GENETICS AND CYTOTAXONOMY IN BIRDSFOOT TREFOIL (*Lotus corniculatus* L.)

by

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INTRODUCTION

Birdsfoot trefoil (Lotus corniculatus L.) is one of our leguminous forage crops. It has gained some importance in North America since 1934, when Professor Johnstone-Wallace of Cornell University found a type adapted to New York State. It appears to be especially suited to long term pasture seedings and to have a very definite place in modern grassland farming and soil conservation practices. But the potential value of this legume will not be realized until plant breeders are able to combine the growth characteristics of some of the more productive European strains with the hardiness of the New York type found by Professor Johnstone-Wallace. It may also be possible to introduce characteristics from related species or to produce new polyploid combinations which may have agronomic value.

Plant breeders are being hampered by a lack of basic information which they need in order to establish effective plant breeding procedures. The objective of the studies reported in this dissertation is to obtain basic cytogenetic knowledge which may lead to more effective breeding programs. It includes a survey of the genetic variation present within Lotus corniculatus L., a discussion of the taxonomic limits of the species and some consideration of its relationships to other species within the genus. The work reported on the latter aspect is of a preliminary nature only as it was not

stressed after it was learned that Dr. Kai Larsen of Copenhagen is also working on this problem for the whole genus. However, the preliminary work did illustrate the extent of the taxonomic problem and the chaotic state of the knowledge of taxonomy of the genus Lotus. This led to the formation of an extensive co-operative research project involving Dr. Larsen of Copenhagen with Drs. E. O. Callen and W. F. Grant and the writer of Macdonald College and having as its objective the preparation of a biological monograph on the genus Lotus.

The genetics of certain segregating characters was investigated to determine the method of inheritance in Lotus corniculatus. The extent of this work was limited by the difficulties in obtaining suitable characters for study. However, some useful evidence on the occurance of tetrasomic inheritance was obtained.

A study of the genetics of self-incompatibility is presented and discussed. This work was emphasized after preliminary work suggested that some genetic mechanism other than those previously proposed for other species might be involved. Attempts to solve this problem led to the discovery of an association between self-incompatibility and the proportion of the available ovules that may be fertilized in different species in the genus Lotus.

These investigations should be considered as an exploration of some of the cytogenetic phenomena of Lotus corniculatus

that may be of some direct interest to plant breeders. They have revealed cytogenetic problems requiring more intensive study.

LITERATURE REVIEW

The genus Lotus

Brand (1898) included only Old World species in the genus Lotus. Ottley (1923, 1944) believed that the New World taxa Hosackia, Acmispon, Anisolotus and Syrmatium, which some authorities consider separate genera, should all be included in the genus Lotus. She emphasized that she could find no acceptable characteristics for separating the Old World and New World species into different genera and that the main reason for the separation was the claim by Brand that this separation would render a general survey of "Lotus" easier.

Isely (1951) considered the Old and New World taxa congeneric and provided the following taxonomic description of the genus:

> "Plants perennial or annual. Leaflets 1-15, pinnately or palmately foliate. 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, Pods oblong, several seeded, dehiscent."

Brand (1898) divided the Old World Loti into two subgenera; Pedrosia and Edentolotus. He subdivided Pedrosia into two sections, Heinekenia and Eupedrosia; and Edentolotus into five sections, Krokeria, Xantholotus, Erythrolotus, Ononidum and Quadrifolium. Brand considered Tetragonolobus, Bonjeania, Dorycnium and Lotus separate genera while Taubert (1894) grouped them all in the genus Lotus. Taubert divided Lotus

into five sections: Eulotus, Lotea, Krokeria, Ononidium and Tetragonolobus and placed most of the species in Eulotus. Senn (1938) considered his cytotaxonomic evidence as support for Taubert while Larsen (1955) found cytotaxonomic evidence to support Brand.

Ottley (1923) divided the American Loti into three subgenera; Hosackia, Acmispon and Syrmatium. Ottley (1944) divided Acmispon into two sections; Simpeteria and Microlotus.

Taubert (1894) recognized the occurrence of about 70 Old World species, Senn (1938) suggested about 80, and Rudorf (1942) estimated about 100 species. Ottley (1944) recognized 28 species growing in California and estimated that there are about an equal number of New World species which do not occur in that state. Isely (1951) estimated a total of 150 species in the whole genus.

Tscherchow and Kartaschowa (1932) demonstrated that the species of the genus Lotus can be classified according to chromosome number and chromosome morphology. Senn (1938) used cytotaxonomic evidence to determine the relationship and possible evolution of the whole family, Leguminosae. Larsen (1954, 1955, 1956) has published short reports of his preliminary findings in a continuing extensive study of the cytotaxonomy of Lotus. Diploid and tetraploid chromosome numbers have been reported in the two cytotaxonomic groups which have respective basic genome numbers of 6 and 7. The majority of the species previously studied have the genome

number $x = 7$. The important economic group which contains L. corniculatus has $x = 6$. Larsen (1956) found some examples of intraspecific polyploidy in some Lotus species and pointed out some of the difficulties in classifying these taxa by their chromosome numbers. Favarger (1953) reported chromosome numbers of $2n = 12$ and $2n = 24$ in different populations of L. corniculatus var. alpinus collected in the Swiss Alps. In discussing this diploid form, he expressed his opinion of the origin of our cultivated tetraploid forms as follows, "... une forme diploïde de Lotus corniculatus, qui pourrait bien être l'ancêtre des formes tétraploïdes qu'on rencontre surtout à des altitudes plus basses." Bonnier (1920) reported transplant experiments in the Alps in which L. corniculatus from lower altitudes was modified in nurseries at high altitudes until it was identical with alpine plants which grow naturally at the altitudes of the nurseries. Tome and Johnson (1945) presented evidence that the narrow-leaf ($2n = 12$) and broadleaf ($2n = 24$) forms in agricultural production are separate species. They based their conclusions on a comparison of induced tetraploid narrow leaf and the normal broadleaf forms. Dr. J. M. Armstrong, Forage Crops Division, Central Experimental Farm, Ottawa, kindly showed the writer some unpublished idiograms of the chromosomes of these two forms, which suggest considerable homology between them.

Inheritance in tetraploids

Little (1945) reviewed the genetic segregations that have

been suggested for tetraploids. He compared the theories presented by Gregory (1914), Muller (1914), Haldane (1930) and Mather (1936). Gregory proposed a disomic inheritance scheme based on the supposition that maternal and paternal chromosomes must associate in pairs during meiosis. Muller presented a tetrasomic inheritance scheme based on the supposition that chromosomes pair at random during meiosis. Haldane, and Mather also presented tetrasomic inheritance schemes based on the supposition that chromosomes pair at random during meiosis. They suggested modifications of Muller's hypothesis which considered multivalent pairing during meiosis in conjunction with crossing over of chromatids and the effects of the distance of a locus from the centromere on segregation ratios. Little (1945) pointed out that Gregory's proposed ratios may apply to allopolyploids and that Muller's may apply to autotetraploids in which multivalents are infrequent. In autotetraploids in which multivalents are formed frequently, Little favoured Mather's hypothesis.

Dawson (1941) obtained discrete segregations for cyanogenetic properties in L. corniculatus and found a good fit to Muller's (1914) tetrasomic inheritance hypothesis. He found that multivalents are rare if not entirely absent during meiosis. Little (1945) cited this work as an example of tetrasomic inheritance occurring in a natural tetraploid.

Muntzing (1936) pointed out that multivalents are a characteristic of artificial autopolyploids and that the maximum

number of chromosomes in these associations corresponds to the number of homologous genomes present. He then stated that he believes that the presence of multivalents in natural polyploids is an indication of autopolyploidy and conversely that their absence indicates allopolyploidy. However, Gilles and Randolph (1951) obtained evidence with artificially induced tetraploid Zea mays that the frequency of multivalents decreased over a period of ten generations. They attributed this to natural selection. Dawson (1941) believed that his evidence for tetrasomic inheritance in L. corniculatus is a good indication of autoploid origin although he found multivalents to be rare during microsporogenesis of the plants with which he found tetrasomic inheritance.

Both tetrasomic and disomic inheritance has been demonstrated in alfalfa (Medicago sativa), by Stanford (1951), Stanford and Cleveland (1954) and by Twamley (1955). Numerous inheritance studies by earlier workers were cited by Atwood and Grun (1951). Twamley (1955) concluded that alfalfa is of autotetraploid origin and is now passing through a transition stage in which segregation for some characters is tetrasomic and for others has become disomic. Stanford (1951) fitted his data to Mather's (1936) hypothesis as quadrivalents are fairly frequent during microsporogenesis in alfalfa.

Self-incompatibility

Incompatibility mechanisms which encourage outbreeding have been found for many higher plants and fungi. Several extensive review articles are available, of which the most

recent is the one by Lewis (1954). The incompatibility mechanisms found in higher plants may be classified according to the associated flower structures into two classes:

A. Heteromorphic, where incompatibility is associated with flower structure types occurring within self-incompatible species.

B. Homomorphic, where incompatibility is not associated with flower structure types within self-incompatible species.

Our discussion will be confined to the latter type as Lotus corniculatus has this type of flower structure.

Lewis (1954) and Bateman (1955) claim that only two genetic mechanisms are necessary to explain incompatibility in homomorphic species for which adequate genetic data have been obtained. These are:

1. The gametophytic oppositional incompatibility system in which an oppositional reaction occurs between the genotype of the pollen grain and the genotype of the plant pollinated. This is the mechanism originally proposed by East and Mangelsdorf (1925) for incompatibility in Nicotiana sanderae. A multi-allelic series of genes at a single locus (S_1, S_2, S_3, ... etc.) is one characteristic of this system. Incompatibility occurs between the pollen and the pistil when the same allele is present in both of them. Self-incompatibility may be weakened with the induction of autopolyploidy, as has been demonstrated by the induction of autotetraploidy of known self-incompatible diploids in which this system was operating. This breakdown in incompatibility has been

attributed to dominance or competition reactions between alleles in the diploid pollen grains produced by the autotetraploids.

2. The sporophytic oppositional incompatibility system in which an oppositional reaction occurs between the genotype of the pollen parent and the genotype of the plant pollinated. This system was proposed by Hughes and Babcock (1950) and by Gerstel (1950). Again, a single locus with a multi-allelic series of genes has been proposed for each instance where this system has been demonstrated. Induced autotetraploidy does not greatly weaken self-incompatibility where this system is operating and Lewis (1954) believes that when induced polyploidy does not produce self-fertility, this negative evidence is a strong indication that a sporophytic system is involved.

Characteristics of the sporophytic system which can be used to differentiate it from the gametophytic system according to Lewis (1954) are:

1. There are frequent reciprocal differences.
2. Incompatibility can occur with the female parent.
3. A family can consist of three incompatibility groups.
4. Homozygotes are a normal part of the system.
5. An incompatible group may contain 2 genotypes.

Bateman (1955) suggested that the sporophytic system occurs throughout the Cruciferae and that Kakizaki's (1930) data on incompatibility in the cabbage fit this hypothesis

better than the hypothesis involving two loci which was proposed by Kakizaki. Elliott (1946) presented evidence for the genetic control of self-incompatibility in Lotus tenuis and modeled his explanation on the hypothesis presented by Kakizaki.

Lundqvist (1956) proposed that a third genetic mechanism is necessary to account for his findings with rye, Secale cereale. He proposed a system involving two genetically separate loci which behave as a physiological unit, with specific incompatibility substances resulting from interactions between alleles at the two loci. A gametophytic system is proposed so that pollen with one gametophytic incompatibility factor matched in the style is compatible, pollen with both gametophytic incompatibility factors (one at each locus) matched in the style is incompatible. This gametophytic system displays features which Lewis (1954) and Bateman (1955) associated with sporophytic incompatibility systems. Lundqvist (1956) pointed out that the criteria used by Lewis and Bateman to differentiate between gametophytic and sporophytic systems apply only when incompatibility is controlled at one locus. Thus, incompatibility in rye is more probably controlled gametophytically than sporophytically, according to evidence presented by Lundqvist, although there are frequent reciprocal differences in seed set, incompatibility does occur with the female parent, there is an absence of "true self-compatibility segregating" and incompatibility does not break down with induced autotetraploidy.

Lundqvist proposed that the complete or almost complete lack of a breakdown of incompatibility in autotetraploid rye can be explained by the fact that the incompatibility alleles work together at the diploid level, so that competition and dominance interactions have been selected against or the diploid would also be self-fertile.

Lundqvist proposed that a similar system may occur in Festuca pratensis and suggested that it may be quite common in grasses. He also suggested that the system proposed by Owen (1942) for Beta vulgaris, which involves two loci and a gametophytic system, may be valid. Lewis (1954) suggested that a sporophytic system may fit Owen's data but it would be difficult to apply. Lundqvist suggested that it may be significant that the above instances with two incompatibility loci occur with naturally wind pollinated plants.

Two controlling reactions for self-incompatibility have been suggested at the biochemical level. These are (1) an oppositional inhibition modeled after the antigen-antibody reaction to give incompatibility and (2) a complimentary stimulation to give compatibility. Lewis (1954) considers that the oppositional reaction is the only controlling reaction possible in multi-allelic systems.

The genetics of incompatibility in polyploids was considered by Lawrence (1930). He gave some preliminary consideration to the possible effects of autopolyploidy but could find no data that would support his hypothesis and he went on to develop an hypothesis for alloploidy which was

essentially the same as the hypothesis developed by Kakizaki (1930). Lawrence applied his hypothesis to data on Verbascum phoeniceum provided by Sirks (1926), and on Cardamine pratensis provided by Correns (1912). Lewis (1954) pointed out that Correns was dealing with a sporophytic system and that his original interpretation was essentially correct.

Lewis (1954) reviewed the recent work on the genetics of self-incompatibility in induced autotetraploids in which competition and dominance of the incompatibility alleles was demonstrated and in which the different effects of polyploidy on the sporophytic and gametophytic systems at one locus were demonstrated. These concepts have been developed with artificially induced autotetraploids and Lundqvist (1956) points out that they will probably not apply to natural polyploids. There are several reports of investigations of self-incompatibility in natural polyploids but no genetic interpretations have been offered although the methods used suggest that a definite genetic interpretation was an objective of at least some of these investigations.

Adams (1949) investigated Bromus inermis, an octoploid, and found no simple, clear cut interpretation for his incompatibility data. He found evidence for both additive and non-additive gene action influencing cross-incompatibility, with non-additive effects relatively more pronounced among plants of inbred origin. He used this evidence to support his claim that an oppositional incompatibility system is involved.

Myers (1942) studied the heritable variations in self-fertility of Dactylis glomerata, a tetraploid, and found no simple Mendelian basis for its inheritance. He found that vigour was not closely correlated with the ability to set seed under a bag within inbred families.

Armstrong (1952) and Picard and Demarly (1952) reported on some aspects of self-sterility in Medicago sativa, which Twamley (1955) considered an autotetraploid, and demonstrated some of the characteristics of an oppositional system but again no discrete genetic mechanism is proposed.

The morphological aspects of pollen germination and growth associated with incompatibility mechanisms were surveyed by Sears (1937). He found that flowering plants can be grouped into three classes according to pollen tube behaviour, as follows:

Class I. Germination of pollen inhibited on the stigma.

Class II. Pollen germination normal, but pollen tubes inhibited in the style.

Class III. Pollen tubes grow normally and reach the ovules, but no seed is formed.

The only example of a class III species that Sears (1937) presented was Gasteria verrucosa var. intermedia, in which incompatibility occurred after fertilization was effected. Brink and Cooper (1938, 1939, 1941) proposed a post fertilization abortion mechanism following inbreeding in Medicago sativa which they called somatoplastic sterility, which could also be included in Sears' class III according

to the original definitions. Post-fertilization abortion should not be included as an incompatibility mechanism as the ovules which are fertilized with self-pollen may be lost, whereas the ovules are not fertilized with self-pollen with a true incompatibility mechanism and they may be fertilized later if compatible pollen becomes available. Giles (1949) found that Lotus corniculatus has a class III pollen tube behaviour in that the pollen tubes appeared to enter the micropyle but the ovules did not become fertilized following incompatible matings. This is the only species in which true self-incompatibility is reported to act after pollen tubes enter the micropyle. Giles also stated that post-fertilization abortion may occur, but he did not consider it the main cause for self-sterility.

Muller (1873) and Darwin (1885) both reported that birdsfoot trefoil does not set seed in the absence of insects. Silow (1931) tested for seed-set in Lotus corniculatus and other Lotus species in the absence of insects, with and without artificial self-pollination. He found that L. angustissimus and L. hispidus were self-fertile without artificial self-pollination in the absence of insects. L. uliginosus set seed freely following artificial self-pollination, although not as many seeds per pod were obtained as from cross-pollination with insects. It set no self-seed without artificial self-pollination. MacDonald (1946) reported that L. corniculatus is "voluntarily" self-sterile,

slightly "artificially" self-fertile and somewhat more "cross-fertile". He found a higher incidence of fertility under field conditions than in the greenhouse. He obtained high seed sets on single birdsfoot trefoil plants isolated under screen cages under field conditions when he self-pollinated with sterilized bumble bees. Giles (1949) was unable to duplicate MacDonald's results and suggested that the "sterilized" bees may have carried viable pollen so that the seed obtained may have been due to cross-pollinations.

McKee (1949) also found that L. corniculatus arvensis is highly self-sterile, that L. uliginosus is less self-sterile and that L. corniculatus hirsutus and L. corniculatus filicaulis are highly self-fertile.

Hagerup (1951) reported that L. corniculatus forma carnosa set seed in the Faroes in the absence of pollinating insects. He suggested that self-compatible forms of self-incompatible species may evolve in the absence of insects. Hagerup reported in a personal communication to the writer that he was unable to confirm his observation on Lotus plants he introduced into the Botanic Garden at Copenhagen and stated that his observation on the incompatibility of Lotus is questionable.

Giles (1949) found that birdsfoot trefoil is highly self-sterile and that seed production under natural conditions is almost entirely the result of cross-pollination. He proposed three levels to self-sterility in L. corniculatus, as follows:

(1) A failure of pollen tubes to penetrate a stigmatic membrane.

(2) A failure of ovules to become fertile even when pollen tubes are abundant in the ovary cavity.

(3) Post fertilization abortion.

He was unable to demonstrate differential rates of pollen tube growth from self- and cross-pollinations but he did find that the stigmatic membrane has to be broken before the stigma can become receptive to any pollen. A stigmatic fluid was also demonstrated. He concluded that the failure of the ovules to become fertilized by self-pollen is the primary factor in self-incompatibility of this species. However, even the most highly self-sterile plants produced some self-seeds.

Growth and development of Lotus corniculatus

Hansen (1953) provided a morphological description of the development of Lotus corniculatus var. vulgaris through a complete life cycle. He described the origin of the ovary in which a row of ovules arises along each edge of the carpel of a one carpellate ovary. The megasporocyte undergoes two divisions and produces a linear quartet of megaspores. The chalazal megaspore then gives rise to the female gametophyte which contains an egg, two synergids, two polar nuclei and three antipodals. He found from 44 to 72 ovules per ovary, with an average of 59 and saw pollen tubes in the micropyle 36 hours after pollination under greenhouse conditions. Giles (1949) observed fertilization in 24 hours under field conditions in the summertime and noted that 8 to 12 hours

longer were required under greenhouse conditions in the wintertime. Hansen found 2 to 35 seeds per pod, with an average of 19.

Giles (1949) gave a detailed description of the pollination process in which the stigmatic membrane is broken by insects. He discussed the mechanics of cross-pollination and described the flower structure and stages in flower development in considerable detail.

Morse (1955) reported on the value of bumble bees as pollinators for L. corniculatus and confirmed the occurrence of insect pollination under field conditions.

MacDonald (1946) gave an extensive review of cultural methods and Hansen (1953) suggested the use of a 24 hour photoperiod to induce flowering, for which he reported reasonably normal development during the winter months under greenhouse conditions.

MATERIALS

Plant materials used in these investigations have been given accession numbers within the system used for forage crops by the Agronomy Department, Macdonald College and details of origin have been entered in the permanent records of that department. The first two digits indicate the year of accession and the remaining digits indicate the specific collections. Identification and taxonomic study of these collections and the preparation of herbarium specimens, which are being done by Dr. E. O. Callen of Macdonald College, were not completed at the time when this dissertation was prepared. The first digits of the numbers used in Dr. Callen's system correspond to those used herein. The scientific names cited in the following list of materials were provided by the various sources.

