diakinesis and metaphase I the chromosomes are most condensed. The chromosome configurations at these stages depend not only on pachytene pairing but also upon the formation of chiasmata which in most cases are confined to homologous regions. A further consideration is the fact that a single chiasma is sufficient to produce bivalents at diakinesis and metaphase I, thereby providing a normal picture of meiosis in a cursory cytological examination. Consequently, the chromosomes of two related species, may have several homologous regions leading to bivalent formation but structural differences in other regions would be obscured and not apparent. Furthermore, the existence of non-homologous pairing (McClintock, 1933) and also of physiological and genetic factors preventing pairing between homologous or homoelogous

chromosomes (Riley and Chapman, 1958) can sometimes complicate the picture. However, in the absence of a more trustworthy criterion, in this study reliance has been placed on chromosome pairing as an index of homology. It has been realized that the study of diakinesis and metaphase I alone could lead to erroneous conclusions concerning chromosome differentiation. Therefore, an examination of stages following metaphase I has been conducted also in order to provide a further interpretation of chromosome relationships in the various Lotus species studied.

Meiosis in the diploid species and the interspecific diploid hybrids

Univalents were observed in the parental diploid species and in the interspecific hybrids at diakinesis but they were more often present at metaphase I. In the diploid species, two univalents were observed frequently in each meiocyte; occasionally four of these entities were seen, for example, in L. filicaulis. In the species hybrids, two to six univalents were detected. In a few cells of the hybrid, L. alpinus x L. filicaulis, as many as eight univalents were scattered on the first meiotic spindle. The behavior of the univalents was variable in the plants in which they were studied. They would reach the pole either intact, or divided, or they would lag at the equatorial plate, or divide later. Certain chromosomes which approached the poles at anaphase I prior to the rest of the complement were presumably univalents which failed to become oriented on the metaphase I plate. The distribution of chromosomes at first division

anaphase was sometimes irregular partly due to such variable behavior of the univalents. The frequency of these unpaired chromosomes was greater in the hybrids than in the parental diploid species.

The occurrence of univalents at diakinesis and metaphase I have also been observed in the diploids, L. japonicus, L. filicaulis and L. tenuis (de Nettancourt, 1963) and in L. pedunculatus (Chen, 1967). Grant (1963), Grant et al. (1962) and de Nettancourt and Grant (1963, 1964a) also found two to six univalents in a number of interspecific hybrids. Grant (1963) considered the presence of the univalents to be the result of precocious separation of the bivalents at diakinesis and metaphase I, that is, desynapsis.

In this study, the occurrence of a higher frequency of bivalents than univalents at both diakinesis and metaphase I in the interspecific hybrids suggests that such univalents are not due to complete lack of homology. Failure of pairing among pairable chromosomes is regarded as being due to structural differences (Darlington, 1937). The presence of univalent chromosomes at early diakinesis and the larger number of these entities observed in some interspecific hybrids suggested to the author that precocious separation of chromosomes alone cannot explain the occurrence of these univalents but that segmental and genetic differences as well as other factors may also play an important role.

The normal association of six bivalents in over 50 per cent of the cells in the interspecific hybrids, indicated that a certain amount

of homology exists between the chromosomes of the seven diploid species. However, the loose bivalents observed in a number of cells at metaphase I, is suggestive of some lack of specificity in pairing of the homologous chromosomes, which might have undergone a certain amount of structural differentiation. Also, it should be noted that the preponderance of bivalent formation in a majority of the pollen mother cells does not in fact prove that the chromosomes are not structurally differentiated. This pairing behavior may suggest that such structural differences may not be very extensive but may be "cryptic" as suggested by Stebbins (1950). According to Stebbins, cryptic structural differences are so slight that pairing at meiosis is not affected. Furthermore, what might appear to be normal bivalent formation at diakinesis and metaphase I, could occur with a minimum of chiasmata per bivalent. In these hybrids, a very high frequency of rod bivalents was apparent; since the presence of rod bivalents suggested that a chiasma is formed only in one arm of the chromosome, it is theoretically possible that some of these rod configurations observed may have only one pair of homologous arms.

Quadrivalents of the closed ring and chain types were observed in a number of hybrids. Also trivalents (predominantly chain types), were detected and many of these are believed to have resulted from the falling apart of one homologue in a quadrivalent association. The presence of quadrivalents is interpreted to mean that these

morphologically closely allied species differ by segmental interchanges. Since meiosis is regular, in general, in the parental species, one of the two parental species, in each case, must be considered to be homozygous for a reciprocal translocation.

Multivalent configurations at metaphase I in Lotus interspecific hybrids were also observed by Grant et al. (1962) and de Nettancourt and Grant (1963, 1964a). These authors also suggested that multivalent pairing may result from translocations through which some of the chromosomes may have differentiated from their homologous partners. Some of the anaphase bridges observed in the diploid species L. japonicus and L. krylovii may be attributed to the arrest of terminalization of chiasmata. Similar types of bridges were observed by de Nettancourt (1963) in L. filicaulis; in the present study, meiosis was investigated in L. filicaulis also, but no anaphase bridges were detected in this species.

In a number of interspecific hybrids, bridges with, and without, acentric fragments were observed both at anaphase I and telophase I, and anaphase II and telophase II. Chromosome stickiness belated separation of chromosomes, and failure of chiasmata terminalization are probably responsible for the presence of some of the bridges without accompanying fragments. The presence of dicentric chromatid bridges with associated acentric fragments, however, indicates that the particular hybrid was heterozygous for an inversion. The bridge and fragment may result from a two-strand cross-over within the

inverted segment. A single bridge and fragment can also arise from a three-strand double cross-over where one exchange occurs in the proximal region and the second within the loop. The detection of inversion heterozygosity in the hybrids has made possible certain deductions relative to the parental species. The occurrence of these inversion bridges would indicate that the homology between chromosomes within inverted segments is strong and that crossing-over between these homologous chromosomes is taking place.

The occasional delayed separation of certain bivalents at anaphase in some of the hybrids may be due to difficulty in movement of chiasmata (terminalization). Late separation of chromosomes in certain Lotus hybrids was also reported by Grant et al. (1962) and de Nettancourt and Grant (1963, 1964a). Magoon, Cooper and Hougas (1958b) observed this type of anomaly in Solanum hybrids and considered it as being due to the arrest of terminalization of chiasmata. Lawrence (1931) suggested that the interference of movement of chiasmata might be due to a change in homology of paired chromosomes.

An accessory chromosome considered to be a B-chromosome was observed in the meiocytes of one genotype (AF-5-2) of the cross L. alpinus x L. filicaulis. No gross phenotypic or microscopic differences have been recognized which could be attributed to the presence of this extra chromosome. Pollen fertility in this genotype was comparable to that observed in other genotypes of this

hybrid combination which did not have the accessory chromosome. It is difficult to explain the presence of this chromosome. In general, the origin of such a chromosome is believed to be associated with structural rearrangements and meiotic abnormalities. Meyer (1944) indicated that B-chromosomes could originate as broken ends of inversion bridges. Although this postulate cannot be discounted the absence of paracentric inversion heterozygosity in this hybrid raises a question as to its validity. Markarian and Schulz-Schaeffer (1958) also suggested that the breakage of secondary constrictions could give rise to the spontaneous production of fragments or accessory chromosomes. So far, only limited morphological studies have been made on chromosomes of the two diploid parental species, and hence, it is difficult to rule out this possibility also. The accessory chromosome observed in this genotype could be a by-product of meiotic irregularity arising from chromosomal instability associated with hybridity (Levin, 1967).

