Suggested short title

A CYTOGENETIC STUDY OF THE SPECIES OF LOTUS IN CANADA

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# A CYTOGENETIC STUDY OF THE SPECIES OF LOTUS IN CANADA

by

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# I. INTRODUCTION

There has been considerable disagreement among systematists as to whether North American species should be placed in the Old World genus Lotus or in a separate genus Hosackia. Ottley (1923), in her monographic revision of the Californian species, recognized only the genus Lotus, since the genus Hosackia was maintained on vegetative characters which to her were of doubtful generic importance. Lotus has now been largely accepted by systematists for the North American species; however, recently without any explanation, Shreve and Wiggins (1964) have retained the generic name Hosackia for their treatment of the flora of the Sonoran desert.

The growing economic importance of Lotus corniculatus as a forage crop has generated interest in the genus as a whole. Therefore, it was thought worthwhile to study Canadian members, which have previously been unsurveyed, and for which even the exact number of species growing in Canada was uncertain.

Nine species of Lotus can be found in Canada. Five of these are native and four are introduced species. The native species range from British Columbia on the

west coast, as far east as Manitoba. The introduced species range right across Canada. Although most species grow in southern Canada, plants belonging to Lotus denticulatus and Lotus corniculatus can be found in the northern half of British Columbia.

Apart from studies on the geographical distribution of species of Lotus, some new chromosome number determinations along with karyotype analysis of eight of the species (excluding L. corniculatus) are presented. The growing interest in chemotaxonomy for the diagnosis and delineation of taxa prompted a study of phenolic patterns of fresh and dried leaf extracts by the use of thin-layer chromatography. It was thought that this particular technique would be a useful aid in resolving the difficulties of assigning specific names to certain herbarium specimens whose characteristics were not readily discernible from the specimen, which might have been initially poorly prepared. A programme of interspecific hybridization was tried to help establish relatedness of the species.

The native species found in Canada are: Lotus pinnatus Hook., Lotus formosissimus Greene, Lotus micranthus Benth., Lotus denticulatus (Drew) Greene and Lotus purshianus (Benth.) Clem. and Clem. Introduced species are: Lotus corniculatus L., Lotus tenuis Waldst. et Kit., Lotus pedunculatus Cav., and Lotus krylovii Schischk. and Serg.

## II. LITERATURE REVIEW

In the last few decades, the genus Lotus has been receiving increased attention, particularly the Old World species. This can be attributed to the fact that among the Old World members are certain species which are of considerable agronomic importance (MacDonald, 1946). Because they are of little economic importance, less is known about the New World Lotus species, although uses in horticulture, in soil erosion, and their use under arid and saline conditions in certain geographic areas, has recently been suggested by Grant (1965).

The first two North American species were described in 1814 and 1816. Hooker, in 1829, described L. pinnatus as belonging to the Old World genus. However, that same year Bentham redescribed and renamed it Hosackia bicolor Dougl., placing it in a new genus. He thought this was justified because of its pinnate leaves and membranous stipules (Bentham, 1834-37). In 1837, Bentham placed five New World species including L. micranthus back into Lotus, creating a section Microlotus for them, while retaining eleven species in Hosackia. The species retained in Hosackia had inflorescences in umbels while

the ones placed in Lotus were uniflorous. Torrey and Gray (1838) favored the generic name Hosackia for the North American species while Greene (1890) favored the generic name Lotus.

Brand (1898), in his monograph of the genus, excluded the New World species from Lotus. He felt this was necessary from a practical as well as a taxonomic point of view, Lotus always having five leaflets and Hosackia having pinnate leaves whose number and position of leaflets vary considerably. Piper (1906), in his "Flora of the State of Washington," follows Brand in using Hosackia. Ottley (1923) makes a firm stand on uniting Old and New World species in the one genus Lotus, since these two groups are not separable from each other by any one, or a combination of characters, including the diagnostic characters of the inflorescence, flower, or fruit. If it is justifiable to split off Hosackia from Lotus, to be consistent, she claims one must put the widely variable North American species into at least five genera. Therefore, she favors putting them all in Lotus, in the subgenus Hosackia. Abrams (1944), however, still uses Hosackia while Munz and Keck (1959) and Hitchcock et al. (1961) use Lotus in their respective treatments of the genus.

Once it is accepted that the North American

species are members of Lotus, there still remains varying opinions as to the subdivisions of the genus. Ottley (1944) divided the genus into three subgenera, namely, Hosackia Bentham, Acmispon Rafinesque, and Microlotus Bentham, and with two sections in the subgenus Acmispon, namely, Simpeteria Ottley and Microlotus Bentham. Of the Canadian species of Lotus, L. pinnatus and L. formosissimus belong to the subgenus Hosackia and L. denticulatus, L. purshianus and L. micranthus belong to the section Microlotus of the subgenus Acmispon. Callen (1959) included Hosackia and Tetragonolobus in Lotus and subdivided the genus according to the character of the style. In this classification, eight of the nine species found in Canada are placed in the subgenus Edentolotus Brand and L. denticulatus is placed in the subgenus Deflectostylus Callen. The species belonging to Edentolotus all have simple, erect styles whereas those belonging to Deflectostylus have a style deflected from the vertical at an obtuse angle.

The various taxonomic surveys done on North American Lotus have been of species growing in the United States, whereas relatively little is known of Canadian species. The distribution of New World Loti has been reported (Ottley, 1923) as mainly western North America from British Columbia to Mexico and Lower California. One

species is found as far south as Chile and two are found in the middle and eastern United States.

A knowledge of chromosome numbers of more Lotus species is necessary. Of the approximately 200 Lotus species distributed throughout the world, only 73 species have chromosome numbers reported (Grant, 1965). Of these 73 species, 16 are North American. Two basic chromosome numbers, both with polyploid taxa, are found in the genus; $\underline{n} = 6$ and $\underline{n} = 7$. Evolution seems to be occurring in the direction of 6 from 7 in a descending aneuploid series from an eight-chromosomed ancestor of the tribe Galegeae (Senn, 1938). The majority of the New World species have a basic number of $\underline{n} = 7$, 13 of the 16 species studied, while three species have $\underline{n} = 6$ (Grant, 1965). No polyploid taxa have been reported in North America (Grant, 1965).

Few chromosome counts have been reported but even fewer karyotype studies are available in the literature. The only karyotype of a native North American species is that presented by Larsen (1956) for L. purshianus. This species has fourteen small chromosomes, two of which have satellites. Larsen (l. c.) concludes that his comparative study of the chromosome morphology of these species supports the inclusion of the New World species into Lotus.

Drawings of the somatic chromosomes of introduced

Canadian species (L. corniculatus, L. tenuis, L. pedunculatus, L. krylovii) have been published by several authors, L. corniculatus by Tschechow und Kartaschowa (1932), Larsen (1954a), Ujhelyi (1960), Larsen and Zertova (1963), and Gilot (1965); L. tenuis by Tschechow und Kartaschowa (1932); L. pedunculatus (uliginosus) by Tschechow und Kartaschowa (1932) and Gilot (1965). Larsen (1958) shows a karyotype of L. heterophyllarius which is taken to be synonymous with L. krylovii, a species which has been introduced into Canada. Most workers, however, have merely reported chromosome numbers (see Grant, 1965).

Natural interspecific hybridization does not seem to be very important in the genus Lotus. In North America, Ottley (1944) reports the occurrence of the only natural hybrids between a few of the southwestern species she has studied. The variability and the difficulty of separating L. corniculatus, L. tenuis and L. pedunculatus (uliginosus) in Southern Europe has led to the suggestion that they have formed hybrid swarms (Larsen, 1954b). This is a possibility, since these species are all obligate outbreeders, allowing the occasional interspecific fertilization to take place.

Experimentally produced interspecific Lotus hybrids are listed in Grant (1965). There is no report of

hybridization involving any of the five native species found in Canada. However, hybridizations using European Lotus species, although difficult, have been effected. Tome and Johnson (1945) attempted to hybridize L. tenuis and L. corniculatus, but were unsuccessful. Autotetraploid L. tenuis crossed with L. corniculatus produced no viable seed. Bent (1962) did produce hybrids of L. corniculatus and L. tenuis (4x), L. pedunculatus (uliginosus) and L. tenuis, L. pedunculatus (uliginosus) (2x and 4x) with L. corniculatus. He found that the success of hybridization appeared more likely if the female parent was self-compatible. The genome of L. tenuis is thought to have been incorporated into tetraploid L. corniculatus, thus rendering the two species closely related. Because of their close relation to one another, an effective isolating mechanism is a necessity for them to remain separate and distinct species. Thus, Bent suggests that perhaps it is easier to hybridize less closely related species since they might not have developed such an effective barrier to hybridization.

Reciprocal differences were found in crosses of L. tenuis and L. corniculatus. Jaranowski and Wojciechowska (1963) reported that embryo development was normal up to the cotyledon stage when L. tenuis was used as the female parent. Breakdown occurred, however, due to a failure in

the endosperm. More pronounced abnormalities occurred in the embryo and endosperm in the reciprocal cross.

The status of L. corniculatus has long been of concern to workers in the field. It was first thought to be an autotetraploid of L. tenuis. However, the artificially produced autotetraploid proved to be different in leaf and stipule shape (Tome and Johnson, 1945). Lotus corniculatus was first considered by Stebbins (1950)[1] to be a segmental alloploid which he defines as "a polyploid containing two pairs of genomes which possess in common a considerable number of homologous chromosomal segments or even whole chromosomes, but differ from each other in respect to a sufficiently large number of genes or chromosome segments, so that the different genomes produce sterility when present together at the diploid level." Wernsman, Keim and Davis (1964) have presented data on chromosome pairing relationships in L. corniculatus, L. tenuis (4x) and interspecific hybrids of these two taxa to support the hypothesis of an autotetraploid origin of L. corniculatus from diploid L. tenuis. Harney and Grant (1964), however, from a chromatographic study of the phenolic properties possessed by these species, considered that the biochemical data supported an allotetraploid origin for L. corniculatus.

Lotus krylovii has been crossed with three diploid

[1]G. L. Stebbins, in Variation and Evolution in Plants. New York: Columbia Univ. Press, 1950, p. 318.

species, L. japonicus, L. krylovii and L. schoelleri (de Nettancourt and Grant, 1964). These hybrids showed hybrid vigour but had greatly reduced seed set. Embryo-culture techniques are necessary for the successful production of these and most other interspecific hybrids in Lotus.

As early as 1912, Armstrong and co-workers, from studies of L. corniculatus over nearly all of Europe, reported that this species sometimes contains a cyanophoric glucoside and a corresponding enzyme. All collections of L. pedunculatus (uliginosus) proved to be negative. Armstrong et al. (1913) early recognized the usefulness of differentiating botanical species by the study of chemical factors such as enzymes and glucosides.

Lotus corniculatus was found to be variable in its HCN content by Dawson (1941) and MacDonald (1946). The former worker reports that while L. pedunculatus (uliginosus) reacts negatively to the picric acid test for the presence of HCN, L. tenuis was found to have both positive and negative members. Lotus krylovii reacts positively for HCN, L. purshianus mostly negatively, with a few positive plants, and L. denticulatus reacts negatively (Grant, unpublished). Fewer of the North American species are found to contain HCN than the Old World species. When they do give a positive reaction, it is always weaker

than the reaction given by the Old World species. Phillips (1963), as well as testing other species, found L. micranthus and L. purshianus to be negative with respect to HCN content. The presence, or absence, of HCN seems to be governed by a single gene, although modifier genes may be affecting its concentration, making it a useful marker in interspecific crosses (Bent, 1962; de Nettancourt and Grant, 1964). Caution must be exercised when testing for HCN as this substance may be detected only at certain seasons and may vary from time to time (Gibbs, 1954). Gibbs tested leaves from herbarium specimens of nine New World species and found them to be negative. However, tests with herbarium material may not be significant.

The idea that a "chemical description" should accompany each description of a new species, or genus, is not new (Greshoff, 1909 in Gibbs, 1954). Gibbs (1954, 1958) stresses the usefulness of a study of the distribution of chemical characters in finally arriving at a true phylogeny of flowering plants.