Accession	Description
5101	L. corniculatus. Seed collected by Mr. A. Charbonneau, Agronome, Joliette Co., P.Q. from an old stand near Ste. Melanie, P.Q.. Seed used to establish this stand originated in New York State.
5401	L. corniculatus. Seed from the Department of Agronomy, Pennsylvania State University. Selections labled C-1 to C-35 were selected from the "Empire" variety, C-36 to C-112 from various collections of L. corniculatus obtained from continental Europe.
5402	L. corniculatus. Plants collected by the writer

from a pasture near Hatley, P.Q. Seed originated in New York State. One plant had a light yellow flower colour.

5403 L. corniculatus. Plants collected by the writer from a pasture near Hatley, P.Q. Seed originated in New York State and was seeded in 1951.

5404 L. corniculatus. Seed obtained from the National Institute of Agricultural Botany (United Kingdom) under their number H. 2440. Origin - Italy.

5405 L. corniculatus arvensis. Seed obtained from Dr. P. Henson, U.S.D.A., Beltsville, Md., U.S.A. under their number F.C.23239.

5406 L. tenuis. Seed from the Division of Forage Crops, Central Experimental Farm, Ottawa.

5407 L. corniculatus. Seed obtained from Campo Experimental Centinela, Chile.

5408 L. corniculatus arvensis. Seed obtained from the National Institute of Agricultural Botany (United Kingdom). Collected on a 100 year old pasture in Kent, England.

5409 L. ornithopodioides. Seed obtained from Dr. P. Henson, U.S.D.A., Beltsville, Md., U.S.A., under their number P.I.121158.

5410 L. angustissimus. Seed obtained from the Forage Crops Division, Central Experimental Farm., Ottawa, under their number 1893-3576. Introduced from Jerusalem.

5411 L. Weilleri. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa, under their number 1893-3836. Introduced from Paris.

5412 L. uliginosus glabrisculus. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa, under their number 1893-2965. Introduced from Astoria, Oregon.

5413 L. uliginosus villosus. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa, under their number 1893-2964. Introduced from Astoria, Oregon.

5414 L. uliginosus. Seed of the "Columbia" variety obtained from the National Institute of Agricultural Botany (United Kingdom). Introduced from Astoria, Oregon.

5415 L. uliginosus. Seed of the "Beaver" variety obtained from the National Institute of Agricultural Botany (United Kingdom). Introduced from Astoria, Oregon.

5416 L. uliginosus. Seed of the "Brauns Weihenstephaner" variety obtained from the National Institute of Agricultural Botany (United Kingdom). Introduced from Germany.

5417 L. hispidus. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa, under their number 1893-3950. Introduced from Copenhagen.

5418 L. palustris. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa, under

their number 1893-3575. Introduced from Jerusalem.

5419 L. creticus var. cytisoides. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa, under their number 1893-3375. Introduced from Portugal.

5420 L. perigrinus. Plant obtained from the Forage Crops Division, Central Experimental Farm, Ottawa. Introduced from Jerusalem.

5421 L. edulis. Seed obtained from Dr. P. Henson, U.S.D.A., Beltsville, Md., U.S.A., under their number P.I.197827. Seed originated in Algeria.

5422 L. canimbriensis. Plant obtained from the Forage Crops Division, Central Experimental Farm, Ottawa. Introduced from Portugal.

5423 L. tetragonolobus. Seed obtained from Dr. P. Henson, U.S.D.A., Beltsville, Md., U.S.A., under their number P.I.67217. Introduced from Gronenger, Germany.

5424 L. jacobaeus. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa, under their number 1893-4538. Introduced from the Botanic Garden, Lisbon, Portugal.

5425 L. seliquosus. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa.

5426 L. parviflorus. Plant obtained from the Forage Crops Division, Central Experimental Farm, Ottawa. Introduced from Portugal.

5427 L. americanus. Seed obtained from Dr. P. Henson, U.S.D.A., Beltsville, Md., U.S.A., under their number F.C.31900. Collected in California.

5428 L. divaricatus. Seed obtained from Dr. P. Henson, U.S.D.A., Beltsville, Md., U.S.A., under their number P.I.109314.

5429 L. filicaulis. Seed obtained from Dr. P. Henson, U.S.D.A., Beltsville, Md., U.S.A., under their number P.I.51864.

5430 L. gebelia. Seed obtained from Dr. P. Henson, U.S.D.A., Beltsville, Md., U.S.A., under their number P.I.210439. Introduced from Iran.

5432 L. scoparius. Seed obtained from the Department of Agronomy, University of California, Davis, Calif., under their number BN-4629. Collected in California.

5433 Hosackia sp. Seed collected by Dr. V. C. Brink, Department of Agronomy, University of British Columbia, Vancouver, B.C.

5501 L. corniculatus japonicus. Seed collected on a river bank, Gifu, Japan, by Professor Isawo Hirayoshi, Kyoto University, Kyoto, Japan.

5502 L. Wrightii. Seed obtained from Mr. Forest Bent, Graduate Student in Plant Breeding, Cornell University. His source was the Soil Conservation Service in California under their number A-11590.

5503 L. arabicus. Seed obtained from Mr. Forest Bent, Cornell University, under the U.S.D.A. plant

introduction number P.I.51858.

5505 L. tenuis. Seed obtained from the Department of Agronomy, University of California, Davis, Calif., as the variety "Los Banos" selected by the Soil Conservation Service for tolerance to alkaline soils.

5506 L. maroccanus. Seed obtained from the Department of Agronomy, University of California, Davis, Calif., under their number K2794. Original source was French Morocco.

5508 L. pedunculatus. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa, under their number 1893-4505. Introduced from Sacaven, Portugal.

5509 L. campylacladus. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa, under their number 1893-4682. Introduced from Coimbra, Portugal.

5510 L. rectus. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa, under their number 1893-4567. Introduced from Pisa, Italy.

5511 L. denticulatus. Seed obtained from the Department of Agronomy, University of California, Davis, California, under their number K-2382. Native of California.

5512 L. conjugatus requienii. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa.

5513 L. suaveolens. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa.

5514 L. maritimus. Seed obtained from the Forage Crops Division, Central Experimental Farm, Ottawa.

5515 L. tenuis. Polyploid A. Seed obtained from the Eastern States Farmers Exchange, West Springfield, Mass., U.S.A., under their number 014072. Originated as a colchicine induced tetraploid by Dr. A. Gershoy, University of Vermont and multiplied about six generations.

5516 L. tenuis. Polyploid B. Seed obtained from the Eastern States Farmers Exchange, under their number 014067. Origin same as for 5515.

5517 L. tenuis polyploid X L. corniculatus. Seed obtained from the Eastern States Farmers Exchange under their number 014064. Hybrid plants between L. tenuis polyploids and L. corniculatus were produced by Dr. A. Gershoy of the University of Vermont. A population consisting of these hybrids and backcross progeny was built up. Dr. Gershoy describes this as a "Synthetic 2"..

5518 L. tenuis. Open pollinated polyploid. Seed obtained from the Eastern States Farmers Exchange, under their number 014071. Origin was the same as for 5515, but it was maintained in a nursery that was not isolated from other Lotus collections.

5621 L. corniculatus. Seed obtained from Dr. Kai Larsen, Copenhagen, who forwarded collections from Czeckoslovakia. Six separate packages were labled as varieties:
1. Maxelovsky. 2. Malejovsky. 3. Vysakomylsky. 4. Francoursky. 5. FC23318. 6. Americky.

5622 Birdsfoot trefoil. Seed obtained from Dr. Kai Larsen, Copenhagen, collected on mountains near Florence, Italy.

5664 L. scoparius. Seed obtained from Dr. R. R. Seaney, Department of Agronomy, Cornell University, under their number 504. Seed originated at the Rancho Santa Ana Botanic Garden, Claremont, Calif., under their number 6449.

5668 L. douglasii. Seed obtained from Dr. R. R. Seaney, Cornell University, under their number 616. Originally from Spokane, Washington, under their number P.I.231-451.

5674 L. lamprocarpus. Seed obtained from Dr. R. R. Seaney, Cornell University, under their number 640. Seed originated in Spain as P.I.214113.

5696 L. corniculatus. Seed obtained from Dr. Kai Larsen, Copenhagen. Collected at Heliopolis, Cairo, Egypt.

5698 L. corniculatus. Plants collected by Mr. I. V. Hunt, West of Scotland College of Agriculture, Auchincruive, Scotland on the College Farm.

5699 L. corniculatus. Plants and seed collected by Mr. I. V. Hunt, in sand by the seaside, Doonfoot, (Mouth of the River Doon) Scotland.

56100 L. corniculatus. Plants collected by Mr. I. V. Hunt on a roadside, near Sherwood, Scotland.

56101 L. corniculatus. Plants collected by Mr. I. V. Hunt, in a marshy area with a low pH, near Rawhill, Scotland.

56102 L. corniculatus. Seed obtained from Mr. I. V. Hunt. Collected from an upland area in Western Scotland.

METHODS

Small populations of plants of various collections were grown in a field nursery during the summers of 1954, 1955 and 1956 and in the greenhouse during the winters of 1953-54, 1954-55, 1955-56 and 1956-57. Seeds were generally germinated on moist blotters in Petri dishes and hard seed coats were broken with a sharp needle. Some collections of old seed, which were difficult to establish, were transferred to a sterile nutrient agar medium until the seedlings were well established. The medium suggested by Randolph and Cox (1943) was prepared and the culture was carried out in Drosophila culture tubes. Equipment was steam sterilized and seeds were sterilized in a weak sulphuric acid solution. Seedlings were transplanted to 1½ or 1 3/4 inch thumb pots, or to 1½ inch plant bands containing the standard Agronomy Department soil mixture of 3 parts sterilized compost and 1 part washed sand. A single seedling was transplanted into each single pot to make certain that only one genotype was growing in each pot.

The established seedlings were either transplanted to 4 inch thumb pots in the greenhouse, or directly into a field nursery where they were grown in rows 30 or 36 inches apart and spaced in the rows at 12, 30 or 36 inch intervals. Detailed nursery planting plans are available in the planting records of the Agronomy Department, Macdonald College. Pots in the greenhouse were individually labelled. All plants were innoculated with the birdsfoot trefoil strain of symbiotic bacteria. Small quantities of 2-12-10 fertilizer were added

to the pots at intervals of six to eight weeks during the wintertime.

Flowering was induced in L. corniculatus, L. tenuis, L. uliginosus and many other Lotus species with artificial illumination turned on about one-half an hour before sunset and turned off about 8 A.M.. The light intensity used was not sufficient to prevent folding of the leaflets and flowering was not entirely normal as some plants produced contorted flowers, some produced very little pollen, some produced abnormally long styles and some flowers on some plants had no styles. These abnormalities were not produced on the same plants in the summertime in field nurseries. Attempts were made to overcome these abnormalities by increasing light intensity and decreasing daylight, but this was not successful within the limits of conditions possible with the available facilities. It was possible to produce fairly satisfactory flowering during December, January, February, March and April.

When more than one plant of any specific genotype was required and when plants were taken from the field to the greenhouse, stem cuttings were taken and rooted in "Vermiculite". Rooting was promoted by applying the rooting hormone "Auxan" to the cut ends and by frequent applications of a fine mist spray. The cuttings were wired directly to pot labels with fine aluminum wire and remained attached to the labels until they were transplanted into 4 inch pots,when each pot was labeled. The chromosome numbers of some cuttings were

determined to make sure that this method of making cuttings did not induce changes in chromosome number.

Cross-pollinations were made to produce seed for compatibility studies and to test for cross-compatibility. Pollen was collected from the plant serving as the male parent by clasping the two lobes of the keel petal with tweezers and pulling it off. The pollen which remained in the end of the keel was squeezed out through the pore in the end of the keel next to the stigma onto patches of fine sandpaper glued onto matchsticks. The keel was removed from the plant to be pollinated in the same way and the exposed stigma was buffeted with the sandpaper loaded with pollen. Emasculation was not possible as the removal of the anthers before they burst destroyed the flowers. All equipment was sterilized in a solution of 3 parts of 95% alcohol and 1 part glacial acetic acid after each pollination. The pollination process is diagramed in Plate 1.

The details of each cross were recorded on a marking tag which was tied to each group of flowers pollinated in the same way at one time. The female and male parents were recorded on the tag, the first number representing the plant pollinated (female), the second the pollen source (male). The number of flowers pollinated at that time, and the date, was also recorded on the tag. The same procedure was used for self-pollinations except the keels were not removed and the stigma was simply forced through the pore in the end of the keel against the sandpaper and allowed to return into the

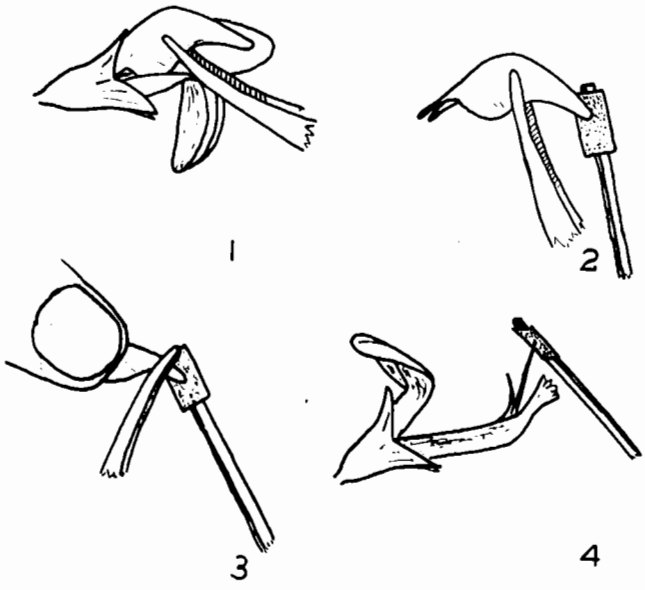

Plate 1. Diagrams of pollination technique.

1. Pollen was collected from the plant used as the male by clasping the lobes of the keel and pulling it off.
2. The keel containing a mass of pollen was placed on a piece of fine sandpaper glued to a matchstick.
3. The keel was clasped between the thumb and forefinger and was stroked with the tweezers away from the thumb forcing pollen onto the sandpaper.
4. The sandpaper loaded with pollen was buffeted against the stigma of the flower used as the female. These flowers were prepared by removing the keels as in figure 1.

mass of pollen.

Pods were collected after about 30 days, when they were ripe. All the pods from a cross together with the tag were put into a coin envelope. The pods were taken to the laboratory and the number of flowers pollinated, the number of pods harvested, the number of seeds in each pod and the date when pollinated were recorded in a record book.

In order to control pollination, it was necessary to isolate flowers from insects under field conditions in the summertime. Glycine bags of the type used on wind-pollinated crops were tried but they were unsatisfactory because the flowers faded, petals dropped and flowers often dropped. Moisture collected inside these bags. Cellophane bags were tried but moisture also collected in them and flowers often dropped although the petals did not fade and drop as readily as in glycine bags. The moisture problem was partly overcome with the cellophane bags by perforating them with a needle. These bags were placed over unopened buds and were removed when the opened flowers were pollinated. The bags were replaced once the pollination was completed. They were removed again about a week following pollination and the pods were allowed to mature in the open. This technique was fairly satisfactory during periods of fine weather, but a lot of flower dropping occurred during rainy weather. This technique was used to establish families for incompatibility studies, but a lot of these crosses could not be used to determine compatibility indices because of variability in

seed set due to weather conditions after the crosses were made.

Also, whole single plants were isolated under 30 inch X 30 inch X 30 inch screened cages. These cages were either placed over the plants before flowering started or all the opened flowers were picked off. These cages provided isolation from the relatively large insects that are generally considered pollinators of L. corniculatus but did not provide isolation from some smaller insects. Flowering was somewhat inhibited, probably because of shading by the cages. These cages were used when compatibility was measured using the caged plant as female and a number of plants as males. When a plant was used as female in only a few compatibility tests, flowers were isolated in nylon net bags. These bags were the most satisfactory insect isolating devices used, although flowering was still not perfectly normal. They were the only isolating devices that were at all satisfactory for L. tenuis. Nylon net deteriorated with exposure to the weather, however the bags lasted about three months under field conditions.

The technical problems of insect isolation in the field made it necessary to make most of the compatibility tests in the greenhouse in the wintertime, where insects were eliminated with insecticides.

Some chromosome numbers were determined and meiosis was studied using standard aceto-carmine squashes of the microsporocytes. Staining was improved by adding ferric

acetate to the fixative. Also, root-tip sections of some materials stained with standard crystal violet techniques were studied.

Ovule development was studied in serial sections of entire ovules cut transversely. The material was fixed in Craf solution, embedded, sectioned at 15 microns, stained with Delafield's haematoxylin and mounted in "Permount". Large slides, 50 X 75 mm, were used in order to get all sections from one ovary on one slide.

Colchicine treatments were used to double the chromosome number of some materials. Seeds were germinated on blotting paper and allowed to grow for six days. The seedlings were then wrapped in cotton batten with the cotyledons protruding and were inverted in a 0.2% solution of colchicine to which a drop of "Ultrawet" was added. This treatment was applied overnight (13 hours) after which the seedlings were transplanted into thumb pots and watered. A similar treatment was applied to some rooted cuttings, from which gigas branches were taken and rooted. Seedlings which exhibited gigas characteristics were selected and root tip chromosome numbers were determined to confirm chromosome number doubling of each plant used to study the effects of the doubling on self-incompatibility. Only plants and parts of plants exhibiting morphological characteristics indicating polyploidy were used in this work.

CYTOTAXONOMY AND CYTOLOGY OF *LOTUS* SPECIES

Cytotaxonomy of *Lotus corniculatus*

L. corniculatus L. was described by Isely (1951) 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 basally placed on rachis, the remaining three apical. Leaflets obovate to lanceolate in shape. Peduncles axillary, exceeding leaves. Umbels 3-8 flowered. Pedicels very short. Flowers usually 12-16 mm. in length. Calyx lobes approximating the tube, appressed in the bud. 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 at maturity. Seeds about 1.5 mm. across, assymetrically rounded, dark- or olive-brown in colour, frequently mottled or speckled."

Plants which conform to the above description, at least vegetatively, and which have a somatic chromosome number of $2n = 24$ are found in collections 5101, 5401, 5402, 5403, 5404, 5405, 5408, 5622, 5698, 5699, 56100 and 56102. Plants of collection 56101 vary from the description provided in that they possess a weak tendency to produce rhizomes in the location where they were collected in Scotland. Otherwise they conform to the description provided by Isely and are very similar in appearance to plants in other collections from Great Britain, that is, to plants in collections 5405, 5408, 5698, 5699, 56100 and 56102. Plants in collection 56101 also have $2n = 24$. Bonnier (1920) discussed *L. corniculatus* var. *alpinus*, which possesses very stout

rhizomes, which gives precedence for including this rhizomatous collection in L. corniculatus. Plants of collections 5515, 5516, and 5518 also have 2n = 24, and fit Isely's description for L. corniculatus. These plants originated as colchicine induced autotetraploids of 2n = 12 plants. Plants in collection 5517 also conform to the description provided by Isely and have 2n = 24. This collection originated from a hybrid between an autotetraploid of L. tenuis and L. corniculatus. Plants of collections 5515, 5516, 5517 and 5518 are easily distinguished from other tetraploid L. corniculatus plants as they have longer, narrower leaflets and longer internodes.

Plants from collections 5406 and 5505 also fit Isely's description of L. corniculatus and have a somatic chromosome number of 2n = 12, These plants have relatively longer and narrower leaflets than plants from the tetraploid collections other than those from 5515, 5516 and 5518. The latter plants differ from plants of collections 5406 and 5505 in that they exhibit gigas characters often associated with induced tetraploidy. Plants of collection 5696 have not flowered, however, they have a leaflet shape intermediate between that of plants from collections 5406 and 5505 and that of plants of collection 5101 and a chromosome number of 2n = 12.

Isely (1951) differentiated L. major Scop., synonymous with L. uliginosus Schukr., from L. corniculatus L. by the presence of rhizomes; by the presence of stronger nerves on the leaflets; by the calyx teeth being more divergent in the

bud; and by the flowers being 8 to 12 in a cluster. Plants from collections 5407, 5412, 5413, 5414, 5415, 5416, 5425 and 5501 can be distinguished from plants from collection 5105 by these characteristics and they all have a somatic chromosome number of $2n = 12$. Plants of collection 5501, obtained from Japan as L. corniculatus japonicus, can be distinguished from plants of other L. major collections as they are highly self-fertile and very prostrate in growth habit.