Meiosis in the autotetraploids

The results of meiotic studies in the induced autotetraploids indicated that the PMC's of these plants were characterized by a high degree of bivalent pairing and a low occurrence of quadrivalent configurations. The highest frequency of quadrivalents was observed in 4x *L. tenuis* which had an average of 1.10 per cell. This is considerably lower than the mean of 2.85 reported by Wernsman *et al.* (1964) for this autotetraploid. Chen (1967) and Gershon (1961) also

reported a low frequency of quadrivalents in the autotetraploid L. pedunculatus. Chen reported the average number of quadrivalents per cell to be 1.80 and Gershon an average number of 2.02 quadrivalents per cell. The findings of Morrison and Rajhathy (1960) that in all autotetraploid plants about two-thirds of the chromosomes are on the average united as quadrivalents do not seem to hold for these derived tetraploids of Lotus. In the present study, only meiocytes were studied in detail when the author was certain of the manner of pairing of each chromosome. Because of the tendency for the chromosomes to adhere to one another, multivalent structures were more difficult to determine than bivalents, hence only those figures were considered which showed recognizable quadrivalent configurations. As a result, an unavoidable bias towards the counting of PMC's with a higher number of bivalents might have occurred.

Also the low frequency of quadrivalents may be attributed to other factors such as the small size of the chromosomes, the low chiasma frequency or genetic factors, all of which affect multivalent formation. It is well known that the shorter the chromosomes, the lower the chance for the occurrence of multivalent pairing and hence, observing quadrivalents in diakinesis and later stages (Darlington, 1958). This is due to the fact that a lower number of chiasmata are found in short chromosomes, since at any given region of the chromosome only two strands can pair. Observations on

chromosome pairing affinities in these autotetraploids indicate that the frequency of chiasma was low as evidenced by the preponderance of rod bivalents and the absence of interstitial chiasmata. Regardless of the considerable homology between chromosomes, the homologues in multivalents might have separated prior to metaphase, or their formation in prophase might have been reduced. Genetic factors may be important in Lotus since the frequency of quadrivalents was observed to vary between genotypes of the same autotetraploid. For example, in L. japonicus, the genotype T-129-1 had 0.38 quadrivalents per cell, whereas, T-129-2 exhibited a quadrivalent frequency of 0.70 per cell. Rees (1961) has shown conclusively, that chiasma frequency is genotypically controlled, and Roseweir and Rees (1962) have shown further that the distribution of chromosome pairing in autotetraploids is dependent on chiasma frequency. Swami and Thomas (1968) indicated that in Avena, quadrivalent frequency was positively correlated with chiasmata frequency. It would seem that the control of multivalent formation in autotetraploids of Lotus species is influenced by chromosome size, frequency of chiasmata and genetic factors. In this respect, it is quite different from the type of genetic control conditioning regular bivalent formation in wheat (Riley and Chapman, 1958).

Meiosis in Lotus corniculatus

In L. corniculatus a high frequency of bivalents and relatively few quadrivalents were observed at diakinesis and metaphase I.

Twelve bivalents and less frequently, 11 II's + 2 I's were detected in the majority of cells examined. These results are in agreement with those of Dawson (1941) and Wernsman et al. (1964). These authors observed that an average of one quadrivalent was observed in every fourth microspore mother cell, that is, a mean frequency of 0.25 per cell. The presence of quadrivalents indicates that the genomes of this species are partially homologous. The high frequency of bivalents and the low occurrence of multivalents provide evidence for the possibility of an allotetraploid origin of L. corniculatus.

Meiosis in the synthetic amphidiploids

Meiosis in the synthetic amphidiploids was characterized by the presence of univalents, bivalents, trivalents and quadrivalents at diakinesis and metaphase I, and by the occurrence of lagging chromosomes and bridges with, and without fragments at anaphase I and II, and telophase I and II.

One amphidiploid (L. alpinus x L. krylovii) appeared primarily desynaptic since there was a high frequency of univalents and very few bivalents observed for this plant. This is not altogether unexpected since there are reports of desynapsis in Lotus. Chen (1967) observed desynaptic behavior in a primary trisomic of L. pedunculatus; Gershon (1961) also found that a hybrid obtained by crossing 2n L. pedunculatus with L. corniculatus exhibited irregular chromosome behavior during meiosis and he attributed this to partial desynapsis. Dyck and Rajhathy (1965) studied desynapsis in an induced autotetraploid

in Avena and concluded that the desynaptic chromosome behavior was conditioned by a single recessive gene that could have resulted from colchicine treatment. It is quite possible that the partial desynapsis exhibited by the amphidiploid in this study, may have resulted from the colchicine treatment.

A comparison of chromosome association at diakinesis and metaphase I in the remaining amphidiploids with that in the induced autotetraploids indicates that chromosome pairing was more regular in the amphidiploids although in some cases, for example in (L. japonicus x L. alpinus) and (L. alpinus x L. filicaulis), the differences were not as pronounced as might have been expected. In the amphidiploids, the mean number of bivalents per cell ranged from 9.40 to 10.50 and the average number of quadrivalents per cell ranged from 0.23 to 0.64; the corresponding figures in the autotetraploids were 8.37 to 9.11 and 0.57 to 1.10, respectively. Although a maximum of 5 IV's was observed at diakinesis and metaphase I in some of the autotetraploids of the diploid species, the amphidiploids exhibited fewer quadrivalents but depending on the species involved, there was considerable variation in the maximum number. Furthermore, more PMC's with 12 II's were observed in all the amphidiploids. This increase in pairing regularity is attributable to differential pairing of structurally similar chromosomes. Since the frequency of homogenetic association as compared to heterogenetic association depends on the degree of genomic affinity (Darlington, 1937), it would follow that the most highly differentiated genomes would

display the highest pairing regularity when combined in an amphidiploid. In this regard, when the percentage of the chromosomes associated as bivalents and quadrivalents in these amphidiploids are considered, it can be postulated that there are varying degrees of structural differentiation between the genomes of the diploid species. For example, a high degree of genomic difference appears to exist between the genomes of L. filicaulis, L. krylovii and L. corniculatus var. minor as evidenced by the low frequency of quadrivalents in (L. krylovii x L. filicaulis) and (L. corniculatus var. minor x L. filicaulis). At the other extreme, the genomes of L. japonicus and L. alpinus seem to be very similar since the frequency of quadrivalents in (L. japonicus x L. alpinus) was 0.64 per cell. This figure is comparable with 0.65 in the autotetraploid of L. alpinus and 0.57 in the autotetraploid of L. japonicus.