Three classes of phenolic compounds are found to have a widespread distribution in the leaves of higher plants--leucoanthocyanins, flavonols, and hydroxycinnamic acids. Every class of phenolic compound is found to be present in the Leguminosae (Bate-Smith, 1958). These

secondary phenolic compounds are thought to be of taxonomic importance and the rarer a compound two species have in common, the more likely they are to be related.

Numerous cases of interspecific hybridization are reported for the genus Baptisia. Sometimes these hybrids are difficult to separate. Alston and Turner (1962) analyzed trihybrid populations of B. leavicaulis, B. leucantha, and B. viridis, both morphologically and by the use of two-dimensional paper chromatography. Some contrary results were obtained but these workers consider the chromatographic results more accurate. Alston et al. (1962), using chromatography alone, identified the hybrids, B. lanceolata x B. alba and B. lanceolata x B. pendula, which were nearly indistinguishable morphologically. The usefulness of paper chromatography for taxonomic purposes in the genus Lotus has been shown by Harney and Grant (1965). The technique is also helpful in the study of hybrids between Lotus species (Harney and Grant, 1964.

In view of the little known about the North American Lotus species, particularly ones found in Canada, it was thought worthwhile and important to conduct a comprehensive survey of Canadian Lotus, utilizing new techniques such as chromatographic analysis and embryoculture, as well as the better known methods of chromosome analysis.

## III. MATERIALS AND METHODS

### 1. Herbarium Specimens

Herbarium specimens of Canadian species of Lotus were borrowed from the following Canadian herbaria: Acadia University, Wolfville (ACAD); New Brunswick Museum Herbarium, Saint John (NBM); Quebec Department of Agriculture, Quebec (QFA); L'Ecole d'Agriculture, Rimouski (RIM); Institut de Technologie Agricol, La Pocatière (ITA); McGill University, Montreal (MTMG); Phanerogamic Herbarium, Department of Agriculture, Ottawa (DAO); National Museum of Canada, Ottawa (CAN); Ontario Agriculture College, Guelph (OAC); University of Toronto, Toronto (TRT); Royal Botanical Gardens, Hamilton (HAM); University of Western Ontario, London (UWO); University of Manitoba, Winnipeg (WIN); The W. P. Fraser Herbarium, University of Saskatchewan, Saskatoon (SASK); University of Saskatchewan Regina Campus, Regina (SASKR); University of Alberta, Edmonton (ALTA); University of British Columbia, Vancouver (UBC); Provincial Museum, Victoria (V).

American Lotus material, representative of Washington State, was borrowed from the University of

Washington, Seattle (WTU), and from Washington State University, Pullman (WS). A total of 367 Canadian herbarium specimens, exclusive of duplications, were examined. Of the 349 Washington State herbarium specimens examined, 239 belonged to species found in Canada.

Distribution maps were prepared by mapping all the herbarium specimens seen, excluding duplications.

Each Canadian herbarium specimen was examined, identified as to species, and measurements of the following characters were made: number of florets per inflorescence, number of leaflets per bract, if present, length of standard, length of calyx tube, length of calyx, length of peduncle, length of legume, length of petiole, length and width of central leaflet. Calyx index was calculated as $\frac{\text{total length of calyx}}{\text{length of calyx tube}}$. Leaflet index was calculated as $\frac{\text{central leaflet length}}{\text{central leaflet width}}$. Fresh material was used in the study of the following characters. Styles and ovaries were drawn with the aid of a camera lucida, pollen measurements were made using an eye-piece micrometer and seed measurements were made with the aid of calipers.

## 2. Root tip squashes

Root tips for cytological examination were taken from plants grown in a growth chamber, greenhouse or in a

cold frame. Pretreatment consisted of placing root tips in 0.002N 8-hydroxyquinoline for one hour. They were then fixed in Carnoy's fluid (3:1 ethanol-glacial acetic acid or 6:3:1 ethanol-chloroform-glacial acetic acid). Staining was by the Feulgen technique according to Darlington and La Cour (1962). Maceration was carried out in 4% pectinase for one to one and a half hours. Slides were prepared by squashing root tip meristems in 45% acetic acid after which they were made semi-permanent by sealing with a mixture of paraffin and vaseline. Photographs were taken of appropriate figures using phase contrast optics.

### 3. Karyotypes and Idiograms

With the aid of a camera lucida, a karyotype of the somatic chromosomes was prepared for each species. For the construction of idiograms, short and long arm measurements of the entire chromosome complements were made on ten cells for each species. Standard deviations were calculated using a 1620 IBM computer.

### 4. Hybridization Studies

Immature flowers were emasculated using the air-suction technique as described by Grant, Bullen and

Nettancourt (1962). They were sprayed immediately with ten parts per million (ppm) 2-4-5 trichlorophenoxypropionic acid (TCPPN) to prevent flower drop and placed in a growth chamber. Pollination was carried out two days after emasculation. Embryos were cultured according to the method described by Grant et al. (1962). In two instances the Randolph-Cox solution was used as the culture medium, as described by Randolph (1955) and in one instance, the culture medium used was as described by Nitsch (1951).

### 5. Chromatography of unhydrolysed phenolic compounds

0.08 gram fresh leaves of the species to be studied was weighed out and placed in 0.5 ml. of 1% hydrochloric acid in methanol. If dried material was to be used, 0.02 gram was weighed out as the leaves were found to contain approximately 75% moisture. The material was left in the extracting solution at room temperature in the dark, overnight. The experimental procedure followed was according to Grant and Whetter (1966). The solvent systems used were cyclohexane-ethyl acetate 1:1 (C.EA 1:1) and methanol-chloroform 30:70 (MCh 30:70). The first solvent was allowed to run up the layer twice to 15 cm. and the second solvent was allowed to run up the layer once to 7 1/2 cm. Before observing the finished plate under short wave ultraviolet (UV) light, it was

exposed to ammonium hydroxide vapour. Results were recorded by mapping the spots on paper and also by photographing the plates through the viewing window of the Chromato-Vue using color (Kodak High Speed Ektachrome) film with the exposure 25 sec./f/8 at a distance of 20.3 cm. from the plate.

## 6. Hydrogen cyanide test

Leaves from fresh material grown from seed and a leaf from each herbarium specimen were tested for the presence of hydrogen cyanide. The procedure followed was according to Dawson (1941). The vials containing the leaves were observed after approximately 24 hours.

## IV. RESULTS

### 1. Key for *Lotus* species found in Canada

Annuals; flowers solitary.
  Stipules reduced to small glands.
    Flower subsessile.
      Bract absent.
        Leaflets 3-4; calyx teeth longer
          than tube; pod hairy, 2-4
            seeded . . . . . *L. denticulatus*
    Flower short pedunculate.
      Bract present, 1 foliolate.
        Leaflets 3; calyx teeth as long
          as corolla; pod 5-10 seeded . . *L. purshianus*
      Bract present, 3 foliolate.
        Leaflets 3-6; calyx teeth
          shorter than the tube; pod 3-6
            seeded, constricted between
              the seeds . . . . . *L. micranthus*
        Leaflets 5; calyx teeth
          equalling the tube; pod 15-30
            seeded . . . . . *L. krylovii*

Perennials; flowers in umbels.

Stipules expanded and membranous.

Flower long pedunculate; leaves 5-7.

Bract absent; 4-9 florets per umbel; corolla with yellow standard and white wings . . . . . L. pinnatus

Bract present, 1-3 foliolate; 3-5 florets per umbel; corolla with yellow standard and purple wings . . . . . L. formosissimus

Stipules reduced to small glands.

Flower long pedunculate; leaflets 5.

Bract present, 3 foliolate.

Leaflets twice as long as wide.

Florets 2-7; calyx teeth apressed in the bud . . . . . L. corniculatus

Florets 4-14; calyx teeth divergent in the bud . . . . L. pedunculatus

Leaflets 4-8 times as long as wide.

Florets 3-5; calyx teeth apressed in the bud . . . . . L. tenuis

TABLE 1.--Sources of Lotus collections grown from seed*

| Accession Number | Source | Chromosome Number (2n) |
|---|---|---|
| L. corniculatus L. | | |
| B-221 | Sidney and Saanichton, Vancouver Island, B. C. Coll. M. C. Melburn; Aug. 28, 1961. | 24 |
| B-535 | Antigonish Co., West River, N. S. Coll. J. E. Langille; June 20, 1966. | 24 |
| B-500 | Cumberland Co., N. S. Coll. F. S. Warren; Feb. 16, 1966. | 24 |
| B-257 | Viking (Cultivar). Received from Dr. J. S. Bubar, Department of Agronomy, Macdonald College. | 24 |
| L. sp.** | | |
| B-181 | Plant Introduction Station, Geneva, N. Y.; Source, Iran. P. I. 251400, 1961. | 24 |
| L. purshianus (Benth.) Clem. and Clem. | | |
| B-318 | Plumas Co., Quincy, Calif. Coll. E. K. Balls; No. 15,568 Aug., 1950. | 14 |
| B-65 | Texas, USDA TO-1912, Cornell Acc. No. 605. | 14 |
| B-489 | Brandon, Manitoba. Coll. J. A. Stevenson; No. 3483; Aug., 1965. | 14 |

(table continued)

TABLE 1 (continued)

| Accession Number | Source | Chromosome Number (2n) |
|---|---|---|
| *L. denticulatus* (Drew) Greene | | |
| B-240 | Victoria, Vancouver Island, B. C. Coll. M. C. Melburn; July, 1962. | 12 |
| B-242 | Kamloops, B. C. Coll. V. C. Brink; Aug., 1962. | 12 |
| B-224 | Quesnel, B. C. Coll. V. C. Brink; July, 1961. | 12 |
| *L. micranthus**** Benth. | | |
| B-243 | Nanoose Bay, B. C. Coll. V. C. Brink; June, 1962. | 14 |
| B-306 | Sooke, Vancouver Island. Coll. V. C. Brink; May, 1962. | 14 |
| B-329 | 7 miles W. of Victoria, Vancouver Island, B. C. Coll. M. C. Melburn; July, 1962. | 14 |
| B-388 | Nanaimo, B. C. Coll. K. I. Beamish; July, 1964. | 14 |
| *L. formosissimus**** Greene | | |
| B-151 | Point Reyes Peninsula, Marin Co., Calif. Coll. Beecher Crampton 5613; June, 1960. | 14 |

(table continued)

TABLE 1.--(continued)

| Accession Number | Source | Chromosome Number (2n) |
|---|---|---|
| *L. pinnatus* Hook.*** | | |
| B-488 | Hook, Benton Co., Oregon. Coll. L. Gottlieb; July, 1965. | 14 |
| B-490 | Nanaimo, B. C. Coll. K. I. Beamish; July, 1965. | 14 |
| *L. tenuis* Waldst. et Kit. | | |
| B-309 | 4 mi. W. of Victoria, B. C. Coll. M. C. Melburn; Aug., 1962. | 12 |
| B-145 | Received from U.S.D.A. Soil Conservation Service, Pleasanton, Calif. P-14496, Sept., 1959. | 12 |
| *L. krylovii* Schischk. and Serg. | | |
| B-226 | White Lake, Oliver, B. C. Coll. V. C. Brink; July, 1961. | 12 |
| B-86 | Received from Hortus Botanicus Universitatis, Uppsala, Sweden. | 12 |
| B-198 | Received from All-Union Institute of Plant Industry, Leningrad, U.S.S.R. No. 31547; June, 1961. | 12 |
| *L. pedunculatus* Cav. | | |
| B-201 | Received from Hortus Botanicus, Coimbra, Portugal; 1964. | 12 |
| B-294 | Received from Plant Introduction Station, Geneva, N. Y.; Source, Austria, P. I. 251829; 1962. | 12 |

(table continued)

TABLE 1.--(continued)

| Accession Number | Source | Chromosome Number |
|---|---|---|
| | | (2n) |
| L. humistratus Greene | | |
| B-154 | Eldorado Co., Calif. Coll. Beecher Crampton; June, 1960. | 12 |
| L. subpinnatus Lag. | | |
| B-160 | Point Reyes Pen., Marin Co., Calif. Coll. Beecher Crampton; June, 1960. | 12 |
| L. angustissimus L. | | |
| B-146 | Received from Museum d'Histoire Naturelle, Paris; May, 1960. | 24 |

*Canadian collections listed here also included in the distribution maps.