MacDonald (1946) pointed out that two other species sometimes included in L. corniculatus L. sens lat., L. angustissimus and L. hispidus are more pubescent annuals and have smaller flowers than L. corniculatus. Plants from collection 5410 of L. angustissimus have $2n = 12$ and plants from collection 5417 of L. hispidus have $2n = 24$. These annuals are morphologically distinguishable from the perennials.

Plants of collection 5429, obtained as L. filicaulis have $2n = 12$ and are easily distinguishable from plants in other diploid and tetraploid collections that fit Isely's description of L. corniculatus by their regular alternate branching of their stems and by their very narrow leaflets. Plants of collection 5428, obtained as L. divaricatus have $2n = 24$ and have a branching habit similar to those of collection 5429. These plants are more pubescent than those of any other collections belonging to the corniculatus group (i.e. the collections in which the plants closely resemble L. corniculatus L.). Also, they have a very prostrate growth

habit. Plants of collection 5418, obtained as L. palustris, have 2n = 12 and are very similar to those from collection 5428 except they are more prostrate and spreading and less pubescent. Plants of these three collections are perennial and do not produce rhizomes. They could easily be included in L. corniculatus according to the description provided by Isely. However, they are readily distinguishable from plants in other collections that conform to Isely's description and look quite distinct from other collections and from each other in nursery rows, so that each can readily be considered a separate species.

No differences in chromosome morphology were observed among the plants of the collections that conformed to Isely's description for L. corniculatus other than differences in chromosome size. Plants of collection 5501, obtained as L. corniculatus japonicus, have visibly larger meiotic chromosomes than plants of other 2n = 12 collections observed in the same stage with the same technique. (See plate 2.)

One possible taxonomic treatment would be to include all the collections so far discussed in one species and to reduce the separate taxa to subspecies, varieties, forma, etc.. This would follow the description provided by Isely for L. corniculatus, except it would have to be modified to include L. major. Another possible taxonomic treatment would be to elevate many of the separate taxa, for example those separated in table 1 except for the induced autotetraploid tenuis and the corniculatus-4x tenuis hybrid, to species and

Table 1. Classification of the corniculatus group of plant collections studied.

Taxa	Collections represented	Chr. No.	Characteristics by which collections differ from corniculatus collection 5101
corniculatus			
Empire type	5101, 5401, 5402, 5403.	2n = 24	
European type	5401, 5404, 5622	2n = 24	More upright, more rapid growth in the spring and after clipping.
British type	5405, 5408, 5698, 5699, 56100, 56101, 56102	2n = 24	Smaller, more prostrate, more compact, some with rhizomes.
tenuis	5406, 5505	2n = 12	Narrower leaflets, longer internodes.
autotetraploid tenuis	5515, 5516, 5518	2n = 24	Narrower leaflets, longer internodes.
corniculatus-4x tenuis	5517	2n = 24	Narrower leaflets, longer internodes.
major	5407, 5412, 5413, 5414, 5415, 5416, 5425	2n = 12	Stronger nerves, broader leaflets, calyx teeth more divergent, more flowers per cluster, more self-fertile.
japonicus	5501	2n = 12	Same as major, except more prostrate, highly self-fertile.
filicaulis	5429	2n = 12	Very narrow leaflets, regular alternate stem branches.
divaricatus	5428	2n = 24	Pubescent, more prostrate, regular alternate stem branches.
palustris	5418	2n = 12	Pubescent, very prostrate, regular alternate stem branches.
angustissimus	5410	2n = 12	Annual, pubescent, small flowers, self-fertile.
hispidus	5417	2n = 24	Annual, pubescent, small flowers, self-fertile.

to limit L. corniculatus to the broadleaf forms with a somatic chromosome number of 2n = 24 and possibly to include some 2n = 12 forms. The group of collections that come under the descriptions provided by Isely for L. corniculatus and L. major could then be considered a section or subgenus within the genus Lotus. This group is hereafter referred to as the "corniculatus group".

The latter alternative is more realistic and more useful for agronomic purposes because plants in the separate taxa listed in table 1, with one exception, are not cross-fertile with plants of collection 5101. In tests made using tester plants of collection 5101 as the female, plants of the taxa listed under the three types in corniculatus, were all cross-fertile with the tester plants and plants in the other taxa were not cross-fertile with the tester plants, except the tester plants were cross-fertile with corniculatus-4x tenuis plants from collection 5517. Cross-fertility between plants of collection 5101 and plants of collection 5517 can be accounted for by the origin of the latter collection and is an indication that tenuis and corniculatus are closely related. The work of Tome and Johnson (1945) provides grounds for separating tenuis and corniculatus. It was not possible to test plants of collection 5696 for cross-fertility as they had not flowered by the time this dissertation was prepared. These plants are 2n = 12 and have leaflet characteristics intermediate between tenuis and corniculatus. Plants of japonicus could be included with

major, on the basis of the characteristics used to separate major and corniculatus by Isely (1951). Further investigation is necessary to determine if major and japonicus are separate species or of the same species. Also, further investigation of cross-fertility relationships between the separate taxa is planned.

Much more material remains to be examined and floral characteristics of plants in collections that have not flowered need to be studied. Original descriptions in literature that are not yet available need to be examined before a satisfactory description of the species corniculatus or of the other species can be arrived at. A continuation of this work has been organized as a co-operative research project involving Dr. Kai Larsen of Copenhagen and Dr. E. O. Callen, Dr. W. F. Grant and the writer of Macdonald College. Dr. Callen is preparing herbarium specimens and identifying materials described in this dissertation.

Meiosis in tetraploid L. corniculatus

Meiosis was observed in plants 40, 47 and 115 of collection 5101 and in the lemon yellow plant of collection 5402, all of which have $2n = 24$. These plants were chosen because they were used for inheritance studies discussed later. No meiotic irregularities were observed. A few cells in first metaphase of meiosis were examined for each of the four plants studied and no definite multivalent configurations were observed. Of course, length differences in the chromosomes may make the identification of multivalents

involving the shorter chromosomes difficult. It was possible, however, to observe 12 distinct associations of chromosomes in some metaphase plates, which is also evidence that bivalent pairings prevail. Two first metaphase plates from a microsporocyte smear preparation from plant 40 of collection 5101 are shown in plate 2. These observations indicate that it is not necessary to become involved in tetrasomic segregation ratios proposed by Haldane (1930) or Mather (1936) since bivalent pairing apparently occurs regularly during meiosis.

Cytotaxonomy of Lotus species

Plants of a number of Lotus collections were grown and chromosome numbers were determined. Data obtained are summarized in table 2 and drawings of chromosome compliments from some of the collections are presented in plates 2 and 3.

Somatic chromosome numbers of $2n = 12$, 24, 14 and 28 were found in the Lotus materials investigated. These numbers are in agreement with those previously reported. The plants in the corniculatus group of collections have $2n = 12$ and 24. Also plants of the Old World collections 5422 and 5674 of L. canimbriensis and L. lamprocarpus and plants of the New World collection 5511 of L. denticulatus have the basic chromosome number of $x = 6$. Plants in the latter three collections are morphologically quite different from those in the corniculatus group and from each other. Also, their chromosomes are visibly morphologically different from those of plants in the

Table 2. Chromosome numbers of plants of collections of *Lotus* studied.

Collection	Name supplied by source	Chromosome number	Remarks
5406	*L. tenuis*	2n = 12	Plate 2, Figure 8.
5407	*L. corniculatus*	2n = 12	Actually *L. major*.
5410	*L. angustissimus*	2n = 12	
5412	*L. uliginosus glabrisculus*	2n = 12	
5413	*L. uliginosus villosus*	2n = 12	
5414	*L. uliginosus*	2n = 12	
5415	*L. uliginosus*	2n = 12	
5416	*L. uliginosus*	2n = 12	
5418	*L. palustris*	2n = 12	Plate 2, Figure 3.
5425	*L. seliquosus*	2n = 12	Actually *L. major*.
5429	*L. filicaulis*	2n = 12	
5501	*L. corniculatus japonicus*	2n = 12	Plate 2, Figure 7.
5505	*L. tenuis*	2n = 12	
5422	*L. canimbriensis*	2n = 12	2 satellites, Plate 3, Figure 5.
5511	*L. denticulatus*	2n = 12	American, Plate 3, Figure 1.
5696	*L. corniculatus*	2n = 12	
5101	*L. corniculatus*	2n = 24	Plate 2, Figures 10, 13 and 14.
5401	*L. corniculatus*	2n = 24	
5402	*L. corniculatus*	2n = 24	
5403	*L. corniculatus*	2n = 24	
5404	*L. corniculatus*	2n = 24	
5405	*L. corniculatus*	2n = 24	
5406	*L. corniculatus*	2n = 24	
5622	*L. corniculatus*	2n = 24	
5698	*L. corniculatus*	2n = 24	

Table 2 continued

5699	L. corniculatus	2n = 24	
56100	L. corniculatus	2n = 24	
56101	L. corniculatus	2n = 24	
56102	L. corniculatus	2n = 24	
5515	Tetraploid L. tenuis	2n = 24	
5516	Tetraploid L. tenuis	2n = 24	
5517	Tetraploid L.tenuis X L. corniculatus	2n = 24	
5518	Tetraploid L. tenuis	2n = 24	
5417	L. hispidus	2n = 24	
5428	L. divaricatus	2n = 24	Plate 2, Figure 5.
5674	L. lamprocarpus	2n = 24	Plate 3, Figure 7.
5409	L. ornithopodiodes	2n = 14	
5411	L. weilleri	2n = 14	Plate 2, Figure 1.
5421	L. edulis	2n = 14	
5423	L. tetragonolobus	2n = 14	Plate 2, Figure 3.
5424	L. jacobaeus	2n = 14	
5427	L. americanus	2n = 14	American, Plate 2, Figure 9.
5430	L. gebelia	2n = 14	Plate 2, Figure 4.
5432	Hosackia sp.	2n = 14	American, Plate 3, Figure 12.
5503	L. arabicus	2n = 14	Plate 2, Figure 12.
5506	L. maroccanus	2n = 14	Plate 2, Figure 7 and Plate 3, Figure 6.
5509	L. campylacladus	2n = 14	Plate 3, Figure 2.
5510	L. rectus	2n = 14	Plate 3, Figure 11.
5512	L. conjugatus requienii	2n = 14	Plate 3, Figure 10.
5513	L. suaveolens	2n = 14	Plate 2, Figure 2.

Table 2 continued

5514	L. maritimus	2n = 14	
5664	L. scoparius	2n = 14	American, Plate 3, Figure 8.
5668	L. douglasii	2n = 14	American, Plate 3, Figure 9.
5419	L. creticus var. cytisoides	2n = 28	
5420	L. peregrinus	2n = 28	
5426	L. parviflorus	2n = 28	Disagrees with Larsen (1956), Plate 3, Figure 4.

corniculatus group and from each other, whereas chromosomes of plants from within the corniculatus group are visibly morphologically similar. Drawings of chromosomes of plants within the corniculatus group are presented in plate 2, figures 3, 5, 7, 8, 10, 13, and 14. Drawings of L. canimbriensis chromosomes, which include a pair with satellites, are presented in plate 3, figure 5. Those of L. lamprocarpus are presented in plate 3, figure 7 and of L. denticulatus in plate 3, figure 1. Plants in collections with 2n = 14 and 28 exhibit the full range of taxonomic variation that is present in the Lotus materials studied. Ottley (1944) pointed out that it is difficult to find a good taxonomic reason for separating the American species as a group from the Old World species. In the present studies, basic chromosome numbers of x = 6 and of x = 7 were found in both Old and New World collections which is in agreement with Ottley's opinion that these two groups should be included in the one genus. These studies also indicate that chromosome

numbers cannot be used by themselves to make a major division within this genus, although chromosome numbers and studies of chromosome morphology together may be very useful in arriving at a classification of species within this genus.

The above observations indicate that the direction of evolution in Lotus has been from a basic chromosome number of $\underline{x} = 7$ to one of $\underline{x} = 6$. From the similarities in the chromosome idiograms of plants within the corniculatus group as well as from taxonomic similarities, it is proposed that the species in this group have a common ancestor with $\underline{x} = 7$ and that the group evolved following the loss of one set of homologous chromosomes. The other three $\underline{x} = 6$ collections are morphologically different from those in the corniculatus group, so it is proposed that they originated from other $\underline{x} = 7$ species on different occasions. Morphological variation of $\underline{x} = 6$ species falls well within the range exhibited by those with $\underline{x} = 7$.

Evolution from a higher to a lower chromosome number has been demonstrated in Crepis (Reviewed by Stebbins, 1950), in which reduction in chromosome number is associated with evolution from more primative to more specialized taxonomic characteristics. Stebbins pointed out that reduction in

chromosome number with evolution has been found in the majority of plant genera investigated. The trend proposed for Lotus, based on the writer's general impression of morphological variation in the genus, is in agreement with the common trend proposed for other plant genera by Stebbins. Further investigation will be necessary to confirm or disprove this hypothesis.

Plate 2. Drawings of meiotic and premeiotic chromosomes prepared from acetocarmine squashes with the aid of camera lucida.

Figure	Collection	Name supplied	Remarks
1	5411	L. weilleri	Anaphase I, polar view, 2n = 14.
2	5513	L. suaveolens	Anaphase I, side view, 2n = 14.
3	5418	L. palustris	Anaphase I, polar view, 2n = 12.
4	5430	L. gebelia	Premeiotic anaphase, 2n = 14.
5	5428	L. divaricatus	Anaphase I, polar view, 2n = 24.
6	5409	L. ornithopodioides	Anaphase I, polar view, 2n = 14.
7	5501	L. corniculatus japonicus	Anaphase I, polar view, 2n = 12.
8	5406	L. tenuis	Anaphase I, polar view, 2n = 12.
9	5427	L. americanus	Anaphase I, polar view, 2n = 14.
10	5101	L. corniculatus	Anaphase I, polar view, 2n = 24.
11	5506	L. maroccanus	Telophase II, n = 7.
12	5503	L. arabicus	Anaphase I, polar view, 2n = 14.
13	5101	L. corniculatus	Polar view of diakinesis, 2n = 24.
14	5101	L. corniculatus	Metaphase I, side view, 2n = 24.

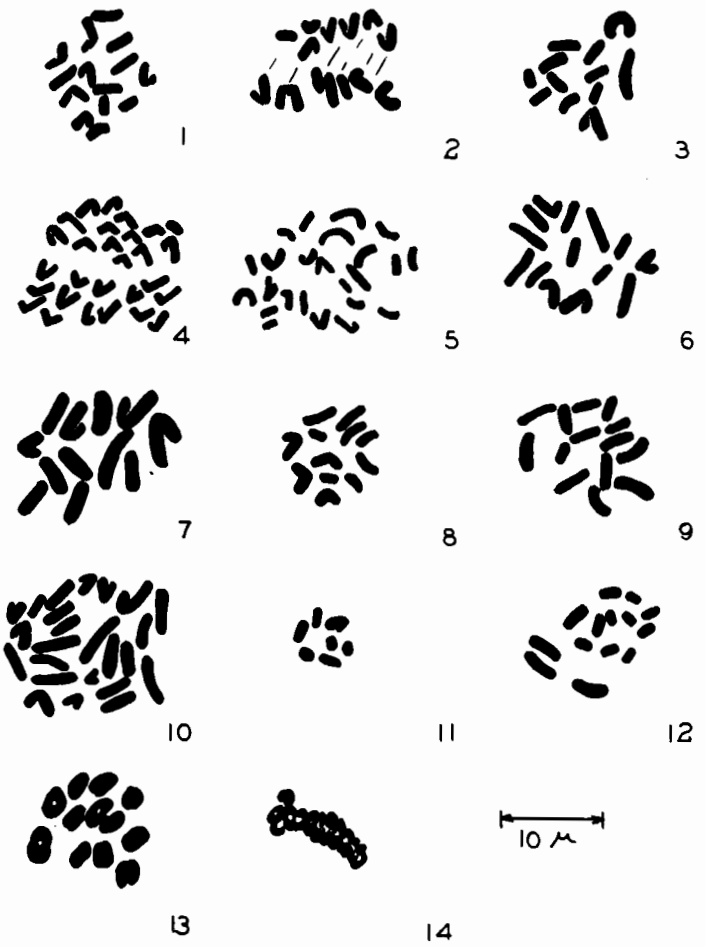

Plate 2.

Plate 3. Drawings of meiotic chromosomes prepared from crystal violet sections of root tips with the aid of camera lucida.

Figure	Collection	Name supplied	Remarks
1	5511	L. denticulatus	American
2	5509	L. campylacladius	
3	5423	L. tetragonolobus	
4	5426	L. parviflorus	
5	5422	L. canimbriensis	Two satellites
6	5506	L. maroccanus	
7	5674	L. lamprocarpus	
8	5664	L. scoparius	American
9	5668	L. douglasii	American
10	5512	L. conjugatus requienii	
11	5510	L. rectus	
12	5432	Hosackia species	American

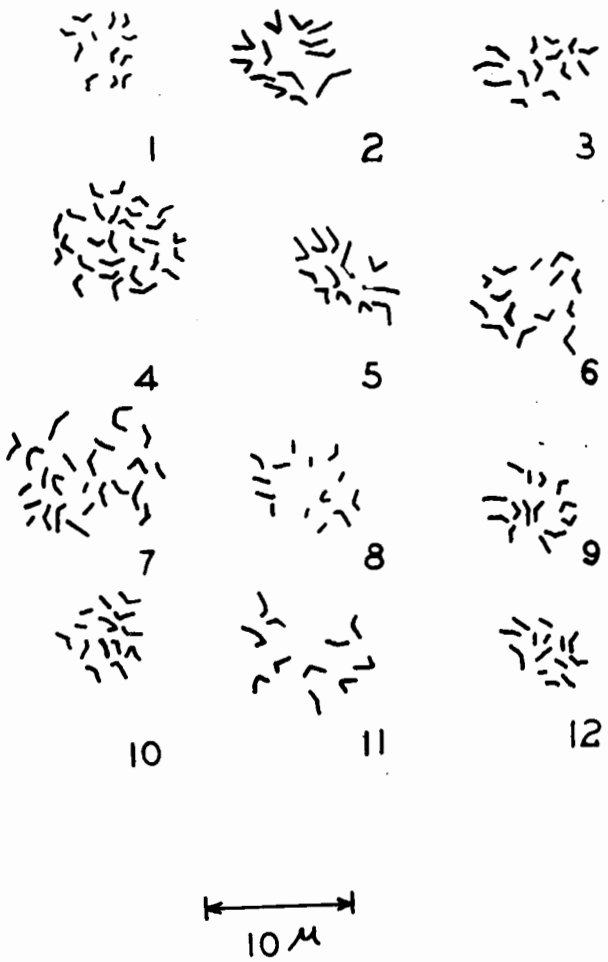

Plate 3.

Plate 4. Lemon yellow flower colour. The plant on the left has lemon yellow flowers and the one on the right has the wild type colour flowers. Plant colours in this print are fairly accurate although the soil colour is inaccurate.

Plate 5. Keel tip colour. The center pair of keels have the red tip phenotype and the outside pair have the yellow phenotype.

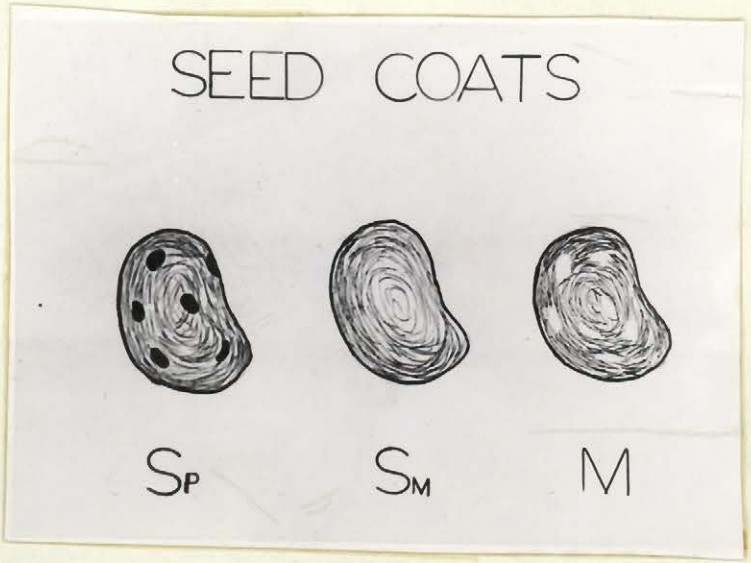

Plate 6. Seed coat characters. Drawings showing the speckled phenotype (Sp) on the left which has black speckles on an olive brown background, the smooth phenotype (Sm) in the center which has a uniform olive brown colour, and the mottled (M) phenotype on the right, which has white mottling in an olive brown background.

