A CYTOGENETIC STUDY ON INTERSPECIFIC DIPLOID HYBRIDS CLOSELY RELATED TO *LOTUS CORNICULATUS* L. (LEGUMINOSAE)

by

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

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April, 1963

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ACKNOWLEDGEMENTS

The writer wishes to express his deep gratitude to Dr. W. F. Grant for his guidance throughout the course of this study and for his help during the preparation of the manuscript. He also expresses his gratefulness to Dr. A. F. Naylor who prepared the IBM statistical program for the leaf and floret analyses.

The author thanks Mr. Ian Ogilvie for technical help received in the maintenance of the collections and in the back cross experiments.

The author also wishes to acknowledge his gratitude to Mr. Ferenc Stefani for developing and printing the microphotographs and to Mrs. Anna Stefani who typed the manuscript.

Ph.D. Genetics

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A Cytogenetic Study on Interspecific Diploid Hybrids closely related to *Lotus corniculatus* L. (Leguminosae)

A cytogenetic study was carried out on 53 interspecific hybrids derived from ten different crosses between six diploid species of *Lotus*, namely, *L. japonicus* (Regel) Larsen, *L. alpinus* Schleich., *L. filicaulis* Dur., *L. schoelleri* Schweinf., *L. krylovii* Schischk. and Serg., and *L. tenuis* Waldst. et Kit., to provide data on crossability, hybrid fertility and the mode of inheritance of phenotypic traits. Red stem colour, presence of cyanogenetic glycoside, black keel tip, floret striping, pod mottling and seed speckling were inherited as dominant characters. Several of the hybrids expressed pronounced heterosis, especially for leaflet and floret size. In spite of cytological irregularities which included cytomixis, desynapsis and multivalent formation, normal chromosome pairing was recorded in at least 60% of the metaphase plates observed for each hybrid. Percentages of stainable pollen ranged from 55 to 99 in the parental species, whereas only 2 to 45% of the pollen stained in the hybrids. Seed set per pod was low in all hybrids.

Inheritance studies in F_2 populations of the hybrid *L. japonicus* x *L. filicaulis* suggested that red stem colour and presence of HCN were each controlled by a dominant gene. These two genes are located on different linkage groups, but both are linked to genes controlling width of the central leaflet. Floret size, which was positively correlated to leaflet width, appeared to be governed by genes located on the stem colour linkage group. In F_2 and back cross progenies of *L. japonicus* x *L. alpinus* floret striping was considered to be controlled by two unlinked genes. Keel tip colour and seed speckling were governed by a single dominant gene, or two closely linked genes. Neither of the two genes controlling floret striping were linked to the seed speckling or keel tip colour markers. A deficiency in the number of homozygous recessive genotypes was observed for several characters.

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INTRODUCTION

Interspecific hybrids are of particular interest to the geneticist, the taxonomist, and the plant breeder. To the former they allow an analysis of chromosomal homology, gene interaction, gene segregation and cytoplasmic inheritance which would be impossible to perform, in most cases, at the intraspecific level. To the taxonomist, on the other hand, interspecific hybrids permit a detailed study of the relationship between species, and eventually, an appreciation of the major phylogenetic pathways. To the agronomist finally interspecific hybrids may lead, after appropriate selection and/or induction of amphidiploidy, to the production of desirable genotypes for plant breeding purposes.

For these reasons considerable attention has been devoted during this century to the production and study of hybrid plants derived from crosses between both wild and cultivated species. It may be recalled here the successful hybridization experiments performed by Baur (1924) in *Antirrhinum*, by Kihara (1925) in *Triticum*, by Karpechenko (1927) in the Cruciferae, by Upcott (1939) in the genus *Primula*, by Kostoff (1938), Goodspeed (1954) and Valleau, Stokes and Johnson (1960) in *Nicotiana*, and the classical analysis carried out by Clausen, Keck and Hiesey (1945) in the tribe Madinae of the Compositae. Keck, Clausen and Hiesey (l.c.) were able to reconstitute, by means of interspecific hybridization, some of the major evolutionary events which took place in the karyotypes of genera of the Madinae. Karpechenko (l.c.) and Upcott (l.c.) secured data of great interest on the various factors controlling hybrid sterility and on the effects of amphidiploidy upon Darwinian fitness. The studies of Kihara (l.c.) and Goodspeed (l.c.) allowed a deeper understanding of intergenomic relationships and of genotypic stabilization in allopolyploids. Valleau, Stokes and Johnson (l.c.) were able to develop, through the incorporation of selected germplasm derived from wild species, a number of commercial varieties of tobacco which proved to be resistant to various diseases such as black shank, wildfire, and mosaic. Many other examples could have just as easily been chosen but suffice it to say here that the study of interspecific hybrids has constituted a significant contribution to the science of cytogenetics and allied fields.

The genus Lotus, because of its increasing importance in providing a type of forage plant adapted to a wide range of environmental conditions, has recently been involved in several interspecific hybridization programs at various research centers. At two of these institutions, namely Cornell University and the University of Vermont, some important advances have been made in combining L. corniculatus (4x), commonly known as birdsfoot trefoil, with induced autotetraploids of L. uliginosus and L. tenuis. In both cases, the hybrids were partially fertile, vigorous and potentially valuable for plant breeding purposes (Keim, 1952; Mears, 1955; Erbe, 1955; Bent, 1958; Fish, 1960). Yet, because of their amphidiploid nature, the hybrids obtained were of limited use for cytological analyses and, like the tetraploid L. corniculatus, proved to be difficult to study from a purely genetic point of view. In order to avoid such difficulties in the cytogenetic evaluation of Lotus hybrids it was decided at this laboratory to initiate a hybridization program involving only diploid species of Lotus. The goal of the project was to analyse pairing relationships of the chromosomes in the hybrids and to study gene segregation in advanced generations. It was hoped that these investigations would provide information on the feasibility and ease of transferring characters on the diploid level and likewise contribute to the knowledge of character relationship in the genus Lotus. At the same time it was considered that such a study, involved with an analysis of the detailed morphological characters, might, in addition to the cytological data, help to clarify the taxonomic status of the species closely related to L. corniculatus.

In the work reported here cytological, morphological and genetical data have been obtained for 53 interspecific hybrids derived from ten different crosses between six diploid species of Lotus, namely L. japonicus, L. alpinus, L. filicaulis, L. schoelleri, L. krylovii, and L. tenuis.

LITERATURE REVIEW *

Lotus is a name not devoid of poetry and history. Theophrastus and Herodotes designated as Lotus the sacred lily of the Nile and India. Dioscorides and Pliny, during the first century of our era, described as Lotus a number of valuable plants including the Italian date palm and several clover varieties. Some fifteen hundred years later Camerarius (1558), followed by Gerard (1633) and Linnaeus (1753), introduced the name Lotus in the Leguminosae family where it holds a generic position in the tribe Loteae.

I. Limits of the genus Lotus

One would think, with such a glorious past and so many centuries of civilization behind it, that the genus Lotus is actually a well defined, readily understood and highly stable taxon. This is by no means the case and even now, at the second half of the twentieth century, taxonomists are still faced with conflicting opinions by eminent authorities as to the true limits of the genus.

In 1837, the European taxonomist Bentham reported that the species of Lotus should not be restricted to the Old World but should embrace the North American representatives as well. One year later Torrey and Gray (1838) indicated their belief that there were sufficient differences between the American and the European species to refer the former to the genus Hosackia. Greene (1890) did not agree with Torrey and Gray and

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*The survey of literature pertaining to this review was completed on November 1, 1962.

relegated all the North American species to the genus Lotus.

Using floral characters as a major tool of classification Taubert, in 1894, distinguished Lotus from Hosackia and other closely related genera such as Tetragonolobus, Bonjeania and Dorycnium. Brand (1898) accepted Taubert's delimitation of the genus and, in his study, recognized three major characters for distinguishing Lotus, namely 1) Pentafoliolate leaves, 2) Pod dehiscence and 3) Absence of foliar stipules. Thus, like almost all European monographers, Brand restricted Lotus to the Old World and placed all North American species in the genus Hosackia. Ottley (1923, 1944) considered, however, that the North American and European species were congeneric and included them together within Lotus. Isely (1951) supporting Ottley's view, not only incorporated Hosackia within Lotus but also the genera Acmispon and Tetragonolobus. He gave the revised genus the following definition:

> "Plants perennial or annual. Leaflets 1-15, pinnately or palmately foliolate. Stipules small or glandlike. Flowers in pedunculate, axillary clusters or solitary, yellow, white or red. Keel petals generally fused both above and below. Stamens diadelphous, style curved. Pod oblong, several seeded, dehiscent."

A few years ago, Gillett (1958) defined a new subgenus in Lotus, namely Dorycnium, and placed in it the Canary Island species which were formerly relegated to the genus Dorycnium. One year later Callen (1959) excluded Dorycnium and Bonjeania from Lotus but retained Hosackia and Tetragonolobus in the genus. This last author is in agreement with Larsen (1958) who, on the basis of limited karyological analyses, accepted the inclusion of Hosackia and Tetragonolobus in the genus and who approximated to 200 the number of species actually composing the genus.

II. Subdivisions of the genus

In his well known monograph of Lotus Brand divided the genus into two subgenera and seven sections which are listed as follows:

A) Subgenus Pedrosia (Lowe) Brand
    1) Section Heinekenia (Webb) Brand
    2) Section Eupedrosia Brand

B) Subgenus Edentolotus Brand
    1) Section Krokeria Muench
    2) Section Xantholotus Brand
    3) Section Erythrolotus Brand
    4) Section Ononidium Boiss.
    5) Section Quadrifolium Brand

As previously stated, Callen has disagreed with Brand's concept of the limits of the genus. Using characters of the style as a taxonomic criterium, Callen subdivided Lotus into four subgenera:

A) Style erect, forked or toothed: Subgenus Pedrosia (Lowe) Brand
B) Style erect, simple: Subgenus Edentolotus Brand
C) Style deflected: Subgenus Deflectostylus Callen
D) Style erect, club shaped: Subgenus Tetragonolobus Callen

From a literature review concerning the cytology of the genus it can be seen that there is a good correlation between chromosome numbers and Callen's classification. Reports by Senn (1938), Darlington and Wylie (1955), Löve and Löve (1961) and Grant (1962) indicate the presence, within the genus, of a polyploid series (2*n* = 12, 14, 24 and 28) with two basic numbers (*x* = 6 and 7). All species with a basic number of 6 are found in either the subgenus *Edentolotus* or the subgenus *Deflectostylus* (Callen, Bubar and Grant, 1959). Furthermore *L. corniculatus* L. and its related species, which have a basic chromosome number of six, all belong to the subgenus *Edentolotus*.

III. Geographical distribution of *Lotus*

The genus *Lotus*, which according to Hertzch (1959) probably originated in the Mediterranean area, is actually naturalized in six different regions of the world:

The Mediterranean area
East Africa
South Africa
North of the Mediterranean sea up to the Arctic circle
Western North America (where all species of *Lotus* were once considered to belong to the genus *Hosackia*)
Australia.

IV. Description of some diploid species closely related to *L. corniculatus*

The delineation of many *Lotus* species is often a matter of considerable difficulty and this is especially true in the case of *L. corniculatus* L. and closely related taxa. *L. corniculatus* is a polymorphic tetraploid species with 24 somatic chromosomes. Isely (1951) described this species as follows:

"Plants perennial from a stout crown; rhizomes not present. Stems decumbent or erect, up to 6 dm. in height, glabrous or pubescent. Leaves pinnately 5-foliolate, the lower pair of leaflets placed on rachis, the remaining three apical. Peduncles axillary, exceeding leaves. Umbels 3-8 flowered. Pedicels very short. Flowers usually 12-16 mm. in length. Calyx lobes approximating the tubes, appressed in buds. Corolla yellow to orange red; standard as broad as long, exceeding wings and keel. Pods 2-3.5 cm long, terete, straight; valves brown, splitting apart and twisting in maturity. Seeds about 1.5 mm across, assymetrically rounded, dark or olive brown in color, frequently mottled or speckled."

The geographic distribution of _L. corniculatus_ was probably limited to Southern Europe before the intervention of man. This species is now widely distributed, occurring throughout Europe, Western Asia, Australia, New Zealand and North America (Isely, 1951) where, in most areas, it has become established with the native populations.

Resembling _L. corniculatus_ in certain morphological characters are a number of taxa with a somatic chromosome number of 12. These entities have been, in most instances, considered as varieties of _L. corniculatus_. Bullen (1960) lists a few of these so called diploid varieties and closely related diploid species, which were initially involved in the interspecific hybridization program at this laboratory, as follows:

L. corniculatus var. _alpinus_ Ser.
L. corniculatus var. _eremanthus_ Chiov.
L. corniculatus var. _heterophyllarius_ Freyn

L. filicaulis Dur.
L. japonicus (Regel) Larsen
L. tenuis Waldst. et Kit.

The nomenclature of some of these taxa has been revised, however, in the course of this research program (Grant, Bullen and de Nettancourt, 1962) and specific rank given to the varieties *alpinus*, *eremanthus* and *heterophyllarius*. An attempt shall be made here to review the literature pertinent to the six species which were used during the present study.

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

This species was discovered by Favarger (1953) in the Western Alps, at an altitude of 2,000 meters. Bullen (1960) described it in the following terms:

> "A perennial species with a slow and somewhat intermittent growth. The flower is of average size with only one or two flowers per inflorescence. Although the flower color is like *L. corniculatus* the keel tip, in the plants observed, were of a very dark brown."

Bullen found plants of this species to have a positive reaction to the cyanogenetic glycoside (HCN) test.

It is interesting to compare *L. alpinus* (2*n* = 12) with *L. corniculatus* var. *glacialis* Boiss. (2*n* = 24) which is the Alpine birdsfoot trefoil. Macdonald (1946) described this latter taxon as follows:

> "Root stalk very short, often woody. Stem usually only 2.5 to 5.0 cm long, prostrate to erect. Leaflets small, bald or slightly

hairy. Inflorescence with only 1 to 3 comparatively large flowers. Occurs in mountainous areas of South and Central Europe, Asia and North Africa. In Switzerland up to 2,900 meters, and in Sierra Nevada up to 3,500 meters. Not found in America."

Information in the literature concerning the possibility that diploid L. alpinus may be involved in the origin of L. corniculatus shall be reviewed later. Suffice it to say here that L. alpinus and L. corniculatus var. glacialis, as they are described above,appear to be very closely related.

2) Lotus japonicus (Regel) Larsen, 2n = 12

Fish (1961) gave a brief description of this Eastern Asiatic Lotus: "Purplish coloration of the slender stems. Diploid plants are procumbent, small, not of agronomic stature. The leaves are pale bluish-green, moderately small, rhomboidal, elliptic, somewhat acute. The flowers are large, distinctly sulphur yellow, the standard having very pale guide lines. The sepals are long, villous, pubescent. The large pods, before maturity have a bright to vivid green color. The plant is self-fertile."

Bullen (1960) indicated that this species is an annual which reacts positively to the HCN test. It is native to Korea and Japan.

3) Lotus tenuis Waldst. et Kit. (syn: L. corniculatus ssp. tenuifolius)2n = 12

An analysis of this perennial species was made by MacDonald (1946) who states:

"Stems usually numerous, decumbent to erect, sometimes 30 inches long, usually quite bare, sometimes strongly branched. Internode long.

> Leaflet linear-lanceolate to linear, seldom wider. Inflorescence o- to 7-flowered. Calyx teeth triangular, abruptly becoming smaller at the tip, as long or shorter than the tube. Flowers smaller than in L. corniculatus var. vulgaris and arvensis. Height of standard equal to its width, wing petal smaller. Petals turn orange red with withering. Chromosome 12 in number. Legumes often depressed."

Isely (1951) underlined the essential differences that exist between L. corniculatus and L. tenuis and stated that leaf width, floret size and broadness of the standard are the most reliable characters for distinguishing these two taxa. Similar conclusions were reached by McKee (1941) who emphasized the lack of pubescence and the length of calyx teeth as ultimate criteria for differentiation.

In spite of these morphological dissimilarities, and the differences in the chromosome numbers between these two taxa, most authors, up to 1954, considered tenuis as a variety or subspecies of L. corniculatus. Larsen (1954), on the basis of cytotaxonomic evidence, reinstated L. tenuis to specific rank. Ujhelyi(1960), who corroborated Larsen's views, noted important differences between L. tenuis and L. corniculatus in relation to the structure of the leaf epidermis. He stated that:

> "La reunion de cette espece au L. corniculatus L. etait causee en partie par l'attachement a la conception lineenne et en partie par la ressemblance de la fleur. Sa genetique autonome est prouvee par son aire geographique distincte quoique en partie couvrant celle du L. corniculatus et surtout par son exigence envers l'habitat. Sa parente doit etre cherchee aupres du L. decumbens Poir."

Zertova (1961), who treats L. tenuis as a subspecies of L. corniculatus, mentioned that in tenuis the form of the leaf and the shape and size of the floret are stable characters. The same author indicated that the broadness of the calyx, as compared to its length, is a reliable taxonomic criterium for distinguishing tenuis from L. krylovii (given by her as L. frondosus Freyn; see Grant, Bullen and de Nettancourt, 1962).

MacDonald (1946) reported what he designated as L. corniculatus var. tenuis as giving a positive reaction to the HCN test but Bullen (1960) observed the species to be polymorphic for this character.

Bubar (1958) observed L. tenuis to be self-sterile, but MacDonald (1946) and McKee (1949) earlier demonstrated that a limited percentage of seed set results after deliberate self-pollination. A similar observation was made by Bullen (1960) who reported foreign pollen to stimulate self-fertility in this species.

L. tenuis is native to Europe and adjacent Africa and is reported to have become naturalized in the United States in several states, including New York, Ohio, Georgia, Oregon, and California (Isely, 1951; Munz and Keck, 1959).

4) Lotus filicaulis Dur. (syn.: L. corniculatus var. filicaulis) $2n = 12$

Only a few comparisons have been made between L. filicaulis and other species reported in this thesis. Brand (1898) wrote that this taxon, which he considers as a variety of L. corniculatus, differs from L. corniculatus var. vulgaris in that filicaulis has very thin, long branches and, in addition, possesses longer petioles. He also stated that filicaulis generally has one or two florets per inflorescence as compared to 3 - 7 in L. corniculatus. Brand did not think, however, that these differences justified raising filicaulis to specific status and maintained this taxon as a variety of L. corniculatus. At that time the cytological differences between these two taxa were not known to Brand.

In a study of cross-pollination in the genus Lotus Fish (1961) compared L. filicaulis to L. tenuis and stated that the two species resemble each other for their narrow leaves, slender stems, small flowers, and pod and seed characters. Fish noted, however, that the leaf of L. filicaulis, which is rounded at the distal end or oblanceolate, is blue green in contrast to L. tenuis. Another difference Fish reported was that L. filicaulis has 1 - 2 florets per umbel as compared to 1 - 7 in L. tenuis. Bubar (1958) indicated that L. filicaulis is self-fertile, and observed 22 ovules per ovary and 23 seeds per pod following cross-pollination. Bullen (1960) obtained, for all plants that he studied, a negative reaction to the HCN test.

The geographical distribution of the species appears to be restricted to North Africa. Jahandiez and Maire (1941) observed scattered populations of L. filicaulis in Morocco and Algeria and believe the latter country to be its center of origin.

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

Gillett (1958), considering the wide polymorphism of L. corniculatus in the Mediterranean area, classified L. schoelleri as a variety of L. corniculatus. He indicated, however, that:

> "This plant, with 1 - 2, rarely 3 inflorescences, small flowers and short, glabrous or almost glabrous peduncles at first sight seems significantly distinct from L. corniculatus as it is known in N.W. Europe."

Gillett also stated that *L. schoelleri* seems especially similar to *L. tenuis*, though its wider leaflets resemble those of *L. corniculatus*. Some of the plants he reported as possessing very minute leaves and presumably lacked growth and vigor. Gillett assumed that the plants were dwarfed by the dry montane environment in which he found them. Since Gillett did not study his material cytologically it may be wondered if the phenotypic variations which he reported are not the result of differences in the level of polyploidy. *L. schoelleri*, which gives a negative reaction to the HCN test (Bullen, 1960), is found in Sudan, Eritrea, Ethiopia, Kenya and Tanganyka (Gillett, 1958).

6) *L. krylovii* Schisch. et Serg. (syn.: *L. frondosus* Freyn, *L. corniculatus* var. *heterophyllarius* Pet.-Stib.) $2n = 12$.

Larsen (1958) was the first to report that *L. corniculatus* var. *heterophyllarius* and *L. frondosus* were quite likely to be identical while Zertova (1961) indicated synonymy between *L. krylovii* and *L. frondosus*. This Eurasian taxon, as described by Kuprianova (1945) has 1-3 florets per inflorescence. These florets are small and turn red with aging. The leaves are narrow and oblanceolate. Zertova (1961) considered that *L. krylovii* differs essentially from *L. tenuis* by the shape of both the leaflet and the calyx. Quoting Kuprianova, she indicated that *L. krylovii* has flowers which are red upon maturing and calyx teeth that are approximately 1.5 times as long as the calyx tube (length to width ratio) as compared to 1.00 in *L. tenuis*. *L. krylovii* has been reported to be self-fertile by Chamberlain (1961) and negative to the HCN test (Bullen, 1960).

V. Cytology of diploid species closely related to *L. corniculatus*

Relatively little information is available on the cytology of this group. Tschechow and Kartaschowa (1932) and Larsen (1954) studied the karyotypes of *L. corniculatus* and some of its related taxa but no idiograms have been published for any of these species.

Observations made by Tschechow and Kartaschowa (1932) for taxa concerned in the present study can be summarized as follows:

L. corniculatus var. *alpestris* Lamotte, 2*n* = 24:

2 pairs of long submetacentric chromosomes and ten pairs of short chromosomes.

L. tenuis Waldst. et Kit., 2*n* = 12:

One pair of long submetacentric chromosomes and five pairs of short chromosomes.

L. filicaulis Dur., 2*n* = 12:

One pair of long submetacentric chromosomes, one pair of metacentric chromosomes, and four pairs of short metacentric chromosomes.

Favarger (1953), observed in *Lotus alpinus*, given as *L. corniculatus* var. *alpinus*, one pair of chromosomes to be much longer than any of the five other pairs. He considered the karyotype of *L. alpinus* to be very similar to that of *L. tenuis*. Mears (1955) reached identical conclusions when she compared the somatic chromosomes of an induced tetraploid of *L. tenuis* with those of *L. corniculatus* as she found no significant differences in karyotypes between the two species. As may be ascertained from the very limited karyological studies in *Lotus*, the karyotypes appear to be relatively uniform from one species to another.

VI. Origin of *Lotus corniculatus*

The origin of *L. corniculatus* is, at the present time, far from being elucidated. Dawson (1941) observed, although very rarely, some quadrivalents at meiosis in *L. corniculatus* and concluded, on the basis of tetrasomic inheritance and its morphological similarity with *L. tenuis*, that *L. corniculatus* arose as an autotetraploid from *L. tenuis* or its prototype. Tome and Johnson (1945) found, however, distinct differences between *L. corniculatus* and the induced tetraploid of *L. tenuis*, the greatest divergences being in the stipule length-width ratio and floret size. Stebbins (1950) interpreted Tome and Johnson, and Dawson's results as evidence that *L. corniculatus* is a segmental allotetraploid. A few years later, Larsen (1954) suggested *L. alpinus* (*2n* = 12) as the ancestral form of *L. corniculatus*, but Favarger (1953) considering this eventuality, pointed out that the reverse may also be true, namely that *L. alpinus* possibly had a parthenogenetic origin. Grant (personal communication) taking both Dawson and Larsen theories into consideration proposed that *L. corniculatus* originated as an amphidiploid of *L. alpinus* X *L. tenuis*.

Attractive as they may be none of these theories is actually supported by sufficient data and further evidence shall be needed in order to elucidate, on a substantial basis, the mysteries concerning the origin of *L. corniculatus*.

VII. Natural hybridization in the genus *Lotus*

Interspecific hybridization may occur in nature after the partial or complete removal of geographical and reproductive barriers. Such a break down of isolating mechanisms appears to be rare in the genus Lotus. Stebbins (1959), who reported that hybrid swarms are unknown in the family Leguminosae, has not observed any hybrids between closely related species of Lotus which grow side by side in California. It should be pointed out, however, that the absence of hybrid populations is not necessarily evidence that natural hybridization does not take place but also a possible indication that individual hybrids are eliminated at an early phase of their life, during the embryonic or seedling stage. Although there is no real evidence that natural hybridization is common in the genus Lotus, several reports have been made suggesting that the wide polymorphism of some Lotus species may be due to natural interspecific crossing. Larsen (1954) stated that L. tenuis, L. uliginosus and L. corniculatus which are not difficult to distinguish in Northern Europe are extremely polymorphic in Southern Europe. He suggested natural hybridization as a possible explanation for the lack of clear boundaries between these three taxa. Gillett (1958) also noted the great variability of these same three taxa in Mediterranean Africa. Ottley (1944) had made a similar statement for some American specimens of Lotus which she considered as interspecific hybrids.

But the best evidence concerning the occurrence of natural interspecific hybridization is probably provided by Mattick (1950) and Seaney (1957-1961). Mattick reported a plant of L. corniculatus with 26 chromosomes which may well have been the F_1 hybrid of a cross between L. corniculatus ($2n = 24$) and a 28 chromosomes species. Seaney made a comparable discovery and found several plants of L. hispidus Desf.

($2\underline{n} = 24$) with somatic chromosome numbers of 25 and 26. Neither of these cases of aneuploidy nor the wide polymorphism met throughout the genus can bring about, however, the conclusion that natural hybridization is a common phenomenum in Lotus. Taking into consideration the self-fertility of a number of Lotus species and the difficulties met with in hybridization experiments one may, perhaps, even conclude that interspecific hybridization, if it takes place, is a rather rare and limited event in the genus.

VIII. Experimental hybridization in the genus Lotus.

With the primary aim of improving cultivated strains of L. corniculatus by the introduction of favourable genes from other species, several experimental programs embodying interspecific hybridization have been carried out at different institutions in North America. At the present time almost all studies concerned with interspecific hybridization have been restricted to crosses involving the tetraploid and diploid taxa closely related to the cultivated species L. corniculatus. Crossing experiments attempted so far can be classified into three main categories:

A) Hybridization between tetraploid species
B) Hybridization between diploid species
C) Hybridization between tetraploid and diploid species.

The data which have been reported concerning these categories will now be briefly summarized.

A) Interspecific hybridization at the tetraploid level: One of the first successful experimental attempts at hybridization between tetraploid species was that of Keim (1952) who obtained hybrid plants from crosses between an induced tetraploid of L. tenuis and L. corniculatus. Some of his hybrids were produced with the aid of embryo culture techniques while others were grown from mature seeds. All of the hybrids were observed to have a phenotype intermediate to both parents. Keim, in some cases, used pubescence, a dominant character, as a marker for the detection of hybrid progeny.

Keim's success in the production of viable hybrids was followed by those of Mears (1955) and Erbe (1955) who also obtained hybrid plants from the cross L. tenuis (4x) X L. corniculatus. In addition Mears managed to produce a hybrid of the reciprocal cross, L. corniculatus X L. tenuis (4x) and indicated that about 25% of the hybrid seeds germinated when L. tenuis was used as the pistillate parent as compared to approximately 1% in the case of the reciprocal cross. Gershoy (1961), who carried out a meiotic study for the hybrid L. tenuis (4x) X L. corniculatus, made the following statement:

> "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. Mears (l.c.) was unsuccessful in attempts to cross L. corniculatus to L. uliginosus (4x). However, in 1958, using embryo culture techniques, Bent (1958, 1962) was able to develop into mature plants nine embryos resulting from a cross between *L. uliginosus* (4*x*) and *L. corniculatus*. The hybrids resembled *L. corniculatus* but a few morphological characters were statistically more like *L. uliginosus*. He found that the reaction to the HCN test and the pubescence of the sepals were inherited as dominant characters. Fertility of the hybrids was variable.

Fish (1961) succeeded to cross a pubescent strain of *L. corniculatus* to *L. japonicus* and reported that the F_1 hybrid was patroclynous in regards to lack of pubescence but appeared intermediate to both parents for the other character investigated. Fish also produced a hybrid from a cross between *L. corniculatus* and *L. filicaulis* (4*x*) and indicated that the hybrid closely resembled *L. filicaulis* with the exception of the florets which were intermediate in size and coloration. From 80 to 85% of the pollen produced by this hybrid was stainable

B) *Interspecific hybridization at the diploid level:* From the foregoing it may be seen that a number of investigators have been successful in their attempts to produce interspecific hybrids between tetraploid species of *Lotus*. The situation is, however, quite different in regards to the various trials which have been made in order to obtain diploid interspecific hybrids.

Dawson (1941) carried out reciprocal crosses between *L. tenuis* ($2n = 12$) and *L. uliginosus* ($2n = 12$) but failed to recover any hybrid seeds from the 425 florets that he pollinated. The presence of a reproductive barrier between these two species was later confirmed by MacDonald (1946) who did not obtain any hybrid seed when he placed *L. tenuis* and *L. uliginosus* in adjacent areas. However, Keim (1952) was somewhat more successful in artificially hybridizing these two species. From the 234 florets that he pollinated, 158 pods were formed and 83 plump seeds collected. Yet, no evidence was provided to verify the hybrid nature of these seeds, since his studies were curtailed at this point. Three years later, Mears (1955) indicated, in the course of a study concerned with species hybridization in the genus, that intercrossing of these same two species resulted in the formation of embryos which aborted at an early stage of development. Mears'results suggested that an isolating mechanism of some unknown nature took place during embryonic growth which prevented further development of hybrid tissue. With the use of embryo culture Bent (1958) managed to overcome this barrier and produced a mature plant from a cross where *L. uliginosus* was the pistillate parent and *L. tenuis* the pollinator. The hybrid was intermediate to the parents in all characteristics studied except the width of the standard petal. In general, however, it resembled *L. tenuis* more closely than *L. uliginosus*. Bent (1958) found that the pubescence of the sepals and the HCN reaction were inherited as semi-dominant characters. The single plant produced was sterile.