**Similar morphologically, but not identical to L. corniculatus.

***New chromosome number determination.

## 2. Morphological description of Lotus species

An illustration for each species is shown in Figures 1 through 9. Results of measurements of herbarium material on which descriptions are based, are shown in Table 2.

Lotus pinnatus Hook.

Perennial, 1.5-4 dm. high, prostrate and spreading or erect, branching from a thickened rootstock; glabrous; stipules membranous; leaflets 5-7, more commonly 7, pinnately arranged, oblong to obovate; central leaflet 15-30 mm. long, 5-15 mm. broad; umbels 4-9 flowered, produced on axillary peduncles 3-10 cm. long; bract absent; standard of flower 13-16 mm. long, standard yellow, wings white; calyx 6-8 mm. long, teeth shorter than the tube; pod 20-40 mm. long, 1.5-2 mm. broad; seeds 10-15, 2-2.5 mm. long, dark olive-brown mottled with black.

Lotus formosissimus Greene

Similar to L. pinnatus Hook. but more slender; perennial, 1-3 dm. high, decumbent, branching from the base; glabrous; stipules membranous; leaflets 5-7, more commonly 5, pinnately arranged, oval to obovate; central leaflet 10-20 mm. long, 5-10 mm. broad; umbels 3-5 flowered, produced on axillary peduncles 2-6.5 cm. long; bract 1-3 foliolate, more commonly 3, just below the

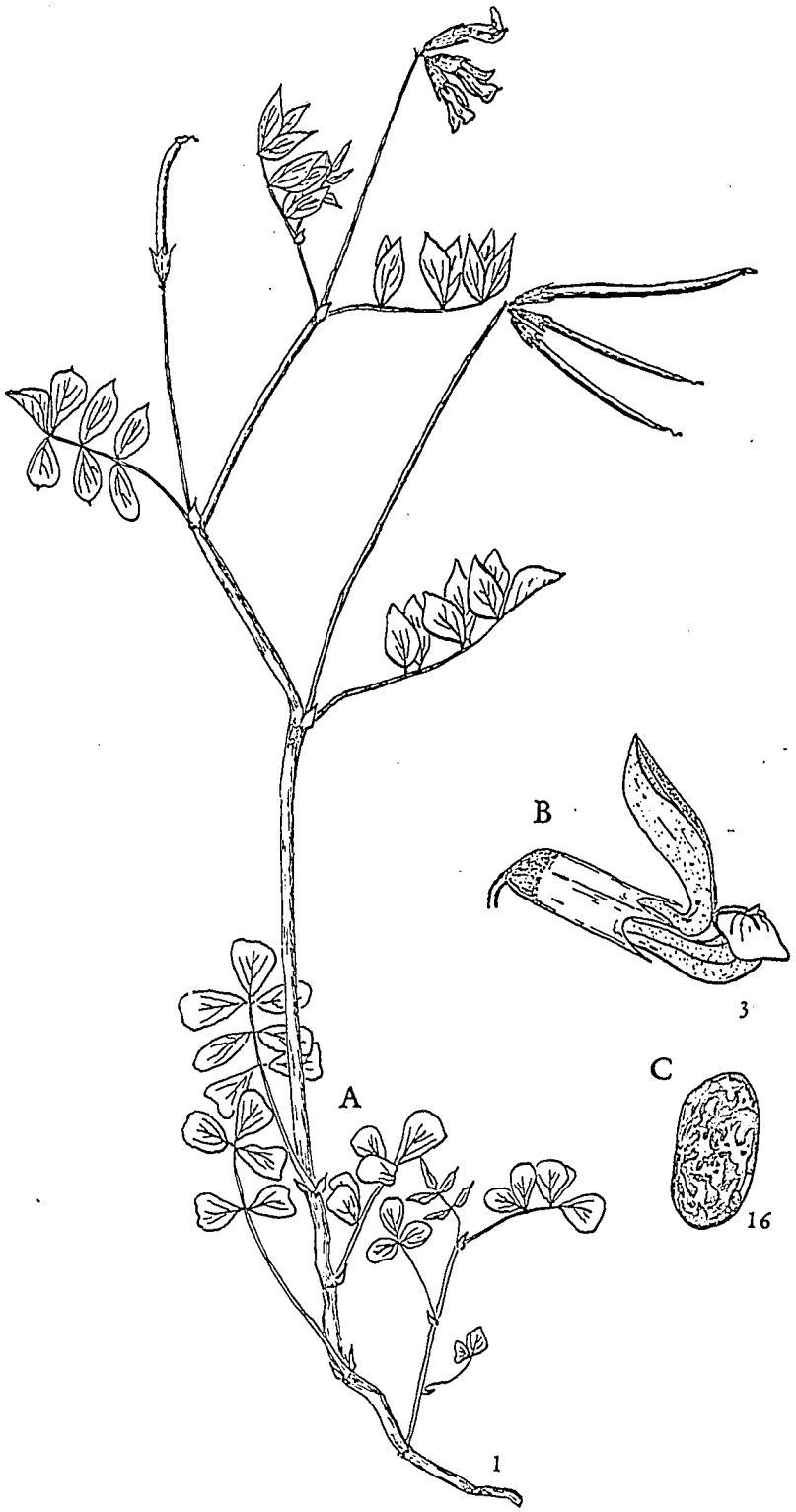



Fig. 1.--Lotus pinnatus

A, plant; B, flower; C, seed.

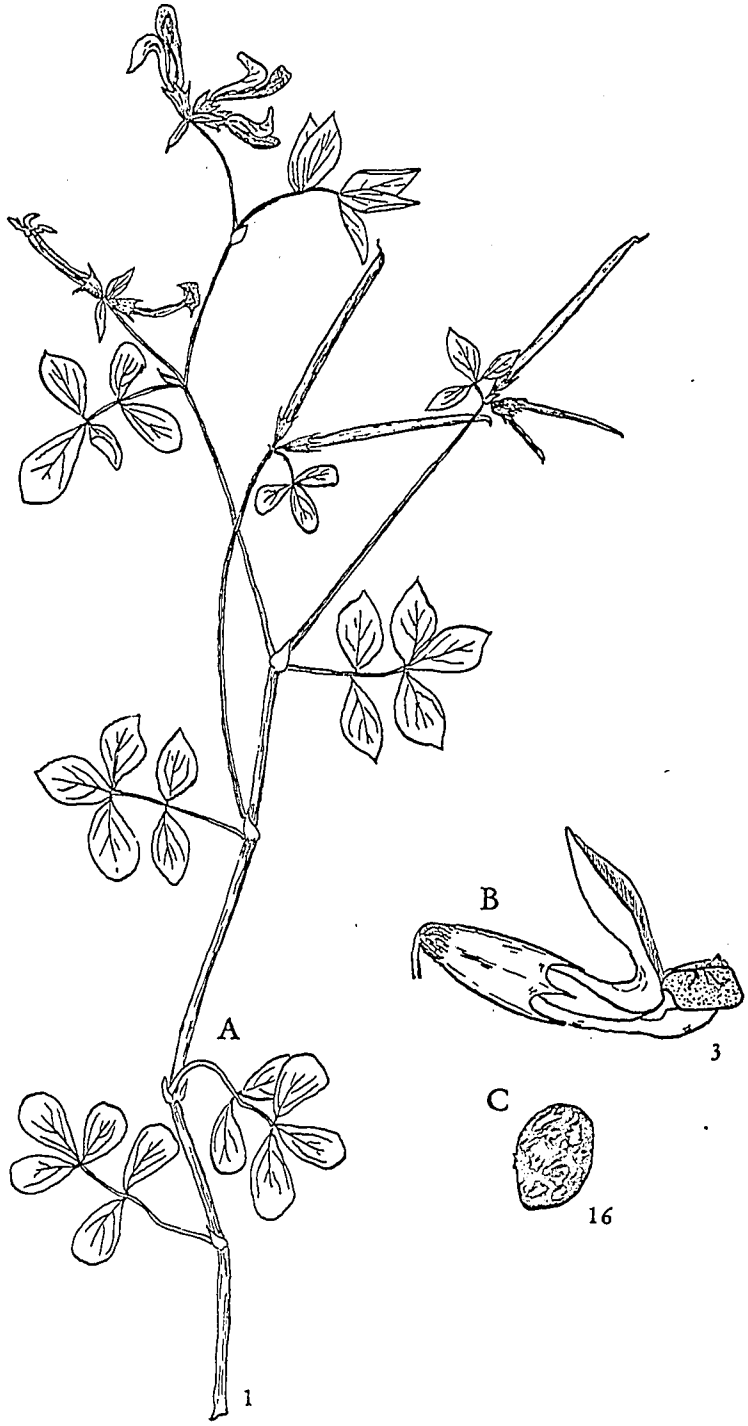



Fig. 2.--Lotus formosissimus
A, plant; B, flower; C, seed.

flower; standard of flower 11-15 mm. long, standard yellow, wings light purple, keel purple-tipped; calyx 4-7 mm. long, teeth shorter than the tube; pod 25-35 mm. long, 1.5-2 mm. broad; seeds 7-15, 1.3-1.8 mm. long, dark brown to black, mottled with olive.

Lotus micranthus Benth.

Annual, 1-3 dm. high, prostrate to erect, branching from the base producing several to many stems; usually glabrous, sometimes glaucous; stipules glandlike; leaflets 3-6, pinnately or unequally arranged on either side of the rachis, oblong to obovate; central leaflet 6-11 mm. long, 3-5 mm. broad; flowers solitary, produced on axillary peduncles 5-25 mm. long; bract trifoliolate, just below the flower; standard of flower 4.5-6.0 mm. long, cream, tinged with pink; calyx 2-3 mm. long, teeth shorter than the tube; pod 10-25 mm. long, 1-2.5 mm. broad, constricted between the seeds; seeds 3-6, 2.0-2.5 mm. long, mottled dark brown to black.

Lotus purshianus (Benth.) Clem. and Clem.

Annual, 1-5 dm. high, erect, profusely branched from a central stem; glabrous to pilose; stipules glandlike; leaflets 3, one central, one on either side, oblong, oval to ovate; central leaflet, 10-20 mm. long, 3.0-7.0 mm. broad; flowers solitary, produced on axillary peduncles 5.0-25 mm.; bract unifoliate, just below the

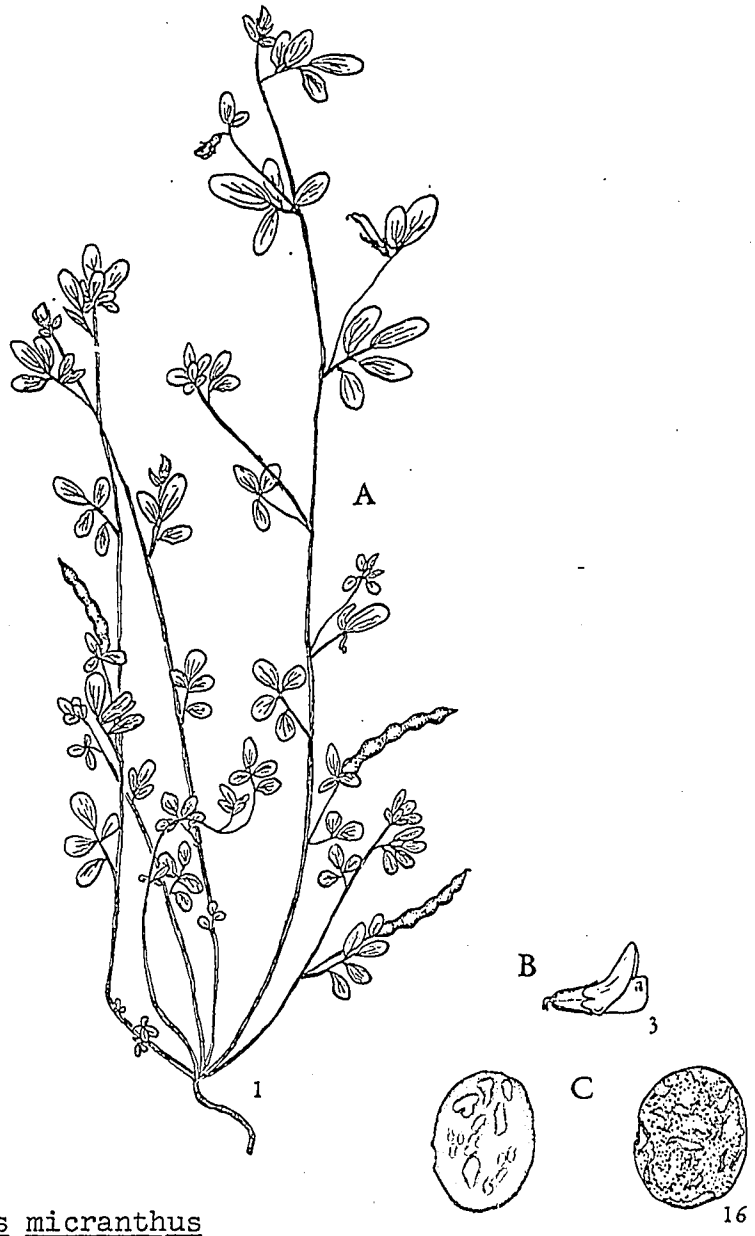



Fig. 3.--Lotus micranthus
A, plant; B, flower; C, seed.