GENETIC STUDIES IN L. CORNICULATUS

In order to obtain some evidence on the prevalence of tetrasomic inheritance in L. corniculatus, available populations were carefully examined to locate characters which appeared to segregate into discrete classes. The characters found were:

A. Lemon yellow flower colour. (Plate 4.) A single plant in collection 5402 possessed a lighter flower colour, which is designated as "lemon yellow" in these investigations. This plant is a tetraploid with a somatic chromosome number of $2n = 24$ and has bivalent pairing during meiosis. (Bivalent pairing in this plant is discussed in the section on meiosis in tetraploid L. corniculatus).

B. Red keel tip. (Plate 5.) Populations of plants from collection 5101, which were used for incompatibility studies, segregated for presence or absence of red colour on the tip of the keel. These plants are tetraploids with a somatic chromosome number of $2n = 24$ and bivalent pairing during meiosis.

C. Mottled seed coat. (Plate 6.) The populations established from collection 5101 also segregated for presence or absence of white mottling on the seed coat.

D. Speckled seed coat. (Plate 6.) The populations established from collection 5101 also segregated for presence or absence of black speckling on the seed coat.

E. Self-incompatibility. Populations from plants selected

for desirable agronomic characters in collection 5101 of L. corniculatus were used for a series of experiments on self-incompatibility. These plants are cross-fertile and self-sterile so that it was possible to discriminate clearly between seed set following self- and cross-pollination.

Considerable variation in leaf size and shape, pubescence, growth habit, red pigment in stems, red venation in petals and redness of the buds was also observed. Preliminary observations indicated that it would be very difficult to classify these plants into discrete classes for these characters, which are required for satisfactory inheritance studies.

Lemon yellow flower colour.

The lemon yellow plant was crossed to a wild type colour plant from collection 5101. Eleven F_1 progeny were grown and they all exhibited the wild type flower colour. A single F_1 plant was selected and backcrossed to its lemon yellow parent and 81 backcross progeny were grown. These plants were grown in a spaced planting of over 600 plants in which they replaced seedlings which died following transplanting. The location of each backcross progeny was recorded in a planting plan. When the nursery was in full bloom, the flower colour of each plant in the whole nursery was compared with that of the lemon yellow parent. Thirteen lemon yellow plants were found, all of them progeny of the backcross. The remaining sixty-eight progeny were not distinguishable

from other plants in the nursery.

These data indicate that the lemon yellow colour is recessive to the wild type colour and that it is inherited tetrasomically at a single locus. These data are in agreement with Muller's hypothesis for tetrasomic inheritance, as is illustrated in table 3.

Table 3. Lemon yellow flower colour inheritance.

Cross $\underset{\text{(wild)}}{YYYY} \times \underset{\text{(lemon)}}{yyyy} \longrightarrow \underset{\text{(wild)}}{YYyy}$

Gametes of $YYyy = 1YY : 4Yy : 1yy$

Backcross $YYyy \times yyyy \longrightarrow \underbrace{1YYyy : 4Yyyy}_{\text{wild}} : \underbrace{1yyyy}_{\text{lemon}}$

wild		lemon	
Obs.	Exp.	Obs.	Exp.
68	67.5	13	13.5

Red keel tip.

This character was observed to be segregating in the plants grown of collections 5101, 5401, 5404, 5405, 5408, and 5622 of L. corniculatus and in Agronomy Department plots of the varieties; Empire, Cascade, Viking, Mansfield and Granger. Data were collected on populations of plants used in incompatibility studies. No crosses were made specifically to study the inheritance of this character.

It was possible to consistently classify plants for the presence or absence of red keel tip, but it was not possible to consistently classify the red keel tip plants according to the intensity of the colour. The data obtained from specific cross- and self-progenies, together with proposed

genotypes, are presented in table 4. Chi square tests are presented in table 5 and a contingency test is presented in table 6.

The segregation data do not indicate a dominance relationship between keel tip colour alleles as self-progenies of plants with and of plants without red keel tip segregated for this character. It was observed that yellow is the background colour of the whole keel and that the red can be scraped off leaving the yellow background. This suggests that the red colour occurs when a pigment is present and yellow when it is absent. Since it has been demonstrated in numerous inheritance studies (for example see Twamley 1955) that the presence of a pigment is generally dominant to its absence, red may be expected to be dominant to yellow in this case.

The ratios obtained do not fit any simple tetrasomic inheritance hypothesis. An hypothesis is proposed involving tetrasomic inheritance at one locus, as was found with lemon yellow flower colour and in Dawson's (1941) investigations. In order to explain segregation in self-progenies from both red and yellow keel tip parents, a dosage effect is proposed so that in the nulliplex or simplex conditions for red, the yellow phenotype is produced and in the duplex, triplex, or quadruplex conditions, the red phenotype is produced. Some of the data deviate significantly from this hypothesis, as is illustrated in tables 5 and 6. It was noted that the data which deviate significantly are associated with inbreeding

Table 4. Keel tip colour inheritance data.

Segregations obtained for keel-tip colour

Self-Pollinations

Parent Plant	Colour	Proposed genotype	Progeny Yellow	Red
40	Yellow (Y)	Kkkk	33	9
45	Yellow	Kkkk	2	3
47	Red (R)	KKkk	26	20
115	Red	KKkk	3	32
133	Yellow	Kkkk	4	3
210	Yellow	Kkkk	4	2

Cross-Pollinations

Plants		Colours	Proposed genotypes	Yellow	Red
40	X 115	Y X R	Kkkk X KKkk	11	8
115	X 40	R X Y	KKkk X Kkkk	14	18
		(Parent X Progeny of above crosses)			
40	X 13-9	Y X Y	Kkkk X Kkkk	15	7
13-12	X 40	Y X Y	Kkkk X Kkkk	60	2
40	X 14-2	Y X R	Kkkk X KKkk	10	0
40	X 14-14	Y X Y	Kkkk X Kkkk	12	5
14-14	X 40	Y X Y	Kkkk X Kkkk	36	2
115	X 14-9	R X R	KKkk X KKkk	3	4
13-12	X 115	Y X R	Kkkk X KKkk	10	7
		(Parent X Progeny of 40⊗)			
40	X 19-4	Y X R	Kkkk X KKkk	5	6
20-7	X 40	Y X Y	kkkk X Kkkk	42	0
20-12	X 40	Y X Y	kkkk X Kkkk	43	0
		(Parent X Progeny of 115 ⊗)			
17-9	X 115	R X R	KKkk X KKkk	8	30
		Other Crosses			
40	X 47	Y X R	Kkkk X KKkk	8	8
115	X 47	R X R	KKkk X KKkk	0	10
47	X 115	R X R	KKkk X KKkk	2	5
102	X 47	Y X R	Kkkk X KKkk	5	4
102	X 40	Y X Y	Kkkk X Kkkk	11	0
102	X 115	Y X R	Kkkk X KKkk	2	1

⊗ = self-pollinated

Y and R designate phenothypic colours, K and k represent genes.

Table 5. Goodness of fit of keel-tip colour inheritance data to ratios obtained using the tetrasomic inheritance at one locus hypothesis.

Cross	Yellow Obs.	Yellow Exp.	Red Obs.	Red Exp.	Yates' corrected Chi-square
40 ♀	33	31.50	9	10.50	0.13
45 ♀	2	3.75	3	1.25	1.67
47 ♀	26	11.50	20	34.50	22.75 **
115 ♀	3	8.75	32	26.25	4.20 *
133 ♀	4	5.25	3	1.75	0.43
210 ♀	4	4.50	2	1.50	0.00
40 X 115	11	9.50	8	9.50	0.22
115 X 40	14	16.00	18	16.00	0.28
40 X 13-9	15	16.50	7	5.50	0.24
13-2 X 40	60	46.50	2	15.50	14.53 **
40 X 14-2	10	5.00	0	5.00	8.10 **
40 X 14-14	12	12.75	5	4.25	0.02
14-14 X 40	36	28.50	2	9.50	6.88 **
115 X 14-9	3	1.75	4	5.25	0.43
13-12 X 115	10	8.50	7	8.50	0.24
40 X 19-4	5	5.50	6	5.50	0.00
20-7 X 40	42	42.00	0	0.00	0.00
20-12 X 40	43	43.00	0	0.00	0.00
17-9 X 115	8	9.50	30	28.50	0.14
40 X 47	8	8.00	8	8.00	0.00
115 X 47	0	2.50	10	7.50	2.13
47 X 115	2	1.75	5	5.25	0.00
102 X 47	5	4.50	4	4.50	0.00
102 X 40	11	8.25	0	2.75	2.45
102 X 115	2	1.50	1	1.50	0.00

* sign. at p = 0.05

** sign. at p = 0.01

Table 6. Contingency test of the significance of a reciprocal difference in keel tip colour.

Cross		Yellow	Red	Total
40 X 14-14	Obs.	12	5	17
	Exp.	14.84	2.16	
14-14 X 40	Obs.	36	2	38
	Exp.	33.16	4.84	
	Total	48	7	55

Chi - square for 1 degree of freedom calculated with Yates' correction for small numbers (Snedecor, 1946, page 22) = 4.184, significant at P = 0.05.

and that in only one case, in a cross that yielded only 10 progeny, is a class missing that is expected with this hypothesis. In this particular cross there was a poor seed set and a large portion of the seedlings died after germination. Also, a significant difference in reciprocal parent X progeny crosses was demonstrated in the 40 X 14-14 and 14-14 X 40 crosses. The significance of this reciprocal difference is illustrated by having one chi-square value significant and the other one not significant in table 5 and is confirmed with a contingency test presented in table 6.

This reciprocal difference and the deviations from random segregations illustrated by significant chi-square values in table 5 could occur if the keel tip colour locus is linked to a locus influencing survival or to a locus influencing breeding behaviour. The data obtained may be accounted for without discrediting the tetrasomic inheritance at one locus hypothesis by assuming linkage to an incompatibility locus or to a locus or loci involving lethals or overdominance heterosis. Reciprocal differences for incompatibility is one characteristic of some oppositional incompatibility systems, which suggests that the keel tip colour locus is linked with an incompatibility locus, as is discussed later in this dissertation.

The hypothesis that this locus is linked to an incompatibility locus is made plausible by the observation that red keel tip is segregating in the populations of L. corniculatus that were used in the incompatibility studies.

This linkage would give the observed heterogeneity for this character within populations a selective advantage although it is hard to propose any reason for this heterogeneity by itself. Bee pollination studies by Morse (1955) suggest that this heterogeneity for a flower colour character might even be at a selective disadvantage.

Although the data obtained do not fit a simple tetrasomic inheritance hypothesis, it is possible to account for the observed deviations by making two reasonable assumptions, (1) a dosage effect with a threshold value before a phenotype appears and (2) linkage to an incompatibility locus. This hypothesis appears more reasonable to the writer than alternative hypothesis involving disomic inheritance with which it is necessary to postulate more than one locus and a reversal of the dominance relationship at different loci. Epistasis also has to be postulated to account for the segregations obtained with a disomic hypothesis.

It is concluded that tetrasomic inheritance is more probable than disomic inheritance for the red keel tip character.

Mottled seed coat.

This character was observed to be segregating in plants of collection 5501 and in seed samples of the named varieties of L. corniculatus; Empire, Cascade, Granger, Viking, and Mansfield. Data were collected in populations of plants used for incompatibility studies and no crosses were made specifically to study the inheritance of this character.

It was difficult to recognize the phenotype of this

character and that of the seed speckling character as these phenotypes could be observed only on mature seeds which had not been weathered. No plants were observed with both these characters present on their seeds, which may have some biological significance or may simply be due to the observer's inability to recognize these characters when they are present together. The data obtained are presented in table 7.

Non-mottled plants crossed with other non-mottled plants produced progenies which segregated for this character. 17-9 (mottled) X 115 (non-mottled plant, of which 17-9 is a self-progeny) segregated 1 : 1 and 222 (non-mottled) X 182 (mottled) also segregated 1 : 1. Non-mottled plants 40 and 115 produced self-progenies which segregated 35 : 1 and 3 : 1 respectively. These segregations fit the hypothesis that the allele M (non-mottled) is dominant to m (mottled) and that they are inherited tetrasomically. Genotype mmmm can be assigned to 17-9 and 182, Mmmm to 115 and 222, MMmm to 40, 15 and 46 and MMMm or MMMM to 47. The one chi-square value, of the seven calculated, that deviates significantly from the hypothesis is from a cross of two plants selected for late flowering habit. No explanation of this deviation can be given at the present time.

A tentative hypothesis of tetrasomic inheritance at one locus is based on data obtained from plant populations produced for purposes other than the study of the inheritance of this character and fits these data fairly well as is shown above. Further investigation of plants selected especially

Table 7. Mottled seed coat inheritance data.

Numbers of mottled : non-mottled plants obtained from various crosses. The data from reciprocals are combined.

Parental plants	40	115	47
40	1:40	5:22	0:16
115	5:22	10:23	0:17
47	0:16	0:17	0:44
102	0:10	0:3	0:10
19-4 (40 ♀)	0:11	-	-
21-3 (47 ♀)	-	-	0:11
17-9 (115 ♀)	-	17:20	-
20-7 (40 ♀)	7:36	-	-
20-12 (40 ♀)	0:42	-	-

Parental plants	40	15	46	56	205	182
222	0:11	2:19	0:9	0:14	0:16	9:11
205	0:32	0:21	0:19	0:15	-	0:23
56	0:24	0:23	-	-	0:15	0:18
127	0:14	0:20	-	0:17	0:12	-
147	-	0:9	-	0:8	-	-
46	-	0:17	-	-	-	7:16

Mottled parents: 17-9, 182.

Non-mottled parents: 40, 115, 47, 102, 19-4, 21-3, 20-7, 20-12, 15, 46, 50, 205, 222, 127, 147.

Table 8. Goodness of fit of mottled seed coat data to ratios obtained using a tetrasomic inheritance hypothesis.

Cross	Mottled Obs.	Mottled Exp.	Non-mottled Obs.	Non-mottled Exp.	Yates' corrected Chi-square
17-9 X 115	17	18.50	20	18.5	0.11
222 X 182	9	10.00	11	10.00	0.05
40 ♀	1	1.20	40	39.80	0.00
115 ♀	10	8,25	23	24.75	0.25
182 X 46	7	2.83	16	20.17	5.43 *
222 X 15	2	1.75	19	19.25	0.00
40 X 15	5	2.25	22	24.75	2.45

* Significant at P = 0.05

Proposed genotypes

mmmm - 17-9 and 182.

Mmmm - 115 and 222.

MMmm - 40, 15 and 46.

MMMm or MMMM - 47.

for the study of the inheritance of this character, especially in a cross of mottled X mottled, is needed to test this hypothesis.

Speckled seed coat.

This character was segregating in all populations in which mottled seed coat was found to be segregating. All plants of collection 5406 of L. tenuis produced speckled seeds. Again data were collected on populations produced for other purposes and no crosses were made specifically to study the inheritance of this character.

The data obtained are presented in table 9. These data are not extensive enough to suggest the mode of inheritance. Dominance cannot be determined as both speckled and non-speckled plants produced self-progenies which segregated for this character. The behaviour of this character appears to be somewhat similar to that observed for red keel tip and may possibly be accounted for in the same way, however more data are needed before a definite hypothesis can be proposed.

Self-incompatibility.

Terminology. In this dissertation, the term "self-sterile" is used in the broad sense when self-seeds are not produced by undisturbed isolated flowers. The sterility may be caused by genetic, environmental or mechanical factors which may act before, during or after fertilization. "Self-fertile" is the antonym of "self-sterile".

A plant may be "self-sterile but mechanically self-fertile" if self-seeds are not produced by undisturbed

Table 9. Speckled seed coat inheritance data.

Numbers of speckled : non-speckled plants obtained from various crosses. The data from reciprocals are combined.

Parental plants	40	115	47
40	9:32	8:19	8:8
115	8:19	12:21	10:3
47	8:8	10:3	22:22
102	1:9	0:3	6:4
19-4 (40 ⊗)	8:3	-	-
21-3 (47 ⊗)	-	-	7:4
17-9 (115 ⊗)	-	5:30	-
20-7 (40 ⊗)	0:43	-	-
20-12 (40 ⊗)	0:42	-	-

Parental plants	40	15	46	56	205	182
222	0:11	0:21	1:8	4:10	2:14	0:20
205	3:29	0:21	0:19	3:12	-	0:23
56	0:24	0:23	-	-	3:12	0:18
127	0:14	0:20	-	0:17	0:12	-
147	-	0:9	-	0:8	-	-
46	-	0:17	-	-	-	0:23

Speckled parents: 47, 19-4, 222.

Non-speckled parents: 17-9, 182, 40, 115, 102, 20-7, 20-12, 56, 127, 205, 46, 15, 147.

flowers but are produced by flowers which are mechanically self-pollinated.

The term "self-incompatible" is used specifically to describe a plant characteristic, controlled by genes, that is manifest by restricted self-fertilization or by restricted cross-fertilization between plants of the same incompatibility genotypes. In some species (see Sears 1937), restricted self-fertilization has been associated with differential pollen tube growth rates, although this association may not necessarily occur as any other factor that can produce differential fertilization may also produce incompatibility. Theoretically, a plant could be self-fertile and at the same time self-incompatible if a mechanism is acting to encourage cross-fertilization but does not prevent self-fertilization. This may be the case in the wallflower, reported by Bateman (1956). "Self-compatible" is the antonym of "self-incompatible."

Genetic studies. This character was studied extensively in plants selected from collection 5101 of tetraploid L. corniculatus. Plants in collections 5401, 5402, 5403, 5404, 5405, 5408, 5611, and 5622 of L. corniculatus were also tested and were observed to be about as self-sterile as plants in collection 5101. Also, plants in collections 5406 and 5505 of L. tenuis, 5515, 5516 and 5518 of tetraploid L. tenuis and 5517 of 4x tenuis X corniculatus were tested and observed to be about as self-sterile as plants in collection 5101. Plants selected from the named varieties of L. corniculatus: Empire, Granger, Cascade, Viking and Mansfield

were also observed to be about as self-sterile as the plants from collection 5101. Further data on self-sterility are presented in table 28, where they are given in conjunction with observations on ovule development.

Experiments were designed to discriminate between gametophytic and sporophytic oppositional systems based on the theoretical considerations presented by Lewis (1954). The experimental design also allowed for possible disomic and tetrasomic inheritance at one or two loci. Backcross and self-progenies were grown which were theoretically large enough to determine if tetrasomic inheritance is occurring at two or possibly three loci, or if disomic inheritance is occurring at a number of loci, provided that alleles at these loci are not selected differentially by inbreeding and that they act in the same way.

Compatibility was evaluated in terms of a "compatibility index" calculated by dividing the total number of seeds produced from a cross or self-pollination by the total number of flowers pollinated in making this cross or self-pollination. This value may have been influenced by uncontrollable variations in technique and by environmental variations, as would an index based on the number of pods produced divided by the number of flowers pollinated. An index based on the average number of seeds per pod may remove some of the effects of variations in technique but was not considered as satisfactory as the index chosen because it is influenced more

by variability in ovule development associated with age of the flower, which is discussed later in this dissertation. Also, it does not make use of the evidence from the proportion of flowers pollinated that set pods which is especially meaningful in crosses which are quite incompatible and in self-pollinations, in spite of uncontrollable variations in technique and environment.

The compatibility index used makes it possible to compare crosses in which different numbers of flowers were used. The number of flowers used was largely determined by the number of flowers available at the time the tests were made. Crosses which failed were repeated one or more times, if materials were available, until some seeds were obtained or until there was good evidence of cross-incompatibility. The number of flowers used to calculate the index included those from attempts that failed as well as from attempts that succeeded. Successful crosses were rarely repeated if more than three pods were obtained. The practice of repeating crosses that failed could easily lead to a bias in the indices compared with values that would have been obtained if equal numbers of flowers had gone into each cross.