L. uliginosus and *L. tenuis* are not, however, the only diploid species which have been used in hybridization programs. Although McKee (1949) did not obtain any pods when he attempted to cross *L. tenuis* and *L. filicaulis*, he succeeded to raise putative hybrids derived from crosses between *L. tenuis* and *L. divaricatus* (2*n* = 12). It should be stated, however, that the hybrid constitution of the plants produced by McKee was never clearly ascertained. Perhaps the most interesting result ever obtained in hybridization experiment conducted at the diploid level was provided by Seaney (1957). Seaney, by means of embryo culture, produced a viable hybrid between *L. coimbrensis* (2*n* = 12) and *L. ornithopodioides* (2*n* = 14)

The hybrid has a somatic chromosome number of 13 and was completely sterile. This case actually represents the only known example of successful intercrossing between diploid species of Lotus having different chromosome numbers. Seaney failed in his attempts to induce amphidiploidy in this hybrid and stated that propagation of the hybrid by means of cuttings was very difficult to achieve.

At this laboratory, with the help of a new emasculation technique and by means of embryo culture, several hybrids have been obtained from various crosses between diploid species closely related to L. corniculatus (Bullen, 1960; Grant, Bullen and de Nettancourt, 1962). It is the purpose of the present study to investigate the morphology, cytology and genetics of these hybrids and of some of their progeny in later generations.

C) Interspecific hybridization between diploid and tetraploid species:

Triploid hybrids are generally completely sterile and, thus, of limited use in applied agriculture. They often prove to be valuable, however, when considered as intermediate vectors for the transfer of desirable genetic traits from one species to another. Their potential value as fertile hexaploids (after colchicine treatment) is also of considerable interest. From a genetic point of view, another quality of the triploid hybrid, in addition to the classical investigations upon aneuploidy and evaluation of linkage groups, is that it may allow, in some instances, the study, at the disomic level, of genes which are segregating tetrasomically in the tetraploid parent. Theoretically, such an analysis is possible each time that the diploid parent is homozygous recessive for the character investigated. It is therefore not surprising to observe that many authors have attempted to produce triploid interspecific hybrids of *Lotus*.

Eliott (1946) intercrossed *L. corniculatus* and *L. tenuis* and obtained fully developed seeds. He did not state, however, if these seeds were viable. McKee (1949) grew to maturity putative hybrids from a cross of the same parentage but did not report any further on them. He also obtained a number of putative hybrids from a cross between *L. corniculatus* and *L. divaricatus* ($2n = 12$).

In the course of his study on pod dehiscence in *Lotus*, Gershon (1962) failed to cross *L. coimbrensis* ($2n = 12$) and *L. corniculatus*. His attempts to hybridize these two species at the tetraploid level were also unsuccessful. Contrasting with those negative or incomplete results the hybridization studies reported by Mears (1958) between diploid and tetraploid species of *Lotus* have been more successful. By means of embryo culture Mears grew to maturity six putative hybrid plants of a cross between *L. uliginosus* ($2x$) and *L. corniculatus* ($4x$). One of the putative hybrids was a triploid while the other five were tetraploid. The triploid produced few mature pods and only an occasional mature seed. The tetraploids set pods abundantly to open pollination but the percentage of fully developed seeds was low.

A few years later, Bent (1958) succeeded, with the aid of embryo culture, to grow to maturity two hybrid plants resulting from a cross between *L. uliginosus* and *L. corniculatus*, the latter species being used as the staminate parent. Both hybrids had a somatic chromosome number of 18. One exhibited a normal growth habit,however, the second was greatly retarded. The hybrids were patroclynous for all characters observed and produced a small amount of stainable pollen. Both plants set a limited quantity of seeds when used as either the male or female parent but Bent failed to obtain successful back-crosses to the parental species.

It seems to the reviewer that a number of concluding statements may be drawn from this brief summarization of the literature which has been reported to date on interspecific hybridization in the genus Lotus. It would appear that interspecific hybridization within the genus Lotus is not common in nature and difficult to succeed under experimental conditions. The reproduction barriers which isolate species of Lotus seem to be operative both prezygotically and postzygotically. It should be said, however, that the use of embryo culture techniques is not an absolute prerequisite to the success of a hybridization program. There is also evidence that interspecific hybridization is generally more successful when attempted at the tetraploid level. Another important fact that is apparent from the literature is that reciprocal hybrids are often difficult to obtain, since some species are cross incompatible when used as the pistillate parent.

IX. Inheritance studies in the genus Lotus

One would believe that a literature review concerned with the genetics of a genus like Lotus should really be subdivided into four major sections designed to account for intraspecific and interspecific inheritance at both the diploid and the tetraploid level. A survey of the literature, however, shows that the great majority of inheritance studies carried on in the genus have been largely confined to L. corniculatus at the tetraploid level. This is by no means surprising if one considers the primary importance of L. corniculatus as a forage and pasture crop.

As may be readily ascertained from a survey of the literature, attention has been largely devoted to those characters which are easily detectable in a segregating population, namely, reaction to the HCN test, floret color, and seed mottling. These characters are therefore among the ones most often investigated. During the past few years, however, some interest has been directed towards the genetics of more complex traits such as leaf size, seed shattering, and root nodulation. It is the purpose of this section of the review to report the results obtained to date by various investigators who, in the course of their studies in Lotus, have given some data for the characters investigated in the present study.

1) Inheritance of cyanogenesis

Cyanogenesis in L. corniculatus was first investigated by Armstrong and others in 1912 and 1913 (cf. Dawson, 1941). Their aim was to correlate the variability in glycoside content of L. corniculatus with climatic and soil conditions. As this correlation was not found to be reliable, Armstrong and his co-workers (l.c.) hinted at the possibility that cyanogenesis was genetically controlled. The hypothesis that HCN production was under gene control proved to be true in another genus of the Leguminosae when Williams (1939) found that in Trifolium repens the presence or absence of cyanogenetic glycoside was determined by a single dominant gene. Three years later, Dawson (1941), in the conclusion of an extensive study involving tetrasomic inheritance in L. corniculatus, reported that cyanogenesis was controlled by a dominant gene. From his segregation data he concluded that the gene controlling HCN production was tetrasomically inherited. Dawson, who had observed sharp segregations into cyanogenetic and acyanogenetic classes, however, also noticed that a small number of plants reacted to the HCN test only slightly positively. Dawson suggested the action of modifying factors as a possible explanation for this unexpected discovery. A number of plants reacting only slightly positively to the HCN test were also reported by Seaney(1957) who classified the responses of the plants that he studied into three distinct groups: positive, slightly positive and negative. Seaney observed that whenever a negative or slightly positive plant was crossed to a positive individual the offspring were all positive, whereas, the progeny of a cross between a negative and a slightly positive plant segregated equally into positive and negative individuals. Seaney observed that a plant must be fully mature and in bloom before the slightly positive HCN reaction would show up. Also, he indicated that slightly positive reacting plants, when selfed, never produced any positive plants in their progeny. Furthermore this author was able to select one individual which appeared homozygous for the slightly positive reaction. All these observations led Seaney to suggest that there is, in L. corniculatus, one gene for the positive reaction which is dominant to both a gene for a slightly positive reaction and a gene for a negative reaction. Slightly positive reaction would be, in turn, dominant over a negative reaction. Seaney did not indicate, however, whether or not he considered these genes to be allelic.

In addition to the discovery of an intermediate phenotype between negative and positive responses, the studies of Dawson and Seaney clearly indicated that the positive reaction to the HCN test is controlled by one dominant gene which is tetrasomically inherited. An elaborated attempt has been made recently by Gershon (1962) to analyze the inheritance of the gene for HCN reaction at the disomic level. This author examined for the presence of HCN in the F_2 and F_3 offspring of six F_1 hybrids from a cross between L. uliginosus (homozygous recessive for HCN) and L. corniculatus (phenotypically positive to the HCN test) and observed that five of these F_1 hybrids bred true for HCN production. The sixth F_1 hybrid, when selfed, had among its progeny a proportion of 3/4 HCN positive plants and 1/4 HCN negative plants. On the basis of this 3:1 ratio and of various intercrossing experiments Gershon confirmed that a single dominant gene was involved in cyanogenesis and that the earlier conclusions of Dawson and Seaney were consistent at both the tetrasomic and disomic level. However, Gershon's results and interpretations only concerned the positive reaction to the HCN test and he did not attempt to elucidate the hereditary mechanism responsible for inheritance of the slightly positive character.

2) Inheritance of flower color

Deep yellow is the flower color common to a large number of Lotus species. Species are known, however, which exhibit florets of quite different colors, ranging from wine red in L. jacobeus to pink in L. arabicus, light yellow in L. filicaulis and white in L. purshianus. Such variations are generally interspecific, the flower color within a species being fairly constant. However, Bubar (1957) working with L. corniculatus crossed a plant with light yellow flowers to one with a normal flower color (deep yellow) and observed that the flowers of the F_1

offspring were all of the normal flower colour. Backcrossing these F_1 plants to the light yellow flowered parent, Bubar secured a population which segregated for floret colour in the ratio of 5 normal to one light coloured plant. This information Bubar considered as evidence for tetrasomic inheritance of the light yellow flower character, postulating that random pairing of the four homologous chromosomes had taken place.

3) Inheritance of keel tip colour

In most species of Lotus the colour of the keel tip does not differ very greatly from that of the rest of the floret. Species are known, however, where the keel tip displays a black, brown or reddish coloration, in contrast to the floret which has a different colour. Inheritance of keel tip colour in L. corniculatus has been reported by a number of authors. Donovan (1957) found that segregation for brown vs yellow keel tip could be explained on either a tetrasomic or disomic basis and he considered this character to be associated with heavy mottling of the seed coat. One year before, Bubar (1956) had reported that keel tip colour was tetrasomically inherited and linked to a locus involved in the self incompatibility mechanism. More recently, Pootschi (1959) supported Bubar's views stating that keel tip colour was tetrasomically inherited in the population he had under study. A similar suggestion was made by Hart and Wilsie (1959) who concluded that brown keel tip in L. corniculatus was conditioned by a single dominant gene, B, which is inherited tetrasomically. Hart and Wilsie postulated that the deficiency of recessive phenotypes which they observed in segregating generations was due to the operation of an independent locus, C, the interaction of the B and C loci being such that the double recessive form bbbb cccc was a zygotic lethal.

4) Inheritance of seed mottling

It has been stated previously that Donovan (1957) considered that the gene controlling keel tip colour was associated with heavy mottling of the seed coat. In the course of this study he investigated the inheritance of two different types of seed mottling. In the first type he found that seed mottling was dominant over non-mottling. He showed this character to be controlled by a small number of genes, the expression of which often appeared confounded by environmental effects. For the second type of mottling, which was also dominant over non-mottling, Donovan postulated a disomic mode of inheritance involving two pairs of genes.

Seaney (195) reported polymorphism for seed mottling in the species *L. angustissimus* and observed that mottling and seed colour were strongly correlated. Heavy mottling was associated with dark gray seeds, intermediate mottling with medium gray seeds and absence of mottling with yellow green seeds. The same author (Seaney, 1961), investigating seed mottling in *L. tenuis* ($2n = 12$), reported this character to be controlled by a single dominant gene.

5) Inheritance of leaflet and floret size

Donovan (1957) found that leaf size in *L. corniculatus* was governed by a single gene and he stated that this gene, which expressed incomplete dominance, was tetrasomically inherited. Pootschi (1959) reported, however, that leaflet length and leaflet width, in the population of *L. corniculatus* that he studied, were controlled by multiple genes which acted in an additive manner. These contradictory results can best be explained by the fact that Donovan and Pootschi did not use the same material in the course of their investigations. In regards to the inheritance of floret size in L. corniculatus, Pootschi (1959) suggested a similar type of polygenic control for both floret length and standard width.

X. Summary

It may be seen from this literature review that the cytogenetics and the taxonomy of the genus Lotus are still in an early phase of development. However, from the various investigations which have been completed in the last two decades, a number of concluding statements can now be made in regards to certain characteristics of L. corniculatus and some of its related diploid species:

Lotus corniculatus is a polymorphic tetraploid species of obscure origin which possibly arose as a result of hybridization between two or more diploid species in the Mediterranean area.

As may be ascertained from preliminary observations, chromosome morphology seems to be somewhat uniform throughout the complex of species closely related to L. corniculatus.

Interspecific hybridization is probably rare in nature and generally leads, when experimentally induced, to sterile or partially sterile hybrids. There seems to be, therefore, in addition to geographic isolation, a number of reproductive barriers between L. corniculatus and its related taxa.

Experimental results reported in the literature indicate that interspecific cross compatibility is generally greater at the tetraploid level, and that successful hybridization often depends upon the proper choice of the species used as the pistillate parent.

Most genetic observations have shown that a number of characters in _L. corniculatus_ are tetrasomically inherited in which it is assumed that there is random pairing of the four homologous chromosomes. Yet, multivalent formations during the first meiotic division have but rarely been observed in _L. corniculatus_.

Certain quantitative traits, such as leaflet size and floret size, appear to be controlled by multiple genes which possess additive effects.

Very little information is available on the genetics of the diploid species of _Lotus_. Although some studies are in progress, especially concerning those diploids closely related to _L. corniculatus_, only a beginning has been made in our understanding of the potential of the diploid species for agronomic utilization.

MATERIAL AND METHODS

I. Lotus species and hybrids used in the study

A) The species

The six species with which successful crosses have been obtained, namely, L. alpinus Schleich, L. filicaulis Dur., L. krylovii Schischk. and Serg., L. japonicus (Regel) Larsen, L. schoelleri Schweinf. and L. tenuis Waldst. et Kit., are all diploid species with 12 somatic chromosomes. The seed was obtained from individual collectors, Plant Introduction Stations and Botanic Gardens, and are accessioned as part of the extensive Lotus collection of this laboratory.

The accession numbers and sources of collection for each species are listed in Table 1. Plants for each species were used from a single collection for the hybridization experiments and the morphological studies. In the case of L. schoelleri, however, plants from a second collection (B 166) were used as the female parent for the cross L. schoelleri X L. krylovii (Table 1). Plants of this latter accession number were considered to be identical to those of accession number B 87 and are believed to be derived from the same origin.

B) The hybrids

A total of 53 hybrids resulting from 10 different combinations of crosses between the six species listed in Table 1 were studied (Table 2). Thirty-six hybrids were produced in this laboratory during the course of an investigation concerned with the techniques required for successful hybridization in the genus Lotus (Bullen, 1960) and were available to the

Table 1

Accession numbers and sources of the species employed

Species	Acc. No.	Source
L. alpinus Schleich	B-77	Institut de Botanique, Universite de Neuchatel. Collector: C. Favarger. Origin: Western Alps. Received as L. corniculatus var. alpinus Ser.
L. filicaulis Dur.	B-37	Dr. P. Henson, U.S. Department of Agriculture, Beltsville, Maryland. P.I. No. 51,864. Origin: Botanic Garden, Madrid, Spain.
L. krylovii Schischk. and Serg.	B-86	Hortus Botanicus Universitatis, Uppsala, Sweden. Received as L. corniculatus var. heterophyllarius Pet.-Stib.
L. japonicus (Regel) Larsen	B-129	Kyoto University. Collector: Isawo Hirayoshi, Origin: Riverbank near Gifu, Japan.
L. schoelleri Schweinf.	B-86	U.S. Department of Agriculture, Ames, Iowa. P.I. No. 226,275. Origin: Kenya, Africa. Received as L. corniculatus var. eremanthus Chiov.
	B-166	Grassland Research Station, Kitale, Kenya. Origin: Kenya, Africa. Received as L. corniculatus var. eremanthus Chiov.
L. tenuis Waldst. et Kit.	B-145	U.S. Department of Agriculture, Soil Conservation Service, Pleasanton, California. P.I. No. P-14,496 Received as L. corniculatus L.

Table 2

Species crosses in *Lotus*,

accession numbers and number of hybrids obtained

Cross	Acc. No. of the species	Acc. No. of the hybrids	Number of hybrids
L. japonicus X *L. alpinus*	B 129 B 77	E 567 E 718 E 752	4 3 2
L. japonicus X *L. filicaulis*	B 129 B 37	E 602 E 1026 E 1030	2 1 1
L. japonicus X *L. schoelleri*	B 129 B 87	E 719 E 720 E 735 H 18	5 3 2 4
L. schoelleri X *L. japonicus*	B 87 B 129	E 1061	2
L. japonicus X *L. krylovii*	B 129 B 86	E 612 E 613 E 1028 E 1035	3 4 1 2
L. krylovii X *L. japonicus*	B 86 B 129	C 64	5
L. krylovii X *L. schoelleri*	B 86 B 87	C 8	2
L. schoelleri X *L. krylovii*	B 166 B 86	C 95	3
L. krylovii X *L. filicaulis*	B 86 B 37	C 20	3
L. tenuis X *L. filicaulis*	B 145 B 37	E 442	1

author at the beginning of the present study. Seventeen hybrids were later obtained. With the exception of four which were grown directly from seeds, all the hybrids were produced by means of embryo culture techniques.

Emasculation and hybridization techniques, environmental control, embryo culture, embryo excision and preparation of nutrient media were performed according to the procedure described by Bullen (1960) and as have been summarized by Grant, Bullen and de Nettancourt (1962).

In the case of embryo-cultured hybrids the frequency of hybrid plants obtained in relation to the number of florets emasculated and the total number of excised embryos were not recorded. As an estimate it can be said that approximately 1,500 florets were emasculated.

The four hybrids grown directly from seeds resulted from the cross H 18 (Lotus japonicus X L. schoelleri). In this case, twenty-six florets from six different plants of L. japonicus were emasculated by means of the air suction method and the whole plants sprayed with 10 parts per million of 2-4-5 trichlorophenoxypropionic acid. Eleven florets dropped off during the two days which separated emasculation from pollination. Before pollination the stigmata were gently rubbed with the end of a flat wooden stick in order to rupture the stigmatic membrane and then covered with large amounts of L. schoelleri pollen. Pods were collected 35 days after pollination and the seeds immediately sown on wet filter paper in Petri dishes. The Petri dishes were kept at a room temperature of 20° C (± 2).

II. Culture

The embryo-cultured hybrids which were obtained during the course of the present study were removed from the culture bottles at the six leaf stage, with the aid of a pair of forceps, and gently washed in water. The seedlings were then planted in three inch pots containing sterile soil and placed in a growth chamber maintained at 16 hours daylength, a temperature of 22°C, and a relative humidity of approximately 80%. One to two weeks later, the plants were transferred into four inch pots.

It was calculated that 13 plants per species and per cross were necessary for the various observations and investigations projected in this study. The appropriate numbers of cuttings from most of the hybrid plants were therefore propagated. Clones of the same origin were distinguished by the addition of an arabic letter placed immediately after the plant number. Plants E 442-1b and E 442-1c are, for example, clones of a hybrid which arose from cross E 422 and from seed lot one. The arabic letter "a" was always given to the mother clone.

III. Morphological and cytological studies of parental and hybrid plants

A) Investigations and observations performed on plants maintained in the growth-chamber and in the greenhouse

For these studies 10 plants of each species and of each of the ten different crosses were raised in the growth-chamber and/or in the greenhouse. In order to make environmental conditions comparable it was attempted to maintain at least three plants per species and per cross in the growth-chamber.

Data were obtained on the following characters:

- growth habit
- leaflet size
- floret size
- bud colour
- floret colour
- floret striping
- keel tip colour
- stem colour
- style curvature.
- seed colour
- pod stippling
- seed speckling
- HCN reaction
- number of florets per inflorescence
- pollen fertility
- chromosome number
- chromosome homology

Growth habit:

Growth habit was evaluated at the time of anthesis and the plants classified into three different categories:

(a) decumbent:- when the stem lay more or less prostrate, immediately surrounding the crown with the branch tips ascending.

(b) procumbent:- when the stem lay prostrate on the ground.

(c) ascending:- when the stem grows arched upwards.

Floret size:

Three florets from each plant of distinct genotype were taken at random and measurements recorded for the length of the floret, width of the standard, and length of the ovary and style of mature flowers. All values were rounded off to 0 or 5 at the second decimal place.

Leaflet size:

Ten central leaflets from each plant of distinct genotype growing in the greenhouse or in the growth chamber were measured at random from the upper half of the plants with a centimeter ruler and all values rounded off to 0 or 5 at the second decimal place. Both the length and width of the central leaflet were measured as soon as the first flowers appeared, and the width to length ratio (leaflet index) was calculated.

Floret colour:

Observations were made when the plants were profusely flowering. The general colour of the floret, keel tip colour and striping on the standard of the buds and the mature florets were the characters recorded.

Stem colour:

Observations were made at the beginning of anthesis.

Style curvature:

Observations were made on young pods which were approximately 20 to 30 days old.

Three different phenotypes were recorded for this character:

(a) straight style:- when the pod axis and the style were in alignment.

(b) curved style:- when the angle made by the style and the pod axis was between 100 and 170 degrees.

(c) strongly curved style:- when the angle formed by the style and the pod was approximately 90 degrees.

No accurate measurements were made of the style curvature, the degree of curvature being only estimated within the above classification.

Pod stippling:

An average of three to five pods for each plant was examined for pod stippling. The observations were made on young pods which were approximately 15 to 25 days old.

Reinforced by the use of various adjectives such as slight, moderate, strong and heavy the following terms were employed to distinguish the various phenotypes observed:

(a) spotting:- portions of the pods, generally close to the dorsal suture, are covered with fine compactly arranged red dots.

(b) mottling:- the dots are so close to each other that they form, for the naked eye, large and uniformly covered red areas.

(c) absence of stippling:- the pod is completely green.

Seed colour and seed speckling:

For each species at least one hundred seeds were examined. In the case of the hybrids, five or six plants for each cross were taken at random and the seeds harvested when the pods had reached maturity. The pods were collected before shattering had started. Observations were made with the aid of a dissecting microscope.

Hydrogen Cyanide reaction:

All the plants, sixty individuals for the species and fifty-three for the hybrids, were tested for their reaction to the presence of cyanogenetic glycoside. The detailed procedure, as reported by Dawson (1941) was followed.

Number of florets per inflorescence:

The number of florets per inflorescence were recorded from approximately ten inflorescences per plant for three to five specimens per species and per cross which were taken at random.

Pollen fertility:

Examinations of pollen grains were made on both the species and the hybrids. Observations were limited to 3 or 4 plants for each species but all hybrid plants of distinct genotype were examined. The number of pollen grains observed for each species and for each cross often reached several thousand and never was less than 800. The pollen was stained with fast green in lactophenol. Various sizes of stained, or partially stained, pollen grains were encountered. Only those grains possessing a diameter in the size range of normal pollen of the species and fully stained were considered normal and tabulated as stainable pollen in order to obtain an estimate of the fertility of the plants. No actual germination tests of the pollen were conducted.

Karyotypic studies:

The chromosome numbers of the species used in the present study had been previously verified in this laboratory. For the hybrids, chromosome number determinations were performed on the meristematic cells of root tips or on pollen mother cells during the first meiotic divisions of microsporogenesis. Root tips were excised from potted plants, pretreated with 0.002 M 8-hydroxyquinoline for one hour and fixed in Carnoy's 6:3:1 (alcohol:chloroform:acetic acid). The root tips were placed, immediately after pretreatment, in a refrigerator, for at least two days, at a temperature of 5°C. The chromosomes were then stained by the Feulgen method (Darlington and La Cour, 1960) with a hydrolysis of the root tips for ten minutes. The stained portions of the root tips representing the meristematic region were squashed in 45% acetic acid on a slide. Flower buds, for the study of meiotic chromosomes behavior, were fixed in alcohol-chloroform-acetic acid (6:3:1) and the anthers were squashed in 15% acetocarmine. Drawings were performed with the aid of a camera lucida. In the case of representative material, slides were made semi-permanent by sealing the edges of the cover slip with a fine layer of nail polish. Such slides, when placed on wet filter paper in a Petri dish and stored in the refrigerator, kept perfectly usable for study for more than two weeks. Some permanent slides were made by the method of Celarier (1956).

B) Observations made on plants growing in the field

Observations concerning growth habit and the number of seeds set per pod were made on each of the plants (3 plants per species and 3 florets per cross) which were planted in the field during the summer months of 1961 or 1962.

The growth habit was evaluated when the plants were in full bloom.

Ten mature pods, taken at random from each of the plants grown in the field, were collected and the number of non-shrunken seeds per pod was recorded to determine the number of seeds set per pod. No attempt was made to hand pollinate or induce pollination. The inflorescences were not bagged. Distances between rows and between plants on the row were maintained at 25 inches. The plants were set out at random among the various species of the *Lotus* collection.

IV. Inheritance studies in second generation and backcross populations

A) F_2 progeny of the hybrid *L. japonicus* X *L. filicaulis*

Two clones from the plant E 1030-1 and three clones from the plant E 602-2 were isolated, shortly before anthesis, in a screened cage during the spring of 1961. In order to increase seed production, approximately 10 florets per plant were tripped and the stigmatic membrane ruptured with the aid of a flat wooden stick.

The F_1 seeds were harvested and sown in a mixture of finely screened soil and sand (in a proportion of 3 to 1) in twelve inch pots. All the seedlings obtained were transplanted to four inch pots at the six leaf stage and were maintained for study in the growth-chamber at the previously stated environmental conditions. As the growth-chamber was limited in size to approximately 100 pots, the seeds were sown at three different periods, namely May 1961, October 1961 and March 1962. Observations performed on the F_2 plants were as follows:

Stem colour:

As a considerable number of different phenotypes were observed for this character the plants were classified into six categories based on intensity and distribution of red stem colouration.

(a) class 0:- stems expressing the filicaulis phenotype, namely, complete lack of red pigmentation.

(b) class 1:- stems exhibiting a slight amount of red colouration

(c) class 2:- stems expressing phenotypes comparable to that found in the F_1 hybrid.

(d) class 3:- stems expressing a japonicus phenotype, namely, a brownish red colouration.

(e) class 4:- stems with a deep red colouration

(f) class 5:- stems exhibiting variegation. A plant was considered variegated when only the upper portion of the stem expressed red colouration while the lower portion remained green.

Observations were made at the beginning of anthesis.

Hydrogen Cyanide reaction:

The HCN test was carried out for all the F_2 plants at the time of anthesis. The detailed procedure as reported by Dawson (1941) was followed.

Leaflet size:

Observations were performed on one central leaflet per plant, an attempt being made to select the leaf most representative of the plant.

Floret size:

A floret was taken at random from each plant and its length determined with a centimeter ruler. One hundred and four plants were observed.

Red striping on florets:

This character was found very difficult to quantitatively evaluate as variations occurred both between and within plants. A floret was classified red whenever any trace of red colour was visible on the standard petal. Plants with at least one red floret were classified as having a red striped phenotype. Ninety-nine F_2 plants were observed.

Pod stippling:

Observations on pod stippling were made on immature pods which were approximately 20 to 30 days old. As pod stippling appeared to be a somewhat variable character, all developping pods from each plant were examined several times. Those plants which expressed a complete absence of stippled pods were classified as having a *japonicus* phenotype.

Seed speckling:

An average of ten seeds per plant were examined. In some instances seed set production had to be initiated through hand pollination by tripping gently the flower and rupturing the stimatic membrane with the aid of a flat wooden stick. Seed spotting was observed for only those F_2 populations grown during the winter of 1962. In all, seeds from 36 F_2 plants were examined for this cross.

Pollen fertility:

A determination of the number of stainable pollen grains were performed on a few plants taken at random. In each case a hundred grains per plant were examined for an estimation of the fertility of the parents.

B) F_2 and backcross populations of the hybrid *L. japonicus* X *L. alpinus*

F_2 plants were grown directly from seeds harvested from F_1 plants of this cross (E 567-1a, E 567-1b and E 567-1c) which were isolated in a screened cage during the summer of 1961.

$B.C._1$ plants were obtained through the aid of the air-suction emasculating technique and the embryo culture procedure, previously described. Only a single F_1 hybrid plant (E 567-1) was used as the female parent. *L. japonicus* was chosen as the back cross parent. The young seedlings were transferred, at the six leaf stage, from the culture bottles to sterilized soil and raised as previously described.

Observations were recorded on each F_2 and $B.C._1$ plant for the following characters:

Keel tip colour

Floret striping

Seed speckling

V. Statistical analysis

Variance analyses, chi square tests and a correlation study were performed in order to ascertain the significance of the results concerning modes of inheritance and linkage relationships in the segregating populations. The significance of the differences recorded for pollen stainability between the hybrids was tested by means of a variance analysis. The standard errors of the means for central leaflet index, leaflet length, floret length, standard width, ovary length and style length were calculated with the aid of the IBM 650 data processing machine at the computing center of McGill University.

RESULTS

I. Morphological study of the parental species and the hybrids

A) Morphology of the parental species

From the descriptions which are given in table 3 it is possible to classify the species used in the present study into two different categories. To the first class belong L. alpinus and L. japonicus which are characterized by a non ascending growth-habit, a high leaflet index, large deep yellow florets, red stems and the presence of cyanogenetic glycoside. In the second category are the species L. filicaulis, L. schoelleri, L. krylovii and L. tenuis which can be described as plants of ascending growth-habit with a low leaflet index, small pale yellow florets, green stems, mottled pods and speckled seeds. As may be readily ascertained from table 3, however, a certain degree of species to species variability is found within each of these two artificial categories for some of the characters investigated. In the case of quantitative traits, the statistical significance of the phenotypic variations recorded between the different species is given in tables 4, 5, 6, 7, 8 and 9 which indicate the difference of the means and the half confidence intervals calculated for central leaflet length, central leaflet index and floret size.