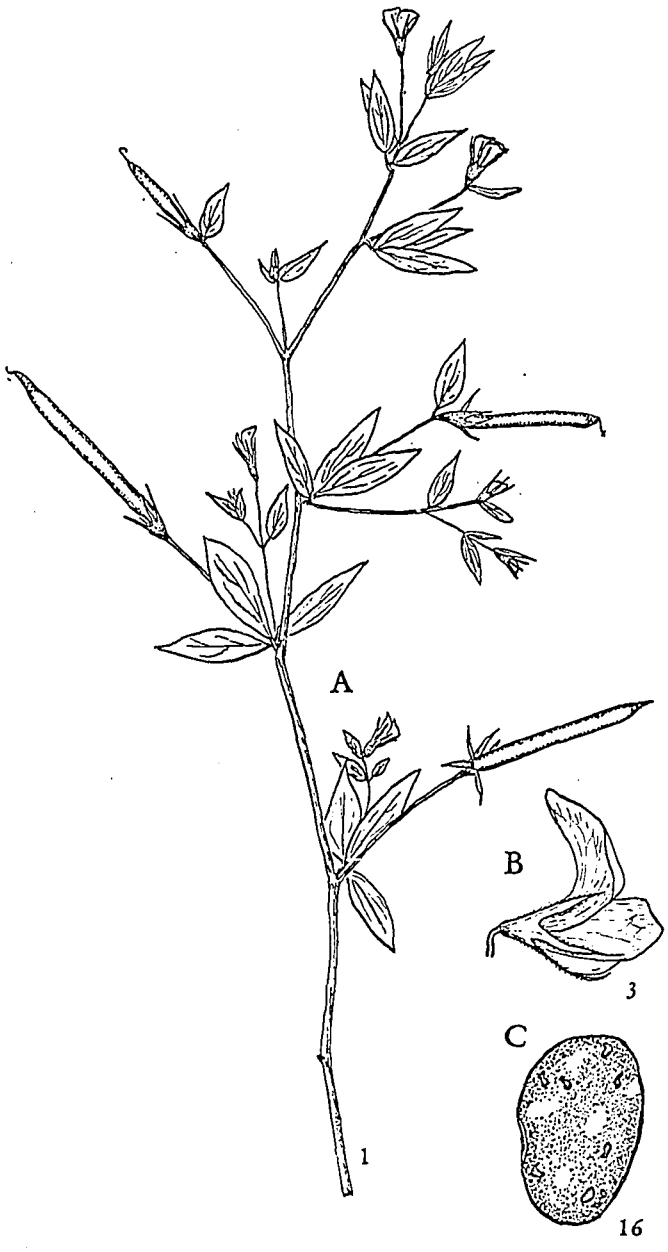



Figure 4.--Lotus purshianus
A, plant; B, flower; C, seed.

flower; standard of flower 6-8 mm. long, white or cream, strongly veined with pink. Calyx 5.0-7.5 mm. long, teeth approximately 1 1/2 times as long as the tube; pod 20-35 mm. long, 2-2.5 mm. broad; seeds 4-9, 2.5-3.5 mm. long, olive-brown, speckled with black.

Lotus denticulatus (Drew) Greene

Annual, 2-5 dm. high, decumbent to erect, sparingly branched from a central stem; leaves and calyx covered with fine hairs; stipules glandlike; leaflets 3-4, asymmetrically arranged, two borne at the tip of a flattened rachis and one or two borne on one side, obovate; central leaflet 12-20 mm. long, 5-10 mm. broad; flowers solitary, subsessile, borne in the leaf axis; bract absent; standard of flower 5-7.5 mm. long, white to pinkish; calyx 4.5-5.5 mm. long, teeth nearly twice as long as the tube; pod, 10-16 mm. long, 3-4 mm. broad, covered with fine hairs, containing 2-4 gray to brown seeds; seeds flattened, 2-4 mm. long.

Lotus corniculatus L.

Perennial, 1-5 dm. high, decumbent or erect, branching from a stout crown, glabrous; stipules gland-like; leaflets 5, 3 terminal, 2 basal, nearly sessile on the stem, oblong, oval to obovate; central leaflet 6-20 mm. long, 2-9 mm. broad; umbels 2-7 flowered, produced on axillary peduncles 3-10 cm. long; bract 3 foliolate, just

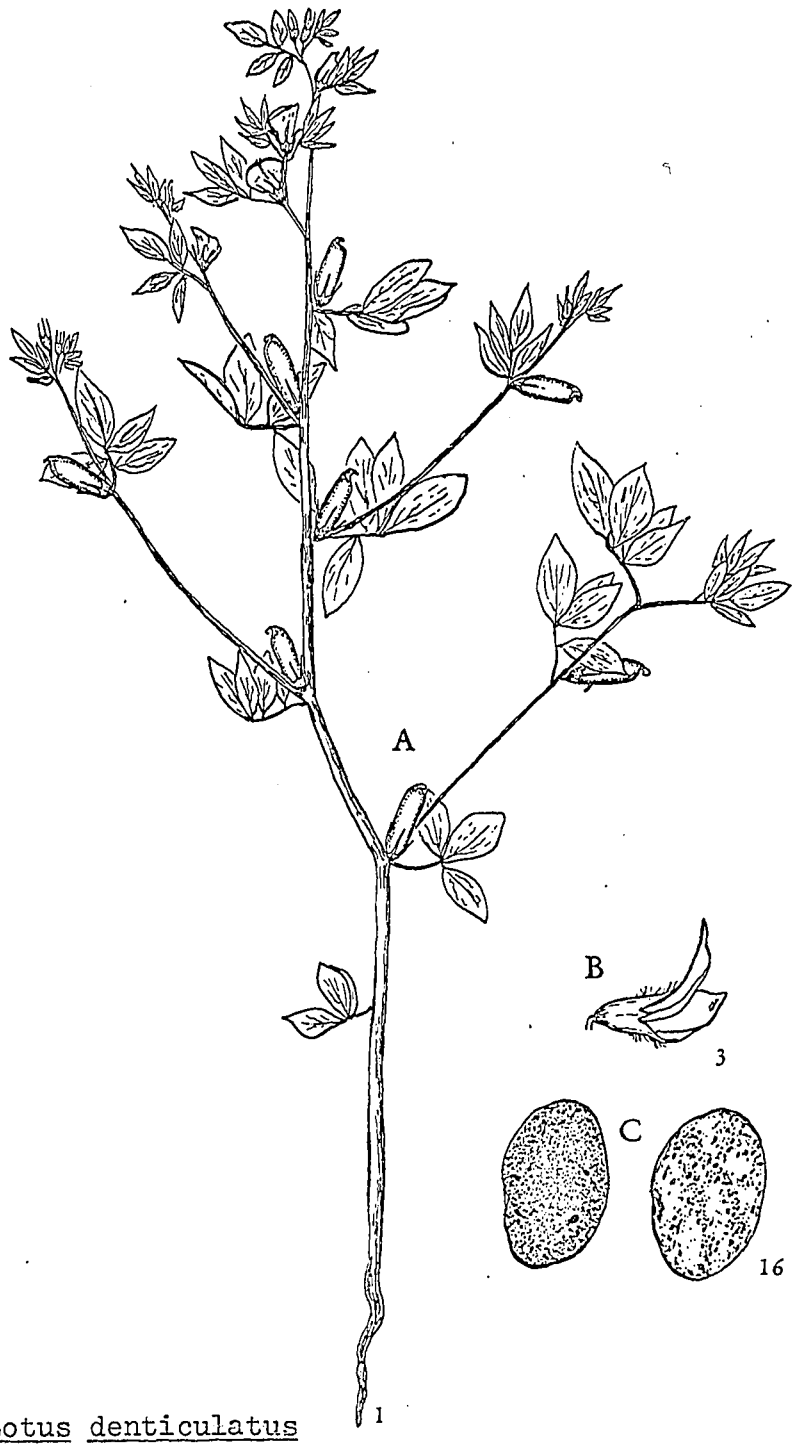



Fig. 5.--Lotus denticulatus
A, plant; B, flower; C, seed.

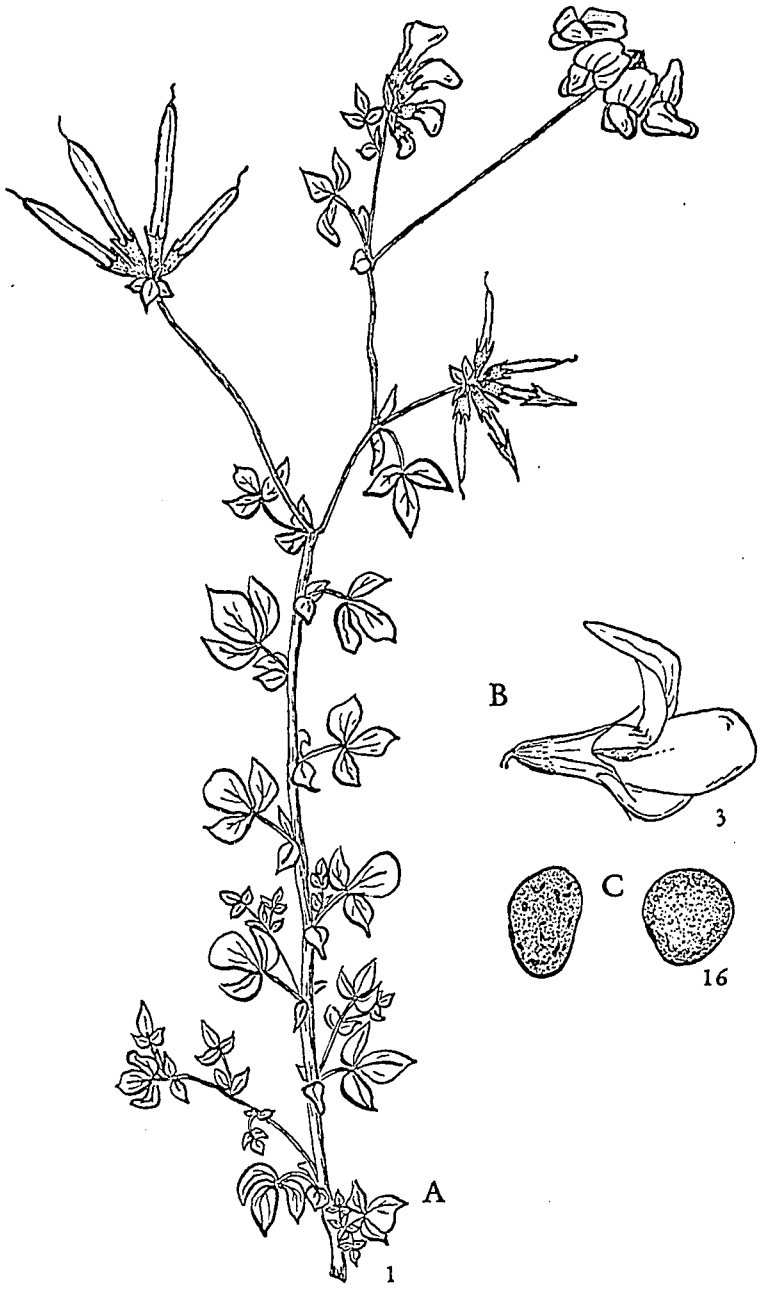



Fig. 6.--Lotus corniculatus
A, plant; B, flower; C, seed.

below the flower; standard of flower 9-13 mm. long, yellow; calyx 5-7 mm. long, teeth equal to or shorter than the tube; pod 15-30 mm. long, 2-3 mm. broad; seeds 5-30, 1-1.5 mm. long, brown, finely spotted.