The tables which follow contain compatibility indices obtained from various experiments involving crosses of L. corniculatus. The very extensive data from which these indices were calculated is not recorded in this dissertation. All the original data are in the possession of the writer and will be kept on record in the Agronomy Department at Macdonald College.

Table 10. Compatibility indices from crosses of plants not known to be closely related.

Cross	Index	
40 X 115 (from collection 5101)	7.05	Average
115 X 40 (" " ")	5.71	6.38
40 X 205 (" " ")	9.50	
40 X 56 (" " ")	12.75	
40 X 15 (" " ")	3.50	
40 X a plant from collection 5404	18.90	
40 X a plant from collection 5405	31.40	
40 open-pollinated by bees in field (seeds per pod)	13.85	

Table 11. Compatibility indices from self-pollinations of plants selected from collection 5101.

Self-pollinations	Index
115 ♀	0.11
40 ♀	0.15
47 ♀	0.39

Table 12. Compatibility indices from parent X progeny crosses between 40, 115 and progeny of 40 X 115 (13-8 to 14-2) and 115 X 40 (14-3 to 14-15) and for the progeny selfed.

Progeny plant number	Selfed	Crossed to parental clones				Sum of crosses
		40 ♀	40 ♂	115 ♀	115 ♂	
13-8	0.00	2.88	4.71	1.87	3.88	13.34
9	-	2.25	4.40	4.56	2.40	13.61
10	-	5.13	3.78	2.33	6.08	17.32
11	-	3.20	3.50	4.17	6.14	17.01
12	0.00	5.46	3.96	1.70	3.79	14.91
13	0.00	3.21	3.82	6.67	0.18	13.88
14	-	2.58	4.76	3.11	7.17	17.62
15	0.00	2.57	4.58	7.15	5.91	20.21
16	-	0.49	4.56	1.73	4.50	11.28
17	-	2.32	9.68	1.90	6.27	20.17
18	0.00	1.22	0.59	4.08	0.21	6.08
20	0.00	4.47	1.11	5.80	6.12	17.50
21	-	0.67	7.50	1.92	2.70	12.79
23	0.00	7.86	3.32	1.95	11.50	24.63
14-1	-	3.91	8.25	2.28	6.90	21.34
2	0.00	2.47	0.59	1.19	1.94	6.19
3	-	0.97	2.83	1.30	6.61	11.71
4	0.00	1.42	8.00	5.10	4.07	18.59
5	-	1.13	4.00	0.00	5.11	10.24
6	0.00	2.67	11.94	2.74	13.08	30.43
7	-	16.60	6.00	3.40	2.86	28.86
8	0.00	2.50	3.47	4.67	4.67	15.31
9	0.00	1.68	2.30	2.21	1.68	7.87
10	0.00	11.80	1.81	4.09	0.47	18.17
11	-	9.14	7.71	4.73	7.71	29.29
12	-	9.78	4.65	3.70	7.53	25.66
13	0.00	0.86	3.42	0.58	10.80	15.66
14	0.00	12.10	6.28	1.98	2.67	23.03
16	-	3.36	5.25	2.23	3.92	14.76
Sum	0.00	124.70	136.75	89.14	146.87	497.46
Mean	0.00	4.30	4.72	2.97	5.16	4.29

Note: a dash signifies that no data were obtained.

Table 13. Analysis of variance of Table 12.

	D.F.	S.S	M.S.	F values
Between compatibility index means for parents.	3	65.54	21.85	2.56 ?
Between compatibility index means for progeny.	28	294.26	10.51	1.23
Remainder	85	726.11	8.54	
Total	115	1085.91		

? significant at P = 0.10

D.F. (degrees of freedom); S.S. (sum of squares); M.S. (mean square).

Table 14. Correlation coefficients for compatibility indices in Table 12.

Between 40 ♂ and 40 ♀, r = +0.053

Between 40 ♂ and 115 ♂, r = +0.624 ***

Between 40 ♂ and 115 ♀, r = -0.052

Between 40 ♀ and 115 ♂, r = +0.092

Between 40 ♀ and 115 ♀, r = +0.134

Between 115 ♂ and 115 ♀, r = +0.005

*** significant at P = 0.001

The analysis of variance (table 13) indicates a trend for plant 115 used as the female and crossed to its progeny from a cross with plant 40 to have lower compatibility indices. It was observed that this plant did not thrive particularily well in the greenhouse during the winter when these crosses were made, therefore these lower values may be a reflection of growth conditions rather than of lower genetic compatibility. This same interpretation may apply to the significant correlation between the compatibility indices obtained when the progeny were used as the female and parents as the male, (columns headed 40 male and 115 male). The indices may indicate the response of these plants to the greenhouse environment, which could be due to inherent differences in seed setting ability between the progenies or to environmental differences in the greenhouse as it was not possible to make all tests at one time because all plants did not flower at the same time.

No self-seed was obtained on any of the progeny of 115 X 40 (table 12) tested by self-pollinating, therefore indices are 0.00. Only one cross had an index value of 0.00 (115 X 14-5). This cross was attempted on two occasions with a total of 18 flowers, hence it is a fair indication of a cross-incompatibility. There are some other low compatibility indices which may also indicate genetic cross-incompatibilities. Only two more of them need to be accepted to have a good approximation to a ratio of 35 compatibilities : 1 incompatibility, which ratio might be

associated with tetrasomic inheritance at one locus. There are a total of eleven compatibility indices of less than 1.00 in table 12, any of which may be accepted as indicating genetic cross-incompatibility.

If we accept any of these indices as indicating true cross-incompatibilities, we admit reciprocal differences in compatibility, which is of criterion that may be used to differentiate gametophytic and sporophytic incompatibility, indicating a sporophytic system if incompatibility alleles occur at only one locus, as is pointed out by Lewis (1954) and Lundqvist (1956).

Another criterion that can be used to differentiate between the two systems, if incompatibility alleles occur at only one locus, is that in the sporophytic system, incompatibility can occur between a progeny and its female parent. This may occur between 115 and 13-13 or 13-18, or between 40 and 14-3 or 14-13.

The data from these crosses are not sensitive enough to definitely indicate how many cross-incompatibilities are present, so they cannot be used to discriminate between possible gene segregation patterns which might indicate disomic or tetrasomic inheritance or the number of loci involved. The data presented in table 12 do not disagree with the hypothesis that incompatibility in L. corniculatus is determined genetically at one locus, if allowance is made for tetrasomic inheritance.

Progeny of selected parent X progeny crosses were grown

Table 15. Compatibility indices from parent by progeny backcrosses between 40, 13-12 and progeny (E) and for progeny selfed.

Progeny plant number	Selfed	Crossed to parental clones			
		40 ♀	40 ♂	13-12 ♀	13-12 ♂
E-1	0.00	4.00	3.91	1.33	3.00
2	0.00	4.00	1.00	0.53	1.91
3	0.00	5.00	9.83	1.00	6.33
4	0.00	6.50	6.50	2.60	7.00
5	0.81	4.13	4.42	3.25	5.10
6	0.00	0.00	8.00	0.00	4.14
7	0.00	4.00	1.00	0.58	7.27
8	0.05	0.00	4.50	0.68	2.22
9	0.00	0.21	10.66	3.50	6.22
10	0.00	0.90	3.12	0.63	8.57
11	0.00	0.00	1.25	0.00	3.05
12	0.00	1.33	5.88	0.00	9.28
13	0.00	3.55	7.22	-	9.62
14	0.19	3.00	9.83	1.75	7.20
15	0.00	3.80	5.75	4.33	15.00
16	0.00	0.00	3.50	-	12.12
17	0.00	0.83	4.20	2.75	4.00
18	0.00	2.50	7.60	1.47	6.60
19	0.00	0.00	6.28	0.28	8.60
20	0.00	0.00	13.87	0.00	2.28
21	-	0.00	7.00	0.00	3.12
22	0.00	0.00	9.20	0.25	1.58
23	0.00	3.42	11.16	0.00	7.00
24	0.00	0.00	0.20	0.58	5.43
25	0.00	0.00	1.88	2.33	10.71
26	0.12	0.33	2.00	0.00	1.14
27	0.00	3.00	3.10	1.00	4.00
28	0.00	-	-	0.00	2.20
29	-	-	3.67	0.00	5.17
30	0.00	-	-	0.00	3.09
31	0.00	2.11	-	0.16	2.20
32	-	-	-	-	10.28
33	0.00	4.89	5.17	0.00	9.14
34	-	-	1.30	0.00	0.00
35	0.00	-	0.47	0.00	3.31
36	0.00	2.00	-	0.00	1.94
37	0.00	10.13	2.24	0.00	4.44
38	0.00	3.73	3.61	0.00	2.73

Table 16. Compatibility indices from parent X progeny backcrosses between 115, 13-12 and progeny (F) and for progeny selfed.

Progeny plant number	Selfed	Crossed to parental clones			
		115 ♀	115 ♂	13-12 ♀	13-12 ♂
F-1	0.00	0.07	6.00	0.00	1.97
2	0.00	12.42	11.50	5.20	5.36
3	0.00	7.25	9.35	1.00	-
4	0.00	0.00	4.75	4.71	2.50
5	0.00	4.10	4.40	0.78	9.67
6	0.00	0.00	8.17	0.00	3.50
7	0.00	2.50	7.57	2.28	6.25
8	0.00	6.57	0.63	-	4.80
9	1.28	3.13	4.40	0.00	7.50
10	0.00	3.15	8.87	-	5.50
11	0.00	-	3.92	0.00	3.38
12	0.12	2.86	10.18	0.00	7.67
13	-	7.38	5.50	0.00	-
14	0.00	2.54	7.12	0.20	2.38
15	0.00	0.00	10.20	-	9.28
16	0.00	0.00	4.50	-	2.17
17	-	-	7.33	0.33	7.67
18	0.00	0.00	-	-	4.25

Table 17. Compatibility indices from parent X progeny crosses between 115 and 115 self-progenies and from self-pollination of the progenies.

Progeny plant number	Progeny selfed	Backcrossed to parental clone 115 ♀	115 ♂
17-8	-	0.19	0.70 *
9	-	1.08	3.84
10	0.00	1.03	1.03
11	0.00	1.42	1.74
12	0.00	0.53	5.69
13	0.00	0.47	1.80
14	0.00	1.61	2.48
15	0.05	0.88	3.66
16	0.00	0.85	4.00
17	0.00	1.00	1.40
18	0.00	0.93	3.45
19	-	0.58	1.75
20	0.00	0.89	0.83 *
21	0.13	0.46	2.78
22	0.08	2.38	3.83
23	0.00	0.00	0.00 *
18-1	0.00	0.92	6.10
2	0.00	0.31	2.72
3	0.00	0.40	1.70
4	0.00	1.43	1.54
5	-	0.17	1.43
6	-	0.42	0.55 *
7	0.00	0.00	5.60
8	0.00	1.00	3.33
9	0.12	0.11	1.83
10	-	0.92	3.11
11	0.00	0.00	1.76
12	0.21	2.76	6.81
13	-	0.00	4.60
14	0.00	1.88	0.60
15	0.00	0.00	2.67
16	0.00	0.00	1.05
17	0.00	1.45	2.17
18	-	1.88	1.28
19	-	3.70	12.41
Average	0.02	0.90	2.85

* = progeny possibly incompatible with parent

Table 18. Compatibility indices from parent X progeny crosses between 40 and 40 self-progenies and from self-pollination of the progenies.

Progeny plant number	Progeny selfed	Backcrossed to parental clone 40 ♀	40 ♂
18-20	-	2.30	3.00
21	-	8.78	5.75
22	0.00	1.71	5.10
23	-	-	4.73
19-1	-	3.62	8.67
2	-	-	10.35
3	0.00	11.00	1.75
4	0.00	4.00	1.93
5	-	12.00	4.90
6	-	8.29	3.00
7	-	10.90	13.00
8	0.00	3.56	9.14
9	0.00	13.40	8.00
10	0.00	2.05	4.69
11	-	2.13	4.28
12	-	0.00	- *
13	0.00	0.10	3.10
14	-	17.44	7.57
15	-	20.44	9.17
16	0.00	0.00	4.50
17	-	10.90	9.64
18	-	4.30	12.40
19	-	6.00	7.07
20	0.00	4.31	0.86
21	-	6.67	5.75
22	-	9.33	7.50
23	-	0.93	3.90
20-1	0.00	5.67	3.19
2	0.00	8.30	1.14
3	-	4.09	5.20
4	-	-	1.18 *
5	-	2.67	1.13
6	0.00	6.20	6.69
7	1.16	5.45	3.26
8	-	3.80	9.50
9	-	12.20	13.28
10	-	14.00	3.88
11	0.00	2.82	0.65
12	0.00	3.96	2.87
13	-	3.50	6.18
14	0.00	0.00	5.00
15	1.33	2.45	3.12
16	0.00	0.30	4.07
17	-	3.08	9.17
Average	0.14	5.88	5.56

* = progeny possibly incompatible with parent.

Table 19. Compatibility indices from parent X progeny crosses between 47 and 47 self-progenies and from self-pollination of the progenies.

Progeny plant number	Progeny selfed	Backcrossed to parental clone	
		47 ♀	47 ♂
20-18	-	1.97	5.93
19	-	3.61	5.42
20	-	1.68	- *
21	-	8.44	21.87
22	-	13.37	11.50
23	-	15.50	2.58
21-1	0.00	11.43	7.75
2	0.00	10.50	11.25
3	2.70	17.14	20.90
4	0.55	7.00	6.10
5	-	12.14	10.56
6	1.24	8.71	8.33
7	0.00	6.38	13.35
8	1.09	7.29	12.20
9	1.08	7.70	9.06
10	-	10.37	4.10
11	-	4.28	-
12	-	4.70	4.80
13	-	6.43	3.08
14	-	4.62	16.57
15	-	3.14	1.50
16	-	3.09	8.50
17	-	3.74	3.60
18	0.00	4.97	5.42
19	2.55	6.30	8.32
20	-	1.63	14.00
21	-	3.19	0.92
22	-	5.29	18.25
23	-	1.94	3.64
22-1	-	2.00	5.82
2	0.00	6.38	8.25
3	-	2.91	9.25
4	0.00	1.62	5.77
5	-	3.66	4.53
6	0.00	4.55	16.27
7	0.00	4.38	9.90
8	1.50	6.00	6.25
9	-	1.29	1.89 *
10	-	4.08	13.14
11	0.07	1.58	9.65
12	-	4.80	0.80
13	0.00	1.81	9.67
14	0.00	5.23	1.94
15	0.18	8.25	8.57
16	1.68	6.06	7.26
17	0.24	0.58	2.22 *
18	-	0.76	12.62
19	3.07	1.74	3.00 *
20	-	1.25	16.00
Average	0.72	5.42	8.35

Table 20. Analyses of variance based on paired reciprocal compatibility indices excluding those in which a compatibility test was made in only one direction.

A. For the 115 family - Table 15

	D.F	S.S.	M.S.	F. value
Between reciprocals	1	66.50	66.50	31.61 ***
Between progeny	34	143.37	4.22	2.00 *
Remainder	34	71.54	2.10	
Total	69	281.41		

B. For the 40 family - Table 16

	D.F.	S.S.	M.S.	F. value
Between reciprocals	1	4.83	4.83	0.41
Between progeny	39	912.39	23.39	1.97 *
Remainder	39	462.50	11.86	
Total	79	1379.72		

C. For the 47 family - Table 17

	D.F.	S.S.	M.S.	F. value
Between reciprocals	1	187.56	187.56	11.47 **
Between progeny	46	1223.63	26.60	1.63 ?
Remainder	46	751.93	16.35	
Total	93	2163.12		

*** significant at P = 0.001
** " " " = 0.01
* " " " = 0.05
? " " " = 0.10

Table 21. Correlation between reciprocal compatibility index values.

Reciprocals compared	D.F.	Correlation coefficient
115 family - Table 15	34	+0.523 **
40 family - Table 16	39	+0.369 *
47 family - Table 17	46	+0.243 ?

** significant at P = 0.01
* " " " = 0.05
? " " " = 0.10

Table 22. Theoretical genotypes expected for incompatibility alleles inherited tetrasomically at one locus in L. corniculatus following a backcross, assumming parents have no alleles in common and no selection.

Parents $R_1\ R_2\ R_3\ R_4$ X $R_5\ R_6\ R_7\ R_8$

One progeny $R_1\ R_2\ R_5\ R_6$

Parental gametes \ Progeny gametes	$R_1\ R_2$	$R_1\ R_5$	$R_1\ R_6$	$R_2\ R_5$	$R_2\ R_6$	$R_5\ R_6$
$R_1\ R_2$	R 1122	R 1125	R 1126	R 1225	R 1226	(R 1256)
$R_1\ R_3$	R 1123	R 1135	R 1136	R 1235	R 1236	R 1356
$R_1\ R_4$	R 1124	R 1145	R 1146	R 1245	R 1246	R 1456
$R_2\ R_3$	R 1223	R 1235	R 1236	R 2235	R 2236	R 2356
$R_2\ R_4$	R 1224	R 1245	R 1246	R 2245	R 2246	R 2456
$R_3\ R_4$	(R 1234)	R 1345	R 1346	R 2345	R 2346	R 3456

Circled genotypes are the same as that of one of the parents.

Table 23. Theoretical genotypes expected for incompatibility alleles inherited tetrasomically at one locus in L. corniculatus following selfing, assuming no selection.

$R_1\ R_2\ R_3\ R_4$ selfed

Gametes	$R_1\ R_2$	$R_1\ R_3$	$R_1\ R_4$	$R_2\ R_3$	$R_2\ R_4$	$R_3\ R_4$
$R_1\ R_2$	R 1122	R 1123	R 1124	R 1223	R 1224	(R 1234)
$R_1\ R_3$	R 1123	R 1133	R 1134	R 1233	(R 1234)	R 1334
$R_1\ R_4$	R 1124	R 1134	R 1144	(R 1234)	R 1244	R 1344
$R_2\ R_3$	R 1223	R 1233	(R 1234)	R 2233	R 2234	R 2334
$R_2\ R_4$	R 1224	(R 1234)	R 1244	R 2234	R 2244	R 2344
$R_3\ R_4$	(R 1234)	R 1334	R 1344	R 2334	R 2344	R 3344

Circled genotypes are the same as those of the parent.

and tested for incompatibility with the recurrent parent and the progeny with which it was crossed. Plants 115, 40 and 13-12 (a progeny of 115 X 40) were used for this work. Plant 13-12 was chosen because it flowered well in the greenhouse and under screened cages in the field.

If tetrasomic inheritance at one locus determines incompatibility, this crossing system should produce some progeny with the same genotype as that of one of the parents, (see table 22) in which case a low compatibility index should be obtained with a test cross made in either or both directions. This appears to happen with the cross of 40 X E-24, which gives a ratio of 1 incompatible cross : 35 crosses which were compatible in at least one direction. This agrees with the hypothesis that tetrasomic inheritance at one locus may be involved. Also, plant E-24 is expected to be compatible with 13-12 if it has the same genotype as 40, as we already know that 40 and 13-12 are compatible. Similarily, one reciprocal incompatibility between a progeny and 13-12 is expected and this progeny is expected to be compatible with 40. E-34 exhibits this behaviour. Table 22 illustrates the expectation with tetrasomic inheritance at one locus and random segregation.

The data obtained from populations established by self-pollinating 40, 47 and 115 are presented in tables 17, 18, and 19. Reciprocal differences are not reliable in these tables as most tests using 40, 47 and 115 as females were made under screened cages in the summertime and their

reciprocals were made in the greenhouse in the wintertime. Extra tests were made under both field and greenhouse conditions where low indices were obtained.

The analysis of variance (table 20) indicates a difference in average compatibility between individual progeny from self-pollinations. It is also of interest that the degree of significance is greater in the two families originating from the more self-sterile parents (115 and 40 with self-compatibility indices of 0.11 and 0.15 respectively) than in the family originating from the less self-sterile parent (47, self-compatibility index 0,39). Although most reciprocal tests were carried out in different environments, as explained above, significant positive correlations were obtained between the reciprocal compatibility indices. The numerical values of the correlation coefficients increase as the degree of self-incompatibility of the parents increase.

In comparison, there are no significant differences either between the average compatibility indices of each of the progeny of 40 X 115 tested reciprocally with both parents or between the average reciprocal compatibility indices of either parent tested reciprocally with its progeny (i.e. table 12 columns headed 40 male and 40 female or 115 male and 115 female). The comparison of the self- and cross-families involving 40 and 115 are especially significant as the same parental genotypes are involved.