Parallel observations were made in Solanum (Swaminathan, unpublished, cited in Swaminathan and Howard, 1953), and Melilotus (Shastry, Smith and Cooper, 1960). Swaminathan (l.c.) conducted similar comparative meiotic studies in auto- and allopolyploids of Solanum species and reported that for two interspecific hybrids, there was no difference in the frequency of quadrivalents in their alloploids and the corresponding autoploids. In five other interspecific hybrids, however, there was a great reduction in quadrivalent frequency in the alloploids in comparison with their corresponding autoploids. He concluded that cryptic structural differentiation between the species involved in the above two hybrids was much less than that

between species in the other five crosses. In their studies of species differentiation on Melilotus, Shastry and his co-workers (l.c.) analysed meiotic chromosome behavior in autotetraploids of M. officinalis and M. alba and in the alloploid M. officinalis x M. alba; they indicated that a similar mean and range of quadrivalent frequency in the auto- and alloploid furnished evidence against chromosomal differentiation in M. officinalis and M. alba.

It should be noted, however, that the presence of multivalent associations in diakinesis and metaphase I plates suggest that a certain amount of homoelogous pairing was taking place in all the twelve amphidiploids and that a certain amount of homology exists between the chromosomes of the diploid species.

Analysis of chromosome behavior at anaphase I and II, and telophase I and II, revealed that inversion bridges with, or without, associated fragments occurred in all the amphidiploids except L. alpinus x L. krylovii. The presence of these inversion bridges is interpreted to mean that crossing-over took place between homologues which differed by a paracentric inversion. A single cross-over in the inverted segment would yield an anaphase bridge, whereas a double cross-over between the centromere and the inversion, and within the inversion, would yield an anaphase II bridge. The presence of these inversion bridges and associated fragments at anaphase I and II constitutes evidence for allosyndetic chromosome pairing in the amphidiploids; they also indicate that inversion is playing an important role in the evolution and differentiation of the diploid

species. The presence of two bridges and fragments (one on each anaphase II plate) in some cells of (L. japonicus x L. alpinus) is interpreted as being due to the occurrence of at least three simultaneous chiasmata in one pair of chromosomes as against a maximum of two chiasmata per bivalent normally noted in these plants at diakinesis. Such a high chiasma formation would seem to suggest that the two diploid taxa, L. japonicus and L. alpinus are somewhat closely related.

Meiosis in the crosses between the synthetic amphidiploids and Lotus corniculatus

From studies on chromosome association at diakinesis and metaphase I in the microsporocytes of crosses between the amphidiploids and L. corniculatus, it was found that univalents, bivalents, trivalents and quadrivalents occurred in all the hybrid combinations. The mean frequency of univalents was lower than those observed for the autotetraploids and many of the synthetic amphidiploids. The percentage bivalents on the whole was greater than those observed in the autotetraploids and those recorded for many of the amphidiploids. This low frequency of univalents and the correspondingly high occurrence of bivalents in these tetraploid hybrids indicate a strong tendency for homogametic pairing. Presumably, a certain amount of bivalent pairing occurred between the L. corniculatus chromosomes. The 12 chromosomes each of the hybrids received from their respective amphidiploids would be expected to pair and produce four to six bivalents

since the chromosomes of the diploid species have been shown to be largely homologous. The 12 chromosomes in the hybrids which came from L. corniculatus would be expected to form zero to 12 univalents if no preferential pairing occurred, or up to six bivalents if preferential pairing occurred. It has been noted above that the frequency of bivalents was high and that of univalents low. This suggests that some homogametic pairing was taking place and that some preferential pairing of the L. corniculatus chromosomes is a possibility. If tetraploid L. corniculatus is a segmental allotetraploid and is represented by the genomic symbols AABB, preferential pairing as suggested above, would mean that strong homology exists between the A and the B genomes, that is, the genomes have almost complete homology. It is important to state at this point, that the genomes of the amphidiploid (L. japonicus x L. alpinus) have been shown in this study, to be almost completely homologous.

Evidence for affinity between the chromosomes of the diploid taxa and those of L. corniculatus is provided by the presence of trivalents and quadrivalents in the meiocytes of these tetraploid hybrids. A relatively high frequency of trivalents were detected in these hybrids. Trivalent configurations in general, are rare in many Lotus tetraploids, probably because of the predominance of symmetrical arrangements of the chromosomes at prophase as evidenced by the preponderance at diakinesis and metaphase I of quadrivalent

types with three or four chiasmata distributed equally in all the chromosome arms. The significantly higher frequency of trivalents in these crosses can, therefore, be regarded as being due to a close homology between the chromosomes of the diploid species and those of L. corniculatus. Quadrivalents were found in all the hybrids and the mean number of quadrivalents per cell for each hybrid combination (0.25-0.80) was greater than that recorded for L. corniculatus (0.24). Further evidence for homology between the chromosomes of the diploid species and L. corniculatus is the presence of inversion bridges at anaphase I and II. These inversion bridges indicate that crossing-over occurred between homoelogous chromosomes.

Homology between the chromosomes of the diploid taxa and those of the cultivated species, combined with recombination, as suggested by the presence of inversion bridges have an important bearing as far as improvement of the cultivated species is concerned. These results suggest strongly that the exploitation of genetic variability in the diploid Lotus species is a possibility.

The presence of multivalent configurations in these hybrids, has made it possible to make certain deductions with regards to the relationship between the synthetic amphidiploids (and hence the diploid taxa) and L. corniculatus. The high frequency of multivalent associations recorded for the hybrid (L. japonicus x L. alpinus) x L. corniculatus and its reciprocal combination, suggests that a very high degree of homology exists between the chromosomes of the

two diploid taxa, L. japonicus and L. alpinus and those of L. corniculatus. The frequencies of the various chromosome associations observed in the L. japonicus and L. alpinus crosses, are comparable with those recorded for the induced autotetraploids of L. japonicus and L. alpinus. In fact, the random pairing of the chromosomes in these crosses, is very similar to that observed in the induced autotetraploids of the diploid Lotus species. These results indicate that L. japonicus and L. alpinus are genetically more closely related to L. corniculatus than the other diploid species that were involved in the production of amphidiploids.

It is difficult to explain the difference in chromosome pairing behavior exhibited by the reciprocal hybrids obtained by crossing (L. japonicus x L. alpinus) and L. corniculatus. The author is aware of no reference in which cytoplasmic differences between different species have been considered to influence chromosome pairing specificity. However, in the present study, it is quite conceivable that a cytoplasmic factor or factors could influence the formation of chiasmata and hence the degree of multivalent association. If this is the case, then, there would seem to be an interaction between the cytoplasm of L. corniculatus and the chromosomes of L. japonicus and L. alpinus which favors a higher frequency of multivalents. Regardless of the difference in chromosome behavior in the reciprocal crosses (L. japonicus x L. alpinus) x L. corniculatus and L. corniculatus x (L. japonicus x L. alpinus), the results support

the view that the most important species from the point of view of the evolution of L. corniculatus, are L. japonicus and L. alpinus.