Lotus tenuis Waldst. et Kit.

Perennial, 1-5 dm. high, decumbent or erect, branching from the base, glabrous; stipules glandlike; leaflets 5, 3 terminal, 2 basal, nearly sessile on the stem, linear, oblong to oblanceolate; central leaflet 8.5-15 mm. long, 1.5-2.7 mm. broad; umbels 3-5 flowered, produced on axillary peduncles 3-10 cm. long; bract 3 foliolate, just below the flower; standard of flower 8.5-12 mm. long, yellow; calyx 4-5.5 mm. long, teeth slightly shorter than the tube; pod 15-30 mm. long, 2-3 mm. broad; seeds 15-30, 1.1-1.8 mm. long, brown, lightly spotted.

Lotus pedunculatus Cav.

Perennial, 1-10 dm. high, stems erect from creeping, scaly rhizomes, glabrous or slightly hairy; stipules glandlike; leaflets 5, 3 terminal, 2 basal, nearly sessile on the stem, oblanceolate, ovate to obovate; central leaflet 12-25 mm. long, 5-9.5 mm. broad; umbels 4-14 flowered, produced on axillary peduncles 4-12 cm. long; bract 3 foliolate, just below the flower; standard of flower 10-13 mm. long, yellow; calyx 5.5-8.0 mm. long,

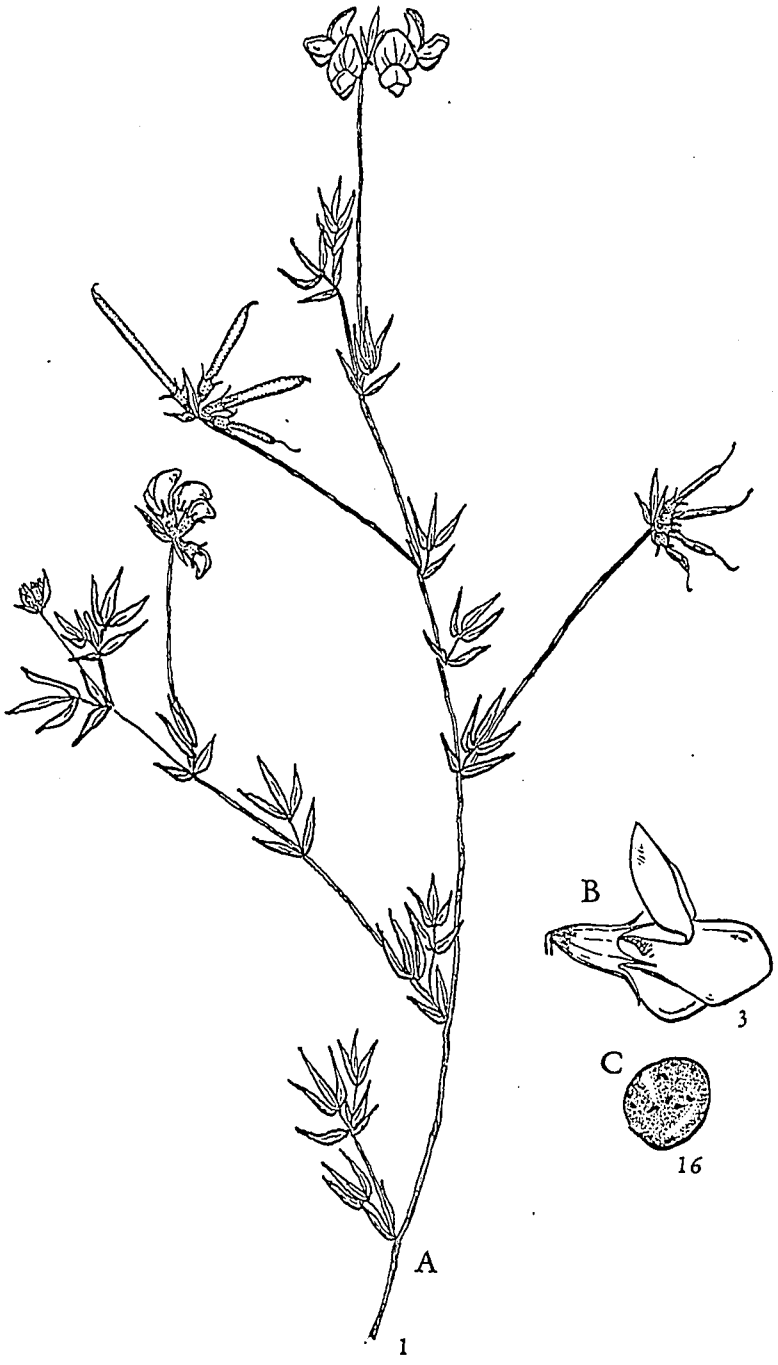



Fig. 7.--Lotus tenuis
A, plant; B, flower; C, seed.


Fig. 8.--Lotus pedunculatus
A, plant; B, flower; C, seed.

teeth equal to or shorter than the tube; teeth divergent in the bud; pod 15-25 mm. long, 1.5-2.5 mm. broad; seeds 15-35, 0.8-1.1 mm. long, olive or yellowish-brown.

Lotus krylovii Schischk. and Serg.

Annual, 1-2 dm. high, decumbent or erect, branching from the base, glabrous; stipules glandlike; leaflets 5, 3 terminal, 2 basal, nearly sessile on the stem, oblong to oblanceolate; central leaflet 9-14 mm. long, 3-5.5 mm. broad; flowers solitary, produced on axillary peduncles 20-45 cm. long; bract 3 foliolate, just below the flower; standard of flower 8.5-15 mm. long, yellowish-pink; calyx 5-7 mm. long, teeth approximately equalling the tube; pod 25-30 mm. long, 2-3 mm. broad; seeds 15-30, 1.2-1.5 mm. long, brown, finely spotted.


Fig. 9.--Lotus krylovii
A, plant; B, flower; C, seed.

Results of measurements of herbarium material for several characters of Canadian Lotus.

Table 2.

| Species | | No. of florets per inflor-escence | Length of standard (cm.) | Calyx* index | Length of peduncle (cm.) | Length of legume (cm.) | Length of petiole (cm.) | No. of leaflets per leaf | Leaf index** | No. of leaflets per bract |
|---|---|---|---|---|---|---|---|---|---|---|
| L. pinnatus | Average | 5.80 | 1.37 | 1.26 | 6.32 | | 3.70 | 6.24 | 2.11 | 0.00 |
| | Range | 4.00-9.00 | 1.19-1.60 | 1.12-1.43 | 3.60-10.80 | | 1.90-7.70 | 5.00-7.00 | 1.15-3.33 | 0.00 |
| L. formosissimus | Average | 3.53 | 1.33 | 1.35 | 4.08 | 3.05 | 2.36 | 5.20 | 2.19 | 2.71 |
| | Range | 3.00-5.00 | 1.11-1.50 | 1.20-1.55 | 2.20-6.20 | 2.70-3.60 | 1.60-3.20 | 5.00-7.00 | 1.42-2.83 | 1.00-3.00 |
| L. micranthus | Average | 1.00 | 0.50 | 1.35 | 1.21 | 1.86 | 0.54 | 4.19 | 2.19 | 3.00 |
| | Range | 1.00 | 0.43-0.59 | 1.16-1.67 | 0.60-2.30 | 0.80-2.40 | 0.35-0.80 | 3.00-6.00 | 1.86-3.00 | 3.00 |
| L. purshianus | Average | 1.00 | 0.67 | 2.48 | 1.29 | 2.77 | 0.36 | 3.00 | 2.99 | 1.00 |
| | Range | 1.00 | 0.60-0.80 | 2.21-2.90 | 0.50-2.20 | 2.10-3.20 | 0.20-0.50 | 3.00 | 2.00-3.66 | 1.00 |
| L. denticulatus | Average | 1.00 | 0.64 | 2.44 | 0.00 | 1.26 | 0.87 | 3.71 | 2.32 | 0.00 |
| | Range | 1.00 | 0.50-0.72 | 2.09-3.47 | 0.00 | 0.90-1.70 | 0.60-1.50 | 3.00-4.00 | 1.89-2.94 | 0.00 |

Table 2 (Cont'd)

| Species | | No. of florets per inflorescence | Length of standard (cm.) | Calyx index* | Length of peduncle (cm.) | Length of legume (cm.) | Length of petiole (cm.) | No. of leaflets per leaf | Leaf index** | No. of leaflets per bract |
|---|---|---|---|---|---|---|---|---|---|---|
| L. corniculatus | Average | 4.37 | 1.16 | 1.82 | 6.41 | 2.28 | 0.54 | 5.00 | 2.45 | 3.00 |
| | Range | 2.00-6.00 | 0.87-1.34 | 1.61-2.24 | 2.80-10.00 | 1.60-2.90 | 0.35-0.85 | 5.00 | 1.75-3.35 | 3.00 |
| L. tenuis | Average | 4.00 | 1.01 | 1.61 | 5.92 | 2.23 | 0.32 | 5.00 | 6.21 | 3.00 |
| | Range | 3.00-5.00 | 0.84-1.19 | 1.32-1.78 | 2.90-8.90 | 1.60-2.90 | 0.20-0.40 | 5.00 | 4.47-9.00 | 3.00 |
| L. pedunculatus | Average | 8.88 | 1.82 | 1.82 | 8.20 | 2.24 | 0.77 | 5.00 | 2.38 | 3.00 |
| | Range | 4.00-14.00 | 1.04-1.32 | 1.45-2.07 | 4.00-11.60 | 1.90-2.50 | 0.46-1.10 | 5.00 | 1.47-3.20 | 3.00 |
| L. krylovii | Average | 1.00 | 1.17 | 1.90 | 3.05 | 2.73 | 0.45 | 5.00 | 2.70 | 3.00 |
| | Range | 1.00 | 0.87-1.60 | 1.70-2.06 | 1.90-4.20 | 2.50-2.90 | 0.30-0.62 | 5.00 | 2.00-3.75 | 3.00 |

*Calyx index calculated as the ratio of total length of calyx to length of calyx tube.

**Leaf index calculated as the ratio of length of central leaflet to width of central leaflet.

### 3. Style and pollen

Illustrations of the ovaries and styles of the nine species of Lotus growing in Canada can be see in Figure 10 and style measurements are given in Table 5. The magnification is approximately 7.5 times the actual size. The five native Lotus species, L. denticulatus, L. purshianus, L. micranthus, L. pinnatus and L. formosissimus (Fig. 10, A to D) all had shorter styles than the introduced species, L. corniculatus, L. tenuis, L. pedunculatus and L. krylovii (Fig. 10, F to G). The style length for the native species varied from 0.7 mm. for L. micranthus to 2.5 mm. for L. purshianus. Lotus denticulatus had a style measuring 2.0 mm. The style length of L. pinnatus and L. formosissimus was the same for both species, namely, 2.3 mm. Lotus corniculatus, L. pedunculatus and L. tenuis had style lengths of 5.2, 5.6 and 5.5 mm., respectively, whereas L. krylovii had a style measuring 4.0 mm.