L. alpinus:

L. alpinus is readily separable from the five other species by its decumbent growth-habit, short stems and very significantly smaller leaves (Table 4 and Figure 2). Its potential value as a forage crop appears to be very low. It can be seen from table 3 that,

Table 3

Description of the parental species

Phenotype	*L. japonicus*	*L. alpinus*	*L. filicaulis*	*L. krylovii*	*L. schoelleri*	*L. tenuis*
Growth habit	decumbent	procumbent	ascending	ascending	ascending	ascending
Leaflet length (cm) (1)	0.9515	0.4130	1.1125	0.9685	0.8803	1.2110
Leaflet index (1)(2)	59.79	50.54	32.95	34.72	48.31	36.64
Floret size	large	large	small	small	small	small
Floret colour	deep yellow	deep yellow	pale yellow	pale yellow	pale yellow	pale yellow
Floret striping	none	orange red	red	red	red	orange red
Keel tip colour	yellow	black	yellow	yellow	yellow	yellow
Stem colour	red	red	green	green	green	green
Style curvature	strongly curved	straight	curved	straight	curved	curved
Pod stippling	none	medium	medium	slight	heavy	medium
Seed colour	chestnut	orange brown	brown yellow	golden brown	brown	light chestnut
Seed speckling	none	heavy	slight	moderate	heavy	medium
Seed set per pod (4)	21.46	2.53	18.20	22.40	20.90	(21.00)
HCN reaction	+	+	-	-	-	+ -
Florets per inflorescence (3)	1-4	1-3	1-3	1-3	1-3	2-8

(1) = average
(2) = (leaflet width/leaflet length)100
(3) = range
(4) = average; as determined in the field under open pollinated conditions.

Table 4

Differences between means ± half confidence interval (± t x SE)(1) for central leaflet length in the parental species

	alpinus $\bar{X}$=0·4130	*filicaulis* $\bar{X}$=1·1125	*schoelleri* $\bar{X}$ =0·8803	*krylovii* $\bar{X}$=0·9685	*tenuis* $\bar{X}$=1·2110
L. *japonicus* $\bar{X}$=0·9515	0·5385 ±0·0441	0·1610 ±0·0756	0·0712 ±0·0504	0·0170 ±0·0588	0·2595 ±0·0735
L. *alpinus* $\bar{X}$=0·4130		0·6995 ±0·0630	0·4673 ±0·0273	0·5555 ±0·0399	0·7980 ±0·0615
L. *filicaulis* $\bar{X}$=1·1125			0·2322 ±0·0672	0·1440 ±0·0745	0·0985 ±0·0872
L. *schoelleri* $\bar{X}$=0·8803				0·0782 ±0·0483	0·3307 ±0·0672
L. *krylovii* $\bar{X}$=0·9685					0·2425 ±0·0735

(1): 95% confidence limits = diff. ± t x SE.

Table 5

Differences between means ± half confidence interval
(- t x SE)(1) for central leaflet index in the parental species

	alpinus $\bar{X}$=50·54	*filicaulis* $\bar{X}$=32·95	*schoelleri* $\bar{X}$= 48·31	*krylovii* $\bar{X}$=34·72	*tenuis* $\bar{X}$=36·64
L. japonicus $\bar{X}$ = 59·79	9·250 ±4·536	26·840 ±4·116	11·480 ±4·662	25·070 ±4·640	23·150 ±4·074
L. alpinus $\bar{X}$ = 50·54		17·590 ±2·457	2·230 ±3·297	15·820 ±3·270	13·900 ±2·394
L. filicaulis $\bar{X}$ = 32·95			15·360 ±2·709	1·770 ±3·717	3·690 ±0·462
L. schoelleri $\bar{X}$ = 48·31				13·590 ±3·465	11·670 ±3·297
L. krylovii $\bar{X}$ = 34·72					1·920 ±2·604

(1): 95% confidence limits = diff. ± t x SE

Table 6

Differences between means ± half confidence interval
(± t x SE) (1) for floret length in the parental species

	alpinus $\bar{X}$=1·1467	*filicaulis* $\bar{X}$=0·8693	*schoelleri* $\bar{X}$=0·8300	*krylovii* $\bar{X}$=0·8317	*tenuis* $\bar{X}$=1·0033
L. japonicus $\bar{X}$ = 1·1100	0·0367 ±0·0470	0·2407 ±0·0424	0·2800 ±0·0573	0·2783 ±0·0431	0·1067 ±0·0491
L. alpinus $\bar{X}$ = 1·1467		0·2774 ±0·0376	0·3167 ±0·0537	0·3150 ±0·0407	0·1434 ±0·0443
L. filicaulis $\bar{X}$ = 0·8693			0·0393 ±0·0498	0·0376 ±0·0353	0·1340 ±0·0401
L. schoelleri $\bar{X}$ = 0·8300				0·0017 ±0·0523	0·1733 ±0·0556
L. krylovii $\bar{X}$ = 0·8317					0·1716 ±0·0433

(1): 95% confidence limits = diff. ± t x SE.

Table 7

Differences between means ± half confidence interval

(± t x SE)(1) for standard width in the parental species

	alpinus $\bar{X}$ =0·8000	filicaulis $\bar{X}$=0·6217	schoelleri $\bar{X}$=0·6317	krylovii $\bar{X}$=0·5733	tenuis $\bar{X}$=0·6667
L. japonicus $\bar{X}$ = 0·8983	0·9830 ±0·0292	0·2766 ±0·0267	0·2666 ±0·0376	0·3250 ±0·0242	0·2316 ±0·0445
L. alpinus $\bar{X}$ = 0·8000		0·1783 ±0·0245	0·1683 ±0·0363	0·2267 ±0·0218	0·1333 ±0·0431
L. filicaulis $\bar{X}$ = 0·6217			0·0100 ±0·0342	0·0484 ±0·0189	0·0450 ±0·0416
L. schoelleri $\bar{X}$ = 0·6317				0·0584 ±0·0323	0·0350 ±0·0494
L. krylovii $\bar{X}$ = 0·5733					0·0934 ±0·0395

(1): 95% confidence limits = diff· ± t x SE·

Table 8

Differences between means ± half confidence interval (± t x SE)(1) for ovary length in the parental species

	alpinus $\bar{X}$=0·6667	filicaulis $\bar{X}$=0·4667	schoelleri $\bar{X}$=0·5117	krylovii $\bar{X}$=0·5233	tenuis $\bar{X}$=0·5583
L. japonicus $\bar{X}$ = 0·6450	0·0217 ±0·0231	0·1783 ±0·0342	0·1333 ±0·0267	0·1217 ±0·0246	0·0867 ±0·0479
L. alpinus $\bar{X}$ = 0·6667		0·2000 ±0·0298	0·1550 ±0·0207	0·1434 ±0·0183	0·1084 ±0·0452
L. filicaulis $\bar{X}$ = 0·4667			0·0450 ±0·0326	0·0566 ±0·0309	0·0916 ±0·0517
L. schoelleri $\bar{X}$ = 0·5117				0·0116 ±0·0244	0·0466 ±0·0470
L. krylovii $\bar{X}$ = 0·5233					0·0350 ±0·0458

(1): 95% confidence limits = diff. ± t x SE.

Table 9

Differences between means ± half confidence interval (± t x SE)(1) for style length in the parental species

	alpinus $\bar{X}$=0·5167	*filicaulis* $\bar{X}$=0·3933	*schoelleri* $\bar{X}$=0·4017	*krylovii* $\bar{X}$=0·4117	*tenuis* $\bar{X}$=0·4417
L. japonicus $\bar{X}$ = 0·5383	0·0216 ±0·0170	0·1450 ±0·0166	0·1366 ±0·0143	0·1266 ±0·0208	0·0966 ±0·0370
L. alpinus $\bar{X}$ = 0·5167		0·1234 ±0·0139	0·1150 ±0·0109	0·1050 ±0·0187	0·0750 ±0·0309
L. filicaulis $\bar{X}$ = 0·3933			0·0084 ±0·0100	0·0184 ±0·0183	0·0484 ±0·0355
L. schoelleri $\bar{X}$ = 0·4017				0·0100 ±0·0160	0·0400 ±0·0344
L. krylovii $\bar{X}$ = 0·4117					0·0300 ±0·0378

(1): 95% confidence limits = diff. ± t x SE.

in spite of its morphological resemblance to *L. japonicus*, *L. alpinus* shares with the four other species a number of somewhat similar phenotypic traits. This is particularly true for the floret striping and seed speckling characters which are similarly expressed in the *L. tenuis* and the *L. schoelleri* phenotypes, respectively. *L. alpinus* is the only species to exhibit a black coloration of the keel tip. This easily detectable character proved to be a very useful marker and an interesting tool in inheritance studies. As can be seen from Tables 6, 7, 8 and 9, floret size in *L. alpinus* resembles that of *L. japonicus* and differs widely from the four other species. Table 7 shows, however, that the width of the standard is significantly greater in *L. alpinus* than in *L. japonicus* ($\bar{X}_1 - \bar{X}_2 = 0.0983 \pm 0.0292$). In all other cases the differences observed in floret size between *L. japonicus* and *L. alpinus* are negligible. One of the ten *alpinus* plants examined expressed strong pubescence on the leaves and on the calyx, suggesting that the species was segregating for this character.

L. japonicus:

L. japonicus is a species which lacks floret striping and pod mottling. Its seeds do not manifest any traces of speckling (Figure 8). In this respect *L. japonicus* is very distinct from the other species involved in the study. Its procumbent growth-habit is also somewhat unique as it constitutes an intermediate position between the postrate growth-habit of *L. alpinus* and the ascending one for *L. tenuis*, *L. schoelleri*, *L. krylovii* and *L. filicaulis*. As may be seen from tables 3, 6, 8 and 9, however, *L. japonicus* resembles *L. alpinus* for several characters, namely stem colour, presence of HCN and floret size. In contrast, *L. japonicus* is strikingly different from the four species, *L. schoelleri*, *L. krylovii*, *L. filicaulis* and *L. tenuis* for all characters investigated (Table 3, Figures 1, 7, 8), with the exception of leaflet length (Table 4) and the number of florets per inflorescence (Table 12).

L. schoelleri:

L. schoelleri closely resembles *L. krylovii*, *L. tenuis* and *L. filicaulis* for a number of characters, namely growth-habit, stem colour, floret size, floret colour and presence of HCN. This species is similar to *L. alpinus*, however, in its central leaflet index ($\bar{X}$ *alpinus* - $\bar{X}$ *schoelleri* = $50.54 - 48.31 \pm 3.297$) and in the heavy speckling of the seed (Figure 9). The central leaflet is approximately the same size as found in *L. japonicus* ($\bar{X}_1 - \bar{X}_2 = 0.0712 \pm 0.504$) and is significantly smaller than those of *L. filicaulis* and *L. tenuis* (Table 4).

L. krylovii, *L. filicaulis* and *L. tenuis*:

As may be seen from table 3, these three species are difficult to distinguish from one another as they exhibit similar phenotypes for growth-habit, stem colour, floret size, floret striping and pod mottling. Plants from these species have narrow leaves (Figures 4, 5 and 6) and a low leaflet index (Table 5). *L. krylovii* and *L. filicaulis*

Figure 1

Leaf of L. japonicus, magnification X ca. 2.

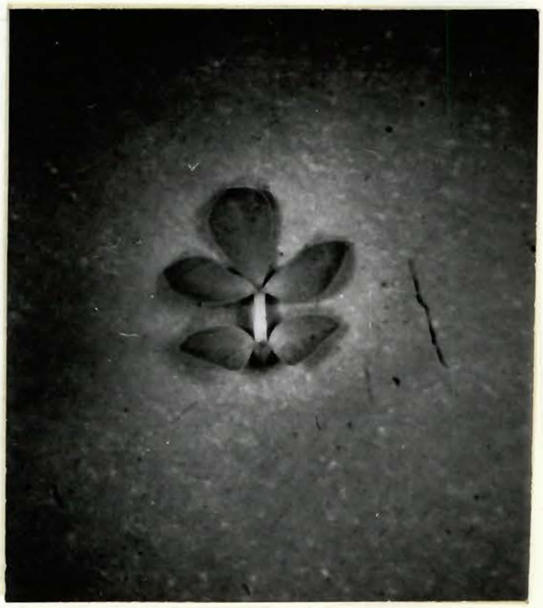

Figure 2

Leaf of L. alpinus, magnification X ca. 2.

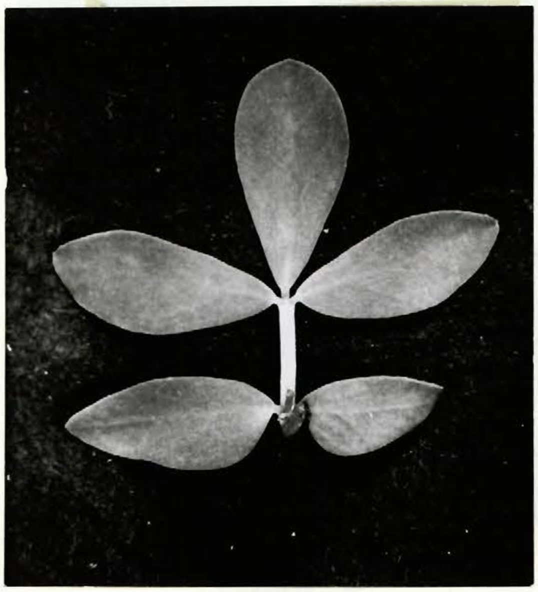

Figure 3

Leaf of L. schoelleri, magnification X ca. 2.

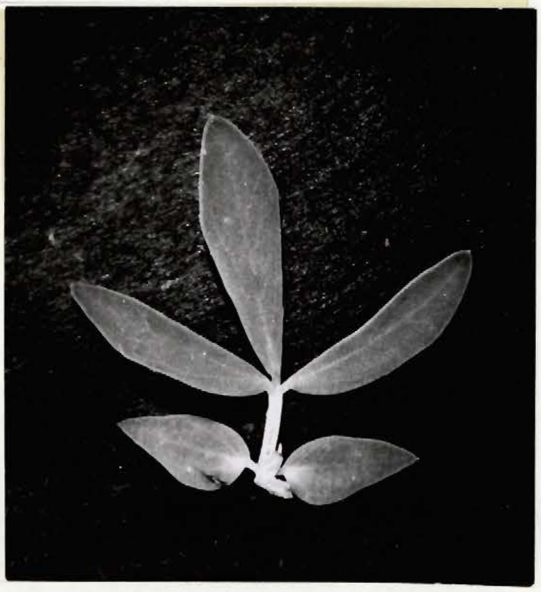

Figure 4
Leaf of L. krylovii,
magnification X ca. 2.

Figure 5
Leaf of L. filicaulis,
magnification X ca. 2.

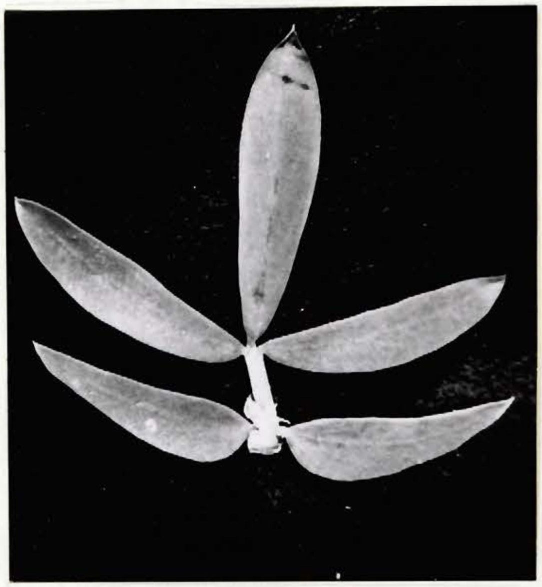

Figure 6
Leaf of L. tenuis,
magnification X ca. 2.

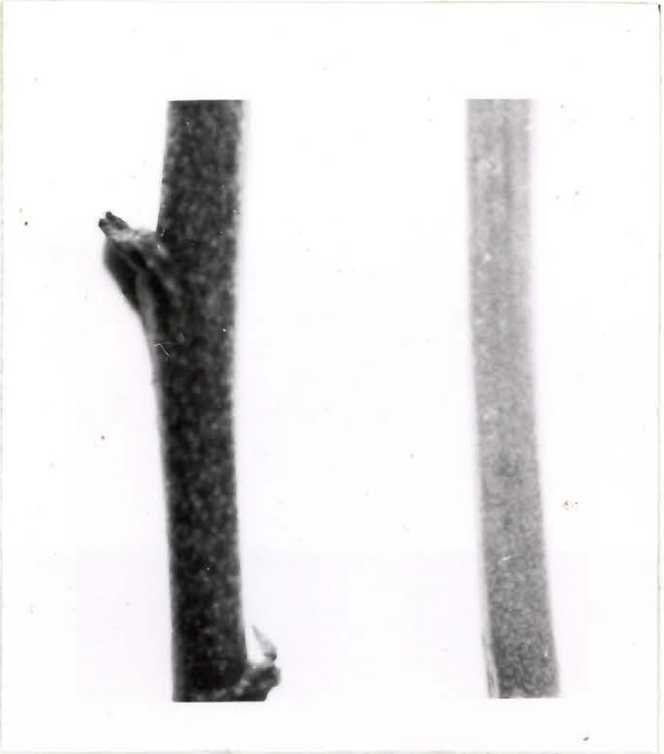

Figure 7

Red stem of L. japonicus (left) and green stem of L. filicaulis (right), magnification X ca. 2.75.

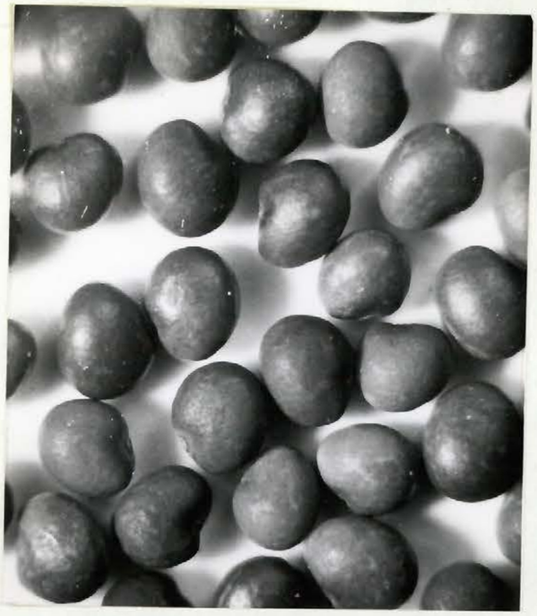

Figure 8

Non speckled seeds of L. japonicus,
magnification X ca. 10

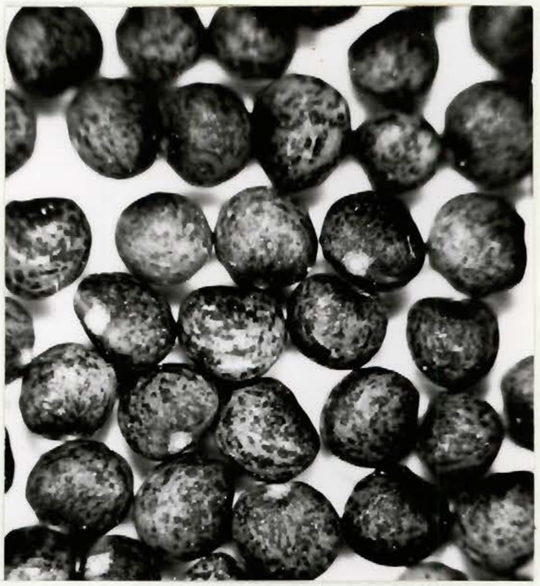

Figure 9

Heavily speckled seeds of L. schoelleri,
magnification X ca. 10

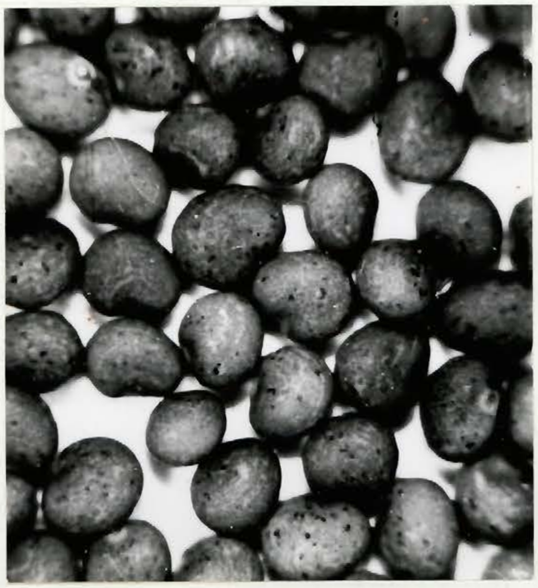

Figure 10

Speckled seeds of L. filicaulis,
magnification X ca. 10

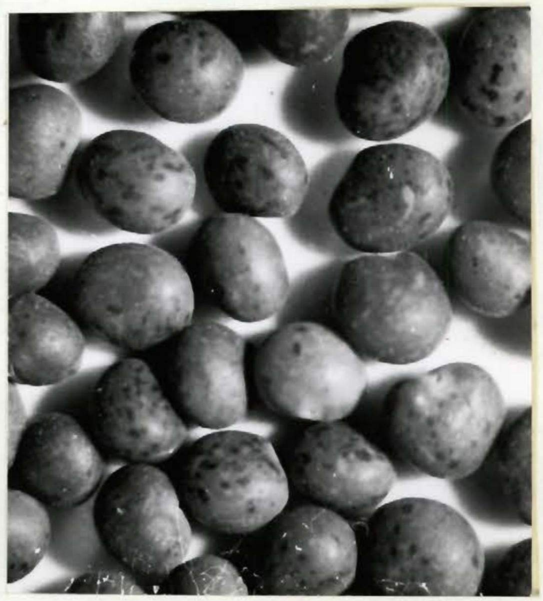

Figure 11

Speckled seeds of L. tenuis,
magnification X ca. 10

react negatively to the HCN test whereas two of the ten plants of *L. tenuis* examined were found to react positively to the test.

L. krylovii differs from the two other species by its shorter leaf length (Table 4) and by the slightly rhomboidal shape of the central leaflet.

Two morphological characters were found useful for distinguishing *L. filicaulis* from *L. tenuis*. The first one, as shown in table 12, concerns the number of florets per inflorescence. In *L. filicaulis* the number of florets per inflorescence ranges from 1 to 3, whereas, in *L. tenuis* there are from 2 to 8 florets in the inflorescence. Seed colour is another character which proved to be valuable for making a distinction between these two species. In *L. filicaulis* the seeds are yellowish brown in colour whereas they are light chestnut coloured in *L. tenuis*. Other morphological criteria which were also used in order to distinguish between these two species were the shape of the central leaflet and floret striping. In *L. filicaulis*, the central leaflet is rounded at the top (Figure 5) and the floret exhibits red stripes when maturing; in *L. tenuis*, the extremity of the leaflet is often acute (Figure 6) and the stripes on the mature floret are orange-red. As previously stated, two plants of *L. tenuis* were found to give a positive reaction to the HCN test, suggesting that the strain used in the present study was polymorphic for this character.

B) Morphology of the interspecific hybrids

In general, the hybrids were intermediate to their parents in morphological attributes and displayed considerable heterosis which was manifested in vigorous growth, profuse branching and flowering. Hybrid vigor was especially pronounced in hybrids from the crosses *L. japonicus* x *L. filicaulis*, and *L. tenuis* x *L. filicaulis*. In contrast, the plants derived from the cross *L. japonicus* x *L. alpinus* did not exhibit such extreme heterotic effects and appeared intermediate in vigor to their parental species.

Growth-habit:

From observations on greenhouse plants, at the beginning of anthesis, ascending growth-habit was determined to be inherited in a dominant manner. However, in the case of the hybrid between *L. japonicus* and *L. alpinus* (decumbent versus procumbent growth-habit) the growth-habit appeared intermediate. In this case, some branches were completely prostrate while the tips of others were slightly ascended. In the field, all hybrid plants and the six parental species were decumbent.

Stem colour (Table 10; Figure 12)

The hybrids derived from crosses between plants of red stemmed and green stemmed species exhibited a pinkish-red stem suggesting semi-dominance of red over green colour.

HCN reaction (Table 10):

The factor responsible for the presence of cyanogenetic glycoside was transmitted as a dominant character. This character proved to

Table 10

Stem colour, HCN reaction, floret colour and floret striping
in parental and hybrid plants

Species	Stem colour	HCN reaction	Floret colour	Floret striping
L. japonicus	red	+	deep yellow	none
L. alpinus	red	+	deep yellow	orange red
L. japonicus X *L. alpinus*	red	+	deep yellow	orange red
L. filicaulis	green	-	pale yellow	red
L. japonicus X *L. filicaulis*	pinkish red	+	yellow	orange red
L. krylovii	green	-	pale yellow	red
L. japonicus X *L. krylovii*	pinkish red	+	yellow	orange red
L. krylovii X *L. japonicus*	pinkish red	+	yellow	orange red
L. schoelleri	green	-	pale yellow	red
L. japonicus X *L. schoelleri*	pinkish red	+	yellow	orange red
L. schoelleri X *L. japonicus*	pinkish red	+	yellow	orange red
L. schoelleri X *L. krylovii*	green	-	pale yellow	red
L. krylovii X *L. schoelleri*	green	-	pale yellow	red
L. krylovii X *L. filicaulis*	green	-	pale yellow	red
L. tenuis	green	+	light yellow	orange red
L. tenuis X *L. filicaulis*	green	+	pale yellow	deep orange red

Figure 12

Red stem in L. japonicus (left), green stem in L. filicaulis (right) and pinkish red stem in the hybrid L. japonicus x L. filicaulis (middle), magnification X ca. 8.

Figure 13

Yellow keel tip in L. japonicus (left), black keel tip in L. alpinus (right) and black keel tip in the hybrid L. japonicus x L. alpinus (middle), magnification X ca. 3.

be of great value for the detection of successful hybridization at early stages of development of the putative hybrids.

Floret colour (Table 10):

Although floret colour in the hybrids derived from crosses between deep yellow (*L. japonicus*) and pale yellow flowered species (*L. schoelleri*, *L. filicaulis* and *L. krylovii*) was considered to be intermediate to the parental phenotypes, the colour was much more intense than in the pale yellow flowered parent and, therefore, was tabulated as yellow (Table 10).

Floret striping (Table 10):

The red stripes on the flower buds and the red or orange-red stripes on the standards of mature florets were slightly modified in the heterozygous condition with the colour intensity and the area occupied by the stripes being somewhat reduced.

Keel tip colour (Plate 3; Figure 13):

The dark keel tip colour, as possessed by *L. alpinus* was transmitted to the hybrid *L. japonicus* x *L. alpinus* in a completely dominant manner. This character served as a useful marker for indicating a successful cross.

Pod stippling (Table 11; Plate 2):

With the exception of *L. japonicus*, all the species concerned in this study are characterized by a certain amount of pod stippling which varies from a very slight mottling, to a heavy mottling, and to a heavy spotting. Stippling was transmitted to the hybrids in an

Table 11

Pod stippling, seed colour and seed speckling in parental and hybrid plants

Species	Pod stippling	Seed colour	Seed speckling
L. japonicus	none	chestnut	none
L. alpinus	mottling	deep orrange	heavy
L. japonicus X *L. alpinus*	mottling	deep orange brown	heavy
L. filicaulis	heavy spotting	yellowish	slight
L. japonicus X *L. filicaulis*	moderate spotting	orange brown	moderate
L. krylovii	slight mottling	golden brown	moderate
L. japonicus X *L. krylovii*	slight mottling	orange brown	moderate
L. krylovii X *L. japonicus*	slight mottling	chestnut	slight to moderate
L. schoelleri	heavy mottling	brown	heavy
L. japonicus X *L. schoelleri*	heavy mottling	orange yellow	medium
L. schoelleri X *L. japonicus*	heavy mottling	brown	medium
L. schoelleri X *L. krylovii*	heavy mottling	brown	medium
L. krylovii X *L. schoelleri*	heavy mottling	brown	medium
L. krylovii X *L. filicaulis*	mottling	brown yellow	medium
L. tenuis	mottling	brown yellow	medium
L. tenuis X *L. filicaulis*	mottling	brown yellow	medium

almost completely dominant manner.

Seed colour (Table 11):

Seed colour varies considerably between the species (Table 3), ranging from a light yellowish-brown in *L. filicaulis* to a deep orange brown in *L. alpinus*. In the hybrid *L. japonicus* x *L. alpinus*, the deep orange colour was transmitted from the male parent in a dominant manner. In all other hybrids seed colour varied, to some extent, from the phenotypes of either parent, presumably as the result of gene interaction.

Seed speckling (Table 11; Plate 1):

With the exception of *L. japonicus*, all the species concerned in the study exhibited a certain amount of speckling on the seed. As a rule, seed speckling was generally transmitted in a dominant manner to the hybrids but in varying degrees of intensity. In some crosses the character appeared over-dominant, whereas, in others, it was recorded as dominant or even semi-dominant (Table 11). Considerable variations in the intensity of seed speckling were also recorded within plants of the same cross and within seeds of the same pod which suggested a strong influence of environmental factors upon the speckling condition.