V. Fertility studies

Fertility in the diploid parental species

Data obtained from studies concerned with pollen stainability in the diploid parental species indicate that pollen formation is not as regular as one would expect in normal diploid species. Only two species, L. japonicus and L. corniculatus var. minor, exhibited over 90 per cent stainable pollen. de Nettancourt (1963) investigated pollen fertility in all these diploid species except, L. corniculatus var. minor and also reported a low percentage of normal pollen in L. alpinus, L. filicaulis and L. krylovii; he, however, found a high per cent pollen stainability in L. tenuis and L. schoelleri. Meiosis was observed to be regular in all these species and this precludes the meiotic process as a factor causing pollen sterility. Pollen abortion in these supposedly good species could be the result of a) environmental factors b) gene interaction or c) hybridization during the early evolutionary history of some of these species. For example, Schark (1957, 1958) showed that high temperature was responsible for nutritional deficiency and plasmolysis of the pollen mother cells in Solanum tuberosum. Schonhorst (1958) indicated also that at least three genes, two of which were additive in effect, controlled pollen abortion in Medicago sativa. He reported that plants which were homozygous recessive for all three genes were more

than 50 per cent pollen sterile and were very sensitive to environmental changes. Species that are of relatively recent origin would be expected to show a certain degree of pollen sterility. Various processes such as hybridization among diploid taxa or parthenogenesis in tetraploid populations would account for pollen abortion. Larsen (1954) suggested that the occurrence of natural hybridization in Lotus species is not a rare phenomenon in southern Europe. The results of interspecific hybridization experiments in this study, also suggest that natural hybridization is a strong possibility. It is likely, therefore, that pollen abortion in some of these diploid taxa may result from the incorporation of foreign germplasm through a process of restricted hybridization that took place during the early history of these species in their natural habitats. In Lotus species, pollen sterility could be due to any of these factors or a combination of two or more of these factors acting in co-ordination.

High seed set was recorded for all the species except L. alpinus. This species also displayed a high per cent of non-stainable pollen and it is possible that pollen sterility may be a contributing factor that influences low seed production in this plant. However, this does not appear to be so in the other species which had a relatively low percentage of stainable pollen and normal seed set. These observations are in agreement with the report of Koffman and Wilsie (1961) who indicated that pollen fertility in a commercial strain of alfalfa showed no relation to fertility as determined by

seed set. It would seem that environmental as well as genetic factors influence seed set in the Lotus species.

Fertility in the interspecific diploid hybrids

The variability in the incidence of stainable pollen observed between the various crosses reflects the difference in the genetic constitution of the parental species. Part of the considerable variability in the percentage stainable pollen between hybrid plants of the same cross combination may be attributable to the fact that not all the hybrids within a cross were derived from the same parental plants. Also it may be that some of the parental species used in this study were segregating for genes controlling pollen sterility. These results are in line with those of Grant et al. (1962) and de Nettancourt (1963) who reported similar variability between crosses and within crosses in interspecific diploid hybrids in Lotus.

The meiotic irregularities recorded for the hybrids do not seem to be correlated with the frequency of non-stainable pollen tabulated for these plants. It is therefore difficult to explain on the sole basis of meiotic irregularities the high percentages of pollen abortion recorded for many of the hybrids studied here. Grant et al. (l.c.) and de Nettancourt (l.c.) also reported that the high degree of sterility observed, was not fully correlated with chromosomal abnormalities at meiosis. This suggests that the parental species may differ in several gene complexes or modifier complexes which

influence pollen fertility. Harland (1936) proposed that such gene complexes and modifier complexes play an important role in affecting pollen fertility and species differentiation in Gossypium. Another possibility is that these species differ by small structural changes which are not extensive enough to disturb pairing between partially homologous chromosomes in the hybrid but at the same time give rise to genetic imbalance which lowers the fertility of hybrids heterozygous for these cryptic structural differences (Stebbins, 1950). An example of this type of chromosomal sterility is that described by Darlington (1937) for the hybrid between Primula verticillata and P. floribunda. In this hybrid, although normal chromosome pairing seems to take place, inviable gametes containing unbalanced and disharmonious combinations of genes are produced. As a result sterility is the rule in this cross. It would seem that an analogous situation is found in this study with these interspecific hybrids. The presence of univalents, and loose bivalents in all these hybrids is highly suggestive of the occurrence of cryptic structural differentiation between the parental species.

Cytoplasmic and environmental factors may be partly responsible for the low percentage stainable pollen observed in these hybrids. de Nettancourt and Grant (1964a) suggested that the differences in pollen fertility seen in reciprocal crosses could be attributable to cytoplasmic influence. In Medicago, it was found that pollen fertility in hybrids derived from crosses between the same species,

depended upon the cytoplasm of the plant which was used as the maternal parent (Lesins, 1961). Webster (1950) and Magoon et al. (1958b) suggested that environmental factors play an important role in the regulation of pollen fertility. They observed that pollen abortion in hybrid plants with a similar cytoplasmic constitution varied from plant to plant and from flower to flower. Webster (l.c.) observed a correlation between the percentage of aborted pollen and the position of the racemes on interspecific hybrids of Melilotus. He also found variation in pollen stainability among plants which had been vegetatively propagated from the same clone. In these Lotus hybrids, therefore, it is reasonable to conclude that pollen sterility may be due to the interaction of several factors such as cytological, genetical, physiological and environmental all acting independently or in co-ordination with each other.

The average seed set per pod was low in all the hybrids. The variability in seed set between the crosses may be ascribable to the difference in the genetic make-up of the parental species. There does not appear to be any correlation between meiotic irregularities, pollen stainability, and the number of seed set per pod in the hybrids. A somewhat similar observation was made by Sprague (1956); he failed to detect a relationship between fertility and production of normal pollen in hybrid plants of Medicago. It is likely that, in addition to abortive gametogenesis, other factors such as gene interaction, unbalanced physiology, and unsuitable environmental conditions may

be responsible for the low seed set recorded.

Fertility in the autotetraploids

The percentage of morphologically good and stainable pollen was determined for each induced autotetraploid and that data indicate that there was a decrease in pollen fertility when a comparison was made with the corresponding diploids. These results are in general agreement with those observed in other induced autotetraploids in that the doubling of the chromosomes of diploid species is usually accompanied by a certain amount of sterility. However, in this study, no precise relationship between the frequency of univalents and multivalents on the one hand, and pollen fertility on the other, was discernable. Autotetraploids with a high multivalent frequency (L. tenuis) were found to be as much pollen fertile as autotetraploids with a lower multivalent frequency (e.g. L. alpinus). This may be due to the fact that there was a high proportion of symmetrically arranged quadrivalents. It, therefore, seems that the types of quadrivalents, rather than their frequency may be important in determining the fertility in these induced autotetraploids. The hypothesis first advanced by Darlington (1937) that sterility in autotetraploids is due to the formation of multivalents which disjoin irregularly at anaphase I to produce unbalanced non-viable gametes does not seem to be borne out in this study. Similar observations were made by Armstrong and Robertson (1956) in alsike clover, and by Chaudhri, Rao and Mehta (1964) in Trifolium alexandrium. These

authors found that the variation in fertility in different autotetraploids was not correlated with cytological behavior. It should be noted that in recently induced tetraploids there is a shift in the nuclear:cytoplasmic ratio which could result in unbalanced physiology. Also changes in size and volume of pollen grains are likely to affect their stainability. Hence, physiological factors must be taken into consideration in explaining pollen fertility. It would seem likely, that, in addition to cytological and physiological factors, gene action and environmental factors are also important in influencing the pollen fertility in these derived autotetraploids.