The analysis of variance data and the correlation coefficients are interpreted to indicate that selection for

self-compatibility is brought about by inbreeding. Also, these data illustrate that greater self-incompatibility of the original self-pollination produces greater selection. A comparison of average self-compatibility indices from families established with various degrees of inbreeding lends further support to the hypothesis that inbreeding leads to selection for self-compatibility. Progenies of 115 X 40 (no inbreeding) have an average self-compatibility index of 0.00, those from 115 X 13-12 and 40 X 13-12 (backcrosses) have average indices of 0.03 and 0.09 respectively, and those from self-pollinations of 115, 40 and 47 have average indices of 0.02, 0.14 and 0.72 respectively.

The analyses of these data do not indicate whether this selection acts on modifying genes which may affect the general compatibility of a plant or on the actual incompatibility alleles, if different alleles have varying degrees of incompatibility associated with them. Also, these data do not indicate the relative importance of selection of various alleles already present and of mutation to produce new alleles.

If selection for increased compatibility occurs during inbreeding, we can see why fewer incompatible matings were obtained than were expected with random assortment of these alleles. Because inbreeding may lead to selection for compatibility, the data from the families that originated

from self-pollinations cannot be used for my original purpose, which was to determine the number of incompatibility loci by determining the number of incompatibilities present with backcrosses of these progenies. Lundqvist (1956) was able to determine the number of incompatibility loci in rye by making diallelic crosses between S_1 progenies, however this technique has not been used yet with L. corniculatus.

The number of incompatibilities found in tables 17, 18 and 19 is too low to fit the hypothesis of tetrasomic inheritance at one locus if selection does not occur. The expected ratio for random assortment with tetrasomic inheritance at one locus is derived in table 23. We see that at least one-sixth of the self-progeny should have exactly the same genotype as the parent and should be incompatible with it in both directions. The actual numbers of crosses between a parent and its self-progeny, which may be reciprocally incompatible, are 4 out of 35 in the 115 family (table 17), 2 out of 40 in the 40 family (table 18) and 4 out of 47 in the 47 family (table 19). These numbers are maximal as further testing would be expected to reduce the numbers of possible reciprocal incompatibilities as crosses are included on which insufficient data were obtained.

Further evidence for differential selection of incompatibility alleles is presented from the studies of inheritance of red keel tip. It was proposed that the locus determining red keel tip is linked to an incompatibility locus

at which mutation and / or differential selection occurs. The evidence for the inheritance of red keel tip comes from the same plants that were used for the incompatibility studies. The very limited data on speckled seed coat were also obtained from these same plants and indicate that this character may also be determined by factors linked with an incompatibility locus.

Although the data on incompatibilities between parents and self-progenies can be accounted for by assuming that inbreeding leads to selection for compatibility, they may also be accounted for by assuming that incompatibility alleles are segregating at two or more loci.

The original plan for the study of incompatibility was to test a one locus hypothesis and to determine if inheritance is disomic or tetrasomic and if it is sporophytic or gametophytic. This plan was used because Lewis (1954) presented theoretical evidence that incompatibility at one locus may be a general rule in angiosperms. The two loci hypothesis proposed by Elliott (1946) appeared to have possible alternative explanations. The work of Lundqvist (1956) indicates that two loci are definitely involved in self-incompatibility in rye. Elliott's data is reconsidered in this dissertation together with additional information and it is proposed that at least two segregating incompatibility loci are active in L. tenuis. Therefore, it now appears to the writer that the theoretical proposal that only one locus determines incompatibility in L. corniculatus is probably

incorrect, however the data obtained do not prove this. This aspect needs further investigation.

If two or more loci are segregating for incompatibility, the evidence presented thus far does not indicate if these alleles act sporophytically or gametophytically in the pollen. It is proposed that the families from 13-12 X 40 and 13-12 X 115 (tables 15 and 16) may be segregating for incompatibility at only one locus, in which case the data obtained do indicate a sporophytic mechanism for the segregating locus.

Further consideration of the possibilities of sporophytic or gametophytic incompatibility follows from the discussion presented by Lundqvist (1956). He implies that he considers negative correlation coefficients between reciprocals in a diallelic crossing experiment evidence for gametophytic incompatibility in S_1 progenies. He does not present his theoretical reasons for this proposal, however, it is reasonable following the considerations given by Lewis (1954). Lundqvist also implies that positive correlations between reciprocals are associated with sporophytic incompatibility where two or more loci are segregating for this character, and where there are no interactions between alleles at each locus or between loci. If interactions occur, he indicates that no correlations are expected. Although these proposals are given for diploids with two segregating incompatibility loci in diallelic crosses, there does not appear to be any theoretical reason why the correlations would not have the same associations in tetraploids, where more than two loci are involved or where

reciprocal backcrosses are compared in progenies of outcrosses, backcrosses of selfs. It has already been pointed out that positive correlations may indicate selection with inbreeding. Also, if reciprocal pollinations were made at the same time and if different reciprocals were made on different days, random variations in seed set due to environmental variations could result in positive correlations.

Correlation coefficients between reciprocal compatibility indices obtained in the present studies are presented in table 24.

Table 24. Summary of correlation coefficients between reciprocals.

Data in table	Family origin	Reciprocals correlated	Coefficient	Environmental conditions for reciprocals
12	40 X 115	40 male & female 115 male & female	+0.053 +0.005	Same Same
15	40 X 13-12	40 male & female 13-12 male & female	-0.093 +0.523**	Same Same
16	115 X 13-12	115 male & female 13-12 male & female	+0.165 -0.126	Same Same
17	115 ♀	115 male & female	+0.523**	Different
18	40 ♀	40 male & female	+0.369*	Different
19	47 ♀	47 male & female	+0.243?	Different

** significant at P = 0.01

* significant at P = 0.05

? significant at P = 0.10

In table 24, the only correlations which are significant are positive. Interactions between incompatibility alleles are not expected if they are inherited tetrasomically at one or more loci or if disomic inheritance occurs at more than one

locus, because the plants in which the interactions occur would be self-fertile. The segregation data for incompatibility indicate that disomic inheritance at one locus does not occur in L. corniculatus. The significant positive correlations indicate sporophytic incompatibility with no interactions between incompatibility alleles. Absence of correlation may also be associated with sporophytic incompatibility if interactions such as dominance occur between these alleles. One interpretation of the correlation coefficients obtained is that weak interactions occur between some incompatibility alleles. These interactions may not lead to a high degree of self-compatibility but may at the same time influence compatibility indices enough to affect correlation coefficients. If we assume weak interactions, increases in self-compatibility with inbreeding may be accounted for by assuming increased interactions between incompatibility alleles.

Another criterion that has been used to discriminate between gametophytic and sporophytic systems in diploids, is the effect of doubling the chromosome number on the incompatibility reaction. Doubling will lead to self-compatibility with gametophytic systems if interactions occur between incompatibility alleles at one locus in the microsporocyte. These interactions occur through dominance or competition interactions of incompatibility alleles which do not normally occur together in the pollen grain. Lundqvist (1956) claimed that these interactions do not occur in rye

because the diploid would be self-compatible if they do occur. In the same way, these interactions should not occur in the natural tetraploid L. corniculatus, hence further doubling should not lead to self-compatibility. Seed from collection 5101 was treated with colchicine to produce autooctoploids. The results of self-pollinating and interpollinating these induced autooctoploids are presented in table 25.

Table 25. Compatibility indices from cross- and self-pollinations of colchicine induced octoploids of L. corniculatus.

Plant	Crossed with other octoploid L. corniculatus	Selfed
1	0.76	0.00
2	0.69	0.14
3	0.00	0.15
4	0.90	0.00
5	--	0.00
6	--	0.09
	91 seeds from a total of 183 flowers	37 seeds from a total of 431 flowers

Doubling the chromosome number did not lead to self-compatibility, therefore interactions do not occur between various incompatibility alleles in these plants. Low seed sets expressed as low compatibility indices were found with both cross- and self-pollinations, probably because of genetic and / or physiological disturbances following colchicine induced autoploidy. Lundqvist's (1956) discussion makes it clear on theoretical grounds that the presence of gametophytic

or sporophytic incompatibility cannot be distinguished when more than one separate incompatibility allele is present in the pollen grain. Lewis (1954) suggested that chromosome number doubling can be used to discriminate between sporophytic and gametophytic incompatibility, however he was considering incompatibility alleles at one locus, as he considered it unlikely that more than one locus was segregating for this character. From the theoretical considerations of Lewis (1954) and Lundqvist (1956), it is clear that a breakdown in self-incompatibility following colchicine induce chromosome number doubling is positive evidence that incompatibility is determined at one locus inherited disomically and that the incompatibility alleles act gametophytically. Therefore, the data presented in table 25 prove nothing about the incompatibility mechanism as the absence of a breakdown does not even eliminate the possibility of the above system, if interactions do not normally occur between incompatibility alleles. However, Lewis (1954) claimed that it is significant that this breakdown occurs in so many species with gametophytic oppositional incompatibility inherited disomically at one locus and that interactions probably will occur if this system is present as there is no selection against interactions.

SELF-INCOMPATIBILITY IN L. TENUIS

Elliott (1946) proposed a gametophytic incompatibility system for L. tenuis with segregating incompatibility alleles at two loci following the model proposed by Kakizaki (1930) for Brassica oleracea, which is essentially the same as the system proposed for rye by Lundqvist (1956). Elliott crossed two plants and studied incompatibility in all possible reciprocal diallelic crosses among twelve progeny and between one parent and the progeny. He found that no two of the progeny were reciprocally incompatible, nor were any reciprocally incompatible with the one parent, hence all the progeny must have different incompatibility genotypes, from each other and from the parent. The writer examined Elliott's data and calculated correlation coefficients between reciprocal values given for the number of seeds obtained per flower pollinated, which correspond to compatibility indices used in the present study. In the diallelic series, a correlation of +0.291 was obtained between reciprocals, which is significant at $P = 0.05$. A correlation of -0.316, which is not significant, was obtained for the reciprocal backcrosses. According to Lundqvist (1956), a positive correlation between reciprocals indicates sporophytic oppositional incompatibility with no interaction between alleles at different loci. Although Lundqvist was applying the use of correlations to indicate the type of incompatibility in S_1 families, they should also apply to the progeny of a cross because the basic premise is the same in cross progenies and in S_1 progenies.

Table 26. Compatibility indices from parent X progeny crosses between L. tenuis 2 and 3 and the progeny of 2 X 3 (N-1 to 18)

Progeny plant numbers	Selfed	Crossed to parental clones		
		2 ♂	3 ♂	3 ♀
N-1	0.03	8.14	3.36	8.33
2	0.00	3.00	4.97	2.64
3	0.00	6.15	0.31	14.75
4	0.28	3.47	4.88	--
5	0.00	4.53	1.30	3.67
6	0.00	1.37	1.22	0.00
7	0.00	0.89	--	--
8	1.73	3.37	1.18	--
9	--	5.00	5.50	--
10	0.00	1.61	3.82	0.00
11	0.00	2.75	--	--
12	0.02	4.68	--	--
13	0.16	--	0.90	--
14	--	9.17	1.23	--
15	--	3.38	7.28	--
16	--	4.43	3.60	--
17	--	3.42	4.43	--
18	0.00	3.50	2.45	--

This premise appears to be that a positive correlation is associated with reciprocal compatibility indices when sporophytic oppositional incompatibility occurs with no interaction between loci because the same sporophytic genotypes oppose each other in each of the reciprocals.

Limited data obtained in the present study, in which two plants were crossed and the progeny backcrossed, are presented in table 26. These data and Elliott's backcross data give some indications of reciprocal differences in compatibility, which Lewis (1954) proposed as a feature distinguishing sporophytic from gametophytic incompatibility. Lundqvist (1956) proposed that no correlation between reciprocals should be expected if there were interactions such as dominance between sporophytic incompatibility alleles. Therefore, the apparent reciprocal differences in compatibility may indicate some interactions between sporophytic incompatibility alleles. Also, colchicine induced autotetraploids of L. tenuis (collection 5406) were examined for cross- and self-sterility. The data presented in table 27 indicate that induced tetraploidy did not overcome incompatibility. It was also observed that self-incompatibility of plants of collections of 5515, 5516 and 5518 of autotetraploid L. tenuis are about as self-sterile as the diploid L. tenuis examined, although these autotetraploid collections are reasonably cross fertile. Indices were not calculated. According to Lundqvist's proposals, failure to obtain compatibility with induced autotetraploidy may indicate

Table 27. Compatibility indices for cross- and self-pollinations of colchicine induced tetraploid L. tenuis.

Plant	Crossed with other tetraploid L. tenuis	Selfed
1	--	0.07
2	0.00	0.00
3	1.50	0.00
4	1.30	0.00
5	0.00	0.00
6	--	0.00
7	--	0.00
8	0.00	0.00
9	0.06	--
10	0.00	0.00
	87 seeds from a total of 150 flowers.	2 seeds from a total of 419 flowers.

that a gametophytic incompatibility mechanism at one locus inherited disomically is not the one involved.

It is concluded from Elliott's diallelic crossing data and from the results with induced autotetraploids that gametophytic incompatibility at one locus inherited disomically does not occur in L. tenuis. Studies of the idiograms of the chromosomes by Dr. J. M. Armstrong (Unpublished) and those presented in plate 2 indicate that L. tenuis is a diploid, hence Elliott's 12 different incompatibility genotypes from one cross indicate that incompatibility alleles occur at more than one locus, provided no mixing of pollen occurred

and there was no mutation of incompatibility alleles. Finally, the positive correlation between reciprocals indicates a sporophytic system, according to Lundqvist's theoretical considerations. There are some indications that the proposed sporophytic alleles are independant and others that they interact.

OVULE DEVELOPMENT AND FERTILIZATION.

A study of ovule development in the ovaries of L. corniculatus and other Lotus species was carried out to determine if an association that was observed between the proportion of ovules forming seeds following cross-pollination and self-sterility could account for some of the variability in compatibility indices obtained in the studies of inheritance of self-incompatibility. These studies involved a survey of self-sterility in Lotus species and of the effect of floral manipulation on self-sterility. The numbers of ovules per ovary and of seeds per pod were also recorded. This was followed by an examination of serial sections of ovaries, both pollinated and unpollinated, to determine the regularity of ovule development and the position of fertilized ovules in ovaries following various degrees of inbreeding.

Survey of self-sterility and associated characteristics in Lotus species.

The results of the survey on the prevalence of self-sterility with and without mechanical manipulation of the flowers, on the numbers of ovules per ovary, and on the number of seeds per pod for the species that were available in the greenhouse during the spring of 1956, are presented in table 28.

The data presented in table 28 indicate that all ovules may be fertilized and may produce seeds for some species of Lotus, while only about one-half or less of the ovules form

Table 28. The number of ovules per ovary and of seeds per pod following cross-pollination and the degree of self-fertility without and with mechanical self-pollination for various Lotus collections.

Name supplied	Collection	Ovules / ovary	Seeds / pod	Self-fertility	
				without manipulation	with manipulation
L. corniculatus	5101	49.0	18.2	none	slight
L. tenuis	5406	42.0	21.0	none	slight
L. uliginosus	5414	48.8	26.4	none	some
L. corniculatus japonicus	5501	30.8	29.4	high	high
L. angustissimus	5410	16.0	16.3	high	high
L. hispidus	5417	13.3	13.0	high	high
L. palustris	5418	11.5	10.0	high	high
L. filicaulis	5429	22.0	23.0	high	high
L. parviflorus	5426	26.8	21.4	high	high
L. divaricatus	5428	19.2	15.8	high	high
L. weilleri	5411	48.8	21.6	none	high
L. perigrinus	5420	19.0	19.3	high	high
L. jacobaeus	5424	76.0	18.2	none	none
L. tetragonolobus	5423	51.3	8.7	high	high
L. suaveolens	5513	32.4	15.7	none	high
Hosackia sp.	5433	2.5	2.2	high	high

seeds with other species. Excluding the New World collection (Hosackia) and the L. tetragonolobus collection, in which some evidence of post-fertilization abortion was observed, the number of seeds per pod ranges between 10 and 30 while the number of ovules per ovary ranges between 10 and 76. These data suggest that during the evolution of this genus, an optimum number of seeds per pod of between 10 and 30 has been selected for the different species and that the species in which a large portion of the ovules do not form seeds have evolved a higher ovule number to compensate for the reduction in the proportion fertilized.

Furthermore, the species in which only about one-half of the ovules form seeds are self-sterile without floral manipulation, although they may differ in the degree of mechanical self-fertility. As Giles (1949) pointed out for L. corniculatus, mechanical manipulation of a flower serves to break a stigmatic membrane and to release fatty materials onto the surface of the stigma. He suggested that these fatty materials are nutrient media in which pollen germinates and grows. He also observed that this membrane is either fragile or not present on the stigmas of the autogamous species, L. ornithopodioides, L. divaricatus and an unidentified species and Elliott (1946) reported that a membrane is present on the stigma of L. tenuis.

The stigmatic surfaces of all the species listed in table 28 were examined during the present study and a complete stigmatic membrane was found on the stigmatic surface of young

flowers or buds with each species. In each case the stigmatic surface was broken with a dissecting needle and sticky materials extruded onto the surface. However, when mature flowers were examined, intact stigmatic membranes were found only on the stigmas of the species which are self-fertile without mechanical manipulation.

Pollinations of plants of L. corniculatus (collection 5101) were made and the stigmas were examined at intervals. It was observed with both self- and cross-pollinations in which adequate masses of pollen were applied, that the sticky substances released onto the surface had disappeared in twenty to twenty-four hours following pollination. Other flowers were manipulated in the same way but were not pollinated. A film appeared to form over the stigmas and some sticky substances were still present after four days. These observations suggest that this sticky substance supplies nutrient material to the pollen and that once it is used up, further pollination will not result in further fertilization to produce increased seed set. This agrees with Giles' (1949) findings with the stigmatic material. A series of self- and cross-pollinations were made in the greenhouse and some of the self-pollinated flowers were cross-pollinated twenty-four hours later (see table 29). Although there were large differences in seed set between the original self- and cross-pollinations, the self-pollinated plants which were cross-pollinated twenty-four hours after the original selfing produced no more seeds than other self-pollinated plants which

did not receive a later cross-pollination, presumably because the nutrient material was exhausted.

Table 29. Seed set following self-pollination, cross-pollination and self-pollination followed by cross-pollination after 24 hours on plant J-10 of collection 5408.

Pollination	Total seeds	Total flowers	Compatibility index
Self	2	34	0.06
Cross	43	8	5.60
Self, followed by cross	0	34	0.00

These experiments demonstrate the role of the stigmatic membrane in allowing the stigma of self-sterile species to be made receptive when the flowers are manipulated by visiting insects and indicate a method by which the time of receptivity of the stigmas of self-fertile species may be controlled in relation to the development of ovules in the ovary. These experiments do not account for the fact that only a proportion of ovules of self-sterile species are fertilized. Further investigations of ovule development, reported below, provide an explanation of this fact.

Ovule development in unpollinated ovules.

Ovule development of unpollinated ovaries was investigated by examining serial sections of ovules stained with haematoxylin. Flowers in various stages of development were collected from plants of L. corniculatus (collection 5101) and the ovules were classified according to their visible characteristics. L. tenuis (collection 5406), L. suaveolens

Stages in ovule development in plate 7.

Figure 1. A young ovule at the four nucleate stage. In ovules in which the nuclei were not observed, it was possible to classify them by observing the condition of other components.

Figure 2. A mature ovule in one of the younger stages. Eight nuclei are present, the polar nuclei have not fused.

Figure 3. A normal developing ovule in which the primary endosperm nucleus has undergone a number of divisions. This ovule was fixed 72 hours after pollination.

Figure 4. A developing ovule in which the integumental tissue has developed rapidly and appears to be crowding the embyro sac. This ovule was fixed 72 hours after pollination.

Figure 5. An old ovule in which the integumental tissue at the micropylar end is starting to disintegrate and a group of dark staining cells are present at the antipodal end of the cavity.

Figure 6. A very old, presumably unfertilized ovule typical of those found among developing ovules 72 hours after pollination.

All microphotographs were taken at the same magnification and contact printed, which gives a magnification of approximately 250 X.