Curvature of the style on the immature pod (Plate 2):

This is another character which appeared to be partially controlled by the environment and, possibly, by the size of the pod. Those species and hybrids which had a low seed set generally possessed a straight or

Plate 1

Seed speckling in the species and their hybrids

1 = *L. japonicus*

2 = *L. alpinus*

3 = *L. japonicus* x *L. alpinus*

4 = *L. filicaulis*

5 = *L. japonicus* x *L. filicaulis*

6 = *L. krylovii*

7 = *L. japonicus* x *L. krylovii*

8 = *L. schoelleri*

9 = *L. japonicus* x *L. schoelleri*

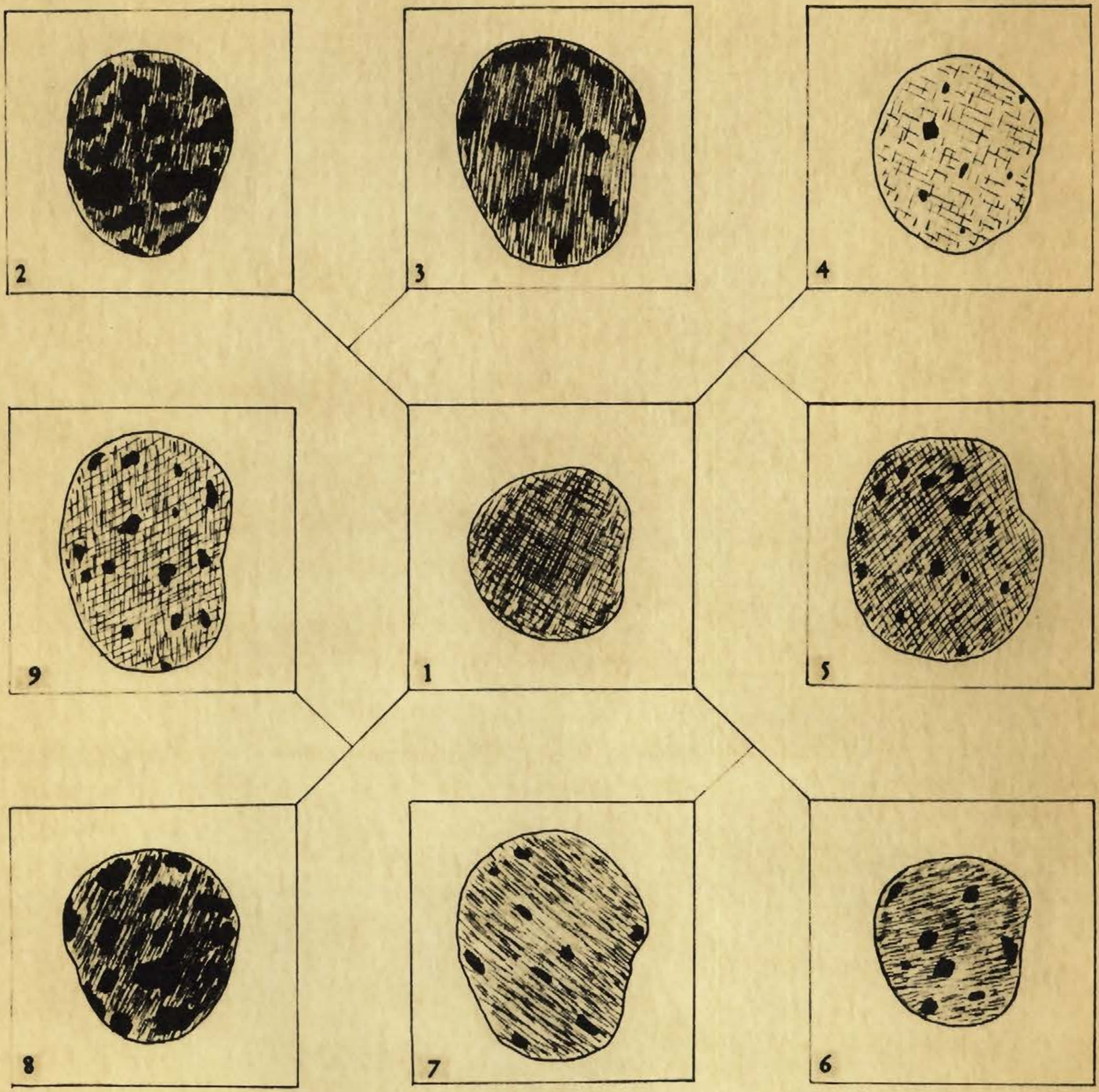
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Plate 2

Pod stippling in the species and their hybrids

1 = *L. japonicus*
2 = *L. alpinus*
3 = *L. japonicus* x *L. alpinus*
4 = *L. filicaulis*
5 = *L. japonicus* x *L. filicaulis*
6 = *L. krylovii*
7 = *L. japonicus* x *L. krylovii*
8 = *L. schoelleri*
9 = *L. japonicus* x *L. schoelleri*

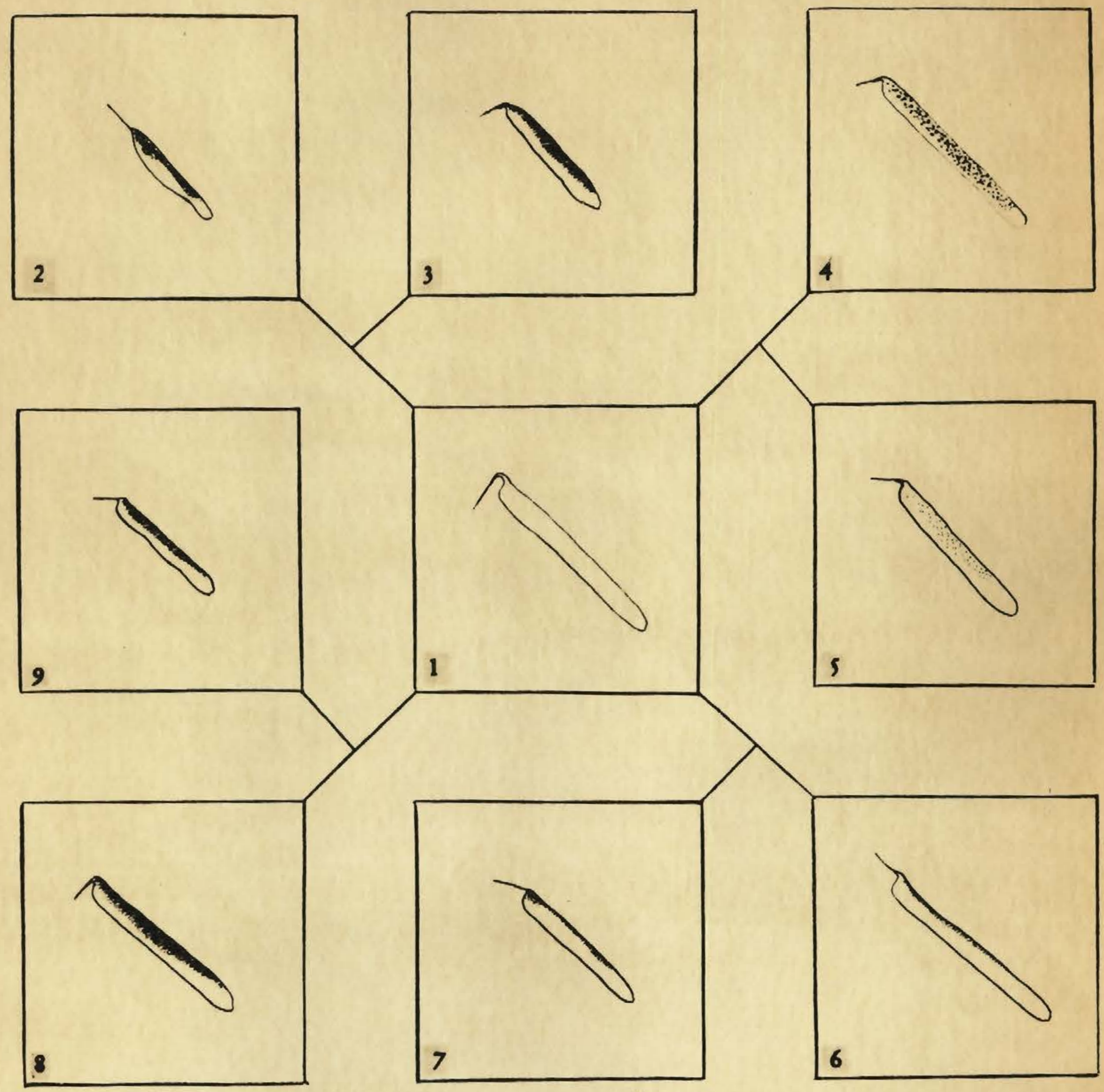
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Plate 3

Shape and size of the standard in the species and their hybrids

1 = *L. japonicus*

2 = *L. alpinus*

3 = *L. japonicus* x *L. alpinus*

4 = *L. filicaulis*

5 = *L. japonicus* x *L. filicaulis*

6 = *L. krylovii*

7 = *L. japonicus* x *L. krylovii*

8 = *L. schoelleri*

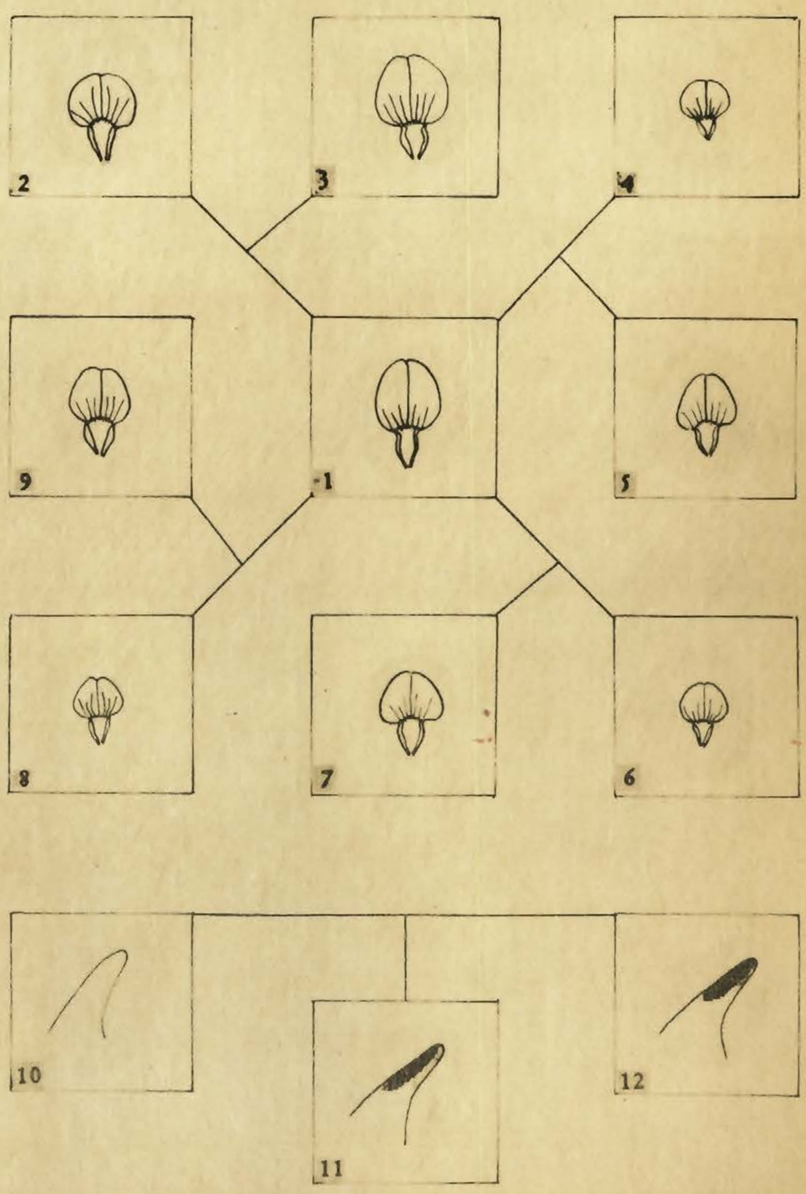
9 = *L. japonicus* x *L. schoelleri*

Keel tip colour in *L. japonicus*, *L. alpinus* and in the hybrid *L. japonicus* x *L. alpinus*

10 = *L. japonicus*

11 = *L. japonicus* x *L. alpinus*

12 = *L. alpinus*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slightly curved style, whereas, the angle of curvature was usually more pronounced when the pod contained a large number of well developed seeds. As a certain amount of variation was observed, both within individual plants and between plants of a species, it would appear that the curvature of the style on the pod should not be considered as a too reliable character. However, as shown in Plate 2, the angles of style curvature were observed to be fairly constant for L. japonicus (style strongly curved), L. krylovii (straight) and their hybrids (slightly curved).

Pubescence:

As mentioned earlier, the leaves and portions of the sepals of some individual plants of L. alpinus used in the cross L. japonicus x L. alpinus were pubescent. Hybrids derived from these crosses exhibited the pubescent character.

Number of florets per inflorescence (Table 12; Figures 14, 15 and 16):

Of the six species used in this study, L. tenuis is the only one which exhibits a high number of florets per inflorescence (an average of 4.13 in L. tenuis as compared to approximately 1.50 to 2.00 in the other species). In the hybrid resulting from the cross between L. tenuis and L. filicaulis the number of florets was found to range from 1 to 5, with an average of 3.03, suggesting a semi-dominant mode of inheritance for this character.

Central leaflet length (Tables 13, 15 and 18; Plate 4):

With the exception of the hybrids derived from the cross L. japonicus x L. alpinus, in which the length of the central leaflet was intermediate to both parents, all of the hybrids derived from other crosses had a central leaflet longer than that possessed by the parent with the greatest leaflet length. It can be seen from Table 15, however, that the reliability of the difference between the mean of the hybrid and the mean of the parent with the longest central leaflet is often questionable since the half confidence interval ($\pm$ t X SE) is, in some cases, almost equal to the difference itself. However, the manifestation of heterosis for central leaflet length appears clearly demonstrated in the case of the hybrids L. krylovii x L. japonicus (X_1 - X_2 = 0.1595 $\pm$ 0.0449), L. japonicus x L. krylovii (X_1 - X_2 = 0.1840 $\pm$ 0.1184) and L. schoelleri x L. japonicus (X_1 - X_2 = 0.2135 $\pm$ 0.1064).

As shown in Table 18, differences between reciprocal hybrids for the leaflet length character were usually very slight. In the case of reciprocal hybrids derived from crosses between L. japonicus and L. schoelleri, however, the difference between means was found to be rather large (X_1 - X_2 = 0.1305 $\pm$ 0.0729).

Central leaflet index (Tables 13, 16 and 19; Plate 4):

The calculated values for central leaflet index in the hybrids fell between the corresponding values of the parental species (Table 13). Table 16 shows, however, that the index values in the hybrids are usually closer to those calculated for the lower parents. This is particularly true in the case of the hybrid plants derived from crosses between L. japonicus and L. schoelleri where the differences between the means of the hybrids and that of the lower parent (L. schoelleri) were found to be smaller than the half confidence interval.

Figure 14

Inflorescence of L. filicaulis, magnification X Ca. 3.

Figure 15

Inflorescence of L. tenuis, magnification X ca. 3.

Figure 16

Inflorescence of the hybrid L. tenuis x L. filicaulis, magnification X ca. 3.

Table 12

Number of florets per inflorescence in parental and hybrid plants(1)

Species	Range	Mean
L. japonicus	1 - 4	2.30
L. alpinus	1 - 3	1.50
L. japonicus X *L. alpinus*	1 - 4	1.66
L. filicaulis	1 - 3	1.46
L. japonicus X *L. filicaulis*	1 - 3	1.50
L. krylovii	1 - 3	1.60
L. japonicus X *L. krylovii*	1 - 2	1.57
L. krylovii X *L. japonicus*	1 - 3	1.70
L. schoelleri	1 - 3	1.50
L. japonicus X *L. schoelleri*	1 - 3	1.66
L. schoelleri X *L. japonicus*	1 - 2	1.37
L. schoelleri X *L. krylovii*	1 - 3	1.66
L. krylovii X *L. schoelleri*	1 - 3	1.46
L. krylovii X *L. filicaulis*	1 - 3	1.50
L. tenuis	2 - 8	4.13
L. tenuis X *L. filicaulis*	1 - 5	3.03

(1) Counts are based on 30 inflorescences.

Table 13

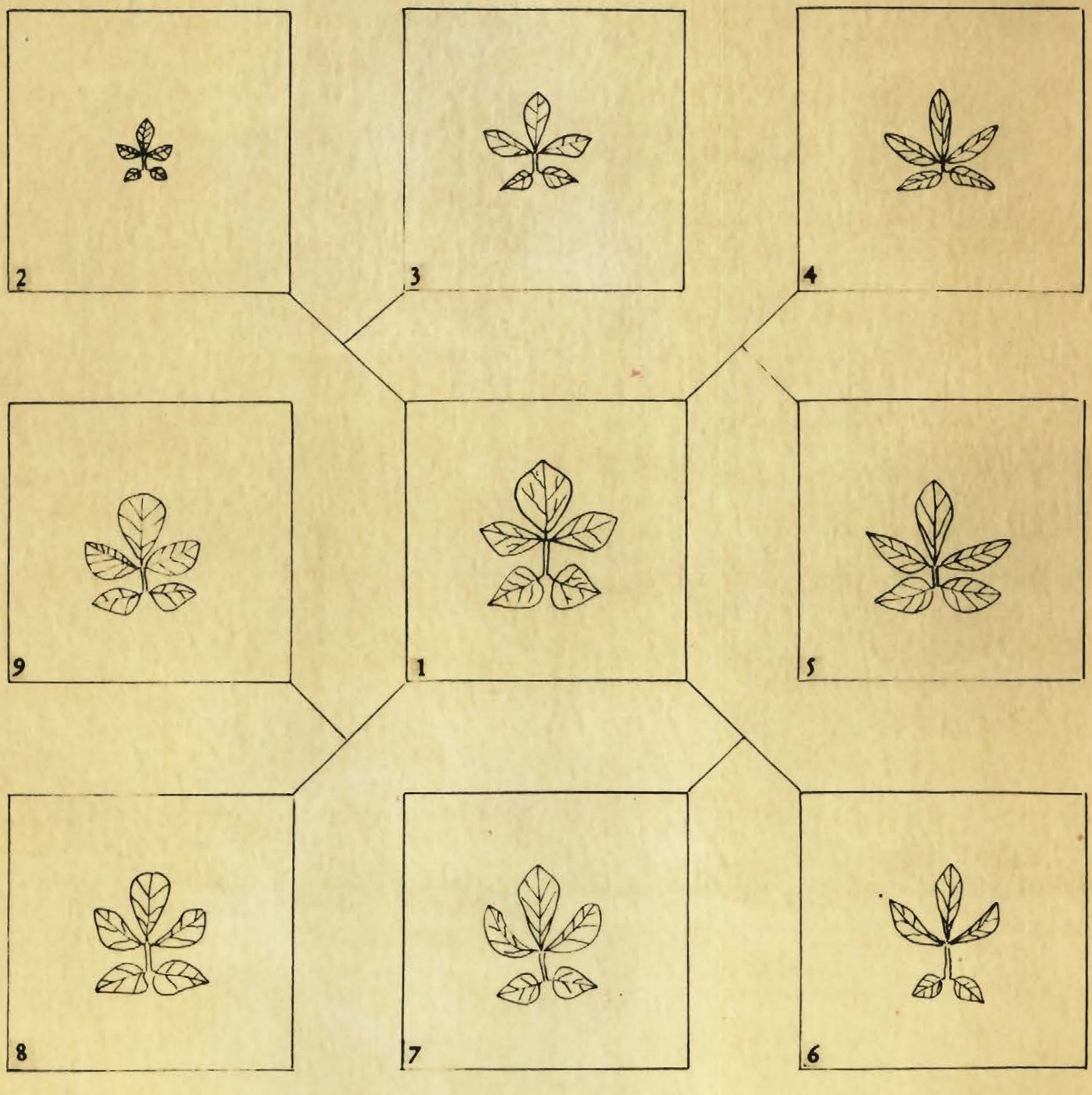
Average values for central leaflet length, central leaflet index and floret length in the hybrids and in the parental species

Species	Leaflet length (in cm)	Leaflet index	Floret length (in cm)
L. japonicus	0·9515	59·7900	1·1100
L. alpinus	0·4130	50·5400	1·1467
L. japonicus X *L. alpinus*	0·6950	46·1778	1·1315
L. filicaulis	1·1125	32·9500	0·8693
L. japonicus X *L. filicaulis*	1·1998	45·4500	1·0167
L. krylovii	0·9685	34·7200	0·8317
L. japonicus X *L. krylovii*	1·1525	46·6000	0·9567
L. krylovii X *L. japonicus*	1·1280	44·6200	1·0067
L. schoelleri	0·8803	48·3100	0·8300
L. japonicus X *L. schoelleri*	1·0345	52·5700	1·0517
L. schoelleri X *L. japonicus*	1·1650	47·7500	1·0583
L. schoelleri X *L. krylovii*	1·0617	40·4000	0·9278
L. krylovii X *L. schoelleri*	1·0825	32·9500	0·9417
L. krylovii X *L. filicaulis*	1·1517	37·8000	0·9556
L. tenuis	1·2110	36·6400	1·0033
L. tenuis X *L. filicaulis*	1·2700	29·4000	0·9333

Plate 4

Leaflet size and leaflet shape in the species and in the hybrids

1 = *L. japonicus*
2 = *L. alpinus*
3 = *L. japonicus* x *L. alpinus*
4 = *L. filicaulis*
5 = *L. japonicus* x *L. filicaulis*
6 = *L. krylovii*
7 = *L. japonicus* x *L. krylovii*
8 = *L. schoelleri*
9 = *L. japonicus* x *L. schoelleri*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Floret length (Tables 13, 17 and 20; Figure 17)

It can be seen from table 17 that whenever a large flowered species is crossed to either a large flowered species or to a small flowered species the hybrid is intermediate to both parents for floret length, whereas, when a small flowered species is crossed to another small flowered species the hybrid expresses greater elongation of the floret. In this last case, the difference between the mean of the hybrid and that of the parent with the greatest floret length is always larger than the half confidence interval.

No significant differences were found in floret length between reciprocal hybrids (Table 20).

Width of standard (Tables 14 and 21; Plate 3):

Whenever a species possessing large floweres was used as one parent in an interspecific cross, the resulting hybrid was intermediate to both of its parents for the width of the standard. Whenever both parental species possessed small flowers, the standard width in the hybrid was larger than that found in the parent with the greatest standard width. This observation is in good agreement with those made on floret length in the hybrids.

Table 21 shows that the differences in standard width between reciprocal hybrids are small and never larger than the half confidence intervals.

Ovary length (Tables 14 and 22 ; Figure 18)

Here, it is also apparent that whenever a large flowered species is crossed to either a large flowered species, or to a small flowered one,

Figure 17

Florets of L. japonicus (left), L. schoelleri (right) and L. japonicus x L. schoelleri (middle), magnification X ca. 3.

Figure 18

Ovaries and styles of L. japonicus (left), L. schoelleri (richt) and L. japonicus x L. schoelleri (middle), magnification X ca. 5.

Table 14

Average values for standard width, ovary length and style length in the hybrids and in the parental species

Species	Standard width (in cm)	Ovary length (in cm)	Style length (in cm)
L. japonicus	0·8983	0·6450	0·5383
L. alpinus	0·8000	0·6667	0·6167
L. japonicus X *L. alpinus*	0·8241	0·6500	0·5685
L. filicaulis	0·6217	0·4667	0·3933
L. japonicus X *L. filicaulis*	0·7833	0·6292	0·4542
L. krylovii	0·5733	0·5233	0·4117
L. japonicus X *L. krylovii*	0·6917	0·5450	0·4333
L. krylovii X *L. japonicus*	0·7033	0·5800	0·4600
L. schoelleri	0·6317	0·5117	0·4017
L. japonicus X *L. schoelleri*	0·7700	0·6250	0·4383
L. schoelleri X *L. japonicus*	0·7417	0·6833	0·4167
L. schoelleri X *L. krylovii*	0·6500	0·5056	0·4056
L. krylovii X *L. schoelleri*	0·6667	0·5417	0·4250
L. krylovii X *L. filicaulis*	0·6889	0·6111	0·4333
L. tenuis	0·6667	0·5583	0·4417
L. tenuis X *L. filicaulis*	0·6500	0·5667	0·4333

Table 15

Differences between means ± half confidence interval (± t X SE)$^{(1)}$ for length of central leaflet in the hybrids and in the parental species

	L· japonicus $\bar{X}$ = 0·9515	L· alpinus $\bar{X}$ = 0·4130	L· filicaulis $\bar{X}$=1·1125	L· krylovii $\bar{X}$=0·9685	L· schoelleri $\bar{X}$ = 0·8803	L· tenuis $\bar{X}$=1·2110
L· japonicus x L· alpinus $\bar{X}$ = 0·6950	0·2565 ±0·0838	0·2820 ±0·0711				
L· japonicus x L· filicaulis $\bar{X}$ = 1·1988			* 0·0863 ±0·1779			
L· japonicus x L· krylovii $\bar{X}$ = 1·1525				* 0·1840 ±0·1184		
L· krylovii x L· japonicus $\bar{X}$ = 1·1280				* 0·1595 ±0·0449		
L· japonicus x L· schoelleri $\bar{X}$ = 1·0345	* 0·0830 ±0·1027					
L· schoelleri x L· japonicus $\bar{X}$ = 1·1650	* 0·2135 ±0·1064					
L· schoelleri x L· krylovii $\bar{X}$ = 1·0617				* 0·0932 ±0·0867		
L· krylovii x L· schoelleri $\bar{X}$ = 1·0825				* 0·1140 ±0·1037		
L· krylovii x L· tenuis $\bar{X}$ = 1·1517			* +0·0392 -0·1263			
L· tenuis x L· filicaulis $\bar{X}$ = 1·2700						* +0·0690 -0·2172

(1): 95% confidence limits = diff· ± t X SE

*: hybrid mean is superior to the mean of the greater parent·

Table 16

Differences between means ± half confidence interval (± t X SE)(1) for central leaflet index in the hybrids and in the parental species

	L. japonicus $\bar{X}$=59.7900	*L. alpinus* $\bar{X}$=50.5400	*L. filicaulis* $\bar{X}$=32.9500	*L. krylovii* $\bar{X}$=34.7200	*L. schoelleri* $\bar{X}$=48.3100	*L. tenuis* $\bar{X}$=36.6400
L. japonicus x *L. alpinus* $\bar{X}$ = 46.1778	13.6122 ± 5.3805	4.3622 ±4.0934				
L. japonicus x *L. filicaulis* $\bar{X}$ = 45.4500	14.3400 ± 8.8726		12.5000 ± 6.1476			
L. japonicus x *L. krylovii* $\bar{X}$ = 46.6000	13.1900 ± 5.8968			11.8800 ± 4.4730		
L. krylovii x *L. japonicus* $\bar{X}$ = 44.6200	15.1700 ± 6.0264			9.9000 ± 3.6936		
L. japonicus x *L. schoelleri* $\bar{X}$ = 52.5700	7.2200 ± 4.6620				4.2600 ±4.6200	
L. schoelleri x *L. japonicus* $\bar{X}$ = 47.7500	12.0400 ± 9.9680				0.5600 ±6.3332	
L. schoelleri x *L. krylovii* $\bar{X}$ = 40.4000				5.6800 ±5.2360	7.9100 ±5.2365	
L. krylovii x *L. schoelleri* $\bar{X}$ = 32.9500				1.7700 ±6.1637	15.3600 ±6.2373	
L. krylovii x *L. filicaulis* $\bar{X}$ = 37.8000			4.8500 ± 2.5300	3.0800 ± 4.9500		
L. tenuis x *L. filicaulis* $\bar{X}$ = 29.4000			3.5500 ± 4.0002			7.2400 ±3.3900

(1): 95% confidence limits = diff. ± t X SE.

Table 17

Differences between means ± half confidence interval (± t X SE) for floret length in the hybrids and in the parental species

	L. japonicus $\bar{X}$=1·1100	L. alpinus $\bar{X}$=1·1467	L. filicaulis $\bar{X}$=0·8693	L. krylovii $\bar{X}$=0·8317	L. schoelleri $\bar{X}$=0·8300	L. tenuis $\bar{X}$=1·0033
L. japonicus x L. alpinus $\bar{X}$ = 1·1315	0·0215 ± 0·0522	0·0152 ±0·0421				
L. japonicus x L. filicaulis $\bar{X}$ = 1·0167	0·0933 ± 0·0828		0·1474 ±0·0674			
L. japonicus x L. krylovii $\bar{X}$ = 0·9567	0·1533 ± 0·0271			0·1250 ±0·0483		
L. krylovii x L. japonicus $\bar{X}$ = 1·0067	0·1033 ± 0·0719			0·1750 ±0·0624		
L. japonicus x L. schoelleri $\bar{X}$ = 1·0517	0·0583 ± 0·0504				0·2217 ± 0·0567	
L. schoelleri x L. japonicus $\bar{X}$ = 1·0583	0·0517 ± 0·1099				0·2283 ± 0·1100	
L. schoelleri x L. krylovii $\bar{X}$ = 0·9278				* 0·0961 ± 0·0615		
L. krylovii x L. schoelleri $\bar{X}$ = 0·9417				* 0·1100 ± 0·0702		
L. krylovii x L. filicaulis $\bar{X}$ = 0·9556			* 0·0863 ±0·0454			
L. tenuis x L. filicaulis $\bar{X}$ = 0·9333			0·0640 ±0·0793			0·0700 ± 0·1191

(1): 95% confidence limits =diff. ± t X SE.

Table 18

Differences between means ± half confidence interval (± t X SE)(1) for central leaflet length in reciprocal hybrids

	L. krylovii X *L. japonicus*	*L. schoelleri* X *L. japonicus*	*L. krylovii* X *L. schoelleri*
L. japonicus X *L. krylovii* $\bar{X}$ = 1·1525	0·0245 ± 0·1657		
L. japonicus X *L. schoelleri* $\bar{X}$ = 1·0345		0·1305 ±0·0729	
L. schoelleri X *L. krylovii* $\bar{X}$ = 1·0617			0·0208 ± 0·1711

(1): 95% confidence limits = diff. ± t X SE.

Table 19

Differences between means ± half confidence interval (± t X SE)(1) for central leaflet index in reciprocal hybrids

	L. krylovii X *L. japonicus* $\bar{X}$ = 44.6200	*L. schoelleri* X *L. japonicus* $\bar{X}$ = 47.7500	*L. krylovii* X *L. schoelleri* $\bar{X}$ = 32.9500
L. japonicus X *L. krylovii* $\bar{X}$ = 46.6000	1.9800 ± 4.2768		
L. japonicus X *L. schoelleri* $\bar{X}$ = 52.5700		4.8200 ± 9.9458	
L. schoelleri X *L. krylovii* $\bar{X}$ = 40.4000			7.4500 ± 8.8086

(1): 95% confidence limits = diff. ± t X SE.