Fertility in the amphidiploids

The results of pollen stainability studies in the synthetic amphidiploids indicate that there was considerable variability in pollen fertility between genotypes as well as between the different amphidiploids. It would seem, therefore, that genetic factors as well as cytological ones influence pollen fertility. Evidence of cytological influence is seen in the desynaptic amphidiploid (*L. alpinus* x *L. krylovii*) which exhibited high pollen sterility and a high frequency of univalents during meiosis. However, there was no apparent correlation between meiotic irregularities and pollen fertility in the remaining amphidiploids. Seed set was unexpectedly low in the amphidiploids and in *L. corniculatus*, and this may be partly due to the fact that pods were harvested during an intermittent

rainy period and there was a high incidence of pod shattering. There was no close association between meiotic chromosome behavior and seed set nor was there any relationship between pollen fertility and seed set. None the less, pollen sterility and abortive gametogenesis must be considered as causative factors for the low seed set recorded. In addition, unbalanced physiological factors and environmental influence must be taken into account.

A comparison of pollen fertility between the amphidiploids and their corresponding interspecific hybrids indicate that all the amphidiploids except (L. alpinus x L. filicaulis) and (L. corniculatus var. minor x L. alpinus) exhibited higher pollen stainability than their corresponding diploid counterparts. Goodspeed and Bradley (1942) stated that when two closely related species are crossed the hybrid is usually fertile but the amphidiploid of such a hybrid would show reduction in fertility. This statement is based on the assumption that multivalent formation and irregular assortment of chromosomes would result in sterility. According to these authors the reduction in fertility in the two above-mentioned amphidiploids is attributable to irregular disjunction of chromosomes and subsequent formation of aneuploid gametes. The other amphidiploids behaved differently with regards to pollen fertility and this would indicate that multivalents alone could not influence pollen fertility. These amphidiploids behaved somewhat like the amphidiploid (Primula floribunda x P. verticillata) in which the diploid hybrid exhibited

a high degree of bivalent pairing but was highly sterile; the amphidiploid, however, was fertile. In this study, the interspecific diploid hybrids were also characterized by a high degree of bivalent pairing and varying degrees of sterility, whereas, the amphidiploids showed a marked improvement in pollen fertility. Thus, it would appear that in addition to irregular assortment of chromosomes resulting from multivalent chromosome disjunction, genetic factors and cryptic structural differences also influence the fertility in these amphidiploids.

Pollen fertility studies also indicate that whereas some of the amphidiploids were superior in the percentage stainable pollen to the autotetraploid of one or both parental species, others were inferior to the autotetraploids of both parental species. Parallel observations have been made in autotetraploids and allopolyploids in some species of Solanum by Westergaard (in Magoon, Ramanujam and Cooper, 1962); Westergaard interpreted his observations to mean that gene substitution as well as cryptic structural differences played a role in affecting pollen fertility.

Fertility in the crosses between the amphidiploids and Lotus corniculatus

The influence of the genetic constitution of L. corniculatus upon pollen stainability was reflected in the relatively high percentage of stainable pollen that was recorded in the hybrids obtained by crossing the synthetic amphidiploids with this cultivated tetraploid species. All the hybrids exhibited over 60 per cent stainable pollen;

in two crosses, (L. corniculatus var. minor x L. alpinus) x L. corniculatus and (L. japonicus x L. corniculatus var. minor) x L. corniculatus, the percentages of stainable pollen were greater than that recorded for L. corniculatus. This suggests that there is a definite possibility of introducing desirable germplasm from the diploid taxa to the cultivated species. Hybrids derived from reciprocal crosses between (L. japonicus x L. alpinus) and L. corniculatus, (L. japonicus x L. schoelleri) and L. corniculatus and between (L. krylovii x L. filicaulis) and L. corniculatus showed appreciable differences in the amount of stainable pollen. These discrepancies may be attributed to cytoplasmic factors. It should be noted, however, that the cytoplasm of L. corniculatus does not necessarily have an adverse effect as might be suggested from the crosses L. corniculatus x (L. japonicus x L. alpinus) and L. corniculatus x (L. japonicus x L. schoelleri) in which the pollen fertility was lower than that in the reciprocal hybrid in each instance. In the cross L. corniculatus x (L. krylovii x L. filicaulis) the per cent stainable pollen was 85.43, whereas in (L. krylovii x L. filicaulis) x L. corniculatus, it was 76.09. In this case, the cytoplasm of L. corniculatus had a beneficial effect. These observations indicate that there may be a nucleo-cytoplasmic interaction that has some bearing on pollen fertility. There are reports suggesting that cytoplasmic factors influence pollen fertility in Lotus (Grant et al., 1962; de Nettancourt, 1963); these authors

observed significant differences in stainable pollen in interspecific diploid hybrids derived from reciprocal crosses. The results of the present study would imply that such cytoplasmic factors may also operate in hybrids at the tetraploid level.

VI. Mechanisms underlying species differentiation in diploid Lotus species

Throughout this study considerable data have been accumulated concerning the genetic and cytogenetical mechanisms underlying species differentiation at the diploid level in Lotus. Studies concerned with the relationships of interspecific cross-compatibility and the cytological relationships of chromosome aberrations found in the interspecific F_1 hybrids have furnished valuable information on these mechanisms.

Evolution of the diploid taxa has involved genic and chromosomal alterations which have led to morphological diversification as well as to barriers of gene exchange between species. Morphological differentiation, however, does not seem to be related to incompatibility. For example, L. japonicus, L. alpinus and L. corniculatus var. minor are morphologically distinct species but never the less hybrids were obtained with ease between these species under artificial conditions. Geographic separation is perhaps the major reproductive barrier in this case. On the other hand, in species which are morphologically similar (e.g. L. tenuis, L. filicaulis and L. schoelleri), the production of interspecific hybrid was more stringent. Genetic

difference between the species are likely to be responsible for the formation of incompatibility barriers and consequent differentiation among some species. Fertility studies in the diploid species and their interspecific hybrids have yielded valuable data on differentiation at the diploid level. In this investigation, high pollen sterility was observed in the absence of extensive meiotic irregularities; one can therefore infer from these observations that sterility is partly genic in nature.

Also, it is evident from the results of this study and from data reported by other workers (cited below), that structural differentiation of chromosomes, both cryptic and patent, plays an important role in the speciation of these closely related species. In the present study, the occurrence in the interspecific hybrids of a quadrivalent, more frequently of a closed ring type indicates that the two parental species concerned, must be considered to be homozygous for a reciprocal translocation. The chromatid bridges with associated acentric fragments observed at anaphase I and II, and telophase I and II in some hybrids suggests that these F_1 hybrids were heterozygous for inversions. Such meiotic irregularities indicate that gross structural differences are operating to isolate the species. However, gross chromosomal repatterning as evidenced by the low frequencies of translocations and inversions, and by karyotypic studies (Zandstra and Grant, 1967; Cheng, 1970), appear to be minimal. There is a considerable amount of evidence suggesting that cryptic structural differentiation has