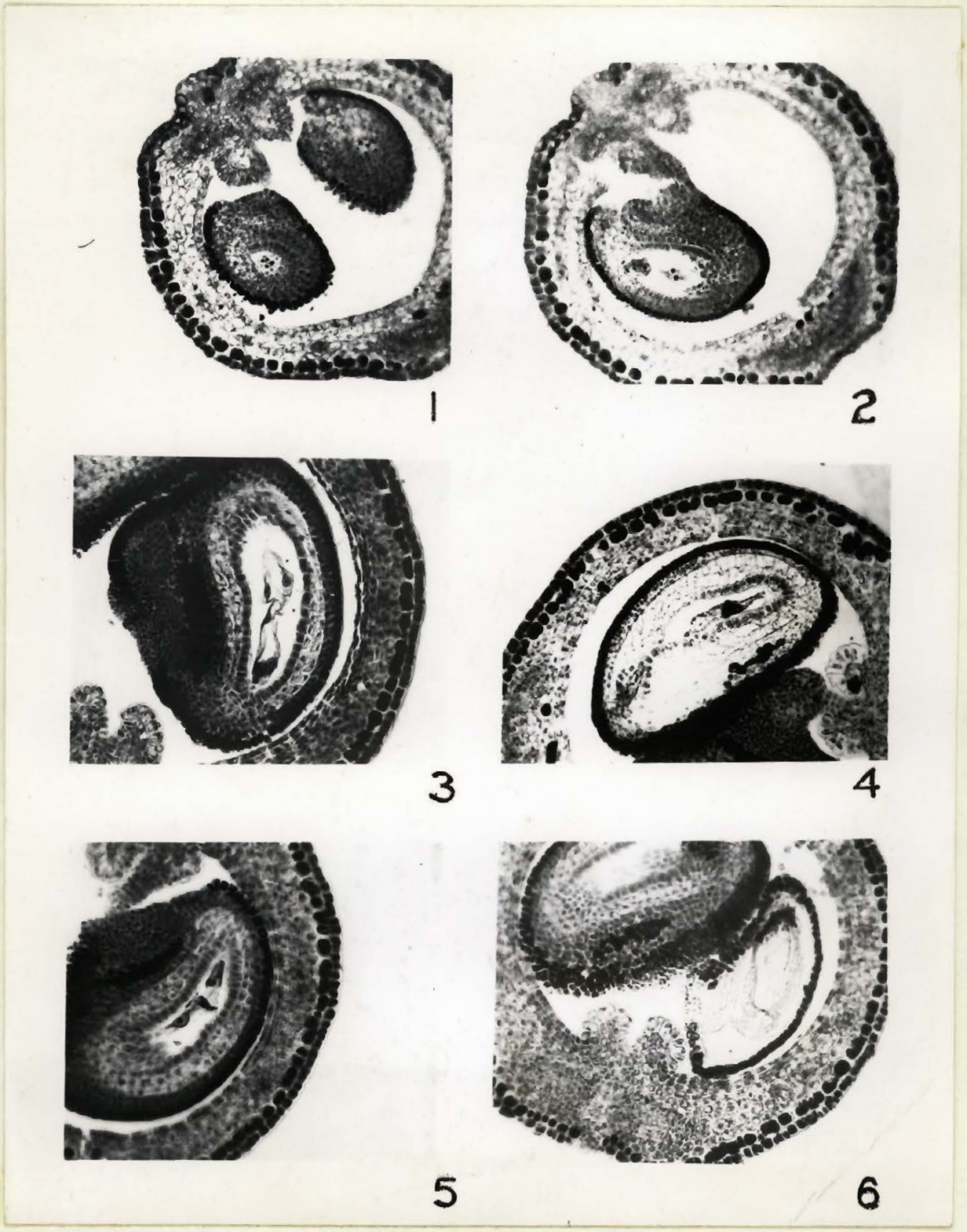

Plate 7. Ovule development. Descriptions on the facing page.

(collection 5513), L. weilleri (collection 5411), L. corniculatus japonicus (collection 5501) and L. divaricatus (collection 5428) were examined and the results are presented in table 30.

The following visible classes of ovules were distinguishable:

(1) Young ovules: The final divisions in the embyro sac had not been completed or the nuclei had not reached their final orientation as found in mature ovules. Plate 7, part 1.

(2) Mature ovules: The nuclei had reached their final normal orientations in the embyro sac, the polar nuclei were sometimes fused but were more often not fused. Plate 7, part 2.

(3) Disintegrating ovules: The nucellar tissue showed signs of disintegration, starting at the micropylar end, and dark staining cells were present at the antipodal end of the ovule cavity. Plate 7, parts 5 and 6.

Because the young ovules are visibly too young to be fertilized and the disintegrating ovules are visibly disintegrating, it is assumed that all ovules in these two classes could not have been fertilized if pollen tubes were present at the time when these ovaries were collected. It is quite possible that other ovules placed in the mature class may have been physiologically too young or too old to be fertilized although all the ovules that could be fertilized should fall into this visible class.

It can be seen from table 30 that all species investigated that are self-sterile without floral manipulation (L.

Table 30. Ovule development within individual unpollinated ovaries.

Species	Flower stage	Ovule development classes			Total ovules
		Young	Mature	Disintegrating	
L. corniculatus	Bud	44	--	--	44
	"	34	--	--	34
	"	50	2	--	52
	"	25	23	--	48
L. corniculatus	Young	14	23	1	38
	"	13	36	--	49
	"	3	37	4	44
L. corniculatus	Medium	3	37	15	55
	"	--	33	18	51
	"	--	31	21	52
	"	--	39	23	62
L. corniculatus	Old	--	27	22	49
	"	--	24	29	53
	"	--	4	51	55
	"	--	1	48	49
L. tenuis	Medium	4	26	3	33
	"	1	28	3	32
	"	--	18	22	40
	"	--	18	23	41
	"	--	7	25	34
L. suaveolens	Medium	4	28	1	33
	"	--	21	7	28
	"	--	20	8	28
L. weilleri	Medium	--	23	10	33
	"	--	18	11	29
	"	--	16	12	28
L. corniculatus japonicus	Medium	--	39	--	39
	"	--	36	--	36
	"	--	29	--	29
L. palustris	Medium	--	14	--	14
	"	--	13	--	13
L. divaricatus	Medium	--	16	--	16
	"	--	13	--	13

corniculatus, L. tenuis, L. suaveolens and L. weilleri) show variation in stage of ovule development within each ovary. On the other hand, the species which are self-fertile without manipulation (L. corniculatus japonicus, L. palustris and L. divaricatus) have all ovules within each ovary in the same stage of development. It is apparent that the self-sterile species set fewer seeds per pod than the number of ovules per ovary because all ovules in each ovary are not in the correct stage of maturity to be fertilized when pollen tubes become available. The self-fertile species set about as many seeds per pod as the number of ovules per ovary because all ovules are at the correct stage of maturity for fertilization when pollen tubes become available. It appears that the stigmatic membrane ruptures and allows self-pollination to take place so that the pollen tubes reach the ovules at a suitable maturity stage for fertilization to take place. Some cross-pollination by insects may occur with these species if bees happen to apply pollen just before the stigmatic membrane would normally break naturally. Emasculation without breaking the membrane will be necessary if such species are to be used in crosses for genetic purposes.

It appears that L. corniculatus ovules vary in stage of development within individual ovaries so that the period during which fertilization is possible will extend over a longer time and will compensate, at least partially, for the capriciousness of insects in their visits. The length of time required for L. corniculatus (collection 5101) flowers to mature through each stage of development under greenhouse conditions was

estimated by observing petal characteristics of some tagged flowers. The bud stage, which corresponds to Giles' (1949) pointed bud stage and may also include his hooded bud stage, matured into the young flower stage, which corresponds to Giles' erect standard stage, in two to three days. The flowers matured from the bud stage into the medium stage in five or six days and were considered old, because the corollas began to wilt, in eight to ten days. Under field conditions, it was observed that corolla development was generally more rapid and that the rate of development was influenced considerably by weather conditions.

The particular stages of development discussed were chosen because it was observed that flowers in each of these three stages could be pollinated and would produce some seeds. Accurate data on relative seed set following pollinations at the different stages were not obtained, however, some observations do suggest that higher numbers of seeds per pod were obtained when young and medium stages were pollinated than when older flowers were pollinated. Bees were seen visiting birdsfoot trefoil flowers in all these stages of development.

The observations on the rate of flower development in L. corniculatus and the data presented in table 30 suggest that individual ovules are fertilizable for a period of only two or three days and that some fertilizable ovules are present in each ovary over a period of eight to ten days. The observed variation in ovule development could have a selective

advantage as it prolongs the time span over which an individual flower may be pollinated effectively by a visiting insect. This may be important as insect flight is considerably influenced by weather conditions which may lead to variations in time of pollination (see Morse 1955).

It is of interest that ovules in the same stages of development are located at random within individual ovaries of the self-incompatible species. No gradients in development and no groupings of ovules in the same morphological stages were observed in the serial sections of individual ovaries. Also, normal seeds and undeveloped ovules appeared to occur at random within mature pods except that groups of undeveloped ovules were frequently observed near the base of pods from pollinations that produced few seeds. Therefore, it appears that rate of development of each ovule is determined autonomously, which may mean that ovules within ovaries are segregating for rate of development. If this random variation is due to genetic segregation, the rate of development of each ovule may be determined by the genotype of the functional megaspore and the products which arise from it. This would require a very early and rapid expression of alleles determining the rate of development in the ovules, which consist mainly of maternal tissue. If genetic segregation determines random variation of ovules within ovaries, an increase in homozygosity through inbreeding should reduce this variation. When progenies arising from a number of generations of self-pollination become available, it will be possible to determine

if inbreeding does reduce random variation within ovaries by comparing development of ovules within ovaries of parental clones and their self-progenies.

Ovule development in pollinated flowers.

Ovule development in pollinated flowers was examined to substantiate the hypothesis of random variation in ovule development within ovaries presented above and to test the conclusions on fertilization following self- and cross-pollinations presented by Giles (1949). Extensive excerpts of Giles (1949) dissertation are presented here as his work is not readily available.

Giles (1949) examined germination and growth of the pollen tubes which he described as follows:

"No germinating pollen was found on stigmas which had not been scratched at the time of pollination. On scarified stigmas of highly self-sterile plants, self-pollen germinated as readily as cross-pollen. Germination began approximately one-half an hour after pollination. Two hours after pollination, tubes were well established in the upper style and the germinated grains had emptied their contents in compatible as well as in incompatible matings."

"In both compatible and incompatible matings some swollen or otherwise misshapen tubes were found on the stigmas. Such tubes usually grew along the surface and some eventually penetrated the stylar tissue for a short distance. Grains from which these tubes arose did not empty their contents. Since this condition occurred in both types of matings, it was not regarded as an incompatibility reaction. The fact that similar malformed tubes were observed in culture supported this conclusion."

The above work by Giles (1949) was not repeated in the present study, other than by a few exploratory observations which did not disagree with his descriptions. He also examined the growth of pollen tubes in the style, which was not observed

in the present study. His description is presented below, in which pollen tube growth after self- and cross-pollination is compared.

"No difference was found in the rate of pollen tube growth nor in the number of tubes penetrating styles from the self-fertile and self-sterile plants following compatible or incompatible matings. There was no evidence that the tubes from compatible pollen were accelerated nor that the tubes from incompatible pollen were retarded in the style. Swollen tube ends were found in two instances near the bases of styles from selfed self-sterile plants. In each case masses of apparently normal tubes accompanied the abnormal ones."

This description indicates that an oppositional reaction does not take place in the style or on the stigmatic surface. He obtained some evidence that an oppositional incompatibility may occur within the ovary cavity as is described below:

"Although incompatible pollen tubes grew through the style as rapidly as compatible ones, this was not true in the ovary. Twelve hours after pollination, numerous tubes were in the upper one-fifth of the ovary regardless of the type of mating; however, few incompatible tubes had grown beyond the tenth ovule, whereas many compatible tubes had reached or passed the twentieth ovule. Based on random samples of ovaries, the mean position of the self-pollen tube tips in the twelve hour collections was at the base of the sixth ovule. In contrast, the position of the cross-pollen tube tips was below the fifteenth ovule and the longest tube had reached the thirty-eighth ovule. Inhibition of self-pollen tubes in the partially self-fertile plant was as strong as in the most highly self-sterile plants."

"Preparations from pistils collected twenty-four hours after pollination revealed that inhibition of self-pollen tubes was by no means complete. In one ovary, five well formed tubes were found below the basal ovule and in each ovary examined at least one tube had reached the last ovule. Although self-pollen tubes were present in the lower half of the ovary, cross-pollen tubes were more abundant in this region."

Giles (1949) observations were made on plants grown in a field at Columbia, Missouri. He noted that pollen tubes penetrated the stigmatic surface in two hours, were half way

down the style in four hours and were in the cavity at the base of the style in eight hours. Growth was noted to be slower under greenhouse conditions but the relative behaviour of self- and cross-pollen was the same.

Giles (1949) work was extended in the present study by observation of ovule development in ovaries collected 72 hours after pollination under field conditions during the summer of 1955.

Pollinated ovules were also classified according to their visible characteristics into the following classes:

(1) Mature ovules: The nuclei had reached their final normal orientations in the embyro sac, the polar nuclei were sometimes fused but were more often not fused. Plate 7, part 2.

(2) Developing ovules: The primary endosperm nucleus had divided at least once. Plate 7, part 3.

(3) Ovules with integument crowding the cavity: Integumental tissue crowded into a major portion of the cavity. This type of development was only found in pollinated ovaries. Plate 7, part 4.

(4) Old ovules: The integumental tissue showed signs of disintegration, starting at the micropylar end, and dark staining cells were present at the antipodal end of the cavity. Plate 7, part 5.

(5) Very old ovules: The ovules were extensively disintegrated. Plate 7, part 6.

Table 31. Ovule development within individual pollinated ovaries of plant 40, collection 5101, of L. corniculatus 72 hours after pollination.

Pollen source	Mature ovules	Developing ovules	Integument in the cavity	Old ovules	Very old ovules
19-13	29	3	--	8	2
(40 ♀	40	2	--	2	6
progeny)	32	--	--	2	5
20-1	26	2	1	4	15
(40 ♀	28	6	6	7	2
progeny)	35	3	1	8	--
20-7	40	1	--	4	--
(40 ♀	25	--	--	11	--
progeny)	31	--	--	3	--
20-12	23	11	--	6	4
(40 ♀	19	2	--	3	20
progeny)	27	6	2	3	5
13-12	34	--	--	10	--
(40 X 115	37	--	--	7	1
progeny)	38	--	--	4	--
13-13	24	--	--	34	2
(40 X 115	28	5	4	2	8
progeny)	29	--	--	3	5
14-3	26	--	--	2	16
(40 X 115	14	--	--	1	26
progeny)					
40	19	1	4	25	5
	25	--	2	15	9
	36	--	1	11	3

The ovaries reported in table 31 were fixed 72 hours after pollination, which would probably be about 60 hours after fertilization according to Giles (1949) observations. Brink and Cooper (1940) were able to observe somatoplastic sterility in Medicago sativa 72 hours after pollination. The ovules classed as having integumental tissue crowding the cavity were probably fertilized but may have aborted in the same way that Medicago sativa ovules abort when the integumental tissue develops too rapidly in respect to the endosperm and embryo tissues. As integumental tissue was not observed crowding the cavity of any of the unpollinated ovules examined and reported on in table 30, it may be assumed that stimulation of the integument only occurs following fertilization. Hence, it may be assumed that both the classes represented as "developing ovules" and as having "integument in the cavity" had been fertilized.

The term "somatoplastic sterility" may be correctly applied to the crowding of the embyro sac by the inner integument in L. corniculatus according to the original application by Brink and Cooper (1939) who used the term when "collapse follows abnormal growth of the somatic tissue adjacent to the embyro sac". Data on the prevalence of somatoplastic sterility following crosses of plants not known to be related were not obtained so its relative importance as a cause of self-sterility cannot be assessed.

Many of the ovules classified as "mature" were probably immature at the time of fertilization, hence could not be

fertilized. Ovules which were mature at the time of fertilization but were not fertilized would probably appear in the "old ovules" class and many which were too old to be fertilized at that time would probably appear in the "very old ovules" class. This is in agreement with the estimation that ovules remain fertilizable for a period of two or three days, which was arrived at with unpollinated flowers.

Failure to obtain any developing ovules in an ovary may have been due to improper pollination or to incompatibility. As special care was taken in making these pollinations and as seeds were often obtained from all flowers pollinated following a cross between plant No. 40 and unrelated plants under the same environmental conditions, at least some of the 10 ovaries which contained no developing ovules may represent incompatibilities. It is unfortunate that comparable data on ovule development following cross-pollinations were not obtained when these crosses were made, however, the high compatibility indices reported in table 10 indicate that these data do represent an expression of lowered seed set following inbreeding. An overall compatibility index for the developing ovules in table 31 would be only 1.83. Inclusion of the other stimulated ovules reported as having "integument in the cavity" with the developing ovules would raise the compatibility index to 2.74 which is still below the lowest obtained from out-crossing and indicates that incompatibility is occurring.

The histogram of the distribution of fertilized ovules (i.e. those developing and those with nucellus in the cavity)

presented in plate 8 is based on the combined data in table 31 and refers to ovules numbered consecutively from the tip to the base of the ovaries. This histogram suggests that fertilization is more frequent at the stylar end of the ovary than further down the ovary for these crosses, all of which represent some degree of inbreeding. This distribution supports Giles' (1949) observation that self-pollen tubes are inhibited, although not completely inhibited, within the ovary cavity. Pods on plant 40 were opened and the distribution of seeds to undeveloped ovules appeared to be completely at random following pollination in the field by bees. A histogram of the distribution of these seeds was not prepared as it was not possible to get accurate data on the relative positions of the seeds and the undeveloped ovules, however, the writer could not observe any tendency for more fertilized ovules to be located at the stylar end of the pod.

These observations support the opinion expressed by Giles (1949), that an oppositional incompatibility occurs within the ovaries of L. corniculatus. This oppositional incompatibility, in conjunction with post-fertilization abortion or "somatoplastic sterility" may account for self-sterility in L. corniculatus.

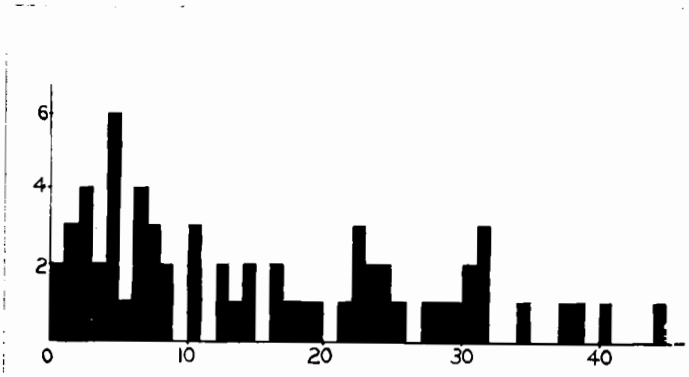

Plate 8. Histogram of the distribution of developing ovules within ovaries recorded in table 31. The height of each column is based on the frequency of "developing ovules" plus those with "integument in the cavity" in each position in the ovary starting from the stylar end. The shortest ovary which contained any ovules considered fertilized contained 42 ovules.

DISCUSSION

The studies reported in this dissertation were planned to obtain genetic and cytotaxonomic information which is needed to facilitate breeding work with L. corniculatus. In the previous sections, the results of the various studies are presented and conclusions are drawn. Some further consideration is given in this section to the applications and theoretical implications of these studies and the conclusions drawn in previous sections are evaluated.

The cytotaxonomical studies are an outgrowth of some unsuccessful attempts at interspecific hybridization, which are not reported on in this dissertation as only negative results have been obtained so far. One of the main reasons that the interspecific hybridization work was postponed was that satisfactory emasculation techniques had not been devised. If anthers are removed before they open naturally, the flowers are so young that they die before the pistils mature. A possible alternative is indicated by the discovery of the role of the stigmatic membrane with the species that are self-sterile in the absence of pollinating insects. Since the stigmatic membrane remains intact until it is mechanically ruptured, it should be possible to prement self-fertilization by cleaning the stigmas before the membrane is broken. Cross-pollination should then be possible without risk of self-pollination. Also, it was observed that stigmatic fluid is consumed by the growing pollen. This suggests that a transfer of stigmatic fluid from the male plant in interspecific crosses to the

stigmas of the flowers pollinated may increase the chances of fertilization, as a supply of the correct nutrient medium would be insured. A few preliminary trials using this technique were made in the spring of 1957. Ovule development was stimulated in some pollinated ovules and did not appear to be stimulated when the same crosses were made without the transfer of stigmatic fluid. The writer's first impressions are that fertilization was accomplished and was followed by post-fertilization abortion, however, further investigations will be necessary before the value of this technique can be determined.