Table 20

Differences between means ± half confidence interval (± t x SE)(1) for floret length in reciprocal hybrids

	L. krylovii X L. japonicus $\bar{X}$ = 1·0067	L. schoelleri X L. japonicus $\bar{X}$ = 1·0583	L. krylovii X L. schoelleri $\bar{X}$ = 0·9417
L. japonicus X L. krylovii $\bar{X}$ = 0·9567	0·0500 ± 0·0756		
L. japonicus X L. schoelleri $\bar{X}$ = 1·0517		0·0066 ±0·0729	
L. schoelleri X L. krylovii $\bar{X}$ = 0·9278			0·0139 ±0·1285

(1): 95% confidence limits = diff. ± t X SE

Table 21

Differences between means ± half confidence interval (± t X SE)(1) for standard width in reciprocal hybrids

	L· krylovii X *L· japonicus* $\bar{X}$ = 0·7033	*L· schoelleri* X *L· japonicus* $\bar{X}$ = 0·7417	*L· krylovii* X *L· schoelleri* $\bar{X}$ = 0·6667
L· japonicus X *L· krylovii* $\bar{X}$ = 0·6917	0·0116 ± 0·0315		
L· japonicus X *L· schoelleri* $\bar{X}$ = 0·7700		0·0283 ±0·1117	
L· schoelleri X *L· krylovii* $\bar{X}$ = 0·6500			0·0167 ±0·0397

(1): 95% confidence limits = diff· ± t X SE·

Table 22

Differences between means ± half confidence interval
(± t x SE)(1) for ovary length in reciprocal hybrids

	L. krylovii X *L. japonicus* $\bar{X}$ = 0.5800	*L. schoelleri* X *L. japonicus* $\bar{X}$ = 0.6833	*L. krylovii* X *L. schoelleri* $\bar{X}$ = 0.5417
L. japonicus X *L. krylovii* $\bar{X}$ = 0.5450	0.0350 ±0.0089		
L. japonicus X *L. schoelleri* $\bar{X}$ = 0.6250		0.0583 ±0.0885	
L. schoelleri X *L. krylovii* $\bar{X}$ = 0.5056			0.0361 ±0.1157

(1): 95% confidence limits = diff. ± t X SE.

the ovary length in the hybrid is intermediate in size to that of the parents. However, when a small flowered species is crossed to another small flowered species, the ovary length is greater in the hybrid. The only exception to this rule was observed in the case of the cross L. schoelleri x L. krylovii where the ovary length in the hybrid was found to be smaller than in either of the two parental species.

Ovary length in the hybrid L. krylovii x L. japonicus appears to be significantly larger than in the reciprocal hybrid L. japonicus x L. krylovii ($X_1 - X_2 = 0.0350 \pm 0.0089$). In all other cases, differences in ovary length between reciprocal hybrids are slight, and smaller than the half confidence intervals.

Style length (Tables 14 and 23; Figure 18):

In general, the length of the style in the hybrids was slightly greater than that found in the smaller parent. In the case of the cross L. japonicus x L. alpinus, however, the style was longer in the hybrid than in either parent.

Table 23 shows that the differences in style length between reciprocal hybrids are small, and never larger than the half confidence intervals.

II. Fertility of Lotus species and their F_1 hybrids

A) Pollen stainability in the species

The percentage of stainable pollen was found to vary from species to species and, to a lesser extent, from plant to plant. As shown in Table 24 the pollen of L. japonicus, L. schoelleri and L. tenuis is completely normal, whereas, the three other species exhibited variable

Table 23

Differences between means ± half confidence interval (± t X SE)[1] for style length in reciprocal hybrids

	L. krylovii X *L. japonicus* $\bar{X}$ = 0.4600	*L. schoelleri* X *L. japonicus* $\bar{X}$ = 0.4167	*L. krylovii* X *L. schoelleri* $\bar{X}$ = 0.4250
L. japonicus X *L. krylovii* $\bar{X}$ = 0.4333	0.0267 ± 0.0264		
L. japonicus X *L. schoelleri* $\bar{X}$ = 0.4383		0.0216 ±0.0290	
L. schoelleri X *L. krylovii* $\bar{X}$ = 0.4056			0.0194 ±0.1014

(1): 95% confidence limits = diff. ± t X SE.

Table 24

Pollen stainability in the parental species

Species	Acc. No.	Pollen condition		% Stainable pollen	
		stained	unstained	Plants	Average
L. japonicus	B 129-1	213	2	99.00	98.70
	B 129-3	204	2	99.02	
	B 129-8	313	5	98.43	
L. alpinus	B 77 -5	208	331	38.59	44.40
	B 77 -6	430	578	42.65	
	B 77 -7	490	503	49.35	
L. filicaulis	B 37 -1	612	277	68.84	76.20
	B 37 -2	910	61	93.72	
	B 37 -3	509	255	66.60	
	B 37 -4	432	176	71.00	
L. schoelleri	B 87 -1	280	1	99.64	99.10
	B 87 -2	240	4	98.36	
	B 87 -3	255	2	99.22	
L. krylovii	B 86 -1	399	45	89.86	85.50
	B 86 -2	776	212	78.54	
	B 86 -3	929	97	90.54	
L. tenuis	B 145-1	218	1	99.54	99.22
	B 145-2	226	4	98.26	
	B 145-3	350	2	99.43	

amounts of aborted pollen. Pollen stainability for these latter species ranged from 85% in L. krylovii to 76.20% for L. filicaulis and 44.40% for L. alpinus. Pollen grains of L. japonicus, L. filicaulis, L. krylovii and L. alpinus are shown in Figures 19, 20, 21 and 22.

B) Pollen stainability in the interspecific hybrids

Table 25 summarizes the pollen stainability data for the F_1 interspecific hybrids. It can be seen that there is a wide difference between the percentage of stainable pollen in any given hybrid and its parental species. The degree of stained pollen can, therefore, be considered as an excellent criterium for identification of hybrid genotypes. Differences in the percentages of pollen abortion between hybrids derived from the same cross, however, are quite appreciable. The greatest variability in pollen stainability was observed in the plants which originated from the cross between L. japonicus and L. schoelleri where stainable pollen ranged from 4 to 24%. A considerable variation in pollen stainability,ranging from 18 to 45%, was also found among plants which were derived from crosses between L. japonicus and L. krylovii. Similar differences were also recorded in the reciprocal hybrids of this cross. An analysis of variance carried out in order to test the significance of these differences between reciprocal hybrids, indicates that the variation in pollen stainability are significant at the 99% level in the reciprocal hybrids L. japonicus x L. krylovii and L. krylovii x L. japonicus, whereas, the differences observed between the hybrids L. japonicus x L. schoelleri and L. schoelleri x L. japonicus are significant at the 95% level only (Table 26). In the case of the reciprocal hybrids L. krylovii x L. schoelleri and

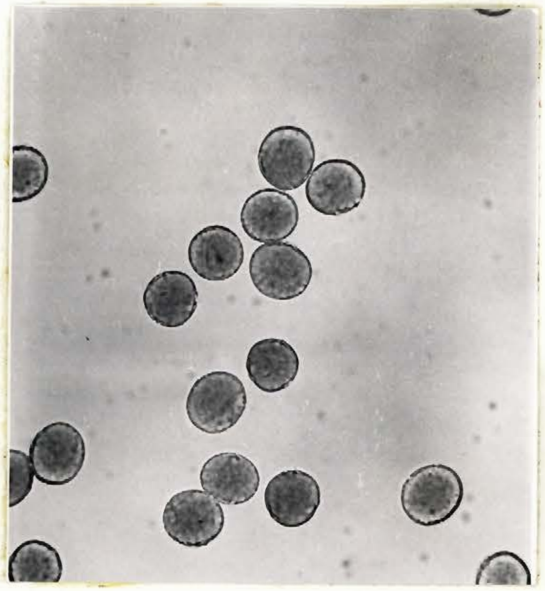


Figure 19
Pollen grains of L. japonicus.
Magnification X ca. 500

Figure 20
Pollen grains of L. filicaulis.
Magnification X ca. 500

Figure 21
Pollen grains of L. krylovii.
Magnification X ca.450

Figure 22
Pollen grains of L. alpinus.
Magnification X ca. 500

Table 25

Pollen stainability in F_1 hybrid plants

Hybrids	Acc. No.	Pollen condition		Stainable pollen	
		stained	unstained	plants	average
L. japonicus X L. alpinus	E 567-2	155	372	29.41	27.37
	E 567-3	257	556	31.61	
	E 567-4	139	447	23.72	
	E 567-8	329	880	27.71	
	E 718-2	196	749	20.74	
	E 752-15	207	449	31.55	
L. japonicus X L. filicaulis	E 602-1	423	702	37.60	33.28
	E 602-2	267	546	32.84	
	E1026-1	242	484	33.33	
	E1030-1	249	600	29.33	
L. japonicus X L. krylovii	E 612-2	218	492	30.70	31.43
	E 612-3	265	622	29.88	
	E 612-4	523	634	45.20	
	E 612-8	250	654	27.65	
	E 612-9	322	772	29.43	
	E 613-2	245	711	25.63	
	E 613-3	281	423	39.91	
	E 613-4	368	692	34.72	
	E 613-6	630	1384	31.28	
	E1028-1	271	487	35.75	
	E1035-4	70	178	28.23	
	E1035-5	74	320	18.78	
L. krylovii X L. japonicus	C 64-1	72	393	15.48	14.67
	C 64-2	45	289	13.47	
	C 64-3	16	84	16.00	
	C 64-4	101	571	15.00	
	C 64-5	31	205	13.13	
L. japonicus X L. schoelleri	E 719-3	40	424	8.62	12.44
	E 719-4	169	515	24.71	
	E 719-11	67	728	8.45	
	E 720-2	34	792	4.12	
	E 735-5	143	735	16.29	
L. schoelleri X L. japonicus	E1061-1a	48	608	7.90	6.46
	E1061-1b	27	425	6.92	
	E1061-2	85	1441	5.89	
L. schoelleri X L. krylovii	C 95-6	6	584	1.02	2.12
	C 95-8	43	1296	3.21	
L. krylovii X L. schoelleri	C 8-1	67	2472	2.71	2.66
	C 8-2a	22	682	3.22	
	C 8-2b	11	578	1.90	
L. krylovii X L. filicaulis	C 20-1	44	1446	2.95	3.43
	C 20-2	89	1439	5.82	
	C 20-3	47	2171	2.12	
L. tenuis X L. filicaulis	E442-1	1220	1626	43.65	43.65

Table 26

Analysis of variance for differences in pollen fertility between reciprocal hybrids

	Sum of squared deviations			Degrees of freedom			Variance		Variance ratio		
	Total	Between groups	Within groups	T	B.G.	W.G.	Between groups	Within groups	F calculated	F table 95%	F table 99%
L. japonicus X L. krylovii L. krylovii X L. japonicus	1,520.292	996.926	523.366	16	1	15	996.926	34.891	28.57	4.60	8.68
L. japonicus X L. schoelleri L. schoelleri X L. japonicus	324.567	39.911	284.565	7	1	6	39.911	47.443	.841	.234	5.86
L. krylovii X L. schoelleri L. schoelleri X L. krylovii	3.578	1.127	2.451	4	1	3	1.127	.817	1.38	10.13	34.12

L. schoelleri x *L. krylovii* the differences in pollen stainability were not significant at the 95% level.

From Figure 26, which depicts in a diagrammatic form the relative pollen fertility relationships in hybrids between the six species involved in the present study, it may be seen that the highest percentage of pollen stainability was observed in the hybrid *L. tenuis* x *L. filicaulis* (43.65%) to be followed by the hybrids *L. japonicus* x *L. filicaulis* (33.28%) and *L. japonicus* x *L. krylovii* (31.43%). The pollen stainability in hybrid plants from crosses between *L. japonicus* and *L. alpinus*, *L. krylovii* and *L. japonicus*, and *L. japonicus* and *L. schoelleri* ranged from 10 to 30 per cent. Pollen fertility in the remaining hybrids, namely *L. schoelleri* x *L. japonicus*, *L. schoelleri* x *L. krylovii*, *L. krylovii* x *L. schoelleri* and *L. krylovii* x *L. filicaulis*, was low and varied from 1 to 8 per cent. Complete sterility, that is, 0 per cent stainable pollen, was, however, never observed. Pictures of normal and empty pollen grans derived from the hybrids *L. japonicus* x *L. filicaulis*, *L. tenuis* x *L. filicaulis* and *L. schoelleri* x *L. krylovii* are shown in Figures 23, 24 and 25.

C) Seed set per pod in the species

Seed set per pod was evaluated in the field under open pollinated conditions. It can be seen from Table 27 that, except for *L. alpinus* which produced very few seeds, approximately 20 seeds per pod were collected from each species. No data were obtained for *L. tenuis*, since through an error, plants of this species were not transferred to the field and were not available at the time these data were

Representative views of pollen showing empty and full grains as observed in different hybrids

Figure 23
Pollen grains of the hybrid
L. japonicus x L. filicaulis.
Magnification X ca. 500

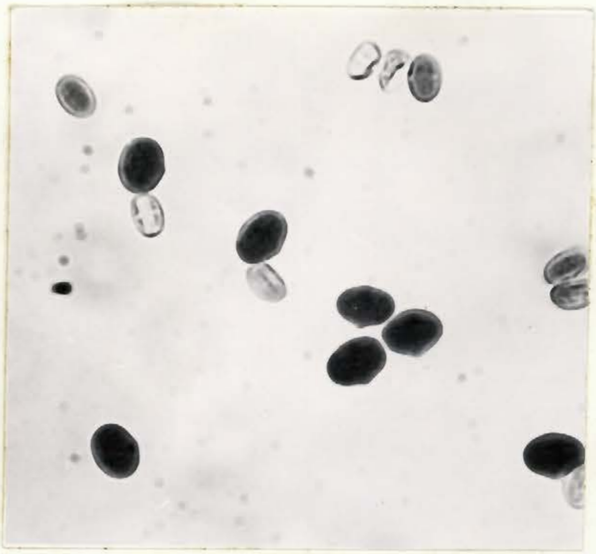


Figure 24
Pollen grains of the hybrid
L. tenuis x L. filicaulis.
Magnification X ca. 500

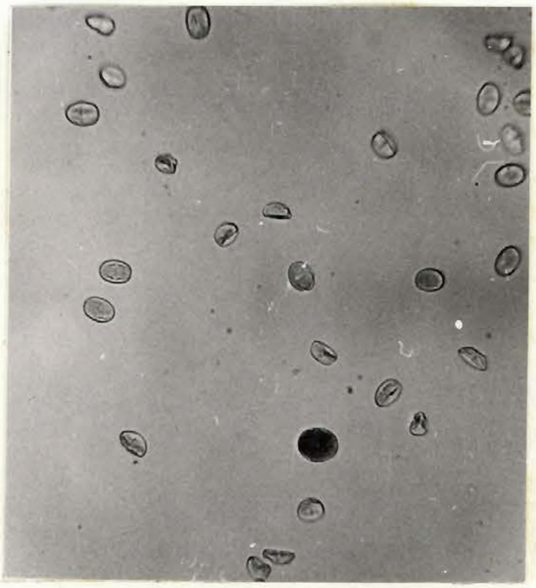


Figure 25
Pollen grains of the hybrid
L. schoelleri x L. krylovii.
Magnification X ca. 320

Figure 26

Pollen fertility relationships in the hybrids between six species of *Lotus*

Diagram illustrating the pollen fertility relationships in the hybrids between six species of *Lotus*. The width of lines drawn between species is directly proportional to the percentage of stainable pollen calculated for the hybrids derived from crosses between the species concerned. The arrow points to the maternal parent in the cross.

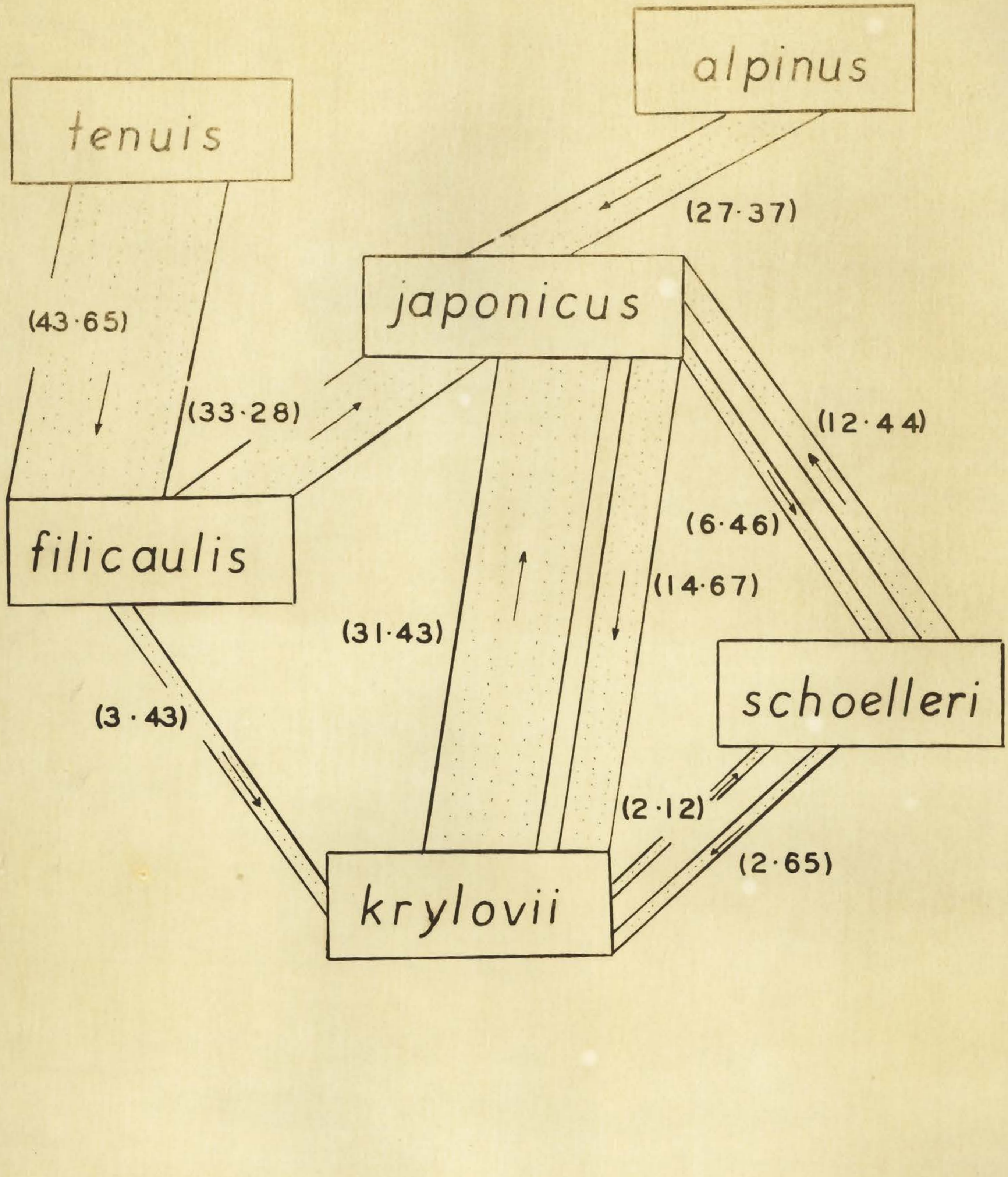

alpinus
tenuis
(27·37)
japonicus
(43·65)
(33·28)
(12·44)
filicaulis
(6·46)
(14·67)
(31·43)
schoelleri
(3 · 43)
(2·12)
(2·65)
krylovii

Table 27

Seed set per pod(1) in parental and hybrid plants

Species	Estimated % normal ovules	Average seed set per pod(2)	Seed set index(3)	Appreciation of seed set index
L. japonicus	98.70	21.46	21.74	high
L. alpinus	44.40	2.53	5.69	low
L. japonicus X *L. alpinus*	27.37	1.60	5.84	low
L. filicaulis	76.20	18.20	23.80	high
L. japonicus X *L. filicaulis*	33.28	5.10	15.32	medium
L. krylovii	85.50	22.40	26.20	high
L. japonicus X *L. krylovii*	31.43	3.47	11.04	medium
L. krylovii X *L. japonicus*	14.67	2.01	13.70	medium
L. schoelleri	99.10	20.90	21.09	high
L. japonicus X *L. schoelleri*	12.44	1.23	9.89	medium
L. schoelleri X *L. japonicus*	6.46	.78	11.61	medium
L. schoelleri X *L. krylovii*	2.12	1.20	56.60	very high
L. krylovii X *L. schoelleri*	2.66	.85	31.95	very high
L. krylovii X *L. filicaulis*	3.43	1.64	47.81	very high
L. tenuis	99.22	(21.00)	21.15	high
L. filicaulis X *L. tenuis*	43.65	2.74	6.28	low

(1) determined from plants growing in the field; no hand pollination performed.

(2) counts are based on 30 pods.

(3) (Seed set per pod/% normal ovules) 100.

obtained for the other species. Bubar (1958), who reports L. tenuis to be highly self-sterile, however, has recorded for this species an average of 21 seeds per pod following cross pollincation. This information is placed between brackets in Table 27.

For each species, the seed set index (seed set per pod/% normal ovules per ovary) 100 was calculated. The percentage of normal ovules per ovary was assumed to be identical to the observed percentage of normal pollen in the hybrid. It is possible, however, that the actual percentage of ovule abortion differs widely from the freqnency of pollen abortion. It can be seen from Table 27 that the seed set index ranges from 20 to 26 for all the species but one, namely, L. alpinus, where a low seed set index of 5.69 was calculated.

D) Seed set per pod in the hybrids

As shown in Table 27, the average number of seeds per pod recorded in the hybrids was low and ranged from 0.75 in the hybrid L. schoelleri x L. japonicus to 5.10 in the hybrid L. japonicus x L. filicaulis. However, all hybrid plants growing in the field produced at least some seeds. The seed set index was found to be very high in the case of hybrids which had a high degree of pollen sterility and low in plants with a relatively high percentage of stainable pollen (Table 27). From these data, the seed set index appears to be negatively correlated to the percentage of stainable pollen recorded in the hybrids.

III. Meiosis in the species and in the F_1 hybrids

A) Meiosis in the parental species

It was considered that the relatively high percentages of aborted pollen observed in some of the parental species may have resulted from irregularities in the meiotic process. It was, therefore, decided to study microsporogenesis in a number of parental plants and to correlate, in each case, irregularities in the meiotic behaviour of the chromosomes with the observed frequency of pollen abortion.

Table 28 is a summary of the observations which were made upon individual plants of L. japonicus, L. filicaulis and L. tenuis. It may be seen from the data that there does not seem to be any correspondence between the frequency of univalent chromosomes observed at metaphase of the first meiotic division and pollen abortion. Plant B 129-3 (L. japonicus), for instance, possessed the largest number of univalents per cell and the highest percentage of pollen fertility while plant B 37-4 (L. filicaulis) exhibited uniform pairing at metaphase I and, yet, was found to have the lowest frequency of stainable pollen. In this latter plant, however, bridge formation was observed to occur somewhat regularly during first anaphase.

B) Meiosis in the interspecific hybrids

Meiosis was observed, for each different cross, in the Pollen Mother Cells (PMC's) of a few hybrid plants taken at random. Table 29 and Table 30 summarize the data that were obtained during these studies. The observations were principally devoted to two stages of meiosis, namely, first division metaphase and first division anaphase.

a) Metaphase I in the hybrids:

It can be seen from Table 29 that normal pairing of the 12 chromosomes of the complement was recorded, for each plant studied,

Figure 27
Metaphase I in L. *japonicus*,
showing 6 II's.
Magnification X ca. 2.760

Figure 28
Metaphase I in L. *filicaulis*,
showing 6 II's.
Magnification X ca. 2.070.

Table 28

Meiotic observations and pollen stainability in three parental species

Species	Plant acc. No.	Stainable pollen %	Metaphase I				Anaphase I						Anaphase II			
			6 II's		5II+2 I		normal		bridges		laggards		normal		bridges	
			No. of Cells	%	No. of Cells	%	No. of Cells	%	No. of Cells	%	No. of Cells	%	No. of Cells	%	No. of Cells	%
L. japonicus	B 129-3	99.02	32	91.43	3	8.57	-	-	-	-	-	-	-	-	-	-
L. filicaulis	B 37-4	71.00	39	97.50	1	2.50	12	75.00	4	25.00	0	0	182	99.45	1	.55
L. tenuis	B145-1	99.54	10	100	0	0	13	92.86	0	0	1	7.14	18	100	0	0

Table 29

Chromosome associations at M I in the hybrids

Hybrid	Acc. no.	No. of cells	6II		5II+2I's		4II+4I's		4II+1III+1I		4II+1VI	
			No.	%	No.	%	No.	%	No.	%	No.	%
L. japonicus X L. alpinus	E567	18	17	94.50	1	5.50	-	-	-	-	-	-
L. japonicus X L. filicaulis	E602	31	26	83.87	4	12.90	1	3.22	-	-	-	-
	E1030	45	34	75.55	9	26.47	2	4.44	-	-	-	-
	Total	76	60	78.95	13	17.11	3	3.94	-	-	-	-
L. japonicus X L. krylovii	E612	16	14	87.50	1	6.25	-	-	1	6.25	-	-
	E1035	18	12	75.00	-	-	2	11.11	3	16.67	1	5.56
	Total	34	26	76.47	1	2.94	2	5.88	4	11.76	1	2.94
L. japonicus X L. schoelleri	E899	19	13	68.42	4	21.05	2	10.05	-	-	-	-
	H 18	13	10	76.92	3	23.08	-	-	-	-	-	-
L. schoelleri X L. japonicus	E1061-1	30	24	80.00	6	20.00	-	-	-	-	-	-
	E1061-2	16	13	81.25	2	12.50	1	6.25	-	-	-	-
	Total	78	60	76.92	15	19.23	3	3.85	-	-	-	-
L. schoelleri X L. krylovii	C95	27	20	74.07	5	18.52	2	7.41	-	-	-	-
L. krylovii X L. filicaulis	C20-1	27	16	59.26	5	18.52	2	7.41	-	-	4	14.81
	C20-3	17	15	88.24	2	11.76	-	-	-	-	-	-
	Total	64	31	70.44	7	15.91	-	-	-	-	4	9.09
L. tenuis X L. filicaulis	E442	155	132	85.16	20	12.90	-	-	2	1.29	1	0.65

in at least 60% of the cells examined. The percentage of cells with six bivalents at first metaphase, when averaged for each cross, never was below 70. Differences in the amounts of normal pairing from plant to plant within a given cross (for instance, plant C20-1 and C 20-3 in the cross L. krylovii x L. filicaulis) were often found to be larger than the differences observed between two hybrid plants of unlike parentage (for instance plant C 20-3 of the cross L. krylovii x L. filicaulis and plant E 612-3 of the cross L. japonicus x L. krylovii).

The highest percentage (94.50%) of cells with 6 II's at M I was observed in hybrid plants derived from the cross between L. japonicus and L. alpinus (Plate 5). However, only 18 cells were examined for this cross since good preparations were difficult to obtain. In some cases, as shown in Plate 5 and in Figure 29, inter pollen mother cell chromatin-like connections, which joined the nuclear material of one cell to that of another, were observed.

From the 155 PMC's examined in the hybrid L. tenuis x L. filicaulis, 85% of the cells were observed to exhibit what appeared to be normal pairing at M I (Plate 6, Figures 34, 35 and 36).

In the remaining hybrids, in which approximately 40 cells per cross were examined, the percentages of PMC's with 6 II's at M I was also high, averaging around 75. The lowest frequency of normal pairing was recorded in the hybrid L. krylovii x L. filicaulis (70.44%).

Normal pairing of the chromosomes in the hybrids is shown on Plates 5, 6, 7, 8, 9 and 10 and in Figures 30, 31, 32, 33, 34, 35 and 36.

In each hybrid, however, a number of M I 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 M I presumably

Figure 29
Inter P.M.C. connections
in L. japonicus x L. alpinus.
Magnification X ca. 480

Figure 30
Six bivalents at M I in
L. japonicus x L. filicaulis
Magnification X ca. 1955

Figure 31
Side view of M I showing 6 II's
in L. japonicus x L. schoelleri.
Magnification X ca. 1955

Figure 32
Polar view of M I showing 6 II's
in L. japonicus x L. schoelleri.
Magnification X ca. 2760

Figure 33
Six bivalents at M I in
L. krylovii x L. filicaulis.
Magnification X ca. 1760

Figure 34
Side view of M I showing 6 II's
in L. tenuis x L. filicaulis.
Magnification X ca. 2760

Figure 35
Side view of M I showing
6 II's in L. tenuis x L.
filicaulis.
Magnification X ca. 2760

Figure 36
Polar view of M I showing 6 II's
in L. tenuis x L. filicaulis.
Magnification X ca. 1955

as a result of desynapsis. It may be calculated from Table 29 that two or four univalent chromosomes were recorded in at least 5% of the total number of PMC's examined for each hybrid plant. The highest percentages of cells showing univalent chromosomes at first metaphase were observed in the hybrid plants derived from crosses between *L. japonicus* and *L. filicaulis* (17.11% of PMC's with 5 II's + 2 I's and 3.94% of PMC's with 4 II's + 4 I's), *L. japonicus* and *L. schoelleri* (19.23% of PMC's with 5 II's + 2 I's and 3.85% of PMC's with 4 II's + 4 I's) and in a plant from the cross between *L. schoelleri* and *L. krylovii* (18.52% of PMC's with 5 II's + 2 I's and 7.41% of PMC's with 4 II's + 4 I's). In the remaining hybrids, the percentage of cells with either two or four univalent chromosomes at first metaphase averaged approximately 12, with the exception of hybrid plants from the cross *L. japonicus* x *L. alpinus* where univalent chromosomes (5 II + 2 I's) were recorded in only 5.50% of the 18 cells examined. Univalent chromosomes observed in the hybrids are shown in plates 8, 9 and 10 and in Figures 38, 39, 40, 41, 43 and 44.