The five North American species were found to have larger pollen than the four species from the Old World. Two different shapes of pollen were observed. Except for L. micranthus, the native Canadian species had pollen which was round to squarish. The introduced species, and also L. micranthus, had oval pollen.

Fig. 10.--Styles and ovaries of Canadian Lotus species, magnifications ca. X 7.5.

A. Lotus denticulatus.

B. Lotus purshianus.

C. Lotus micranthus.

D. Lotus pinnatus.

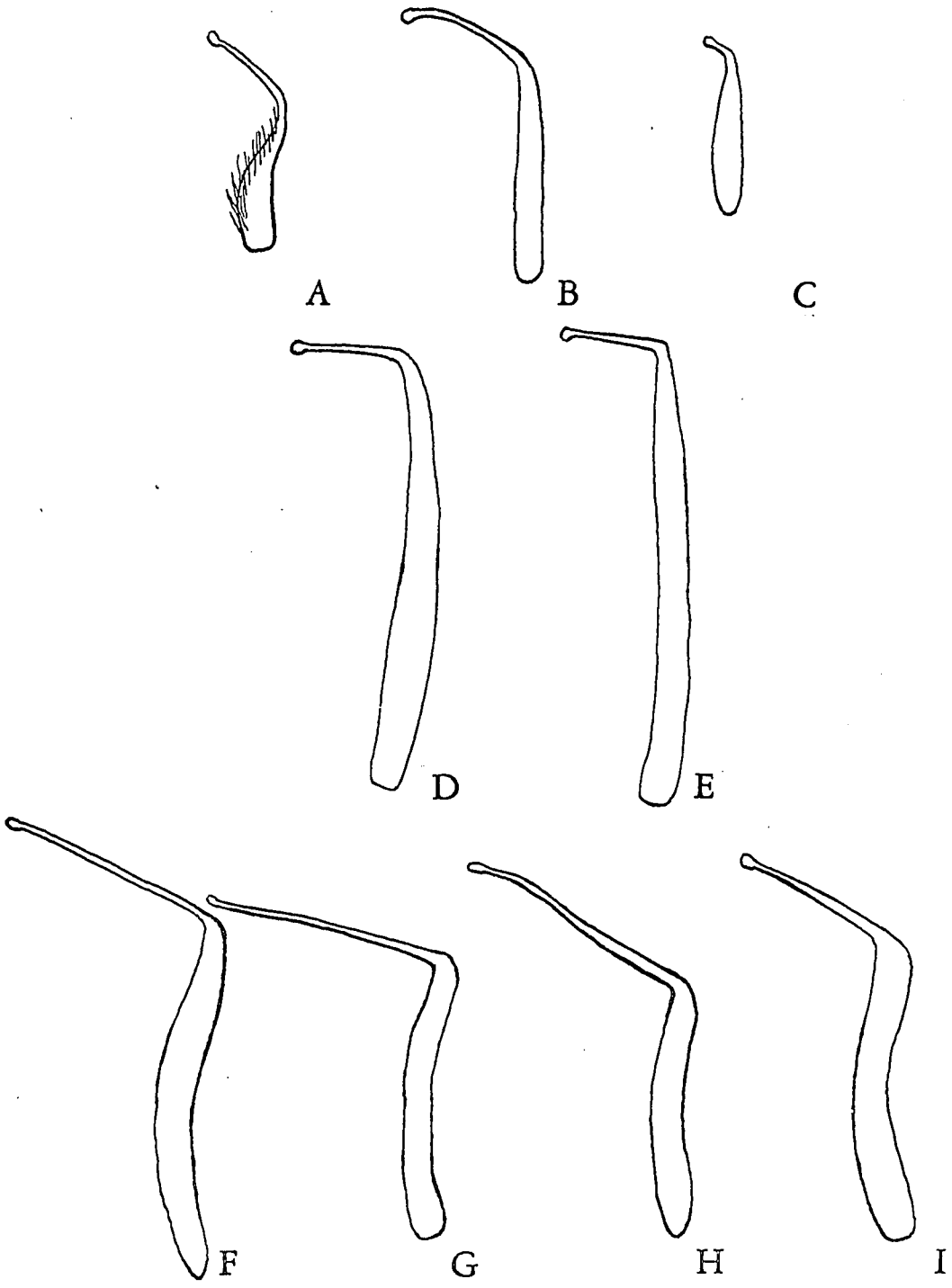
E. Lotus formosissimus.

F. Lotus corniculatus.

G. Lotus tenuis.

H. Lotus pedunculatus.

I. Lotus krylovii.


A
B
C
D
E
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I

## 4. Geographical distribution of species

The geographical distribution of Canadian species of Lotus was studied and a distribution map was prepared for each species (Figures 11 to 18). Comparisons were made with Lotus specimens from Washington State, U.S.A., immediately south of British Columbia, in order to see if the same species are found there as in Canada. Each dot on the maps represents one herbarium specimen, or seed collection, exclusive of duplications. However, it was not always possible to be certain that duplications had been excluded, since many herbarium sheets did not carry sufficient information.

Probably due to its northern position, Canada does not host an abundance of Lotus species. Four of the five native species are limited to British Columbia, with L. purshianus, a morphologically variable species, being found as far east as Manitoba. The species introduced into Canada for agronomic purposes can be found from coast to coast. In certain places these have become naturalized. Lotus krylovii, whose areas of distribution according to Kuprianova (1945) include European Russia, Caucasus, Western Siberia and Central Asia, was probably introduced into Canada accidentally via the Pacific Ocean. One herbarium specimen and one seed collection, both from White Lake, Oliver, British Columbia, were seen as representative of this species.

Lotus pinnatus Hook.

Lotus pinnatus has a very limited distribution in Canada (Fig. 11). Ten collections of this species were among the Canadian material. It is found only on Vancouver Island, British Columbia, in the Nanaimo district. This species prefers wet, coastal areas, although some specimens are from Mt. Benson, Nanaimo. In comparison, L. pinnatus has quite a widespread distribution throughout Washington State, U.S.A., where it can be found as far inland as the eastern border of the state, as well as coastally.

Representative material seen. BRITISH COLUMBIA: Nanaimo, John Macoun, June 13, 1887 (MTMG); Nanaimo, J. W. Eastham, June 8, 1941 (UBC); Nanaimo, J. W. Eastham, June 28, 1939 (UBC); Nanaimo, J. W. Eastham, June 2, 1939 (UBC); Mt. Benson, W. R. Carter No. 3018, June 1, 1918 (V); Mt. Benson, W. R. Carter No. 3017, June 1, 1918 (WS); Mt. Benson, W. R. Carter No. 105954, June 1, 1918 (CAN); Nanaimo, Macoun No. 5275, June 13, 1887 (CAN); Mt. Benson, W. R. Carter, May 31, 1915 (CAN); Nanaimo, K. Beamish, 1965 (UBC).

Lotus formosissimus Greene.

Lotus formosissimus, similar to L. pinnatus in morphology and habitat, is also limited to Vancouver Island, British Columbia (Fig. 11). Most of the 16

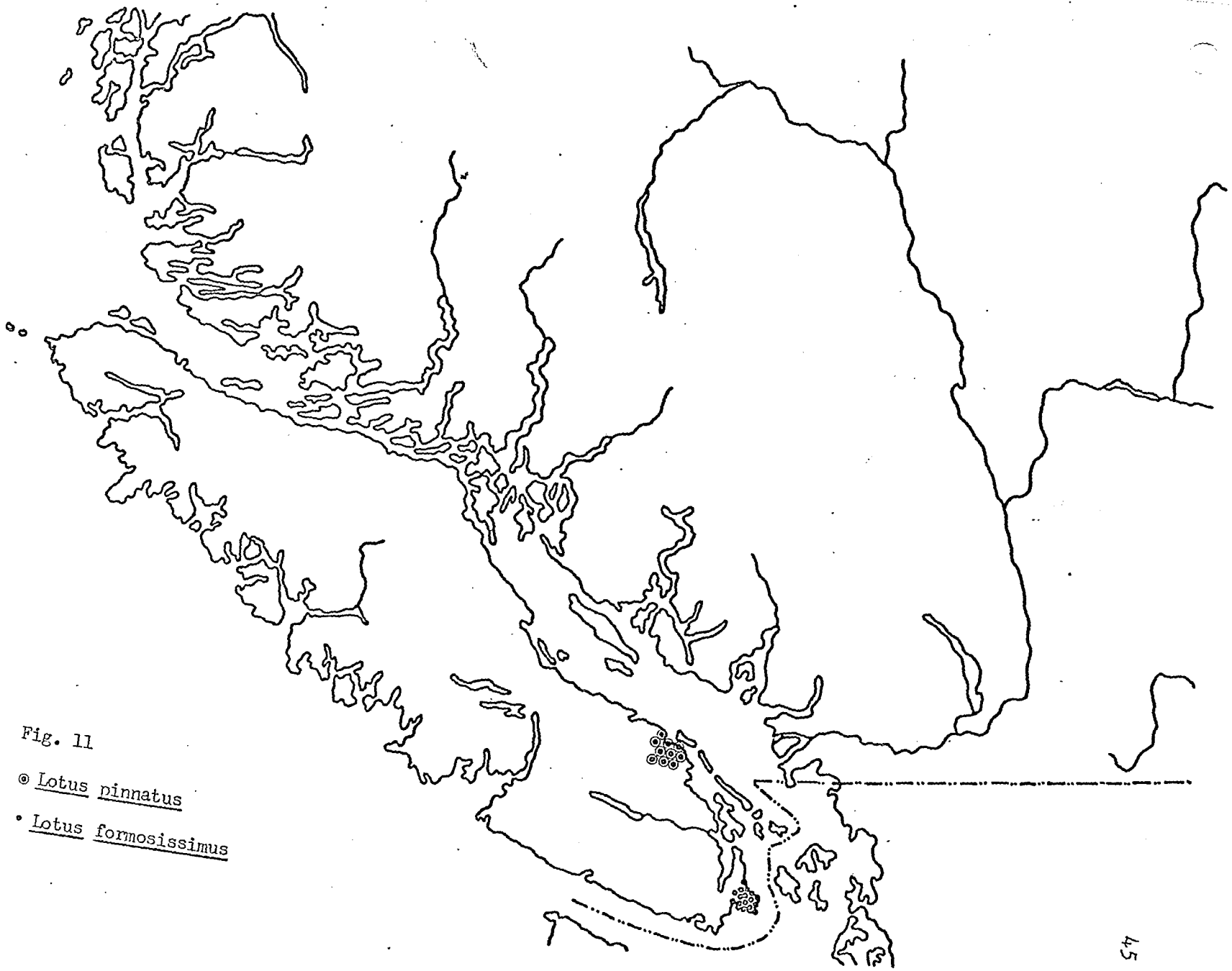


Fig. 11

◎ Lotus pinnatus

• Lotus formosissimus

specimens seen were collected at or near Oak Bay, near Victoria. One collection came from Trial Island, a small island just off Victoria and one from Williams Head, a few miles further south. L. formosissimus from Washington State is not as widespread as is L. pinnatus. There were only four herbarium sheets of this species among the material from the United States. The collections all originated from one county, Gray's Harbor County, Washington.

Representative material seen. BRITISH COLUMBIA: Foul Bay, Victoria, J. K. Henry, July 21 (UBC); Victoria, W. Taylor, June 12, 1913 (UBC); Victoria, W. Taylor, June 13, 1913 (UBC); Oak Bay, Victoria, W. C. McCalla, June 21, 1921 (ALTA); Oak Bay, Victoria, W. R. Carter No 5582, May 29, 1919 (V); Victoria, C. F. Newcombe No. 5577, May 26, 1919 (V); Oak Bay, J. R. Anderson No. 2920, June 14, 1896 (V); Trial Island off Victoria, J. A. Calder and K. T. MacKay No. 28910, May 10, 1961 (DAO); Oak Bay, W. R. Carter No. 105955, May 29, 1917 (CAN); Oak Bay, Victoria, C. F. Newcombe, June 16, 1924 (UBC); William Head, J. R. Anderson, June 29, 1920 (WS); Oak Bay, C. F. Newcombe, June 16, 1924 (WS); Oak Bay, John Macoun, June 5, 1908 (CAN); Oak Bay, J. R. Anderson, June 14, 1896 (V); Oak Bay, J. R. Anderson, No. 130, June 11, 1896 (WS); Foul Bay, Vancouver Island, Prof. Henry, 1912 (V).