The cytotaxonomical studies aid in grouping species for interspecific crossing and in classifying interspecific relationships. The work so far indicates a dire need for a taxonomic investigation of the genus Lotus and has already led to the initiation of a Macdonald College research project for this purpose. A monograph on the genus is planned for which plant materials used in the present studies are being maintained and additional materials are being collected. This investigation should provide basic information of value in planning interspecific hybridizations.

As this thesis study is especially concerned with L. corniculatus, some consideration is given to the range of morphological types that belong within the genus, to chromosome numbers and to relationships with other Lotus species. A tentative hypothesis is presented to the effect that the

direction of evolution in the genus has been from a basic chromosome number of 7 to one of 6. This hypothesis is based on the writer's general impression of variation within the genus. Because a fairly continuous spectrum of morphological types was observed in the collections with $2n$ = 14 and 28 and because they appeared to represent the extreme types in the genus, it is assumed that they represent the more primative condition. The $2n$ = 12 and 24 collections studied represent three segments of the entire spectrum; so it is postulated that evolution through the loss of a homologous set of chromosomes occurred on at least three separate occasions. This hypothesis needs further investigation. It receives some support from the observation that some very successful forms, for example L. corniculatus, that have spread over large areas of the earth in historic times, belong to a group which is considered modern under this hypothesis.

The one previous inheritance study on tetraploid L. corniculatus (Dawson, 1941) demonstrated that tetrasomic inheritance occurs in this species. An objective of the present studies was to establish whether or not tetrasomic inheritance is commonly found. Meiosis was studied in the plants used for genetic studies and it was found that bivalent pairing commonly occurs during meiosis. This observation is important in the study of tetrasomic inheritance because multivalents affect segregation ratios. These observations are in agreement with those of Dawson except that he did observe a very few multivalents whereas none were observed in the

present study. Dawson's material would probably belong to the "English pasture type" class discussed in the section on cytotaxonomy and the materials used in the present inheritance studies belong to the "Empire type" class.

Five genetic characters were studied. (Lemon yellow flower colour, red keel tip, speckled and mottled seed coat and incompatibility). It is possible to reconcile the ratios obtained with four of them to a tetrasomic hypothesis if certain assumptions are made. These are:

(a) A dosage effect in conjunction with a threshold value is acting so that the nulliplex or simplex conditions produce one phenotype, the duplex, triplex or quadruplex conditions produce an alternative phenotype.

(b) Differential selection of incompatibility alleles results from inbreeding.

(c) Linkage between incompatibility alleles and keel tip colour alleles.

Very few data were collected on the fifth character studied so that no conclusions are possible other than that simple disomic inheritance at one locus is probably not occurring. Within the limits of the data obtained, the hypothesis that inheritance in tetraploid L. corniculatus is generally tetrasomic was not disproven.

Of the characters studied, one flower character, the two seed coat characters and self-incompatibility are commonly heterogeneous in plant populations and may be expected to have a more complex inheritance due to genetic modifications that may occur to help maintain these heterogeneities, if they have

selective advantages. Therefore, the assumptions made so that the ratios obtained can be fitted to those expected on a tetrasomic inheritance hypothesis are considered reasonable. The remaining character gave a simple and clear cut segregation indicating tetrasomic inheritance.

This information has a ready application in plant breeding work. Since tetrasomic inheritance may be expected, plant populations from a cross have to be considerably larger than for species in which disomic inheritance prevails, to allow the same probability of obtaining the desired combinations. As L. corniculatus is similar to Medicago sativa in many agronomic characteristics and as both species exhibit some tetrasomic inheritance, breeding programs for L. corniculatus may be modeled on those which have been used successfully with M. sativa.

The available evidence provides some grounds for proposing that L. corniculatus originated as an autoploid rather than as an alloploid. The presence of tetrasomic inheritance and the lack of evidence for other types of inheritance indicates complete homology between the basic sets of chromosomes, which is more probable in autoploids than in alloploids. Chromosome drawings presented in this dissertation and by Dr. J. M. Armstrong (unpublished) of the Forage Crops Division, Central Experimental Farm, Ottawa, indicate that L. corniculatus possesses six sets of four morphologically homologous chromosomes, also indicating autoploidy. Although presence of multivalents is evidence for autoploidy, it was pointed out in the literature

review that rarety or absence of multivalents does not indicate alloploidy. Therefore, the observed rarety or absence of multivalents in L. corniculatus does not contradict the hypothesis that it is an autotetraploid.

Although the evidence presented by Tome and Johnson (1945) indicates that cultivated forms of tetraploid L. corniculatus probably did not originate directly from cultivated forms of diploid L. tenuis, other plant materials have been described in the literature that may represent the source of the original stock. One probable parental stock is discussed by Favarger (1953), who found apparently identical diploid and tetraploid populations of L. corniculatus in the Alps. He proposed that this diploid is the parental stock of this tetraploid. Transplant experiments reported by Bonnier (1920) also provide some evidence that alpine diploids may be parental to tetraploid L. corniculatus found in the surrounding lowlands. Chromosome numbers were not reported in his paper in which it is claimed that normal plants from lower altitudes were modified in alpine nurseries until they were phenotypically identical with alpine forms found at the same altitudes. Bonnier claimed that the results he obtained support the Lamarchian theory of evolution, however, they may also be accounted for if it is assumed that the diploids and tetraploids are phenotypically identical at higher altitudes, as Favarger proposed, and that the tetraploids are at a selective advantange in the surrounding lowlands. It may be assumed that the plants Bonnier found at the lower altitudes

were tetraploids, since all materials that are taxonomically L. corniculatus, that have been collected in this region and that have been studied cytologically, have been tetraploids. Larsen (personal communication) was not able to maintain alpine diploids in the Botanic Garden at Copenhagen, which again suggests that the diploids are at a selective disadvantage at lower altitudes.

Favarger's suggestion that the alpine diploid of L. corniculatus is parental to the tetraploids found in the surrounding lowlands is one of several examples that he cites to support his theory that the Alps are the center of origin (in the sense that this term was used by Vavilov) for many species that are found in the surrounding lowlands.

Although a lot of effort was devoted to an investigation of the genetics of self-incompatibility in L. corniculatus, the results of the various experiments on this problem are inconclusive. These experiments were planned to determine the number of segregating loci that are involved, to reveal the presence of disomic or tetrasomic inheritance at these loci and to establish the presence of sporophytic or gametophytic action of incompatibility alleles with dominance, competition or independant action. Compatibility indices from crosses with unrelated plants and from selfs using the same plants rarely overlap but many indices that were obtained from backcrosses are intermediate and they range in magnitude continuously between the two extremes. It is not possible to

classify these values in discrete groups, therefore ratios of compatible : incompatible crosses cannot be determined and these data do not directly indicate the nature of the genetic mechanism that determines self-incompatibility. However, the data do indicate that the number of incompatibilities within families are lower than expected with random segregation of incompatibility alleles at one locus inherited tetrasomically. The exact number of incompatibility classes was not determined with the backcross technique that was used so the number of segregating incompatibility loci was not determined. It may be possible to determine the number of incompatibility classes in a self-progeny by using a diallelic crossing technique, as Lundqvist (1956) did with rye. This technique should be satisfactory where incompatibility alleles do not segregate at random, as is proposed for L. corniculatus, unless mutation of incompatibility alleles is a major cause of this non-random segregation.

The evidence presented to the effect that incompatibility alleles do not segregate at random in the progeny of crosses of related plants can account for the low numbers of incompatibilities that were obtained. The claim that selection occurs with inbreeding is based on comparisons of average self-compatibility indices following various degrees of inbreeding, on differences in average compatibilities of progenies backcrossed to parents, on correlations between reciprocal backcrosses and on the observation that inbreeding affects segregation at the red keel tip locus in a manner that

can be accounted for if linkage is assumed to an incompatibility locus at which selection occurs. Because of this selection, the low frequencies of incompatible combinations do not prove that more than one locus is segregating for incompatibility in this species.

Several papers by Lewis and Bateman, for example those of Lewis (1954) and of Bateman (1955) and several cited therein, present evidence that incompatibility data on several species that were originally interpreted to demonstrate segregation of incompatibility alleles at more than one locus have alternative interpretations that require segregation at only one locus. However, data on several other species indicate that incompatibility alleles segregate at more than one locus and the writer cannot forsee any one locus interpretation that may be valid. These studies include one by Lundqvist (1956) on rye, one by Owen (1942) on the sugar beet and one by Elliott (1946) on Lotus tenuis. Therefore, there is precidence for proposing that incompatibility in L. corniculatus may be determined at more than one locus, especially in the light of the evidence on L. tenuis, although it has not yet been satisfactorily proven.

It was not necessary to carry out an extensive investigation of pollen growth and of other morphological aspects of incompatibility in L. corniculatus as much of the required information was presented by Giles (1949). Some observations were made to confirm Giles' observations and some additional information was obtained. Extensive excerpts of Giles

dissertation have been quoted as his report is not readily available. Giles' observation that opposition to incompatible pollen occurs within the ovary cavity was confirmed in the present studies by determining the relative positions of developing ovules within ovaries.

The observations that differential fertilization occurs because of incompatibility and that inbreeding leads to selection for compatibility raises some questions about the effects of inbreeding on the breeding behaviour of populations of inbred progenies. Bateman (1956) described differential pollen tube growth in the wallflower, a species which is apparently attractive to insects because it has conspicuous flowers, which is highly self-fertile in undisturbed isolated flowers. He demonstrated that at least 70% of the seeds set in a small planting of these plants were from cross-pollinations. He also demonstrated, with the aid of suitable genetic markers, the presence of a weak self-incompatibility which encourages outbreeding. These findings imply that the increase in self-incompatibility that is associated with inbreeding in L. corniculatus should not be expected to reduce the efficiency of incompatibility as an outbreeding mechanism to an appreciable degree. Therefore, the plant breeder can safely use inbreeding to obtain desired genotypes without much risk of upsetting the breeding structure in subsequent varieties.

Bateman's observations on wallflowers suggest to the writer that species may be mechanically self-fertile and at

the same time self-incompatible. This may apply to L. weilleri, L. suaveolens and any other Lotus species in which the stigmatic membrane does not break automatically when the ovules become fertilizable. Suitable marker genes have not yet been found to determine if these species are self-incompatible.

If this proposal is correct, the first step in the evolution of self-incompatibility may be that the stigmatic membrane loses the ability to break automatically when ovules mature. Following the development of a stigmatic membrane which does not rupture until the flower is visited by insects, both self-incompatibility and variability in ovule development should be at a selective advantage and they could then evolve.

No reports of a relationship between the stigmatic membrane, if present, and ovule development or self-sterility were found in the literature reviewed. However, reports in the literature of several studies on other Leguminosae suggest to the writer that variability in ovule development, which may serve to prolong the period during which ovules may be fertilized, may be quite common. Cram (1955) reported that Caragana arborescens has an average of 14.2 ovules per ovary and of 3.4 seeds per pod following open-pollination. Dessereaux (1951) found that Trifolium repens produces less seeds per pod than there are ovules and that selection for higher numbers of seeds per pod sometimes increased the numbers of ovules per ovary and sometimes increased the proportion fertilized. Cooper and Brink (1940) found that an average of

of 66.2% of the ovules in Medicago sativa became fertile following crossing. On the other hand, Dr. Bronys Povilaitis, Department of Genetics, Macdonald College (unpublished) informed the writer that although the ovaries of Trifolium pratense contain two ovules and generally produce only one seed, he is not able to observe differences in maturity between ovules within each ovary.

An examination of ovule development of these and other self-incompatible legumes is needed to determine the prevalence of random variation in ovule development within ovaries so that the importance of this mechanism can be evaluated. If this type of variation is common in other species and if the significance of this mechanism is correctly evaluated, it may be of considerable importance in plant breeding. Dessureaux (1951) selected for higher numbers of seeds per pod in order to obtain better seed yields in Trifolium repens. If he selected plants with higher numbers of ovules per ovary he should obtain higher numbers of seeds per pod under all comparable environmental conditions. However, if he selected plants which set higher numbers of seeds per pod under certain conditions because a greater portion of the ovules were fertilized as a result of a reduction in variation in maturity within ovaries, a reduction in average seed set could occur under environmental conditions in which pollination did not occur at the same stage of development. From this example, it is obvious that this mechanism may be of considerable practical significance to plant breeders working with Lotus corniculatus

and other species in which this random variation in ovule development occurs.

The results of the experiments presented and discussed in this dissertation do not in general lead to firm conclusions, however, they provide a lot of information on the genetics and cytotaxonomy of L. corniculatus that should be useful in planning further experiments which in their turn may lead to more definite conclusions. Although many of the fundamental questions remain unanswered, the data obtained are directly useful in planning breeding work as they give some indications of population sizes that need to be grown to find desired combinations and they show that it is possible to reduce self-incompatibility by inbreeding. The conclusions which are drawn also aid in breeding work as they indicate similarities with findings in other species for which breeding techniques have been worked out. Further work on this species is warranted to increase our knowledge of the theoretical genetics of autotetraploids, especially with characters that are heterogeneous in populations, such as incompatibility, as practically nothing is known of allelic relationships where more than two different alleles are present on homologous chromosomes. This problem has received some consideration in relation to incompatibility in induced tetraploids, however, it is clear from the considerations of Lundqvist's (1956) work that the findings in the induced tetraploids may not apply to natural autotetraploids. As Lotus corniculatus has tetrasomic inheritance without the complications of double reduction, it

may be useful for these investigations. It does have the disadvantages that the time required to produce a generation is rather long, that self-incompatibility makes inbreeding difficult and may prevent certain recombinations and that highly inbred lines have not yet been produced. However, it is easily maintained and propagated, easily cross-pollinated and valuable agronomically.

SUMMARY

1. Somatic chromosome numbers of 2n = 12 and 24 were found in the plant materials studied which conformed to Isely's (1951) description of L. corniculatus L. and in some other taxonomically similar materials.

2. The chromosomes in the corniculatus group of collections appear to be morphologically homologous.

3. The corniculatus group of materials studied can be classified into taxonomically distinguishable cross-sterile taxa that may be considered as separate species. (See table 1.)

4. Contrary to Isely's description, it is proposed that some materials which possess a weak tendency to produce rhizomes belong to the species corniculatus.

5. All the materials studied in the inter-fertile group of morphologically similar plants that are included in L. corniculatus are tetraploid with 2n = 24. The collections in this taxon are subdivided into three type classes; the European type, the Empire type and the English Pasture type. Some materials with 2n = 12 have been included in L. corniculatus by other workers, however, none of these materials were obtained for the present studies.

6. Meoisis was studied in some tetraploid L. corniculatus plants that were used as parents for genetic studies. Bivalent pairing appears to occur regularily during meiosis in these plants.

7. The Lotus materials studied had 2n = 12, 14, 24 and 28, which is in agreement with previous reports.

8. In addition to the collections in the corniculatus group, three collections which are taxonomically distinct from L. corniculatus and from each other have the basic number of x = 6. Their chromosome morphologies appear quite different from L. corniculatus and from each other.

9. From observations on taxonomic variation and on chromosome morphologies, it is proposed that the direction of evolution in Lotus has been from a basic number of x = 7 to one of x = 6.

10. A plant of L. corniculatus with a light yellow flower colour was crossed with one having the darker, wild type flower colour. The light colour is recessive and is inherited tetrasomically at one locus giving a 5:1 backcross segregation.

11. Inheritance of red keel tip in L. corniculatus also fits a tetrasomic inheritance hypothesis at one locus if a quantitative effect is assumed so that the nulliplex or monoplex for red produces the yellow phenotype and the duplex, triplex or quadruplex produces the red phenotype and if it is also assumed that there is a selective pressure for heterozygosity at this locus.

12. Inheritance of mottled seed coat in L. corniculatus was also found to fit a tetrasomic inheritance hypothesis at one locus.

13. Inheritance of speckled seed coat in L. corniculatus appears to be somewhat similar to that for red keel tip, however, the data that were obtained are not extensive enough to allow any firm conclusions.

14. Inheritance of self-incompatibility in L. corniculatus was investigated extensively by using a series of backcrosses between parents and their self-, cross- and backcross-progenies out of collection 5101.

15. Compatibility was evaluated in terms of a compatibility index based on the number of seeds obtained from a cross divided by the number of flowers pollinated.

16. Compatibility indices involving inbreeding are considerably lower than indices from crosses of plants not known to be closely related.

17. Compatibility indices from backcrosses within families do not segregate into discrete classes and do not indicate the number of incompatibility loci present in L. corniculatus.

18. The frequencies of reciprocal incompatibilities in backcrosses between self-progenies and their parents are well below the expectation for disomic or tetrasomic inheritance at one locus. However, there is independant evidence that inbreeding leads to selection for compatibility so these data alone do not prove that incompatibility alleles in L. corniculatus are segregating at more than one locus.

19. Selection for increased self-compatibility with inbreeding is indicated by a comparison of F values in analyses of variance in which the significance of differences in mean compatibility indices for progenies used in backcrosses are compared. The degree of significance of these F values increases as the compatibility of the parental pollination decreases.

20. Selection for increased self-compatibility with inbreeding is also indicated by a comparison of correlation coefficients between reciprocal compatibility indices from backcrosses. The magnitude and the degree of significance of the correlation coefficients increase as the compatibility of the parental pollination decreases.

21. Selection at an incompatibility locus may account for the data obtained on the inheritance of red keel tip, if linkage is assumed.

22. Sporophytic incompatibility with independant gene action is indicated by positive correlations between reciprocal compatibility indices. Lack of correlation between reciprocal compatibility indices may indicate that interactions do occur between incompatibility alleles.

23. Independant gene action is indicated by the presence of incompatibility in the natural tetraploids and in the induced autooctoploids of L. corniculatus and by the correlation coefficients obtained. With independant gene action, it may be possible to account for increased compatibility following inbreeding by assuming that selection occurs for increased interaction between incompatibility alleles.

24. The incompatibility data do not indicate the number of segregating incompatibility loci involved in L. corniculatus, however, it is suggested that more than one locus is probably involved.

25. These incompatibility data are in better agreement with tetrasomic than with disomic hypotheses.

26. Limited data on the inheritance of incompatibility in

L. tenuis (2n = 12) indicate that more than one locus is involved and that the alleles act sporophytically and independantly.

27. A survey of sterility and seed set in various collections of different Lotus materials indicates that all ovules in an ovary may be fertilized and produce seeds in some species, while only a fraction of the ovules in each ovary are ever fertilized in other species. This is shown to be associated with the behaviour of the stigmatic membrane. In self-fertile species, the membrane ruptures at a specific stage of maturity so that self-pollination can occur and all the ovules in an ovary can be fertilized. In other species, the membrane is broken by visiting insects before pollination can occur and the ovules in the fertilizable stages of development can be fertilized, however, only a portion of the ovules are fertilizable at any one time in these species.

28. It was demonstrated that a medium is released onto the stigmatic surface when the stigmatic membrane is ruptured and that this medium is used up by growing pollen. When this medium is exhausted, repeated pollinations do not lead to fertilizations.

29. Random variation in the rate of ovule development is associated with the requirement for insect visitation to break this membrane under natural conditions. Species in which the membrane breaks automatically have all ovules in each ovary in the same stage of development.

30. Observations on ovule development following self-

pollination indicate that proliferation of nucellar tissue may lead to abortions, like those which Brink and Cooper (1939) designated as the result of "somatoplastic sterility".

31. Observations on the distribution of developing ovules in ovaries examined after pollinations by related plants indicate a tendency for fertilization to occur more frequently near the stylar end of the ovary. This is in agreement with Giles' (1949) observation that pollen tubes are inhibited in the ovary cavity.

32. Applications and theoretical implications of these studies are considered in a "discussion" section. In this section, it is postulated that L. corniculatus is an autotetraploid.

33. A general evaluation of the experimental work and conclusions drawn is also presented in this discussion. It is pointed out that many of these experiments do not lead to firm conclusions, however, they do provide a basis for further investigations.

34. It is concluded that further work is warranted as L. corniculatus can be used to investigate the theoretical genetics of autotetraploids without the added complications of double reduction and subsequent disturbed segregations.

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