Multivalent associations were occasionally observed in the hybrids *L. japonicus* x *L. krylovii*, *L. krylovii* x *L. filicaulis* and *L. tenuis* x *L. filicaulis*. It was often difficult to distinguish general stickiness of the chromosomes, clearly observed for instance in the hybrid *L. japonicus* x *L. filicaulis* (Plate 6 and Figure 56), from multivalent associations determined by pairing of homologous segments. It can be seen from Table 29 that the highest percentage of MI plates exhibiting multivalent configurations was observed for the hybrid *L. krylovii* x *L. filicaulis* (9.09%), whereas, average frequencies of

Figure 37
3 II's + 3 loose II's in
L. schoelleri x L. japonicus
Magnification X ca. 1553.

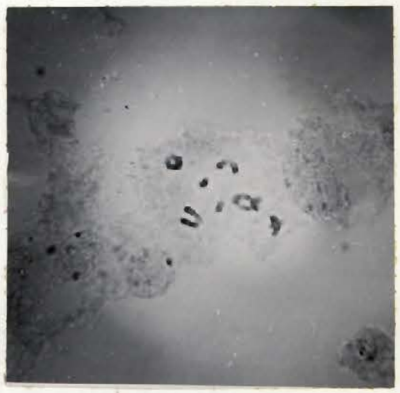


Figure 38
5 II's + 2 I's in
L. schoelleri x L. japonicus
Magnification X ca. 1553.

Figure 39
5 II's + 2 I's in
L. japonicus x L. schoelleri
Magnification X ca. 1760.

Figure 40
5 II's + 2I's in
L. tenuis x L. filicaulis
Magnification X ca. 2760.

Figure 41
5 II's + 2 I's in
L. krylovii x L. schoelleri.
Magnification X ca. 2760.

Figure 42
4 II's + 2 loose II's in
L. krylovii x L. filicaulis.
Magnification X ca. 2760.

Figure 43
5 II's + 2 I's (right) in
L. japonicus x L. schoelleri
Magnification X ca. 1760

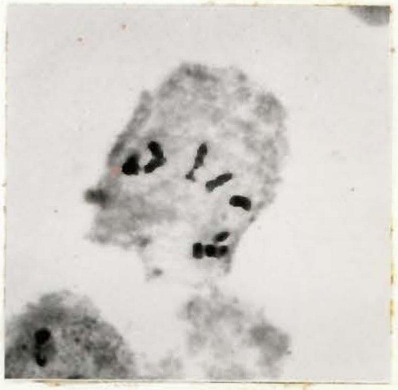


Figure 44
4 II's + 4 I's in
L. tenuis x L. filicaulis
Magnification X ca. 1760

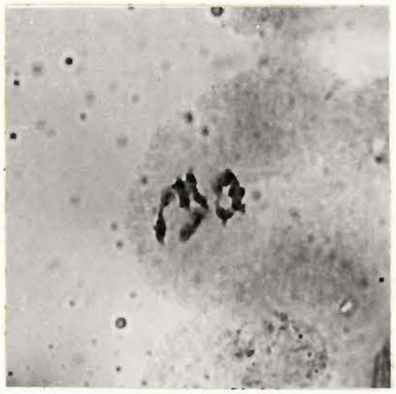


Figure 45
4 II's (left) + 1 IV (right)
in L. krylovii x L. tenuis.
Magnification X ca. 1760

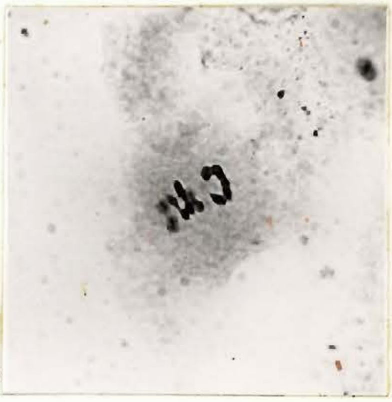


Figure 46
4 II's (left) + 1 IV (right)
in L. krylovii x L. tenuis.
Magnification X ca. 1760.

Figure 47
4 II's + 1 III (right) + 1 I
or, possibly, 5 II's + 2 I's in
L. tenuis x L. filicaulis.
Magnification X ca. 2360.

2·94% and 0·65%, respectively, were recorded in the hybrid L. japonicus x L. krylovii and L. tenuis x L. filicaulis.

Drawings and microphotographs of multivalent associations are shown in plates 6, 7 and 9 and in figures 45, 46 and 47. In figure 47, however, it is not possible to ascertain whether the chromosome association on the right hand side of the picture is composed of two overlapping bivalents or of a chain of three chromosomes.

b) First division anaphase in the hybrids:

It can be seen from Table 30 that at least 60% of the A I cells observed for each cross were devoid of bridges and lagging chromosomes. The average percentages of cells tabulated as normal equals 96 and 91, respectively, in the hybrids derived from the cross between L. krylovii and L. filicaulis and from the cross between L. krylovii and L. schoelleri. In the remaining hybrids, the percentages of normal anaphases averaged between 80 and 90, with the exception of the cross L. japonicus x L. alpinus where only 64 per cent of the cells were recorded as normal.

As can be observed from Plates 5, 6 and 7 and from Figures 57 and 59, one of the medium to large sized chromosome pairs sometimes remained on the equatorial plate at first anaphase. In some cases, the lagging chromosomes were identified as those of a small pair which had earlier separated and which presumably failed to travel to the poles at the beginning of A I (Figures 58, 60 and 65).

It can be seen from an examination of the results presented in Table 29 and Table 30 that there is no correlation, as might be expected, between the percentages of abnormalities observed at first metaphase and the number of irregularities recorded at first anaphase. Asynchronous separation of the chromosomes in first anaphase was observed in all hybrids examined with a frequency ranging from 1.82 per cent in hybrids for the cross L. japonicus x L. krylovii to 21.88 per cent for the cross L. japonicus x L. alpinus (Table 30). In a few cells of the hybrid L. japonicus x L. krylovii (Plate 7) an irregular distribution of the chromosomes was observed in which five chromosomes had gone to one pole and seven to the other at anaphase I. Although bridges at A I were not observed in hybrid plants derived from crosses between L. japonicus and L. schoelleri, they were found in all other hybrids. The highest percentages of bridges were recorded in hybrid plants between L. japonicus and L. krylovii (17.52 per cent) and in those between L. japonicus and L. alpinus (14.06 per cent). In the remaining hybrids, the frequency of cells exhibiting a bridge was low and ranged from 1 to 9 per cent. Drawings and microphotographs of first anaphase bridges are given in Plates 5, 6, 7, 9 and 10, and in Figures 56, 57, 59, 61, 62 and 63.

c) Second division anaphase in the hybrids:

As only a very limited number of cells were examined for second division anaphase in the hybrids, no analysis of the observations were made. However, micronuclei, lagging chromosomes and bridges were observed and these abnormalities are shown in Plates 5, 6, 7, 9 and 10.

Table 30

Chromosome associations at A I in the hybrids

Hybrid	Acc. No.	No. of cells	Normal No. of cells	Normal %	Bridges No. of cells	Bridges %	Laggards No. of cells	Laggards %
L. japonicus	E567	23	15	65·22	3	13·04	5	21·74
X	E868	41	26	63·41	6	14·63	9	21·95
L. alpinus	Total	64	41	64·06	9	14·06	14	21·88
L. japonicus	E1030	105	89	84·76	6	5·71	10	9·52
X	E602	56	37	66·07	8	14·29	11	19·64
L. filicaulis	Total	161	126	78·26	14	8·70	21	13·04
L. japonicus	E613	104	84	80·77	19	18·27	1	0·96
X	E1028	170	137	80·59	29	17·06	4	2·35
L. krylovii	Total	274	221	80·66	48	17·52	5	1·82
L. japonicus								
X	H 18	10	7	70·00	-	-	3	30·00
L. schoelleri								
L. schoelleri								
X	E1061	56	46	82·14	-	-	10	17·86
L. japonicus								
	Total	66	53	80·30	-	-	13	19·70
L. krylovii								
X	C 8·1	57	53	92·98	-	-	4	7·02
L. schoelleri								
L. schoelleri								
X	C 95	56	50	89·92	3	5·36	3	5·36
L. krylovii								
	Total	113	103	91·15	3	2·65	7	6·19
L. krylovii	C20·1	81	80	98·77	-	-	1	1·23
X	C20·2	199	190	95·48	4	2·01	5	2·51
L. filicaulis	Total	280	270	96·43	4	1·43	6	2·14
L. tenuis								
X	E442	91	81	89·01	3	3·30	7	7·69
L. filicaulis								

Figure 48
Early A I in L. krylovii x L. tenuis. The two chromosomes on the right separated before the others. Magnification X ca. 2500.

Figure 49
A I in L. japonicus x L. filicaulis, showing 6 chromosomes at the upper pole. Magnification X ca. 3100.

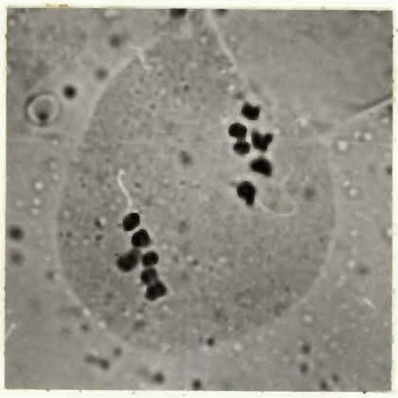


Figure 50
A I in L. tenuis x L. filicaulis, showing 6 chromosomes at each pole. Magnification X ca. 2360.

Figure 51
A I in L. japonicus x L. schoelleri, showing 6 chromosomes at the upper pole. Magnification X ca. 2070.

Figure 52
A I in L. krylovii x L. filicaulis, showing 6 chromosomes at one pole.
Magnification X ca. 3100.

Figure 53
A I in L. krylovii x L. filicaulis, showing 6 chromosomes at each pole.
Magnification X ca. 2360.

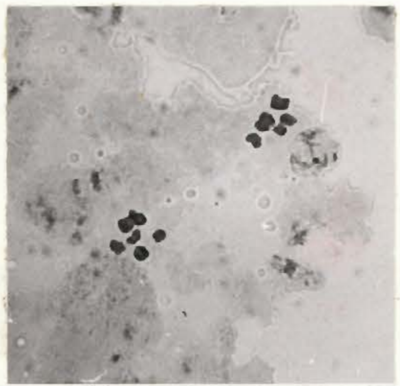


Figure 54
A I in L. krylovii x L. filicaulis, showing 6 chromosomes at each pole.
Magnification X ca. 1760.

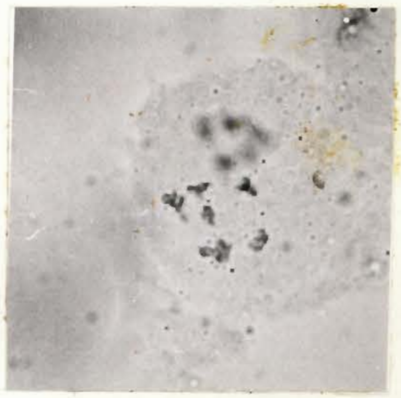


Figure 55
M II in L. krylovii x L. filicaulis.
Magnification X ca. 2070.

Figure 56
A I bridge in L. tenuis x L. filicaulis.
Magnification X ca. 2070.

Figure 57
A I in L. japonicus x L. filicaulis: left, normal; right, bridge.
Magnification X ca. 1553.

Figure 58
A lagging chromosome during A I in L. japonicus x L. filicaulis.
Magnification X ca. 2760.

Figure 59
A I bridge (left) in L. japonicus x L. filicaulis.
Magnification X ca. 2360.

Figure 60
T I in L. *japonicus* x L. *alpinus*, with two chromosomes not incorporated in restitution nuclei.
Magnification X ca. 2070.

Figure 61
A I bridge in L. *japonicus* x L. *filicaulis*.
Magnification X ca. 1553.

Figure 62
A I bridge in L. *japonicus* x L. *filicaulis*.
Magnification X ca. 1553.

Figure 63
A I bridge in L. *japonicus* x L. *schoelleri*.
Magnification X ca. 1553.

Figure 64
T I in L. krylovii x L. tenuis.
Magnification X ca. 1760.

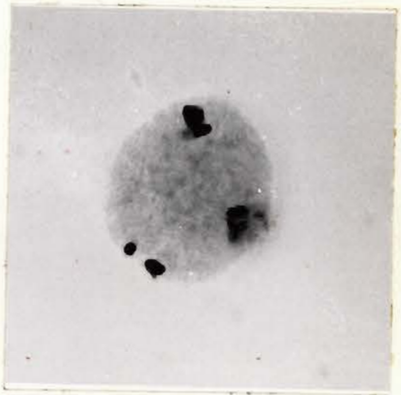


Figure 65
T I in L. japonicus x L. filicaulis, with chromosomes not incorporated in restitution nuclei.
Magnification X ca. 1955.

Figure 66
M II in L. japonicus x L. alpinus (in two nuclei)
Magnification X ca. 2360.

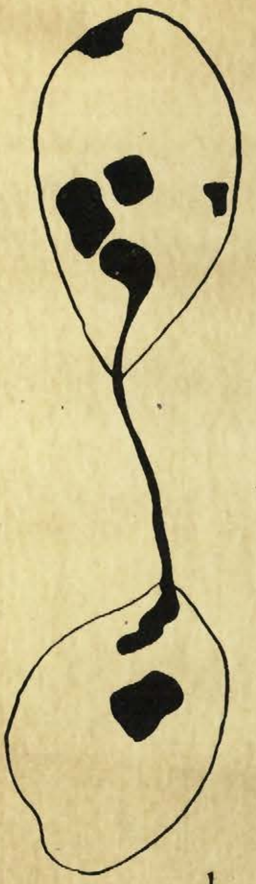
Figure 67
T I (right) and T II (left) in L. schoelleri x L. krylovii.
Magnification X ca. 1350.

Plate 5

Microsporogenesis in the hybrid *L. japonicus* x *L. alpinus*

(X ca. 3,000)

1. Inter PMC's chromatin-like connections.
2. Inter PMC's chromatin-like connections.
3. Six bivalents at first metaphase.
4. Side view of M I showing five bivalents on the equatorial plate and one isolated bivalent.
5. First anaphase with six chromosomes at each pole.
6. Delayed separation of chromosomes of one bivalent at anaphase I
7. Delayed separation of chromosomes of one bivalent at anaphase I
8. First telophase bridge with chromosome or fragment excluded from restitution nuclei.
9. Pollen (X ca. 750)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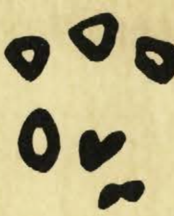
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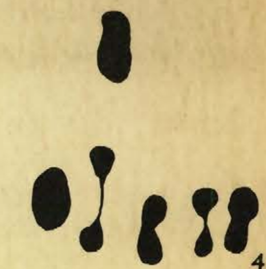
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7


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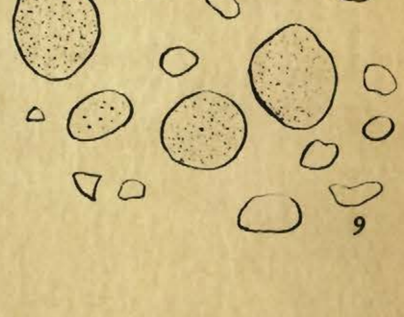
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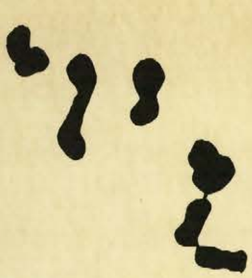
Plate 6

Microsporogenesis in the hybrid L. *japonicus* x L. *filicaulis*

(X ca. 3,000)

1. Side view of M I showing six bivalents on the equatorial plate.
2. Six bivalents at M I: thread-like connections join three bivalents (right).
3. First metaphase: multivalent formation appears to result from chromosome stickiness.
4. Generalized stickiness of the chromosomes during first anaphase.
5. First anaphase with six chromosomes at each pole.
6. Delayed separation of chromosomes of one bivalent at A I.
7. First anaphase bridge.
8. A single univalent lags between the poles at first anaphase.
9. Second anaphase with six chromosomes at each pole.
10. Some chromosomes lag between the poles at second anaphase.
11. Pollen grains (X ca. 750).


1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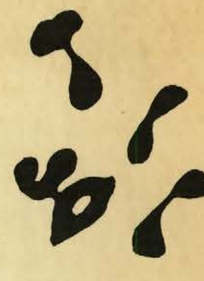
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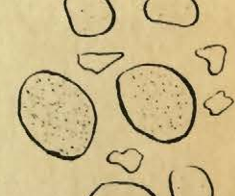
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Plate 7

Microsporogenesis in the hybrid *L. japonicus* x *L. krylovii*

(X ca. 2,800)

1. Six bivalents at first metaphase.
2. Polar view of M I showing five groups of chromosomes.
3. First anaphase with six chromosomes at each pole.
4. First anaphase with five chromosomes at one pole and seven at the other.
5. Chromosomes of one bivalent, excluded from restitution nuclei at T I.
6. A quadrivalent association of chromosomes lagging between the poles at first anaphase.
7. First anaphase bridge in which three chromosomes appear to be associated.
8. First anaphase bridge.
9. Second anaphase.
10. T II in which one (or more) chromosome has been excluded from the restitution nuclei.
11. Bridge at A II involving two of the four nuclei.
12. Bridge at A II involving two of the four nuclei.
13. Pollen grains (X ca. 750).


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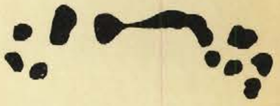
3


4

2


5


6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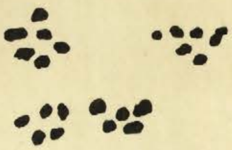
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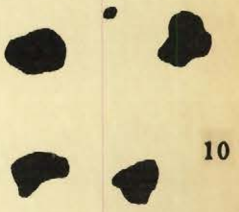
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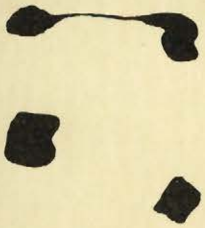
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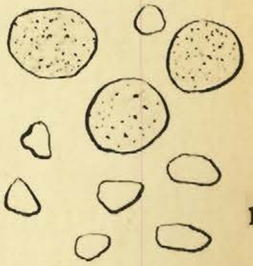
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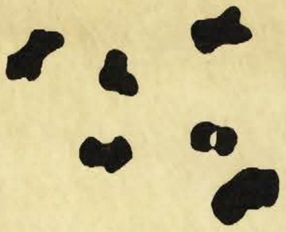
Plate 8

Microsporogenesis in the hybrid _L. japonicus_ x _L. schoelleri_

(X ca. 2,800)

1. Six bivalents at M I.
2. Six bivalents at M I.
3. Five bivalents + two univalents at M I.
4. Four bivalents + four univalents at M I.
5. Eight groups of chromosomes at first metaphase (possibly 4 II's + 4 I's).
6. First anaphase with six chromosomes at each pole.
7. Second anaphase with six chromosomes at each pole.
8. Pollen grains (X ca. 750).



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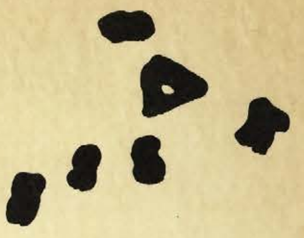

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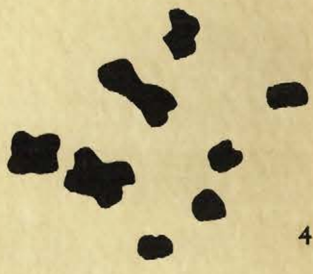

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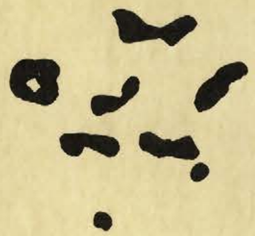

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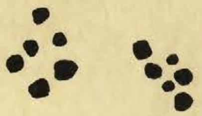

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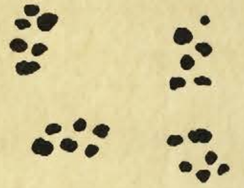

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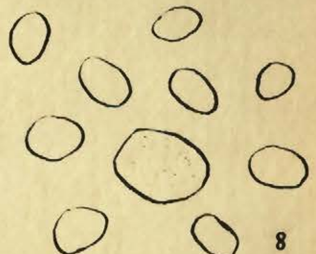

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Plate 9

Microsporogenesis in the hybrid <u>L. krylovii</u> x <u>L. filicaulis</u>

(X ca. 3,000)

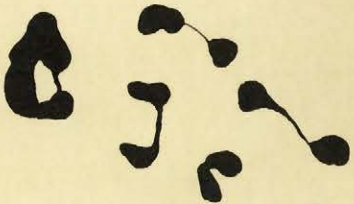
1. Six bivalents at M I.
2. Three bivalents + six univalents at M I (the two univalents in the middle appear to form a loose bivalent)
3. Four bivalents + one quadrivalent at M I.
4. First anaphase bridge.
5. Four bivalents + one quadrivalent at M I.
6. First anaphase bridge.
7. First anaphase showing six chromosomes at the upper pole.
8. First anaphase with five chromosomes at one pole and seven at the other.
9. Pollen grains (X ca. 650).


1


2


3


4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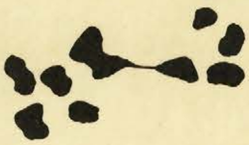
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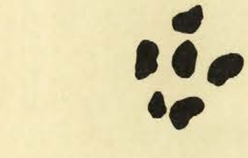
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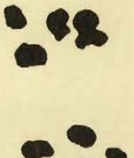
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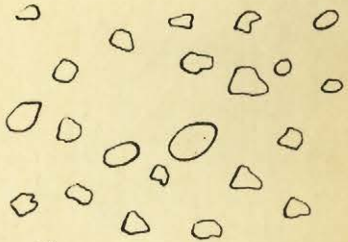
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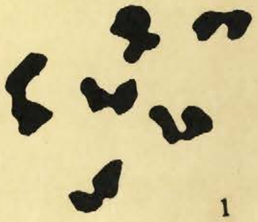
Plate 10

Microsporogenesis in the hybrid *L. tenuis* x *L. filicaulis*

(Magnification X ca. 3,000)

1. Six bivalents at M I.
2. Six bivalents at M I.
3. Six bivalents at M I.
4. Five bivalents + two univalents at M I.
5. Four bivalents + one trivalent + one univalent at M I.
6. First anaphase with six chromosomes at each pole.
7. Chromosomes excluded from restitution nuclei in T I.
8. Pollen grains (X ca. 650).



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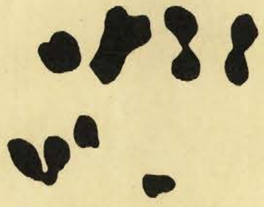

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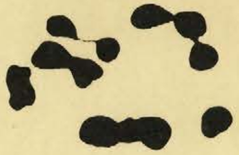

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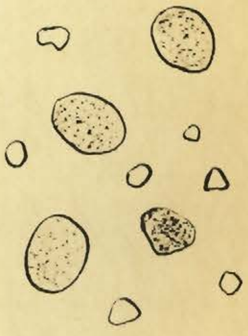

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IV. Inheritance studies in F_2 and $B.C._1$ progenies of the hybrids

As previously stated in the Materials and Methods, a number of segregating characters were studied in the progenies of two F_1 hybrids, namely, *L. japonicus* x *L. filicaulis* and *L. japonicus* x *L. alpinus*. In the case of the hybrid *L. japonicus* x *L. filicaulis* only F_2 plants were observed whereas both F_2 and $B.C._1$ populations were obtained from the hybrid between *L. japonicus* and *L. alpinus*. Inheritance studies on these crosses will be presented here.

A) F_2 progeny of the hybrid *L. japonicus* x *L. filicaulis*

A total of 170 plants were grown from 285 seeds harvested on five clones of two F_1 hybrids (E 602-2 and E 1030-1). The following characters were studied:

HCN reaction
Stem colour
Leaflet size
Floret colour
Pod mottling
Seed speckling
Pollen stainability
Chromosome numbers.

Because the five clones derived from E 602-2 and E 1030-1 had been placed together in the screened cage it was not possible to ascertain whether the seeds produced were the result of selfing or of cross fertilization between the clones of these two hybrids. For this reason it was not attempted, except for the HCN reaction, to analyze separately the segregation ratios observed in the F_2 progeny of each hybrid with a distinct genotype.

a) HCN reaction in the F_2 progeny of L. japonicus x L. filicaulis

As shown in Table 31 the phenotypes observed for the presence or absence of cyanogenetic glycoside were classified into two categories, HCN positive and HCN negative. No attempt was made to separate the plants in relation to reaction intensity. It should be said, however, that a number of plants which were considered as reacting positively to the HCN test gave only a weak response and therefore appeared to resemble the slightly positive reaction of plants observed by Seaney (1957) in populations of L. corniculatus.

Table 31 indicates that 145 F_2 hybrids reacted positively to the HCN test whereas the 25 remaining plants did not give any response. The ratio of positive to negative reaction is equal to 5.8:1.

b) Stem colour in the F_2 progeny of the hybrid L. japonicus x L. filicaulis.

As shown in Table 31 the F_2 hybrid segregated for stem colour in a ratio of 5.54 red : 1 green. As may be seen from Table 32 and from Plate 11 a number of different phenotypes were observed. These ranged from deep red to medium red, light red, dotted, variegated and green. The classification of these various phenotypes was arbitrary as it is somewhat difficult to evaluate colour intensities and pigment distribution. All stems that showed red coloration were classified as red and it is on this basis that the 5.54:1 segregation

Table 31

HCN reaction and stem coloration in the F_2 progeny of the hybrid *L. japonicus* x *L. filicaulis*

	HCN reaction		observed ratio
	+	-	
F_2 of E 602	86	13	6·62 : 1
F_2 of E 1030	59	12	4·99 : 1
Total	145	25	5·80 : 1
	Stem colour		
	Red	Green	
F_2 of E 602	84	15	5·60 : 1
F_2 of E 1030	60	11	5·48 : 1
Total	144	26	5·54 : 1

Table 32

Stem coloration and HCN reaction

in the F_2 progeny of the hybrid L. japonicus x L. filicaulis

Stem coloration		Number of plants observed +	−	Total
Red	very deep	7	0	7
	L. japonicus phenotype	16	3	19
	F_1 hybrid phenotype	54	9	63
	Pale	45	5	50
	Variegated	3	2	5
Green	L. filicaulis phenotype	20	6	26
Total	All phenotypes	145	25	170

Table 33

Summary of results for stem coloration and HCN reaction

in the F_2 progeny of the hybrid L. japonicus x L. filicaulis

Phenotypes	Number of plants observed +	−	Total
Red stem	125	19	144
Green stem	20	6	26
Total	145	25	170

Plate 11

Stem coloration in parental species and F_2 progeny of the hybrid *L. japonicus* x *L. filicaulis*

L. japonicus : red stem

L. filicaulis : green stem

F_1 hybrid : pinkish red stem

F_2 progeny

0 : *L. filicaulis* phenotype (green stem)

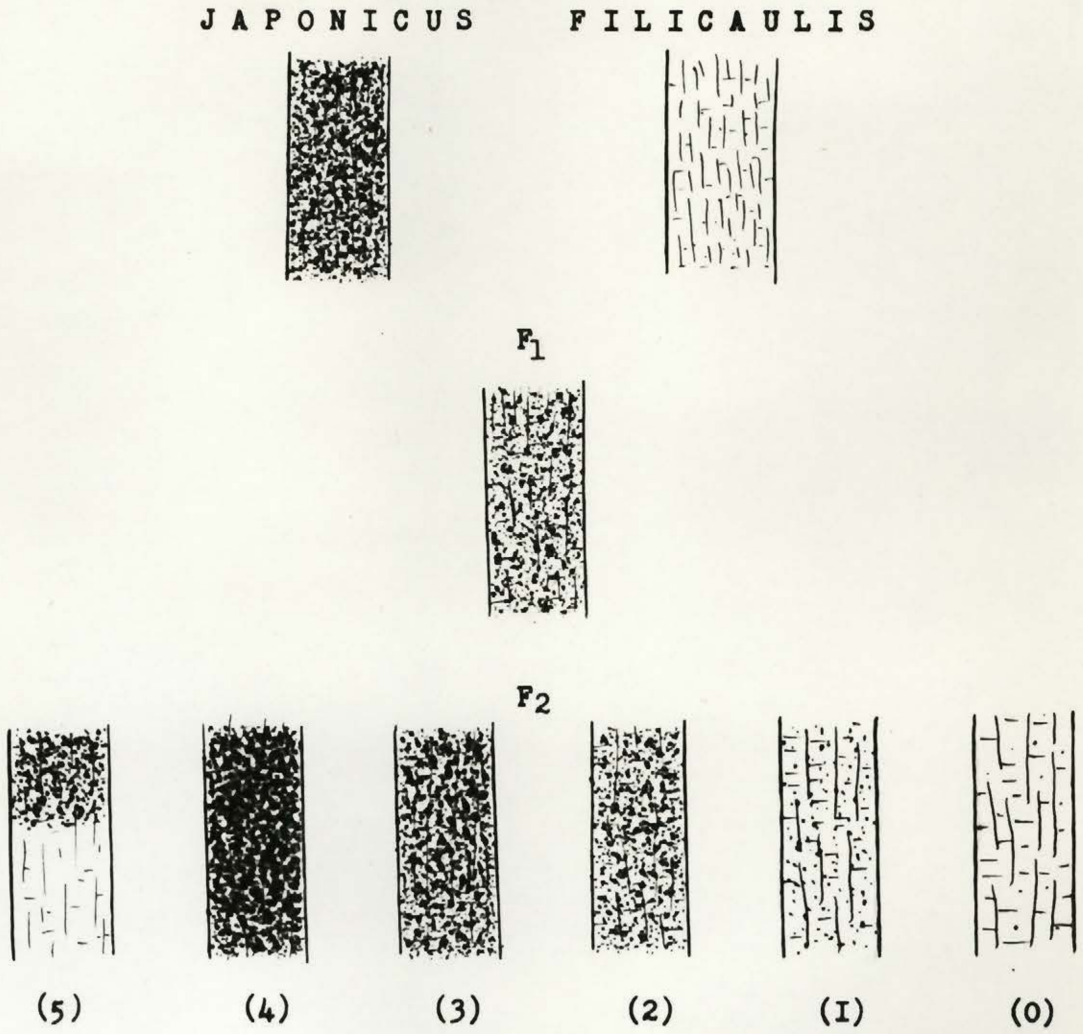
1 : the stem is green with red dots

2 : F_1 phenotype

3 : *L. japonicus* phenotype (red stem)

4 : deep red stem

5 : variegated stem


JAPONICUS
FILICAULIS
F_1
F_2
(5)
(4)
(3)
(2)
(I)
(0)

ratio was calculated and that all analyses of correlation were performed.