Lotus micranthus Benth.

The majority of the plants belonging to L. micranthus are found in British Columbia on Vancouver Island itself or on smaller islands just off Vancouver Island (Fig. 12). Most of the 102 collections are from the southern one-third of the Island, around Nanaimo and Victoria, although one collection is from Savary Island, between Vancouver Island and the main land, and one is from as far north as Telegraph Bay, in the northern one-third of Vancouver Island. The few collections from the mainland are all from the Vancouver area. Lotus micranthus enjoys a wider distribution in Washington State, being found from north to south in the western half of the State.

Representative material seen. BRITISH COLUMBIA: Trial Is., off Victoria, Calder and MacKay No. 28942, May 10, 1961 (DAO); Skutz Falls, 5 mi. SE. of Cowichan L., Calder and MacKay No. 29763, May 29, 1961 (DAO); Sproat R. Falls, N. of Alberni, Calder and MacKay No. 30156, June 7, 1961 (DAO); Savary Is., W. Taylor, May, 1914 (UBC); Ten Mile Point, Victoria, J. Munro, May 9, 1938 (UBC); Caulfields, W. Vancouver, K. Beamish and Vrugtman No. 60504, May 27, 1960 (UBC); Esquimalt, No. 79,709, June 12, 1908 (CAN); 4 mi. NW. of Nanaimo, Mulligan and Woodbury No. 1603, July 7, 1955 (DAO); Mts. above Fisherman's Cove, Vancouver, H. Groh, May 23, 1931 (DAO);

Ganges, H. Groh, May 28, 1931 (DAO); Nanaimo, J. Eastham, June 2, 1939 (UBC); Cowichan L., D Buckland, June 22, 1939 (UBC); Caulfields, J. Davidson, July 8, 1911 (UBC); Caulfields, J. Davidson, July 14, 1912 (UBC); Vancouver Is., A. Hill, June, 1896 (UBC); Mayne Is., A. Hill, June, 1895 (UBC); Saltspring Is., T. Ashlee, June 12, 1955 (UBC); Portage Inlet, Victoria, L. Holm No. 119, May 22, 1959 (UBC); Wellington, W. Carter No. 219, June 1, 1917 (UBC); Caulfields, F. Perry, July 15, 1916 (UBC); Departure Bay, T. Ashlee, June 3, 1916 (UBC); Departure Bay, T. Ashlee, June 3, 1924 (UBC); 1st. Nanaimo L., Krajina, Spitsbury and Szczawinski No. 4582, June 25, 1950 (UBC); between Englishman R. and Parksville, Vancouver Is., Krajina, Spitsbury and Szczawinski No. 4234, May 30, 1950 (UBC); East Sooke, Vancouver Is., J. Hett, May 8, 1962 (UBC); Caulfields, F. Perry, July 5, 1916 (UBC); Goldstream, J. Eastham, June 12, 1939 (UBC); Caulfields, J. Davidson, July 8, 1911 (UBC); Goldstream, J. Eastham, June 12, 1939 (UBC); Gabriola Is., Taylor and Pillsbury No. 46122, June 15, 1946 (UBC); near Victoria, Macoun No. 124, July 8, 1893 (ALTA); Mt. Douglas, near Victoria, W. McCalla, May 11, 1921 (ALTA); Caulfields, K. Beamish and Vrugtman No. 60504, May 27, 1960 (WIN); Cowichan L., Vancouver Is., I. Cowan, June 4, 1940 (V); Saanich, Vancouver Is., J. Shenstone No. 13,660, May 18, 1941 (V);

Beacher Bay, Vancouver Is., C. Newcombe No. 7207, July 9, 1924 (V); Goldstream, G. Hardy No. 15,528, June 2, 1943 (V); Goldstream Dist., Mt. Finlayson, W. Newcombe No. 8778, May 19, 1929 (V); Esquimalt, J. Anderson, June 6, 1896 (V); Oak Bay, E. Eldridge No. 8232, May 24, 1927 (V); Mill Hill, J. Anderson, May 31, 1898 (V); Mt. Benson, Vancouver Is., J. Anderson, June 25, 1898 (V); Saltspring Is., T. Ashlee, May 5, 1957 (V); Bald Mt., Cowichan L., N. J. G., July 15, 1930 (V); Saltspring Is., Ganges, V. Goddard, May 10, 1935 (V); Sproat Lake Falls, W. Carter, May 26, 1917 (V); Vane Is., All Bay, J. Macoun No. 86,975, June 10, 1913 (V); Skirt Mtn., J. Anderson No. 121, May 24, 1896 (V); Wellington, W. Carter, June 1, 1917 (V); Mt. Benson, Vancouver Is., J. Anderson No. 2917, June 27, 1898 (V); Nanoose, Vancouver Is., G. Emerson No. 15,392, July 24, 1942 (V); Nanaimo, J. Eastham, June 2, 1939 (DAO); Nanaimo, M. Gordon, Sept. 18, 1894 (DAO); Victoria, J. Tolmie No. 414,1897 (DAO); Cowichan L. Rosendahl and Butters No. 1463, June 21, 1906 (DAO); Nanoose Bay, Calder and MacKay No. 29104, May 12, 1961 (DAO); Bodega Hill, SE. of N. Galiano, Calder and MacKay No. 28853, May 8, 1961 (DAO); Beaver Pt., S. end of Saltspring Is., Calder and MacKay No. 29570, May 26, 1961 (DAO); N. of Victoria, Calder and MacKay No. 30868, June 22,1961 (DAO); Nanaimo Lakes Rd., Calder and MacKay No. 29,353, May 20, 1961 (DAO); between Nanaimo and Parksville,

Calder and MacKay No. 31147, July 2, 1961 (DAO); Mt. Finlayson, N. of Victoria, Calder and MacKay No. 29422, May 23, 1961 (DAO); Mt. Douglas, N. of Victoria, Calder and MacKay No. 29553, May 25, 1961 (DAO); 3 mi. NE. of Duncan, Calder and MacKay No. 30746, June 19, 1961 (DAO); Victoria, W. Scott, June, 1893 (DAO); 4 mi. NW. of Victoria, Calder, Parmelee and Taylor No. 16342, May 15, 1956 (DAO); 2 mi. ESE. of Langford, Calder, Savile and Taylor No. 20805, May 12, 1957 (DAO); W. Vancouver, P. Henson, April 20, 1934 (DAO); Malahat, Vancouver Is., P. Henson, Aug. 6, 1932 (DAO); 4 mi. SW. of Saanichton, Calder, Parmelee and Taylor No. 16379, May 15, 1956 (DAO); Telegraph Bay, Eastham, June 8, 1939 (DAO); Mt. Newton, Vancouver Is., W. Newton, May (DAO); Victoria, W. Colgrove, June 18, 1941 (UWO); Clark Co., Vancouver, W. Suksdorf No. 6177, June 4, 1908 (WTU); Nanaimo Dist., Esquimalt, J. Anderson No. 121, June 6, 1896 (WS); Nanaimo Dist., Cedar Hill, J. Anderson No. 121, May 8, 1912 (WS); near Victoria, Macoun No. 79,712, May 13, 1908 (CAN); Oak Bay, Macoun No. 86969, June 17, 1913 (CAN); Wellington, Vancouver Is., Carter No. 94649, June 1, 1917 (CAN); near Victoria, Macoun, May 11, 1875 (CAN); Goldstream, Macoun No. 79,710, June 8, 1908 (CAN); Esquimalt, Macoun No. 79,711, June 12, 1908 (CAN); Nanaimo, Protection Is., Macoun No. 123, July 14, 1893 (CAN); Victoria Arm, Macoun No. 124, June 6, 1893 (CAN); Gabriola Is., Taylor and

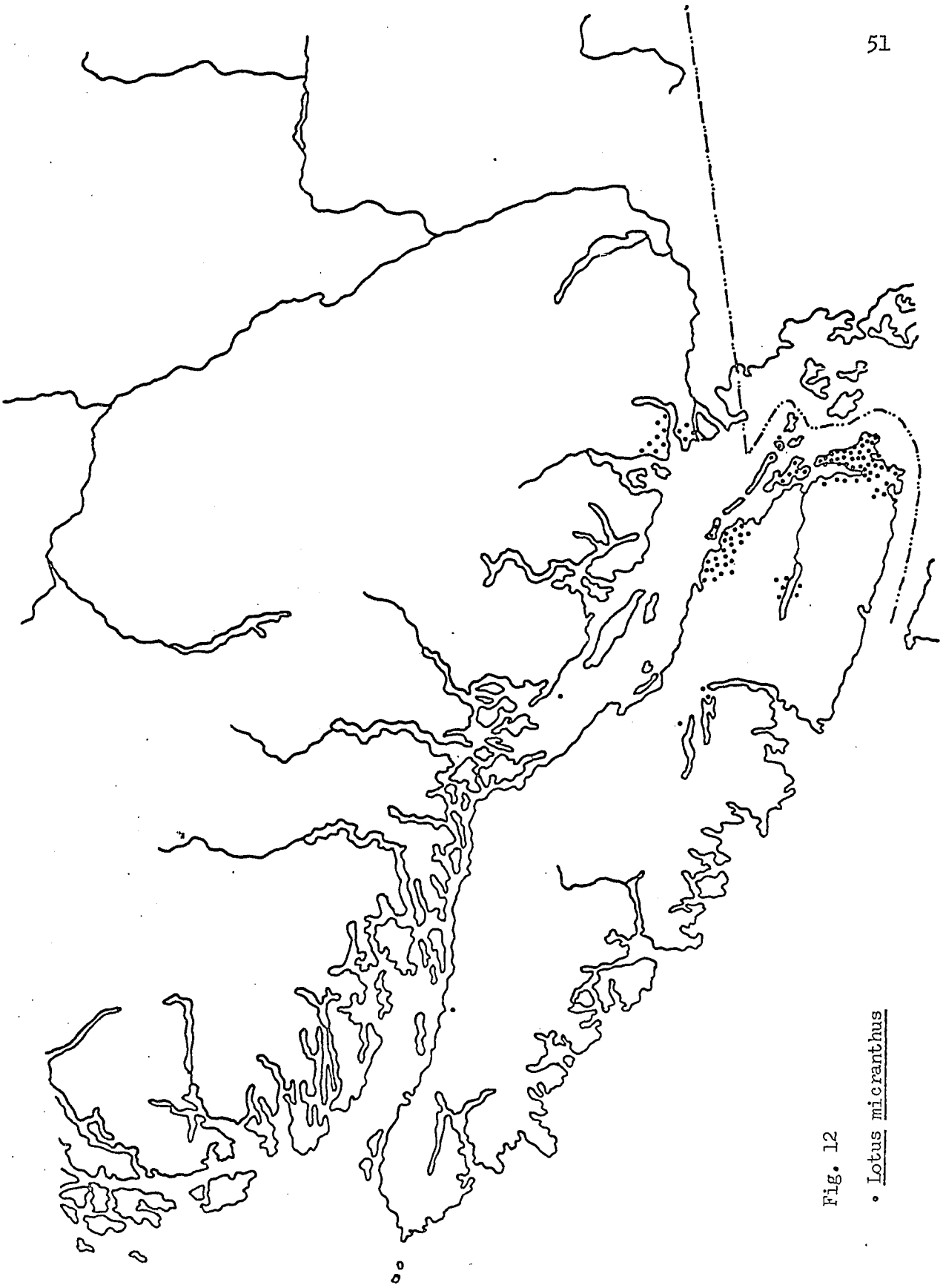


Fig. 12

• Lotus micranthus

Pillsbury No. 46122, June 15, 1946 (CAN); Mayne Is., Macoun, May 21, 1914 (CAN); Goldstream, Macoun, May 21, 1887 (CAN); Goldstream, Macoun, June 27, 1887 (CAN); Departure Bay, C. Berkeley, June 22, 1855 (MTMG); Macoun, 1872 (MTMG); Vancouver Is., Macoun and Survey No. 269, May 7, 1875 (MTMG); near Victoria, J. Fletcher, April 27, 1885 (MTMG); Green Mt., Vancouver Is., Macoun, May 21, 1887 (MTMG); Cowichan Dist., Domvile Is., F. Tomka, May, 1963 (UBC); Sproat River Falls, N. of Alberni, Calder and MacKay No. 30156, June 7, 1961 (WTU); Vancouver, C. Piper No. 4980, June 6, 1904 (WS); near Sidney, Vancouver Is., Macoun, June 3, 1913 (CAN); 4 mi. NW. of Nanaimo, Mulligan and Woodbury No. 1603, July 7, 1955.