proceeded to a greater extent. This supposition is based on the following observations. 1) Loose bivalents and precocious desynapsis were detected in all the F_1 hybrids but were not evident in the synthetic amphidiploids. 2) Reduced chiasma frequency in the F_1 hybrids was apparent by the high occurrence of rod bivalents. The presence of rod bivalents indicates that one chiasma is formed in one chromosome arm whereas none is formed in the other. Stephens (1950) suggested that the replacement of a ring bivalent by a rod bivalent in interspecific hybrids may indicate that an entire chromosome arm has failed to pair because of reduced structural homology. 3) Delayed separation of certain bivalents, a phenomenon which is quite distinct from inversion bridge formation, occasionally occurred during anaphase in the hybrids. Such belated separation of bivalents is believed to be the consequence of a change in homology (Lawrence, 1931). 4) Preferential pairing of chromosomes in the synthetic amphidiploids, as evidenced by comparative meiotic studies between the amphidiploids and induced autotetraploids of the concerned parental species, suggests that the chromosomes of the diploid taxa are structurally differentiated. 5) Distorted segregation ratios were consistently observed in diploid F_2 plants and in backcross progenies by de Nettancourt (1963) for such characters as HCN production, stem color, keel tip coloration, bud striping, and seed speckling. Stephens (1949) explained such distorted ratios in Gossypium, in terms of selective elimination of genes from the donor

parents. He stated that selective elimination of genes requires some form of balanced "polygenic complexes" which may be none other than structurally differentiated chromosome segments. 6) The interspecific diploid hybrids were highly pollen sterile. If the parental species concerned, differed by a number of cryptic structural differences then the distribution of chromosomes at anaphase would be expected to result in a random assortment of these tightly linked segments to the gametes. If such a divergent genic evolution had occurred, which is an essential step in species differentiation, then, it should be expected that the random distribution of these various complexes to the gametes would lead to unbalanced combinations. Thus, in the light of the relatively poor pollen stainability in the Lotus diploid species hybrids, it is considered that cryptic structural differences between many of the species may be fairly extensive.

Stebbins (1947) was the first person to point out that structural differences between chromosomes could conceivably co-exist with a high degree of regular meiotic pairing. He further pointed out that such cryptic differences may be able to effectively prevent the free exchange of genes located within or very close to such regions and lay the foundation for the differentiation of taxa into different species. In Lotus, it seems probable that such cryptic differentiation is acting in co-ordination with other factors to differentiate the species and to make them more distinct from one another. A survey of the literature indicates that small structural changes of the

chromosomes is a common mode of speciation in many plant genera including Melilotus (Kita, 1965; Shastry et al., 1960), Solanum (Magoon et al., 1962), Gossypium (Stephens, 1950) and Phlox (Levin, 1966).

From a consideration of the factors underlying species differentiation in the diploid Lotus species, it is evident that gene substitution, and gross and cryptic structural differentiation of the chromosomes have played an important role in the evolution of the diploid species.

VII. Species relationships and the origin of Lotus corniculatus

The diploid species used in this study exhibit considerable morphological similarity which has perplexed taxonomists of this group for a long time. All of these closely related species, except L. filicaulis, have at one time been considered taxonomically as varieties of L. corniculatus; this implies the existence of a certain amount of morphological affinity between these diploid species and the cultivated tetraploid species. An analogous situation is found when one considers the complex cytological relationships that exist between these various diploid taxa and L. corniculatus. A study of the chromosome pairing relationships in the interspecific diploid hybrids indicates that a high degree of homology exists between the chromosomes of these diploids. Also, meiotic studies on the tetraploid hybrids, obtained by crossing the synthetic amphidiploids and L. corniculatus, suggest that chromosome homology exists between

the diploid species and L. corniculatus. Furthermore, there are reports of chromosome homology between three other diploid species (L. tenuis, L. pedunculatus, L. coimbrensis) and the cultivated tetraploid (see Literature Review). On the basis of these observations some speculations can be made with regards to the evolutionary history of these species. It is the opinion of this author that the progenitors of the present-day species intercrossed freely and that these progenitors probably belonged to one, or a few, polymorphic species. Such a postulate would explain 1) the close morphological resemblance, and the distinct chromosomal relationships that exist between the various diploid species, and 2) the similarity in certain morphological attributes and the chromosome homology between the diploid species and L. corniculatus (assuming that the cultivated species arose from one or more of these diploid species by hybridization and chromosome doubling).

A similar hypothesis was put forward by Sprague (1959) to explain species relationships in Medicago. He conducted meiotic studies in interspecific and trihybrids of diploid M. sativa, M. falcata and M. gaetula and observed normal pairing in all the hybrids; in addition, he noted that the genomes of the three species were morphologically identical. Sprague concluded that these three taxa were not true species but variants of a polymorphic species from which cultivated alfalfa must have arisen.

With regards to the origin of L. corniculatus, the results of

the present study are in agreement with Favarger's (1953) hypothesis which implicates L. alpinus as a putative diploid ancestor of the cultivated tetraploid. In addition, the findings of the present investigator support an allotetraploid origin of L. corniculatus. This view is substantiated by hybridization data, comparative morphological studies, and chromosome pairing relationships. It has been shown that there is little or no incompatibility barrier between the amphidiploid (L. japonicus x L. alpinus) and L. corniculatus; crosses were obtained without resort to embryo culture. Morphological studies indicate a close affinity between 4x L. japonicus, 4x L. alpinus and the cultivated tetraploid. The amphidiploid is not exactly a duplicate of the L. corniculatus phenotype in that small differences, such as stem coloration, slightly larger flowers, and fewer flower per umbel, are readily detected. However, some divergences are to be expected between a newly synthesized tetraploid individual and the "natural" tetraploid L. corniculatus, because if the latter originated during the early history of the genus from the prototypes of L. japonicus and L. alpinus, both L. corniculatus and the two diploid species would be expected to have undergone considerable genic changes in the intervening years. Also, it should be noted that considerable intraspecific hybridization, selection and introduction into new environments have gone into the evolution of the cultivated species.

Cytological data suggesting that L. japonicus and L. alpinus are

putative parents of L. corniculatus are as follows: 1) Considerable homology exists between the chromosomes of these two species. In the interspecific diploid hybrid between L. japonicus and L. alpinus a high degree of bivalent pairing was recorded. The frequency of multivalent configurations found in the meiocytes of the amphidiploids of this hybrid was comparable to that exhibited by 4x L. japonicus and 4x L. alpinus. In fact, this amphidiploid behaved cytologically like an autotetraploid. 2) A high degree of homology exists between the chromosomes of L. japonicus, L. alpinus and L. corniculatus. Meiotic studies of the hybrids obtained by crossing the amphidiploid (L. japonicus x L. alpinus) and L. corniculatus revealed a high frequency of multivalent associations comparable with that seen in 4x L. japonicus, 4x L. alpinus and the amphidiploid (L. japonicus x L. alpinus). 3) Autotetraploid L. japonicus exhibited a low frequency of multivalents when compared with the other autotetraploids. It would appear that L. japonicus has a gene, or a combination of genes, which influence the formation of chiasmata. If such genes are closely linked to form a gene complex, then this would be of special value in the evolutionary development of any tetraploid in which such a complex is found. Stephens (1949) indicated that block transference of linked complexes is a common occurrence in species which exhibit cryptic structural differentiation of their chromosomes.