It may be observed from Table 31 that the segregation ratios obtained for HCN reaction and stem colour are very similar in that 25 individuals appeared homozygous recessive for the HCN character as compared to 24 in the case of stem colour. Tables 32 and 33, which show the number of recombinant phenotypes obtained in the F_2 population, and Table 34, which expresses the results of a test carried out for linkage between the genes controlling HCN reaction and stem colour, however indicate that these two characters are governed by genes which are probably not located on the same chromosome. From Table 34 it can be observed, also, that the genes involved are not completely independent from one another and may well be partly associated through some mechanism concerned with chromosome affinity and selection during gametogenesis and embryonic development.

c) Central leaflet index values in the F_2 progeny of the hybrid *L. japonicus* x *L. filicaulis*

The central leaflet index (ratio of width to length in percentage) was calculated for each of the F_2 plants. Values obtained ranged from 26 to 80 percent with an average of 46.65 percent for the total population. Table 35 shows that the central leaflet index values in both the F_2 and the F_1 populations are intermediate to the parental species and that both the F_1 and F_2 have a very similar average value (F_1, 45.45; F_2, 46.65). Plant to plant variability, however, was, as might be expected, much greater in the F_2 population than in the

Table 34

F_2 progeny of the hybrid *L. japonicus* x *L. filicaulis*:
test for linkage between genes controlling stem colour and HCN reaction

	Phenotypes				Chi square		
						Table	
Segregation ratio	red stem: +	green stem: -	red stem: -	green stem: +	Calculated	5%	1%
observed	125	6	19	20			
Expected if only one linkage group and 10% crossing over	114·75	38·25	8·5	8·5	56·82	7·82	11·34
Expected if two independent linkage groups	95·67	10·63	31·89	31·89	20·65	7·82	11·34

Table 35

Comparison of leaflet indexes in the parental, F_1 and F_2 populations of the hybrid L. japonicus x L. filicaulis

	Number of plants observed	Leaflet index	
		range	mean
L. japonicus	10	40 - 91	59·79
L. filicaulis	10	22 - 44	32·95
F_1 hybrids	4	26 - 69	45·45
F_2 hybrids	170	26 - 80	46·65

first generation hybrids. It may also be seen from Table 35 that the range of the central leaflet index in the F_2 population overlaps that of the F_1 hybrids as well as that of the one parent (L. filicaulis) but remains below the largest index values obtained for the other parent (L. japonicus).

Attempts were made to establish evidence of linkage between the genes responsible for the width of the central leaflet and those controlling other characters investigated in this study. Table 36 lists the range and mean values for leaflet index of the F_2 plants and data on HCN reaction and stem colour for this population. It may be seen that leaflet indexes are greater in populations which express the red stem character and which give a positive reaction to the HCN test. The highest mean index value ($\bar{X} = 48.37$) was calculated for those plants that possessed both the positive HCN reaction and the red stem character, whereas the double recessive individuals (negative reaction to the HCN test and green stem) were found to have the lowest leaflet indrexes ($\bar{X} = 41.33$). The results of an analysis of variance, given in Table 37, indicates, however, that not all variations in the leaflet indexes between F_2 populations of different phenotypes are statistically reliable. Differences in leaflet index are significant at the 99% level when only one variable (HCN reaction or stem colour) and the total of the F_2 population are considered at a time. On the other hand, when populations homozygous recessive for any of the two characters (stem colour or HCN reaction) are compared, the statistical analysis shows that presence or absence of a dominant phenotype for the second character has no effect upon the variation in leaflet index.

Table 36

Values of leaflet indexes in the F_2 progeny of the hybrid *L. japonicus* x *L. filicaulis* segregating for HCN reaction and stem colour

Phenotype		Number of plants observed	Leaflet index	
HCN	stem		Range	Mean
+	all types	145	26 - 80	47·45
-	all types	25	26 - 64	42·00
all types	red	144	26 - 80	47·56
all types	green	26	33 - 54	41·62
+	red	125	26 - 80	48·37
+	green	20	33 - 54	41·70
-	red	19	26 - 64	42·21
-	green	6	34 - 50	41·33

Table 37

F_2 progeny of the hybrid L. japonicus x L. filicaulis:
significance of variations for central leaflet index in relation
to HCN reaction and stem colour

Phenotypes HCN	Phenotypes stem	Number of plants observed	Central leaflet index mean	Sum of squared deviations Total	Sum of squared deviations Between groups	Sum of squared deviations within groups	Degrees of freedom T	Degrees of freedom B·G·	Degrees of freedom W·G·	Variance B·G·	Variance W·G·	Variance ratio F· calculated	F table 95%	F table 99%
+	all types	145	47·45**	15,818·83	631·25	15,187·58	169	1	168	631·25	90·402	6·98	3·89	6·76
-	all types	25	42·00											
all types	red	144	47·56**	15,818·83	837·98	14,980·85	169	1	168	837·98	89·17	9·40	3·89	6·76
all types	green	26	41·62											
+	green	20	41·70	794·16	·63	793·53	25	1	24	·63	33·06	·02	·25	6·23
-	green	6	41·33											
-	red	19	42·21	2,100·00	2·66	2,097·34	24	1	23	2·66	91·19	·03	·25	6·23
-	green	6	41·33											

** significant difference at the 99% level·

d) Floret length in the F_2 progeny of the hybrid L. japonicus x L. filicaulis.

Floret length was measured on 104 F_2 plants. Values obtained ranged from 0.80 cm to 1.20 cm with an average of 1.02 cm for the total population. It may be seen from Table 38 that floret lengths, in both the F_1 and the F_2 populations, are intermediate to the parental species and that the F_1 and F_2 floret lengths are very similar to one another (1.02 cm in the F_2 progeny as compared to 1.01 cm for the F_1 hybrids). The range of the F_2 hybrids overlaps that recorded for both the F_1 plants and the L. japonicus parent.

Attempts were made to relate the observed variations in floret lengths of the F_2 plants to the presence or absence of cyanogenetic glycoside and stem colour. Table 39 shows that when either the whole F_2 populations, or the green stem phenotype, is considered the differences observed in floret lengths between plants segregating for the HCN factor are not statistically significant at the 95% level. The same Table indicates, on the other hand, that the variations in floret lengths are statistically significant, at the 95% level, when populations with stem coloration are considered. However, in no instance were the differences observed to be significant at the 99% level.

Since at least some of the genes controlling floret length and central leaflet index appeared to be linked to the genes responsible for stem colour it was decided to calculate the least square line of relationship and the correlation coefficient of leaflet index to floret length in the F_2 population. It may be seen from Table 40 that the

Table 38

Comparison of floret lengths in parental F_1 and F_2 populations of the hybrid *L. japonicus* x *L. filicaulis*

	Number of plants observed	Floret length	
		range (in cm)	mean (in cm)
L. japonicus	10	1.00-1.20	1.11
L. filicaulis	10	.70-1.00	0.87
F_1 hybrids	4	.90-1.20	1.01
F_2 hybrids	104	.80-1.20	1.02

Table 39

F_2 progeny of the hybrid <u>L. japonicus</u> x <u>L. filicaulis</u>:

significance of floret length variations in relation to HCN reaction and stem colour

Phenotypes		Number of plants observed	Mean in mm	Sum of squared deviations			Degrees of freedom			Variance		Variance ratio		
CNH	stem			Total	Between groups	Within groups	T	B.G.	W.G.	B.G.	W.G.	F calculated	F table 95%	F table 99%
+	all types	93	10.276	809.150	16.823	792.327	103	1	102	16.823	7.768	2.17	3.94	6.90
-	all types	11	9.791											
all types	red	84	10.332*	809.150	47.659	761.490	103	1	102	47.659	7.466	6.40	3.94	6.90
all types	green	20	9.775											
+	red	77	10.36*	663.660	24.809	415.570	81	1	80	24.809	5.194	4.77	3.96	6.96
-	green	4	9.500											
+	green	16	9.844*	102.375	4.569	97.806	19	1	18	4.569	5.433	.841	.25	6.21
-	green	4	9.500											

* significant difference at the 95% level.

Table 40

Calculation of the least-squares line of relationship for central leaflet index and floret length in the F_2 progeny of the hybrid *L. japonicus* x *L. filicaulis*

Number of plants = 104	Solution of Y = a + bX
Floret length	
X = 1,062·00	104 a + 1,062 b - 4,645 = 0
X^2 = 10,925·50	
	1,062 a +10,925·50 b -47,487 = 0
Central leaflet index	
Y = 4,645·00	a = 37·7916
Product-sum	b = 0·6729
XY = 47,487·00	

central leaflet index value in any F_2 plant can be approximated from the equation $Y = 37{\cdot}39 + 0{\cdot}67X$ where Y is the estimated average for central leaflet index and X the value considered as average floret length in the sample. It may be noted that the choice of floret length as the dependent variable is purely arbitrary since no relation of causability can be expected between floret size and leaf narrowness. For this reason it appeared preferable to determine the correlation coefficient (r) of leaflet index to floret length. The r value, calculated by the product-moment method, was found to be equal to 0·228. Such a value of r shows that there is a positive correlation between central leaflet index and floret length and that this correlation, for $n = 100$, is statistically significant at the 95% level and almost significant at the 99% level (calculated value of $r = 0{\cdot}2282$ as compared to 0·2301 on Fisher's table).

e) Orange-red coloration and red stripes on mature florets in the F_2 progeny of the hybrid *L. japonicus* x *L. filicaulis*.

Each one of the 99 F_2 plants observed, in the growth chamber, for floret colour was found to have at least slight traces of red, or orange-red, striping on the standard of the mature floret.

Table 41 gives the results which were obtained in an attempt to classify the F_2 plants in respect to orange and red colour intensities of the florets.

Table 41

Striping on floret in the F_2 progeny of the hybrid

L. japonicus x *L. filicaulis*

Phenotype	Number of F_2 plants observed	Fraction in % of the total number of F_2 plants
Deep red (*filicaulis* phenotype)	6	6.06
Orange red (F_1 hybrid phenotype)	40	40.40
Light orange-red (recombinant phenotype)	41	41.41
Traces of orange-red (recombinant phenotype)	12	12.12
Absence of stripes (*japonicus* phenotype)	0	0.00

Measuring the colour intensity of a floret was difficult as a certain degree of variation is often observed from floret to floret on the same plant and from day to day for the same floret. The *japonicus* phenotype was defined as a state of complete absence for orange, or red, colour on all the mature florets of the same plant.

f) Pod mottling in the F_2 progeny of the hybrid *L. japonicus* x *L. filicaulis*

Pod mottling was recorded for the F_2 plants which were grown during the winter of 1962. In all, immature pods of 40 plants were examined. As pod mottling appeared to be a variable character, all developing pods from each plant were observed several times. Varying degrees of mottling of the pods were recorded on all but two of the plants, the latter expressing what I am calling the *japonicus* phenotype for pod colour, namely, immature pods completely green and devoid of mottling.

The results of an attempt of classification for the F_2 plants in respect to pod pigmentation are given in Table 42.

Table 42

Pod mottling in the F_2 progeny of the hybrid

L. japonicus x *L. filicaulis*

Phenotype	Number of F_2 plants observed	Fraction in percent of the total number of F_2 plants observed
Heavy mottling	7	17.50
Moderate mottling	24	60.00
Slight mottling	7	17.50
Absence of mottling	2	5.00

The two plants showing complete absence of pod mottling were observed to have the following phenotypes in regards to stem colour, HCN reaction, leaflet index and floret length:

Plant acc. no.	stem colour	HCN reaction	Leaflet index	Floret length
F_2 E 1030-56	red	+	48	.95 cm
F_1 E 1030-41	variegated	+	40	1.15 cm

It can be seen that the two plants with the japonicus phenotype for pod colour are similar in regards to their reaction for the HCN test and in their leaflet index while they differ somewhat from each other in respect to floret length and stem colour. The segregation ratio for pod mottling to non mottling equals 20:1.

g) Seed speckling in the F_2 progeny of the hybrid L. japonicus x L. filicaulis

From the 40 plants observed for pod colour, 34 produced seeds in sufficient quantity for an analysis of seed speckling. Approximately 10 seeds per plant were examined.

As indicated in Table 43 four different phenotypes for seed speckling were recorded. The segregation ratio of seed speckling to non speckling equals 10.33:1.00. It may also be seen from Table 43 that the japonicus phenotype for seed pigmentation (absence of speckling) was never observed in plants homozygous recessive for the HCN reaction (filicaulis phenotype) whereas, in one case, absence of speckling was recorded for a plant with the filicaulis phenotype for stem colour.

Table 43

Seed speckling, stem colour and HCN reaction in F_2 progenies of the hybrid

L. japonicus x *L. filicaulis*

Seed speckling	Red stem HCN +	Red stem HCN -	Green stem HCN +	Green stem HCN -	Total
Heavy	5	--	--	1	6
Medium	13	3	1	1	18
Slight	6	--	1	--	7
None	2	--	1	--	3

h) Pollen fertility in the F_2 progeny of the hybrid *L. japonicus* x *L. filicaulis*

As may be seen from Table 44 the percentages of stainable pollen for the 46 F_2 plants examined ranged from 6 to 80 with a mean of 41.50. This last number is higher than the average percentage obtained for the F_1 hybrids (33.28) but well below the values recorded for the parental species.

Table 45 lists the average percentages of stainable pollen in relation to phenotypic expression for the HCN factor and stem colour character in the population studied. It may be seen that pollen fertility, in the F_2 plants examined, remains somewhat constant for any given phenotype, the highest amounts of stainable pollen (49.14% and 50.23%) being recorded from plants expressing red stem coloration and lacking cyanogenetic glycoside.

i) Chromosome numbers in the F_2 progeny of the hybrid *L. japonicus* x *L. filicaulis*

Chromosome numbers were determined for the F_2 plants which expressed any one of the following characters:

-dwarfism or very slow growth

-high pollen sterility

-stem variegation

-very large leaflet size

A somatic number of 12, the same as that of the parental species, was found for each of the nine plants which were examined. In the F_2 plant E 1030-41 (variegated stem) thread like connections between

Table 44

Percentage stainable pollen in the F_2 progeny of the hybrid *L. japonicus* x *L. filicaulis*

Populations	Range	Mean	No. of plants observed
L. japonicus	98 - 99	98.70	3
L. filicaulis	66 - 93	76.20	4
F_1 hybrid	29 - 38	33.28	4
F_2 progeny	6 - 80	41.50	46

Table 45

Percentage stainable pollen in relation to the HCN and stem colour factors in the F_2 progeny of the hybrid *L. japonicus* x *L. filicaulis*

Phenotype		Number of plants observed	Average stainable pollen %
HCN	Stem colour		
+	all types	39	40·13
-	all types	7	49·14
all types	red	37	41·62
all types	green	9	41·00
+	red	31	39·94
+	green	8	40·88
-	red	6	50·33
-	green	1	42·00

some chromosomes of the complement were observed at metaphase in the archesporial cells (Figure 68). The karyotypes of the other plants studied appeared normal. As can be seen from Figures 68, 69, 70 and 71, it is particularly difficult to distinguish between the karyotypes of the F_2 plants and those of the parental species involved in this study. Yet, it may be noticed that the chromosomes observed in archesporial cells appear somewhat thicker and more contracted than the chromosomes examined in root tip cells. These differences in chromosome morphology from one tissue to another may be due to a process of karyotypic differentiation but the possibility exists that the variations observed resulted from the cytological techniques since the root tips were hydrolized and stained by the Feulgen method, whereas, the anthers were squashed, immediately after fixation, in a few drops of aceto-carmine.

B) F_2 and B.C.$_1$ progenies of the hybrid L. japonicus x L. alpinus

Observations were recorded for keel tip colour and bud striping on each of the 21 F_2 and 33 back cross plants which were raised to maturity. The results obtained are summarized in Tables 46 and 47. The results presented on seed speckling were obtained from the examination of five to six seeds per plant from the 14 plants which produced a sufficient quantity of seeds for observation.

a) Keel tip colour in the F_2 progeny of the hybrid L. japonicus x L. alpinus

As may be seen from Table 46 the 33 B.C.$_1$ plants segregated for keel tip colour in an approximately 1:1 ratio (1.36 black keel tip to

Somatic chromosomes of <u>Lotus</u>

from an archesporial cell and from root tips

Figure 68
Metaphase in an archesporial cell of the F_2 plant E 1030-41 from the hybrid <u>L. japonicus</u> x <u>L. filicaulis</u>
Magnification X ca. 2,400.

Figure 69
Metaphase in a root tip cell of the F_2 plant E 1030-41 from the hybrid <u>L. japonicus</u> x <u>L. filicaulis</u>.
Magnification X ca. 2,400.

Figure 70
Metaphase in a root tip cell of <u>L. japonicus</u>.
Magnification X ca. 2,400.

Figure 71
Metaphase in a root tip cell of <u>L. krylovii</u>.
Magnification X ca. 1,875.

Table 46

Keel tip colour in the B.C.$_1$ and F$_2$ progenies of the hybrid L. japonicus x L. alpinus

	Number of plants observed	Phenotype		Observed ratio
		Black keel	Yellow keel	
B.C.$_1$	33	19	14	136 : 1
F$_2$	21	16	5	3.20 : 1

Table 47

Bud colour in the B.C.$_1$ and the F$_2$ progenies of the hybrid L. japonicus x L. alpinus

	Number of plants observed	Phenotype		Observed ratio
		Orange-red bud	yellow bud	
B.C.$_1$	33	25	8	3.13 : 1
F$_2$	21	18	3	6 : 1

1 yellow keel tip) whereas the data obtained for the F_2 plants gave approximately a 3 : 1 ratio (3•20 black keel tip to 1 yellow keel tip). The calculating of chi square values (X^2 = •76 for the backcross and X^2 = •006 in the F_2 plants) indicated that the observed segregating distributions are not significantly different from those which should be expected if black keel tip was controlled by one dominant gene.

b) Orange-red striping on buds in the F_2 progeny of the hybrid *L. japonicus* x *L. alpinus*

Twenty five plants in the backcross population and 18 F_2 plants expressed orange-red coloration of the flower bud. Segregation ratios for flower bud coloration were calculated to be 3•13 : 1 and 6 : 1 for the backcross and F_2 crosses respectively (Table 47).

The chi-square test indicated that the 3•13 : 1•00 ratio, obtained for the $B•C•_1$ plants, is not significantly different from a 3:1 ratio whereas the 6 : 1 segregation distribution observed for the F_2 plants differs very significantly (X^2 = 5•40) from the 15 : 1 ratio which should be expected if two pairs of dominant genes were controlling flower bud coloration.

An attempt was made (Table 48) to find out if the gene for keel tip colour was linked to the gene controlling flower bud coloration. However, as the segregation ratio observed for the latter character in the F_2 population was considered to be somewhat distorted and possibly misleading, only $B•C•_1$ plants were used in a test for linkage. The *alpinus* phenotype (black keel tip - red bud coloration) was observed

Table 48

Test for independent assortment between genes controlling keel tip colour and bud colour in the backcross progeny of the hybrid *L. japonicus* x *L. alpinus*

Segregation ratio	Phenotype				Chi square		
	Black keel red bud	Yellow keel yellow bud	Yellow keel red bud	Black keel yellow bud	calculated	Table	
						95%	90%
observed	14	3	11	5	·520	·352	·584
expected if 3 linkage groups(1)	12·375	4·125	12·375	4·125			

(1) assuming complete dominance

in 14 B.C.$_1$ plants whereas the japonicus phenotype (yellow keel tip - yellow bud coloration) was recorded in only 3 plants. Among the 16 remaining plants expressing a recombinant phenotype, 11 possessed red bud - yellow keel tip coloration and 5 yellow bud - yellow keel tip coloration. This corresponds to a distribution of 14:3:11:5 which is not significantly different, at the 90% level, from the 3:1:3:1 ratio which should be expected if the genes controlling keel tip colour and flower seed colour are located on three different linkage groups.

c) Speckling on seeds in the F$_2$ progeny of the hybrid L. japonicus x L. alpinus.

The seeds of 6 F$_2$ and 8 B.C.$_1$ plants were examined with the aid of a dissecting microscope. From five to six seeds were observed for each plant. In each case an association was recorded between seed speckling and keel tip colour. Ten of the 14 plants examined expressed the alpinus phenotype for both characters (black keel tip and speckled seeds) whereas the japonicus phenotype (yellow keel tip and non speckled seeds) was observed on the four remaining plants. These results suggested that a single dominant gene, closely linked to the locus controlling keel tip coloration, was responsible for the seed speckling condition.

DISCUSSION

I. Morphology of the parental species and their hybrids

A) Morphology of the parental species

It has been shown that, morphologically, the six parental species involved in the present study closely resemble one another. Yet, for almost all characters studied, small variations to occasional large differences were observed between the species which confirmed the morphological distinctness of each taxon. Since all the plants were observed under identical environmental conditions in the greenhouse or in the growth-chamber it is, furthermore, quite evident that the morphological interspecific differences observed were actually reflecting the genotypic distinctness of each species. It is however possible, as has been pointed out by Larsen (1954) for certain species in Southern Europe, that a different environment might increase the polymorphism of each species and consequently would decrease their morphological distinctness. Nevertheless, it should be remembered that what Larsen considered as a large polymorphic species might in fact be a number of separate taxa.

A perusal of the results shows that a number of quantitative characters, in addition to the classical markers such as floret striping, keel tip colour and HCN reaction,proved to be extremely valuable for the identification of the parental species. These quantitative characters were floret size, central leaflet length, central leaflet index and the number of florets per inflorescence. On the basis of the results obtained it is suggested that the following key for distinguishing between the six species that were used in the study be adopted:

a) The number of florets per inflorescence ranges from
one to four . . . . *L. japonicus*
L. alpinus
L. schoelleri
L. krylovii
L. filicaulis

b) Deep yellow florets of large size . . . . *L. japonicus*
L. alpinus

c) Absence of floret striping, absence of pod mottling and absence of seed speckling; central leaflet length approximately equal to floret length . . . . *L. japonicus*

cc) Striped floret, mottled pod and speckled seed; central leaflet length approximately equal to one third of the floret length . . . . *L. alpinus*

bb) Pale yellow florets of small size . . . . *L. schoelleri*
L. krylovii
L. filicaulis

c) Seed very heavily speckled; average central leaflet index between 40 and 50 . . . . *L. schoelleri*

cc) Seed not very heavily speckled; average central leaflet index between 30 and 40 . . . . *L. krylovii*
L. filicaulis

d) The central leaflet is narrowly-rhomboidal *L. krylovii*

dd) The central leaflet is narrowly-elliptical *L. filicaulis*

aa) The number of florets per inflorescence ranges from two to eight . . . . *L. tenuis*

It may readily be seen from this key that several of the parental species differ from one another in only minute characters such as floret size or leaflet shape. Such differences may appear of very limited value from a practical or agronomic point of view. It should be remembered, however, that small taxonomic variations have often been found to be correlated to characters of greater importance in plant breeding. Suffice it to recall here the studies of Clausen and Hiesey (1958) who reported, in Potentilla, a significant correlation between a number of taxonomic attributes, such as petal width, sepal length, petal colour, presence of anthocyanin and leaf length, and various agronomic characters like frost resistance, winter dormancy, crown height and flowering time. Similar studies have not been carried out for the species involved in the present study but the possibility exists that taxonomic and agronomic characters may be correlated in diploid species of Lotus.

B) Morphology of the interspecific hybrids

In addition to more vigorous growth and profuse branching hybrid vigor was demonstrated for one or more of the several quantitative characters evaluated in the F_1 hybrids. Heterosis was considered evident, however, only when the half confidence interval did not exceed the difference between the hybrid mean and the mean of the parent exhibiting the greatest measurement for the character under consideration. In regards to central leaflet length, hybrid vigor was expressed in all hybrid plants of the crosses L. japonicus x L. krylovii, L. krylovii x L. japonicus and L. schoelleri x L. japonicus. On the basis of one of G.H. Shull's (1952) definitions of heterosis, namely, "Heterosis is recognized as the interaction of unlike gametes", this finding is not surprising since both *L. schoelleri* and *L. krylovii* were found to be morphologically distinct from *L. japonicus* for a large number of characters.

It must be said, however, that the statistical computations performed for the floral characters, namely floret length, standard width, ovary length and style length, seem to contradict the rule that hybrid vigor becomes more evident when the morphological differences increase between the parental species. Whenever a large flowered species (*L. japonicus*) was crossed to a small flowered one (that is to say, when the cross involved species showing considerable morphological differences) no heterosis for floret size was detected in the F_1 hybrid; when, on the other hand, a small flowered species was crossed to another small flowered species (that is to say, to a species closely similar in overall morphology) the manifestation of hybrid vigor for floret size in the F_1 hybrid was clearly demonstrated. This information seems to indicate that heterosis for floret size in the F_1 hybrids is not entirely dependent upon heterozygosity but possibly reflects, as it has been suggested by Bruce (1910) to explain hybrid vigor, the result of the combined action of specific dominant factors. The fact that a significant difference in the expression of hybrid vigor was found for central leaflet length and ovary length between reciprocal hybrids of the cross *L. japonicus* x *L. krylovii* and of the cross *L. japonicus* x *L. schoelleri* might be interpreted as an argument favouring A. F. Shull's (1912) early hypothesis, namely, that heterosis could be attributed to the interaction of a changed nucleus with a relatively unaltered cytoplasm. However, with the exception of the two characters listed above, differences between reciprocal hybrids were small and not significant. It is therefore difficult to propose a single explanation for the origin of heterosis in the Lotus hybrids. As shall be discussed later, the possibility also exists that heterozygosity, as observed for stem coloration in some advanced progenies of the hybrid L. japonicus x L. filicaulis, allowed the production of alternative genetic pathways from within the hybrid genotype. Such a finding, if it was confirmed, would support Lewis' hypothesis (1954) that heterosis is due to the capacity of the heterozygote to use alternatively the product of two different alleles.

In regards to qualitative characters, the F_1 hybrids generally exhibited, with slight variations in intensity, the phenotype of one of their two parents. Scott-Moncrieff's rule (1936) that the presence of a pigment is usually dominant over its absence was verified in all cases since red stem colour, floret striping, black keel tip, pod mottling, and seed speckling were found to be inherited in a dominant manner. The results obtained corroborated the work of several authors who had previously reported that presence of HCN (Dawson, 1941), keel tip colour (Bubar, 1958) and seed speckling (Donovan, 1957) were governed by dominant factors. In the case of seed colour, however, the phenotype of the F_1 hybrids was often found to be different from either parent, suggesting the possibility of gene interaction. In spite of the uniform environmental conditions in which the hybrids were studied, some characters, like style curvature and pubescence, were found to vary from plant to plant within a given species and within a given cross. This polymorphism suggests that the parental species were segregating for the pubescent and the style curvature characters. For all other qualitative characters observed, the differences between hybrid plants derived from the same cross were slight and non indicative of gene segregation in the species. From the fact that no differances were observed between reciprocal hybrids for the characters controlling coloration of stems, florets, pods and seeds it can also be concluded that cytoplasmic inheritance was not involved in the transmission of pigment-producing factors.

The F_1 hybrids were generally intermediate to their parents in overall morphology. In the case of the cross L. japonicus x L. alpinus , however, the F_1 hybrid was found to resemble the male parent for most characters investigated. In the case of the cross L. japonicus x L. filicaulis, which may be considered as a representative sample of the hybrids examined in the present study, both parental species contributed dominant characteristics to the F_1 hybrid, two from L. japonicus and four from L. filicaulis.

II. Fertility in the parental species and in the hybrids

A) Pollen stainability in the species

At least three suggestions can be made to explain the relatively high frequency of aborted grains observed in L. alpinus, L. filicaulis and L. krylovii. Pollen abortion, in these species, could be the result of environmental factors such as light, temperature and humidity.

This possibility has been advanced by a number of investigators (Webster, 1950; Magoon et al., 1958; Howard, 1960) to account for pollen abortion in supposedly good species of plants. Schark (1957 and 1958), who suggested that pollen sterility in Solanum tuberosum could be due to the plasmolysis of the cells of the inner tapetum and the pollen mother cells, has shown that this plasmolysis was the result of a nutritional deficiency caused by high temperatures. A second possible explanation may be that pollen abortion in these species is governed by a number of recessive genes, the expressivity of which would be increased by close inbreeding. This hypothesis would be consistent with the fact that the self-sterile species, namely L. tenuis, produces pollen which stains close to one hundred per cent. Schonhorst (1958) in studies with the related genus Medicago, has shown that at least three genes controlled pollen abortion in M. falcata, two of which were additive in effect. Schonhorst found that plants homozygous recessive for all three genes were more than 50% pollen sterile and very sensitive to environmental changes. Pollen abortion, as observed in M. falcata, was not related to ovule abortion, meiotic irregularities, or plant vigor. It is possible that a number of sterility genes, similar in effect to the ones described by Schonhorst, are actually responsible for pollen abortion in the species reported here. A third hypothesis that can be suggested to explain pollen abortion in these species is that they are of relatively recent origin. Various processes such as hybridization among diploid taxa, or, parthenogenesis in tetraploid populations, would account for such pollen abortion. This last assumption appears reasonable, at least in the case of *L. alpinus* where the highest percentage of pollen abortion was recorded, if Favarger's hypothesis (1953) is correct, namely, that *L. alpinus* is a parthenogenetic haploid derived from the tetraploid *L. corniculatus* var. *glacialis*. Whether or not this theory can be extended to the two other species, *L. filicaulis* and *L. krylovii*, is not known. Larsen (1954) has suggested that the occurrence of natural hybridization among various species of *Lotus* is not a rare event in Southern Europe. It is therefore possible that pollen abortion in *L. filicaulis* and *L. krylovii* may result from the incorporation of foreign germ plasm through a process of restricted hybridization which took place, in the past, in the natural habitats of these two species. The limited observations of meiosis made for *L. filicaulis* and *L. tenuis* at any rate would indicate that pollen sterility, if genotypically controlled in the species, is due to gene action and not to irregularities in the meiotic process.

B) Pollen stainability in the interspecific hybrids

The influence of the genetic constitution of the parental species upon pollen abortion in the hybrids is very evident from an examination of the high percentages of pollen sterility recorded within each cross and the differences observed from cross to cross. From the meiotic studies, which shall be discussed in another section, it seems that pollen sterility in the hybrids is not exclusively due to cytological abnormalities but, possibly, also the result of gene action.

Moreover, the analysis of variance carried out in order to test the significance of the differences observed in pollen stainability between reciprocal hybrids revealed the presence of some kind of cytoplasmic control upon pollen abortion. As can be seen from Table 28 the hybrid plants with a krylovii x japonicus constitution have a significantly greater pollen fertility when L. japonicus is used as the female parent. This suggests, in turn, that the presence of a japonicus genome in a foreign cytoplasm (in this case a cytoplasm from either L. krylovii or L. schoelleri) actually increases the percentage of pollen abortion.

It is, however, more difficult to explain the plant to plant variability observed for pollen abortion within each cross. It may perhaps be that some of the parental species used in the study were segregating for genes controlling pollen sterility. As can be seen from Table 25 this seems particularly true in the case of the hybrid plants derived from crosses between L. japonicus and L. schoelleri, and L. japonicus and L. krylovii. It is also possible that cytoplasmic factors are involved in the variations recorded for pollen stainability within each cross. Lesins (1961), in his study of hybridization between various species of Medicago, found that fluctuations in the fertility of hybrids derived from crosses between the same species depended upon the cytoplasm of the plant which was used as the maternal parent. A similar situation may be involved here. Yet, it should be remembered that pollen abortion in hybrid plants with a similar cytoplasmic constitution has often been reported to vary from plant to plant and, for a given plant, from flower to flower (Webster, 1950; Magoon, Cooper and Hougas, 1958). An excellent example of this variability is given by Webster (l.c.) who observed, in interspecific hybrids of Melilotus, a correlation between the percentage of aborted pollen and the position of the raceme on the plant. Webster also noted some variations in pollen stainability among plants which had been vegetatively propagated from the same F_1 clone. It is possible, therefore, that some of the differences observed in the present study between hybrid plants derived from the same cross are due to the action of environmental factors.

C) Seed set per pod in the species

L. alpinus is the only species which does not produce a high number of seeds per pod under open pollinated conditions. It is tempting to correlate the low seed set to the high percentage of pollen abortion recorded for this species. However, it is possible that the low seed set recorded for L. alpinus is not entirely due to abortive gametogenesis but may also result from incompatibility mechanisms or embryonic lethality. In the case of plants of L. filicaulis and L. krylovii, which produced a small quantity of non stainable pollen but at the same time produced high numbers of seeds per pod, it would seem that pollen abortion is not correlated to the number of seeds per pod. This finding is in agreement with the work of Koffman and Wilsie (1961) who indicated that pollen fertility in a commercial strain of alfalfa showed very little relation to fertility as determined by seed set.

D) Seed set per pod in the interspecific hybrids

Since the average seed set per pod was low in all hybrid plants observed, one might consider that the production of F_2 populations would be a rare event if the F_1 hybrids were allowed to grow, in nature, under open pollinated conditions. Yet, it must be remembered that each hybrid plant produced at least a few seeds which proved to be viable under artificial conditions. It is therefore not possible to state conclusively that the low seed set observed for the hybrids constitutes an absolute reproductive barrier between the parental species.

The finding that the seed set index increased when pollen stainability decreased seems to indicate that either the percentage of normal ovules does not correspond to the percentage of stainable pollen or that other factors, in addition to gametic abortion, are actually controlling seed set in the hybrids. A somewhat similar conclusion was reached by Sprague (1956) who failed to detect, in hybrid plants of *Medicago*, a relationship between fertility and production of normal pollen. In the present study it is obvious, however, that pollen abortion contributes to decrease the fertility of the hybrids. The point debated here concerns the possibility that other factors, in addition to abortive gametogenesis, are actually responsible for the low seed set recorded.

III. Meiosis in the parental species and in the hybrids

A) Meiosis in the species

The few meiotic irregularities recorded for the species *L. japonicus*, *L. tenuis* and *L. filicaulis* do not seem to be correlated to the frequencies of non stainable pollen tabulated for these three species. This information is important as it implies that pollen abortion in the species is probably under gene control. The possibility remains, however, that pollen sterility is the result of irregularities in chromosome behaviour during the stages of microsporogenesis which were not examined here. There does not seem to be, for the time being, any "a priori" reason to favour this hypothesis. In regards to the meiotic abnormalities recorded at first metaphase and first anaphase it is interesting to note that Webster (1950) reported similar irregularities in recognized species of Melilotus, a closely related genus in the Leguminosae. This author did not attempt, however, to account for the possible causes of the cytological aberrations, that he observed. Datta (1955), in a cytogenetic study of Lathyrus, another genus of the Leguminosae, recorded a high percentage of pollen sterility and several meiotic abnormalities, such as inversion bridges, non disjunction and laggards in L. sativus and L. odoratus. It would appear, therefore, that pollen abortion in these latter species was the direct consequence of irregularities in the meiotic process. In Lotus, however, the data available do not support this view.

B) Meiosis in the interspecific hybrids

If one recalls that at least 60% of the cells observed at MI and AI were tabulated as normal it is difficult to explain, on the sole basis of meiotic irregularities, the very high percentages of pollen abortion recorded for some of the hybrids studied here. Factors possibly contributing to the pollen degenerescence observed in these hybrids may be small deletions and/or duplications resulting from the presence of minute chromosomal differences in the parental species. A classical example of this type of chromosomal sterility is that described for the hybrid between *Primula verticillata* and *P. floribunda* (Darlington, 1937). In this hybrid the chromosomes can pair and form chiasmata in an apparently normal fashion. Nevertheless, the gametes produced are inviable because they contain unbalanced and disharmonious combinations of genes. The situation in the *Lotus* hybrids may possibly be analoguous to that described above for the *Primula* hybrid. If this was the case, one could expect that the amphidiploids, eventually obtained after somatic doubling, would be fertile. Such amphidiploids, developed from *Lotus* hybrids, may prove to be valuable for agronomic purposes.

It is even more difficult to explain, on the sole basis of the meiotic irregularities observed during microsporogenesis, the low seed set recorded for the F_1 hybrids. In his studies of British Primulas, Woodell (1960) has advanced the hypothesis that seed failure in interspecific hybrids could be due to quantitative differences, resulting from gene duplication, in a genome of one of the parental species. Woodell considers that the results of crosses between species with the same chromosome number but possessing quantitatively different genotypes can be regarded as similar in effect to those between species with different chromosome numbers. Since there is, at present, no evidence to substantiate this theory in *Lotus* it seems more reasonable to simply conclude that seed failure in the F_1 hybrids is not entirely the result of gross structural abnormalities in the meiotic process.

The presence of meiotic irregularities in the hybrids, as observed in 10 to 40 per cent of the cells examined, indicates, however, that the chromosomes of the parental species are not completely homologuous and are undergoing a process of differentiation. This seems particularly true in the case of the smallest chromosomes which were often observed as univalents during MI. Multivalent pairing, as occasionally observed in some of the hybrids, may represent the visible remnants of ancient translocations by means of which some of the chromosomes differentiated from their homologuous partners.

It is difficult to explain the presence of the inter PMC bridges which were observed in the hybrid *L. japonicus* x *L. alpinus*. Gates, in 1911, observed a frequent migration of chromatic material from one microspore into another and called it cytomixis. Since then, cytomixis has been reported in several other instances (Gates and Latter, 1927; Gelin, 1934; Sarvella, 1958). According to Sarvella (1956, 1958) cytomixis in species and hybrids of *Gossypium* is due to incomplete cell wall formation and leads to binucleate PMC's or to PMC's with an increased number of chromosomes. These latter abnormalties were not observed in the cells examined of the hybrid *L. japonicus* x *L. alpinus* in which the inter PMC bridges were found. It should also be noted that Woodworth (1931) artificially induced cytomixis when he applied extra pressure on the pollen mother cells in their preparation for cytological examination. The same author observed that cytomixis was more frequent in hybrids than in other plants and attributed this to a state of unbalance in the heterozygous cytoplasm which made the pollen mother cells more sensitive to pressure. Since in the case under consideration the inter PMC bridges were recorded in only one species cross, it is not likely that the phenomenon observed in *Lotus* was caused by faulty preparation. It is considered, therefore, that cytomixis occurred in the hybrid *L. japonicus* x *L. alpinus*.

The fact that there was a greater number of chromosome irregularities observed in the hybrids L. krylovii x L. filicaulis and L. schoelleri x L. krylovii than in the hybrids L. japonicus x L. alpinus and L. tenuis x L. filicaulis may indicate that L. krylovii is somewhat more distantly related to L. filicaulis, L. tenuis and L. schoelleri, whereas, L. tenuis and L. alpinus would appear to be more closely related to L. filicaulis and L. japonicus, respectively. Such speculations are in agreement with the diagram given in Figure 26 to indicate relationships between species in terms of pollen stainability recorded for the interspecific hybrids.

IV. Inheritance studies

A) F_2 progeny of the cross L. japonicus x L. filicaulis

The 5.5 : 1 ratio recorded for the segregation of cyanogenetic glycoside suggests that either more than one gene is involved in the inheritance of this character or that HCN production is governed by a single dominant gene, the recessive allele of which has been eliminated, or modified, in certain genotypic backgrounds. Preference is given here to the hypothesis that some of the HCN negative genotypes were eliminated at an early stage, possibly during microsporogenesis, since more than 65% of the pollen grains in the F_1 parent were tabulated as abnormal, and therefore, a single dominant gene could be responsible for the HCN character. It must be remembered, furthermore, that a number of weakly reacting F_2 plants, similar to the slightly positive plants described by Dawson (1941) and Seaney (1957), were observed in the present study and recorded as reacting positively to the HCN test. The classification of these slightly positive phenotypes into a third category would have reduced, to some extent, the numerical differences between the expected 3:1 (positive to negative) distribution and the observed distribution for the HCN character. The deviation observed from the expected ratio is however too great to be explained on this basis alone. If a single gene controls the positive reaction to the HCN test one would have to conclude, in order to account for the deficiency of recessive phenotypes, that the presence in double dose of the HCN character is lethal in certain genetic backgrounds. A similar conclusion was reached by Hart and Wilsie (1958) in their study on the inheritance of brown keel tip in *L. corniculatus*. These authors observed a deficiency of recessive phenotypes in a number of progenies and explained this deficiency by assuming that the homozygous recessive genotypes for keel colour were lethal when combined with another homozygous recessive locus. Another comparable finding was made in the closely related genus of *Melilotus* by Baenziger and Greenshields (1958) who reported that a number of genotypes homozygous for low coumarin content were eliminated in the offspring of interspecific hybrids. The situation described by these authors and by Hart and Wilsie (l. c.) appear to be strikingly similar to the one investigated in this *Lotus* cross.

The segregation ratio obtained for stem colour is almost identical to that found for the HCN character, suggesting that, here also, a number of homozygous recessive genotypes had been eliminated or prevented in being formed. Yet, in this case, the possibility that only a single dominant gene actually controls stem colour is perhaps more challengeable as at least five different phenotypes were observed for stem colour in the F_2 progeny investigated (Plate II). In this respect the finding that five F_2 plants expressed what I am calling stem variegation, that is to say a band of green from 3-5 centimeters at the base of the stem and red coloration above this band, is of particular interest as it is indicative of instability in gene action and suggests the presence of modifying factors. It is interesting to note that Jaranowski (1962) observed a comparable situation in advanced progenies of the interspecific hybrid Melilotus polonica x M. alba where a number of plants exhibited leaf variegation at the seedling stage. In respect to the situation observed in Lotus it seems that stem variegation may be due to the failure of the gene responsible for red stem colour to produce the red pigment in the lower portion of the stem whereas environmental conditions or enzymatic activity at other loci may have induced the appearance of red pigmentation in the upper part of the stem. This phenotypic instability could be due to a number of causes. For instance, it may be that the plants expressing variegation are heterozygous at the stem colour locus and that, for some unknown reason, only the recessive allele expresses itself in the lower portion of the stem. It is possible that environmental variations in the growth-chamber modified gene action at certain developmental stages and that a number of genotypes reacted to these changes by producing a variegated phenotype. In spite of the fact that the parental species were not observed to segregate for stem colour, the possibility remains, however, that a multiallelic locus was controlling the presence, distribution and intensity of red coloration on the stems of the F_2 plants. Finally, as a last eventuality, it may be suggested that the stem colour character is governed by more than one locus. On the basis of the segregation ratio observed and of the apparent uniformity of the parental populations the hypothesis preferred here, however, is that a single diallelic gene controls stem coloration in the F_2 progeny and that the expressivity of this gene is very sensitive to the genotypic background and/or environmental influence.

The chi square analysis, which indicated that the stem colour and the HCN genes were not located on the same linkage group, also suggested that a number of homozygous recessive genotypes for both HCN reaction and stem colour were eliminated in certain genetic backgrounds. Such an hypothesis is consistent with the distorted segregation ratios which were observed when each character was considered separately.

The leaflet index values of the F_2 plants derived from the cross L. japonicus x L. filicaulis were significantly higher in populations expressing either the HCN or the red stem phenotypes. This would suggest that genes controlling central leaflet size are located on both the stem colour and the HCN linkage groups. Yet, no cumulative effect was observed in plants with the dominant phenotype for both stem colour and HCN reaction. It is possible that the homozygous dominant genotypes for these two characters were eliminated through a mechanism similar to the one previously suggested to explain the deficiency of homozygous recessive genotypes. Such an elimination of the parental genotypes would maintain heterozygosity in the F_2 population and would provide a suitable explanation for the absence of any cumulative effect in plants expressing the dominant phenotype for both stem colour and presence of HCN.

The absence of clear cut segregation ratios for width of the leaflet in the F_2 plants coupled with the fact that the range of the leaflet index in the F_2 population did not overlap that of the parental species appears to indicate that leaflet width is probably governed by a large number of genes. Scossiroli, Ferrari and Haussman (1960) estimating heritability and environmental variations for length and breadth in *Medicago sativa* have reached a somewhat similar conclusion, namely, that leaflet size was governed by a large number of genes and subject to environmental variations. The hypothesis that several genes are involved in the control of leaflet size is in disagreement, however, with Donovan's report that the inheritance of leaf size in tetraploid *L. corniculatus* is chiefly governed by a single gene with incomplete dominance. Yet, it should be remembered that Donovan studied intraspecific segregation at the tetraploid level whereas the present study is concerned with interspecific gene inheritance at the diploid level. Furthermore, Donovan expressed his results in terms of leaf areas while the central leaflet index was used to represent leaflet size in this investigation.

Similarly, from the absence of clear cut segregation for floret length as observed in the cross *L. japonicus* x *L. filicaulis*, it would seem that many genes are involved in the control of this character.

The analysis of variance for floret length in populations segregating for the stem colour and the HCN genes indicates that the HCN linkage group does not carry any gene responsible for floret length. In addition, these data suggest that the stem colour factor does not segregate independently from floret length. Since evidence had been obtained for linkage between leaflet width, as expressed in terms of leaflet index, and stem colour it became logical to expect, in turn, a correlation between floret length and leaflet index. This was actually demonstrated by means of the correlation analysis which indicates that floret length and leaflet index are positively correlated in the F_2 progeny. Therefore, it is considered that at least some of the genes controlling floret length are located on the linkage group containing those responsible for leaflet width and stem colour.

Several explanations may be suggested to account for the absence of japonicus phenotypes for floret striping in the F_2 populations of the cross L. japonicus x L. filicaulis. For example, it may be considered, as in the case of HCN and stem colour characters, that the japonicus phenotype for floret striping is governed by genes which are linked to one or more recessive lethals. Another possible explanation for the complete absence of the japonicus phenotype for floret striping is that the number of genes responsible for striping is so large that the homozygous recessive phenotypes (florets not striped) did not occur in the limited sized population which was examined. This hypothesis would be consistent with the fact that several phenotypes, ranging from extremely light orange-pink coloration to a deep red colour, were recorded for floret striping in the F_2 progeny. Further support for this hypothesis is provided by the studies of Twamley (1955) and Lepper and Odland (1939) in the inheritance of flower colour in the genus *Medicago*. These authors indicate that at least four different non allelic genes may be involved in the control of purple and yellow flower colour in alfalfa. Lepper and Odland (l.c.) have postulated the presence of two dominant complementary genes that would influence, in varying degrees, the action of two unlinked loci responsible for purple and yellow flower colour, purple being epistatic to yellow. There is, however, a third explanation for the absence of *japonicus* phenotypes in the F_2 population, namely, a maternal effect. The mechanism suggested here would be somewhat similar to the one reported by Kuhn (in Sinnot, Dunn and Dobzhansky, 1958) for the moth *Ephestia kuhnniella* in which the F_1 hybrid, heterozygous for a colour factor, produces pigments which are transmitted through the cytoplasm of each egg. The subsequent embryos develop into individuals which exhibit, to a variable degree, the F_1 phenotype and this independently of their genetic constitution. However, there is insufficient evidence, at the present time, to indicate which one of these three hypotheses for explaining the absence of floret striping is the most plausible.

From the limited data recorded for pod mottling in the F_2 progeny of the cross *L. japonicus* x *L. filicaulis* it is considered that two pairs of dominant genes control inheritance for this character. The fact that the only two plants which exhibited the *japonicus* phenotype for pod mottling (complete absence of mottling) also expressed the *japonicus* phenotype for stem colour and the presence of HCN may indicate that one of the dominant genes controlling pod mottling is linked to the HCN locus, whereas, the other gene would be located on the stem colour linkage group. Additional data shall be required, however, in order to verify this hypothesis.

The approximately 10:1 segregation ratio obtained for the seed speckling character in the F_2 population of the cross *L. japonicus* x *L. filicaulis* is significantly different, at the 95 per cent level, from the 3:1 distribution that would be expected if, as indicated by Seaney (1961) in *L. tenuis*, seed speckling is governed by a single dominant gene. It is therefore possible that the segregation ratio obtained for the seed speckling character is under monogenic control but was modified by some process similar to the one suggested for the stem colour and the HCN characters. Such a hypothesis is consistent with the results of several investigators who have reported seed speckling to be inherited as a single mendelian dominant in other genera of the Leguminosae. In *Melilotus officinalis*, for instance, Stevenson (1937) observed seed speckling to be governed by a single dominant gene, the expression of which appeared to be partially under environmental control. In *Phaseolus lunatus*, a similar report was made by Allard (1953) who categorized three different phenotypes - restricted mottling, diffuse mottling and absence of mottling - which were governed, respectively, by the three alleles of a single gene. The possibility remains, however, that more than one gene controls seed speckling in the cross *L. japonicus* x *L. filicaulis*. Donovan (1957) investigated two different types of seed speckling in *L. corniculatus*. In the first type he found that speckling was controlled by a limited number of genes, the expression of which could not be clearly distinguished from environmental effects. In the second type, he postulated a disomic mode of inheritance involving two pairs of genes. An even more complex type of inheritance was reported by Smith (1939) for seed speckling in *Phaseolus vulgaris*. This author observed that the speckling phenotype was influenced by a second dominant gene, located at another locus, which had no effect upon the recessive phenotype for speckling. Additional studies shall be required, however, in order to determine the exact number of genes controlling the seed speckling character for the species involved in the present study.

The possibility exists that there may be linkage between the seed speckling and the HCN characters since absence of speckling (*japonicus* phenotype) was never observed for F_2 plants which reacted negatively to the HCN test (*filicaulis* phenotype). In contrast, the stem colour factor appears to segregate independently of the seed speckling character.

The average increase in pollen stainability recorded over the F_1 parental hybrid for the F_2 populations of the cross *L. japonicus* x *L. filicaulis* as well as the high percentage of stainable pollen and the general vigor observed in some segregates underline the potential value of the hybrid plants as material for agronomic studies.

The hypothesis that the high frequency of pollen sterility noted in some of the F_2 plants may be due to a state of aneuploidy is not considered to be applicable in this case as in the individuals examined which expressed a very low percentage of pollen stainability, the normal somatic chromosome number of 12 was recorded in each case. It is therefore considered more likely that pollen abortion in the F_2 plants resulted from meiotic abnormalities or from segregation of sterility genes. Concerning the latter possibility it must be remembered, however, that it has not been possible to find any correlation between the variation in percentage stainable pollen and other morphological characters.

Since a normal somatic number of chromosomes was found in the F_2 plants which expressed an abnormal phenotype it seems appropriate to consider that stem variegation, dwarfism and extreme vigor resulted from gene or nucleocytoplasmic interaction. However, the interchromosomal connections which were observed at metaphase in the archesporial cells of one of the plants with a variegated stem suggested the possibility that stem variegation may actually be due to some irregularity in the mitotic process. Yet, it should be remembered that all the somatic metaphase plates examined in the other individuals with variegated stems were devoid of any such interchromosomal connections and tabulated as normal. Hence stem variegation is more likely to be the result of some environmental condition.

B) F_2 and $B.C._1$ progenies of the cross *L. japonicus* x *L. alpinus*

The modifying factors which were considered to be responsible for the distorted segregation ratios recorded in the F_2 populations of the cross *L. japonicus* x *L. filicaulis* did not seem to operate upon the segregates of the cross *L. japonicus* x *L. alpinus*. This appears to be particularly true for the keel tip character which segregated in clear-cut ratios suggestive of single dominant gene inheritance. It is interesting to recall, in this respect, the work of Hart and Wilsie (1958) who observed a deficiency of recessive genotypes in a number of progenies of L. corniculatus segregating for brown keel tip. No deficiency of recessive phenotypes for the keel colour character was observed in the present study.

Floret striping, on the other hand, seems to be governed by more than one gene. In this case, however, the situation appears to be more complex since the segregation frequency obtained for the F_2 population is significantly smaller than the 15:1 ratio which should be expected if, as suggested by the back-cross ratio, two pairs of dominant genes actually control the inheritance of floret striping. It is possible that one or several mechanisms, such as ovule abortion or embryo lethality, operated against randomized segregation in the F_2 plants. Bubar (1958) has reported that the keel tip colour alleles in L. corniculatus may be linked to an incompatibility locus. The results found for floret striping in this study may be comparable to those found for keel tip colour by Bubar in that the genes responsible for floret striping are possibly linked to one or several loci influencing breeding behaviour. It may also be assumed, in order to account for the aberrant ratio observed in the F_2 population, that a number of very faintly striped phenotypes were erroneously tabulated as lacking stripes. This eventuality may have actually occurred since the plants were examined in the field under light conditions which varied from day to day, and the intensity of striping was observed to be under environmental control.

The data suggest very strongly a probability of linkage between keel tip colour and seed speckling, as non speckled seeds (japonicus phenotype) were never collected from plants exhibiting the alpinus phenotype (black keel tip) for keel tip colour, nor speckled seeds from plants which expressed the japonicus phenotype (yellow keel tip) for keel tip colour. Such a hypothesis is in agreement with data of Donovan (1957) who showed that brown keel tip in L. corniculatus was associated with heavy mottling of the seed coat.

CONCLUSION AND SUMMARY

With the increasing importance of L. corniculatus L., a tetraploid species ($2n = 24$), as a forage crop, it became clear that a better understanding of the diploid species of the genus was needed both for forage improvement in determining the feasibility of transferring desirable characters from wild species, and for answering questions regarding the genetic relationships among the species. In order to elucidate these questions, the cytogenetics of 53 interspecific hybrids derived from ten different crosses between six diploid species of Lotus, closely related to L. corniculatus, was investigated and a detailed study of the inheritance of some specific characters in F_2 and back cross progenies of the interspecific hybrids was performed. The six species used in the study were L. japonicus (Regel) Larsen, L. alpinus Schleich., L. filicaulis Dur., L. schoelleri Schweinf., L. krylovii Schischk. and Serg. and L. tenuis Waldst. et Kit.

1) Morphology of the parental species: The different morphological characters studied, namely, growth-habit, stem colour, HCN reaction, floret colour, floret striping, keel tip colour, pod mottling, seed speckling, style curvature, pubescence, central leaflet length, central leaflet index, central leaflet width, floret length, standard width, ovary length and style length varied between each of the six taxa employed in this study from minor to highly significant major differences, indicating the distinctness of each parental species. On the basis of the morphological comparison carried out on the plants growing in the greenhouse, the six species can be classified into two main groups:

the first group includes two species, namely, L. japonicus and L. alpinus, which both possess the largest florets, red stems and a decumbent or procumbent growth habit. The second group comprises the four other species which are characterized by small florets, green stems, and an ascending growth habit. While it is relatively easy to separate L. japonicus from L. alpinus in the first group, it is much more difficult to distinguish between L. schoelleri, L. tenuis, L. krylovii and L. filicaulis of the second group. However, each of these latter species may be distinguished from the other three by at least one or more morphological traits. L. schoelleri is characterized by a larger central leaflet index and a very heavy speckling of the seed. L. tenuis is the only species to exhibit more than three florets per inflorescence. L. filicaulis can be differentiated from L. krylovii by the shape of the central leaflet which is elliptical in the former species and rhomboidal in the latter one.

2) Pollen stainability in the parental species: Whereas essentially complete stainability of the pollen was recorded in L. japonicus, L. schoelleri and L. tenuis, a relatively high frequency of aborted pollen grains, ranging from 10 to 50%, was observed in L. alpinus (46%), L. filicaulis (24%) and L. krylovii (15%). The limited observations of meiosis which were made for these latter species indicated that pollen sterility, if genotypically controlled in the species, was due to gene action and not to irregularities in the meiotic process.

3) Seed set per pod in the parental species: With the exception of L. alpinus which produced a very limited number of seeds, seed set

in the parental species ranged from 18 to 22 seeds per pod. Seed setting in the species was not considered to be correlated to the percentage of aborted pollen grains.

4) Morphology of the interspecific hybrids: Presence of HCN, red stem colour, floret striping, black coloration of the keel tip, pod mottling and seed speckling were found to be transmitted to the F_1 hybrids in a dominant manner whereas floret colour appeared intermediate to the parental species.

5) Pollen stainability in the interspecific hybrids: A very high percentage of pollen sterility, ranging from 55 to 98%, was observed in the F_1 hybrids. Pollen stainability was the highest in the hybrids *L. japonicus* x *L. krylovii* (31%), *L. japonicus* x *L. filicaulis* (33%), and L. tenuis x L. filicaulis (43%) whereas the lowest percentages of stainable pollen were recorded in the hybrids *L. krylovii* x *L. schoelleri* (2.50%) and *L. schoelleri* x *L. krylovii* (2%). From the relatively low frequency of cytological irregularities which were found in the hybrids in contrast to the high pollen sterility it would appear that pollen abortion cannot be explained on the sole basis of the meiotic abnormalities. Moreover, the analysis of variance carried out in order to test the significance of the differences observed in pollen stainability between reciprocal hybrids indicated that, in some cases at least, pollen abortion partly resulted from a nucleocytoplasmic interaction.

6) Seed set per pod in the interspecific hybrids: Seed set in the F_1 hybrids was low and averaged between 1 and 3 seeds per pods. However,

each F_1 plant produced at least a few seeds which proved to be viable. In addition to abortive gametogenesis, as a result of 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.

7) Meiosis in the interspecific hybrids: A normal six bivalents at first division metaphase and a normal separation of the chromosomes at AI were observed in at least 60 per cent of the PMC's in each of the hybrids studied. However, the presence of univalent chromosomes at MI, presumably as a result of desynapsis, and lagging chromosomes at AI indicated that the chromosomes of the parental species were not completely homologous. Multivalent pairing of the chromosomes was occasionally observed in some of the hybrids which presumably represents the visible remnants of ancient translocations that took place in the ancestral populations of the parental species. Cytomixis was observed in the hybrid *L. japonicus* x *L. alpinus*.

8) Character inheritance and linkage relationships in the hybrid *L. japonicus* x *L. filicaulis*. The segregation ratios obtained appeared distorted, suggesting that a number of homozygous recessive genotypes had been eliminated during gametogenesis or during embryo formation. The observed ratios were the following: stem colour, 5.54 red : 1 green; HCN reaction, 5.80+ : 1-; floret striping, 99 : 0; pod mottling, 19 : 1; seed speckling, 12 : 1. Red stem colour and presence of HCN were unlinked and each character was considered to be controlled by a single dominant gene. Each of these two dominant genes were found to be linked to genes responsible for central leaflet

width. Floret size was positively correlated to leaflet width and seemed to be governed by genes located on the stem colour linkage group. The *japonicus* phenotype for floret striping was not found among the 99 plants which were examined for this character. Several hypotheses were proposed to explain this result as well as the segregation ratios observed for the pod mottling and the seed speckling characters. No correlation was found between the percentage stainable pollen and the presence of other morphological characters in the F_2 populations. All F_2 plants which possessed an aberrant phenotype, such as stem variegation, dwarfism or extreme vigor, were found to possess the normal number of 12 somatic chromosomes.

9) *Character inheritance and linkage relationships in advanced progenies of the hybrid L. japonicus x L. alpinus:* Floret striping appeared to be controlled by two unlinked dominant genes. Keel tip colour and seed speckling were governed by either a single dominant or two closely linked genes. Neither of the two genes controlling floret striping were considered to be located on the seed speckling-keel tip colour linkage group.

CLAIM TO ORIGINAL RESEARCH

This is to certify that the study reported in this thesis "A cytogenetic study on interspecific diploid hybrids closely related to *Lotus corniculatus* L. (Leguminosae)" constitutes the original work of the author and is his contribution to knowledge.

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