It should be noted that a relatively high frequency of multivalents have been detected in the amphidiploid (L. japonicus x L. alpinus)

compared with that observed in L. corniculatus. If it is assumed that L. japonicus and L. alpinus are the progenitor species, then, it is likely that a process of diploidization must have occurred during the evolution of L. corniculatus. Gilles and Randolph (1951) reported that the frequency of quadrivalents in autotetraploid maize after a ten year period was reduced from 8.47 to 7.46 quadrivalents per cell. From the results of their study, these authors concluded that autoploids which form multivalents with a relatively high frequency at the time of their origin may shift to bivalent pairing during their subsequent evolutionary history; they considered such a shift in pairing behavior to be the result of a selection for gene, or genes, that influence chromosome pairing. Wernsman et al. (1964) compared the frequency of quadrivalents in the first generation autotetraploids of L. tenuis with that of autotetraploids of L. tenuis which had undergone a number of generations of random mating. They observed a significant reduction in the frequency of quadrivalents in the autotetraploids which were subjected to random mating. These workers also concluded that there was selection for gene, or genes, that influence chromosome association. It is possible that a similar process of meiotic regularization must have occurred during the evolutionary history of L. corniculatus, if this species arose from L. japonicus and L. alpinus.

In the present study there is indirect evidence also implicating L. japonicus as a putative parent. This diploid species was observed

to hybridize easily with many of the other diploid species. If such hybridization had occurred, during the early evolutionary history of the genus, as previously suggested by this author, and if many of the present-day species are the outcome of such crossing, then many of these species would be expected to have a certain amount of L. japonicus germplasm. If it is presumed that L. japonicus is one of the putative ancestors of the cultivated species, then, the other diploid species would exhibit certain morphological attributes of L. corniculatus, and furthermore, there would be evidence of chromosome homology between the diploid species and L. corniculatus. The existence of such a morphological similarity and chromosome homology has been demonstrated by the results of this study.

Inheritance studies by a number of workers (cited below) also provide indirect evidence for the origin of L. corniculatus from L. japonicus and L. alpinus. Because the chromosomes of these two species are largely homologous, tetrasomic inheritance would be expected for many characters in the amphidiploid (L. japonicus x L. alpinus). Tetrasomic inheritance was reported for a number of characters in L. corniculatus; these include the presence of cyanogenetic glycoside (Dawson, 1941; de Nettancourt and Grant, 1964c), light-yellow flower color (Bubar, 1956), leaf size (Donovan, 1957), brown keel tip (Hart and Wilsie, 1959) and dark-green leaf color (Poostchi and MacDonald, 1961). Studies by these various authors indicate that tetrasomic inheritance following chromosome segregation in L. corniculatus is frequently observed.

Thus, the hypothesis that L. japonicus and L. alpinus are the putative parents of the cultivated tetraploid L. corniculatus is supported by evidence drawn from crossing relationships, and morphological data, in addition to that from genetical and cytogenetical studies.

These results concerned with the origin of L. corniculatus are not conclusive and final. Additional studies on crossing relationships between 4x L. japonicus, 4x L. alpinus and L. corniculatus should be investigated. Also, further information regarding the center of origin and the subsequent ecological and geographical distribution of these three species would help to further clarify their relationships.

SUMMARY AND CONCLUSIONS

Within the last decade or so, Lotus corniculatus L., or Birdsfoot Trefoil as it is commonly called, has gained increased importance as a forage crop in Canada and in the Eastern United States. Since it is very likely that this species will become an even more important crop in the near future, the studies reported in this dissertation have been undertaken to provide basic cytogenetic information relating to breeding and agronomic improvement of this forage legume. Morphological, cytological and fertility characteristics of interspecific diploid Lotus hybrids, their induced autotetraploids and synthetic amphidiploids were investigated. In addition, cytological and fertility studies have been carried out on tetraploid hybrids obtained by crossing the synthetic amphidiploids with the natural tetraploid L. corniculatus. The results obtained and the conclusions drawn from these studies are as follows:

1. In an interspecific hybridization program, 44 different cross combinations between 10 diploid species (L. alpinus Schleich., L. japonicus (Regel) Larsen, L. filicaulis Dur., L. schoelleri Schweinf., L. krylovii Schischk. et Serg., L. tenuis Waldst. et Kit., L. corniculatus var. minor Baker, L. pedunculatus Cav., L. palustris Willd., and L. borbasii Ujhelji) closely related to L. corniculatus were made. Emasculation and pollination of about 3000 flowers and the culturing of about 800 embryos were carried out. These crossing experiments resulted in the production of 16 hybrid combinations and

117 diploid interspecific hybrids. Nine of these hybrid combinations (L. japonicus x L. corniculatus var. minor, L. alpinus x L. krylovii, L. alpinus x L. filicaulis, L. alpinus x L. schoelleri, L. krylovii x L. corniculatus var. minor, L. krylovii x L. tenuis, L. filicaulis x L. schoelleri, L. corniculatus var. minor x L. alpinus and L. corniculatus var. minor x L. filicaulis) were produced for the first time. The data derived from these hybridization studies suggested to this author that intercrossing between certain taxa during their early evolutionary history could have occurred and that some of the diploid species might have originated from interspecific hybridization and subsequent gene differentiation.

2. A total of 208 seedlings of L. alpinus, L. japonicus, L. filicaulis, L. schoelleri, L. krylovii and L. corniculatus var. minor were treated with 0.2 per cent colchicine solution. One hundred and eighteen seedlings survived the treatment and 15 autotetraploids were secured. For the production of amphidiploids, 313 seedlings of 14 different interspecific diploid hybrids were treated with colchicine solution. From the 119 plants which survived the treatment, 40 amphidiploids from 12 different interspecific hybrids were obtained.

3. Twenty-two different cross combinations were attempted between the 12 synthetic amphidiploids and L. corniculatus. A total of 236 plants were procured from 15 different cross combinations. The culturing of embryos was not a prerequisite for making these crosses.

The results of these hybridization studies indicate that the transference of desirable germplasm from diploid taxa to the cultivated tetraploid by means of derived amphidiploids can be accomplished without too much difficulty. From the crossing relationships of the various amphidiploids and L. corniculatus, it is concluded that L. japonicus and L. alpinus are more closely related to L. corniculatus than the other diploid taxa. The highest percentage of successful crosses (85.15) was realized when the amphidiploid (L. japonicus x L. alpinus) was crossed with L. corniculatus.

4. A comparative morphological study between L. corniculatus var. minor and six diploid species (L. alpinus, L. japonicus, L. filicaulis, L. schoelleri, L. krylovii and L. tenuis) showed that this taxon, while having some phenotypic characters in common with these other species, possessed certain distinctive morphological characteristics for which this taxon could be recognized. Therefore, it is suggested that L. corniculatus var. minor should be considered a separate species and be accorded specific rank.

5. The meiotic behavior of the chromosomes of the diploid species was quite regular. The homologous chromosomes usually formed bivalents at diakinesis and metaphase I, and a regular disjunction at anaphase I. A few lagging chromosomes were detected at anaphase I and II and telophase I and II which were presumably due to the few univalent chromosomes detected at diakinesis and metaphase I.

6. In pollen fertility studies, over 90 per cent stainable pollen was observed for L. japonicus and L. corniculatus var. minor. The

other five diploid species, however, exhibited varying degrees of pollen abortion which ranged from 32.81 to 12.96 per cent. In meiosis regular chromosome behavior was observed in all these species; therefore, it is most likely that pollen sterility is genotypically controlled. Seed set was high in all the diploid species except L. alpinus which had an average of 9.26 seeds per pod. The average in the remaining diploids was never below 20 seeds per pod. With the possible exception of L. alpinus, seed setting in the diploid species was not considered to be correlated to the percentage of aborted pollen.

7. In addition to visual observations of growth habit, number of florets per umbel, flower color expression, and the presence of HCN, 15 metrical traits were compared in a morphological study of the interspecific diploid hybrids. In general, the F_1 hybrids resembled one or the other parent in regards to some morphological characteristics but were intermediate for other characters. However, a tendency towards heterotic vigor was observed for one or more of the quantitative traits in all of the hybrids.

8. Close chromosome homology with a normal association of six bivalents were observed in at least 57 per cent of the PMC's in each of the 16 different hybrids studied. However, the presence of loose bivalents, and univalent chromosomes at diakinesis and metaphase I, presumably a result of desynapsis, and lagging chromosomes at anaphase I and II, indicated that the chromosomes of the parental species are structurally differentiated. Multivalent pairing of the chromosomes

occasionally observed in a number of hybrids and the presence of inversion bridges at anaphase I would suggest that gross structural changes are also important in chromosome differentiation. An extra chromosome, considered to be a B-chromosome, was observed in one genotype in the cross L. alpinus x L. filicaulis.

9. A high degree of pollen sterility was observed in most of the hybrids studied. Pollen stainability ranged from 86.11 to 16.63 per cent. No correlation was observed between meiotic irregularities and pollen abortion. The variability of pollen stainability between some genotypes of the same cross and between the different cross combinations, could be explained in terms of gene action as well as on the basis of cytological, physiological, and environmental factors. Seed set in these interspecific hybrids was low and the average ranged between 0.66 and 9.03 seeds per pod. In addition to abortive gametogenesis resulting from cytological irregularities, it is likely that other factors such as genic incompatibility and embryonic lethality were responsible for the low seed set recorded in the hybrids.

10. The induced autotetraploids were slower in development and flowered later than the corresponding diploids under similar growing conditions. The autotetraploids possessed thicker stems, larger leaves and bigger flowers. In a comparative morphological study between the induced autotetraploids and L. corniculatus, it was found that each of these derived tetraploids resembled the cultivated species in one to seven phenotypic traits. Morphologically, L. alpinus (4x)

and L. japonicus (4x) showed the closest resemblance to L. corniculatus.

11. Meiocytes of the autotetraploids were characterized by a high frequency of bivalents and a low occurrence of quadrivalent configurations. The mean number of bivalents per cell ranged from 8.37 to 9.11 and the average number of quadrivalents per cell ranged from 0.57 to 1.10. It is considered that the low frequency of quadrivalents may be attributed to the small size of the chromosomes, the low chiasma frequency, or genetic factors.

12. The percentage of stainable pollen in the autotetraploids was lower than in the corresponding diploids. No correlation between meiotic chromosome behavior and pollen fertility was discernable. It appears, that, in addition to cytological and physiological factors, gene action and environmental factors are also important in influencing pollen fertility in these derived autotetraploids.

13. In the PMC's of L. corniculatus, a high frequency of bivalents and relatively few quadrivalents were seen at diakinesis and metaphase I. The presence of quadrivalents indicates that the genomes of this species are partially homologous. The preponderance of bivalents and the rare occurrence of multivalents provide evidence for the possibility of an allotetraploid origin of L. corniculatus.

14. Morphologically, the amphidiploids were intermediate between their diploid progenitor species and were quite distinct from one another. They were slower in development and flowered later than the corresponding diploid interspecific hybrids under similar growing conditions. In a comparative morphological study between these

alloploids and cultivated L. corniculatus, varying degrees of resemblance to the latter species were noted. On the basis of morphological affinity, the amphidiploid (L. japonicus x L. alpinus) appears to be closely related to L. corniculatus.

15. The variable and low number of multivalents and the relatively higher frequency of bivalents in the meiocytes of many of these amphidiploids, suggest that some preferential pairing occurred. Homology of the chromosomes of the parental species involved in the amphidiploids is evident by the presence of trivalents and quadrivalents and the occurrence of inversion bridges with accompanying fragments at anaphase. The amphidiploid (L. japonicus x L. alpinus) exhibited a high frequency of quadrivalents in comparison with the number of quadrivalents observed in L. japonicus (4x) and recorded for L. alpinus (4x). These observations indicate that the chromosomes of the diploid species, L. japonicus and L. alpinus, are largely homologous. One amphidiploid (L. alpinus x L. krylovii) appeared to be desynaptic, since there was a high frequency of univalents and relatively few bivalents observed in PMC's of this plant.

16. Pollen stainability in the synthetic amphidiploids was variable between genotypes as well as between the different amphidiploids. There was no apparent correlation between meiotic irregularities and pollen fertility. Generally, these alloploids exhibited higher pollen fertility than their corresponding interspecific

diploid hybrids. It seems that in addition to cytological influences, gene substitution and cryptic structural differences of the chromosomes play a role in affecting pollen fertility. Seed set was unexpectedly low in all the amphidiploids; it is considered that environmental factors as well as pollen sterility and abortive gametogenesis may be responsible for the low seed set recorded.

17. Meiotic studies in the tetraploid hybrids, obtained from crossing the synthetic amphidiploids and L. corniculatus, indicated a strong tendency for homogametic pairing. The predominance of bivalents suggests that some preferential pairing was taking place between the chromosomes of L. corniculatus. Evidence for affinity between the chromosomes of the diploid species involved in the amphidiploids, and L. corniculatus, is provided by the presence of trivalents and quadrivalents. Meiotic chromosome behavior in these tetraploid crosses support the view that the most important diploid species from the point of view of the evolution of L. corniculatus are L. japonicus and L. alpinus.

18. All these tetraploid hybrids exhibited a relatively high number of stainable pollen grains. In two crosses, the percentages of stainable pollen were greater than that observed for L. corniculatus. This suggests that there is a definite possibility of introducing desirable germplasm from the diploid taxa to the cultivated species. Differences in pollen fertility in certain reciprocal crosses imply that cytoplasmic factors may play a role in influencing pollen fertility.

19. From a study of the factors underlying species differentiation in the diploid Lotus species, it is considered that gene substitution and structural differentiation of the chromosomes, both cryptic and patent, have played an important role in the evolution of the diploid taxa. There is a considerable amount of evidence suggesting that cryptic structural differentiation has proceeded to a greater extent, and that gross repatterning of the chromosomes appears to be minimal.

20. In order to explain (a) the close morphological resemblance and the distinct chromosomal relationships that exist between the various diploid species and (b) the similarity in certain morphological characteristics and the close chromosome homology between the diploid species and L. corniculatus, it is postulated that the progenitors of the present-day species intercrossed freely. It is also, the opinion of this author that these progenitors belonged to one, or a few, polymorphic species.

21. With regards to the origin of L. corniculatus, it is suggested that this cultivated tetraploid is a segmental allotetraploid and that the diploid species L. japonicus and L. alpinus are its progenitor parents. This conclusion is supported by evidence drawn from the morphological data and the crossing relationships of the species, in addition to the data from the cytogenetical studies reported in this dissertation.

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