Lotus purshianus (Benth.) Clem. and Clem.

Thirty-two different herbarium specimens were mapped for this wide-ranging species (Fig. 13). On the west coast it is reported on Vancouver Island, mainly around Oak Bay - Victoria and Cowichan Lake. Two specimens come from Telegraph Bay, at the northern end of the Island. Eastward, no collections come from mainland British Columbia or from Alberta. Lotus purshianus is, however, found in the eastern half of southern Saskatchewan and throughout southern Manitoba as far east as Domain. This species is also found throughout Washington State.

Representative material seen. MANITOBA: Virden, Burman, Sept. 1, 1886 (MTMG); Oak Lake, 30 mi. W. of Brandon, H. J. Scoggan No. 11142, July 5, 1953 (ALTA); S. of St. Pierre toward Carey, Frère Jean-Paul Bernard No. 295, July 24, 1954 (WIN); 10 mi. W. of Brandon, B. Boivin, H. H. Marshall, E. Laishley No. 13223 (ALTA); 2 mi. N. of Deerwood, H. H. Marshall No. 35, July 24, 1950 (DAO); Domain, W. G. Dore No. 9228, Aug. 24, 1948 (DAO); Virden, Wm. A. Burman No. 415 1/2, July 18, 1886 (DAO); Morden, H. Groh, July 16, 1921 (DAO); Griswold, Rev. A. Burman No. 5274, 1887 (CAN); Souris Co., Walker No. 5273, May 13, 1889 (CAN); Stockton, E. Criddle, July 31, 1928 (CAN); Fortier, A. and D. Love No. 5652, July 23, 1952 (DAO). SASKATCHEWAN: Gainsborough, H. Sweet, Aug. 15, 1941 (SASKR); Gainsborough, J. H. Hudson No. 1877, July 21, 1956 (SASKR); Bromhead, J. H. Hudson No. 1891, Aug. 4, 1956 (DAO); Carievale, J. H. Hudson No. 1874, July 21, 1956 (DAO); Carlyle Lake, N. Tripp, July, 1921 (UWO); Gainsborough, H. Sweet, July, 1941 (SASK). BRITISH COLUMBIA: Esquimalt, J. Macoun, June 23, 1887 (MTMG); Cowichan Lake, D. Buckland, July 1, 1939 (UBC); Cowichan Lake, Garnett No. 2743, July 25, 1930 (OAC); Cowichan Lake, J. W. Wogg No. 21,224, Aug. 11, 1947 (V); Cowichan Lake, D. C. Buckland, July 1, 1939 (V); Cowichan Lake, W. J. G., July 25, 1930 (V); Metchosin, W. A. Newcombe No. 9046, June 7, 1931 (V); near Victoria, C. F. Newcombe

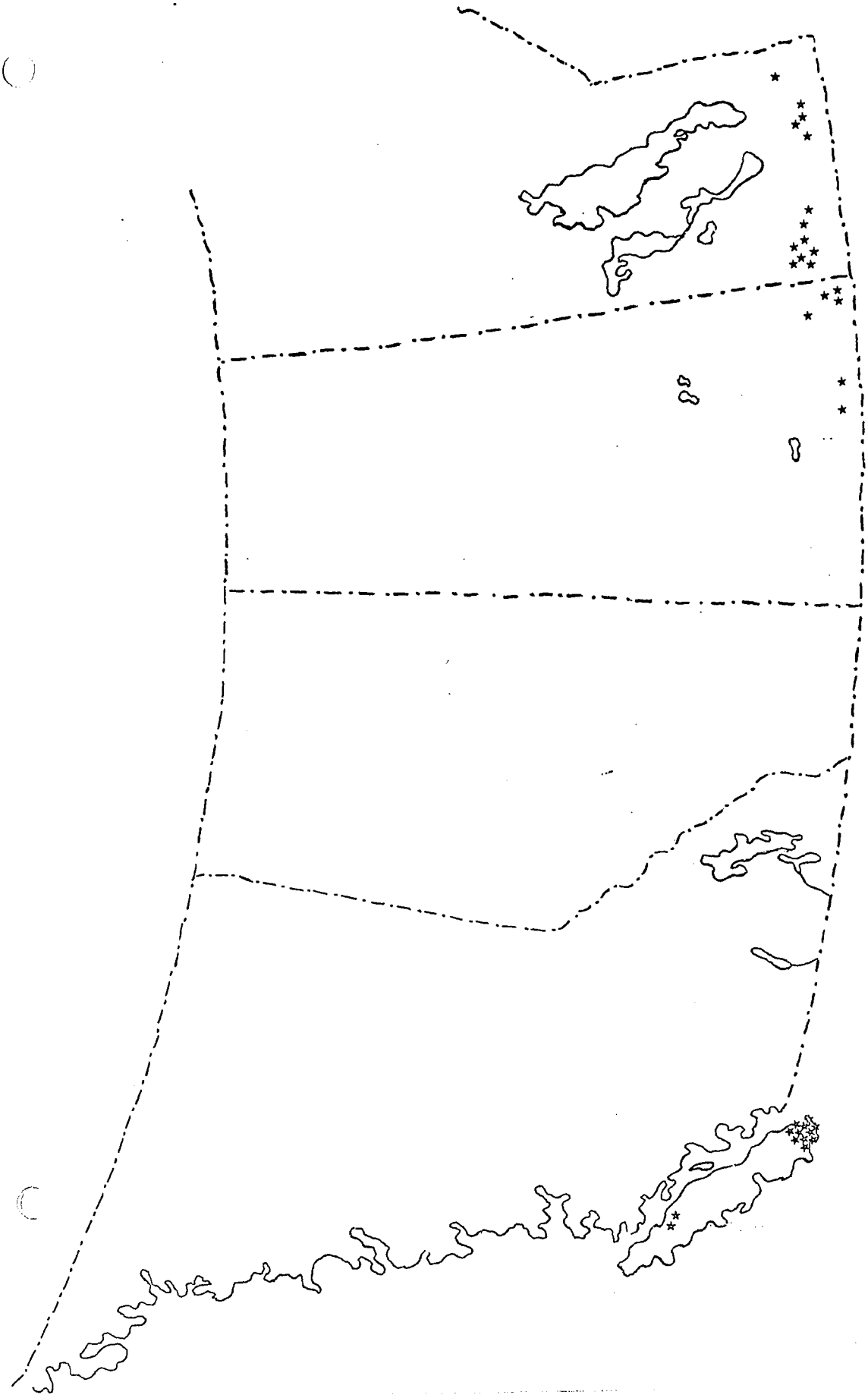


Fig. 13

* Lotus purshianus

No. 6395, June 24, 1923 (V); Albert Head Lagoon, W. A. Newcombe No. 8847, July 27, 1930 (V); Harling Point, Victoria, J. A. Calder and K. T. MacKay No. 30772, June 21, 1961 (DAO); Oak Bay, C. F. Newcombe, June 16, 1924 (WS); Telegraph Bay, G. V. Copley, June 3, 1922 (V); Telegraph Bay, G. V. Copley No. 1, June 3, 1922 (V).

Lotus denticulatus (Drew) Greene

Lotus denticulatus, although confined to British Columbia, has a fairly widespread distribution (Fig. 14). Ninety-three herbarium specimens and three seed collections representative of this species were seen. It has been collected in two areas on Vancouver Island, around Alberni and around Oak Bay, Victoria. It has also been found on Saltspring Island and on some other small islands between Vancouver Island and the United States border. On the mainland, this species is found as far east as Ymir and Rykerts in southern British Columbia and as far north as Smithers where it was grown on the Experimental Farm. When Washington State is taken into consideration, this species can be found throughout the entire state.

Representative material seen. BRITISH COLUMBIA: Vancouver Island, Macoun and Survey, May 9, 1875 (MTMG); Oak Bay, Vancouver Island, Macoun, May 31, 1887 (MTMG); Oak Bay, Macoun, June 18, 1887 (MTMG); Goodfellow Creek,

Underhill No. 913, Aug. 12, 1961 (V); Monte Creek, Eastham No. 13,061, June 12, 1940 (V); Skagit Valley, G. Hardy No. 20474, May 31, 1947 (V); Barkerville, Eastham No. 18,712, July 14, 1945 (V); Lillooet, F. Kermode No. 2914, June 10, 1916 (V); Alberni Canal, W. Carter No. 2913, July, 1916 (V); Oak Bay, Macoun No. 86973, June 17, 1913 (V); 108 Mile Lake, Cariboo, Munro No. 15,663, July 24, 1943 (V); Victoria, D. Newton, June 6, 1928 (V); Saltspring Island, T. Ashlee, June 17, 1956 (V); Oak Bay, G. Hardy, May 23, 1925 (V); Salmon Arm, C. Tice, July 7, 1933 (V); 108 Mile Lake, J. Munro No. 15,662, July 24, 1943 (V); Chain Islands, Hardy No. 21615, May 10,1949 (V); Sulivan Valley, Copley No. 6, July 8, 1921 (V); Armstrong, Tice, July 17, 1939 (V); Victoria, J. Travis (V); Swan Lake, N. of Vernon, Calder and Savile No. 10176, July 6, 1953 (V); E. Manning Park, Underhill No. 738, July 7, 1959 (V); 6 mi. E. of Williams Lake, Calder, Savile and Ferguson No. 12323, July 1, 1954 (DAO); Tranquille Range, Kamloops, Tisdale No. 40-516, July, 1939 (DAO); 5 1/2 mi. SSE. of Kamloops, Calder and Savile No. 10354, July 11, 1953 (DAO); North Thompson River, Macoun, June 12, 1889 (DAO); Spallum Chasm, D. Graham, Sept. 1894 (DAO); Coyle, Merritt, V. Krajina, Aug. 20, 1950 (DAO); Kelowna, Warren, July, 1909 (DAO); Oak Bay, Victoria, Eastham, June 2, 1938 (DAO); Exptl. Farm, 5 1/2 mi. SE. of Smithers, Ashford No. 117, July 19, 1957 (DAO);

Cariboo Hwy., towards Barkerville, Eastham No. 13,528, July 14, 1945 (DAO); Stamp Falls, Alberni, Eastham, July 1, 1939 (DAO); 1 mi. S. of Osoyoos, Calder and Savile No. 9873, June 30, 1953 (DAO); 8 mi. W. of Keremeos, Calder, Parmelee and Taylor No. 19509, July 30, 1956 (DAO); 14 mi. N. of 132 Mile House, Calder, Parmelee and Taylor No. 19007, July 16, 1956 (DAO); Kamloops, F. Hermann No. 12939, July 24, 1956 (DAO); Summerland, J. Bostock, June, 1925 (DAO); near Victoria, Macoun No. 79,714, June 11, 1908 (CAN); Oak Bay, Macoun No. 86,973, June 17, 1913 (CAN); Colquitz, Macoun No. 79,716, July 21, 1908 (CAN); Oak Bay, Macoun No. 79,715, June 5, 1908 (CAN); near Victoria, Greene, July 18, 1890 (CAN); Victoria, M. Malte, July 6, 1913 (CAN); 2 mi. E. of Fort St. James, T. McCabe No. 7610, June 27, 1940 (WTU); between Quesnel and Barkerville, Eastham No. 13,528, July 14, 1945 (WS); Oak Bay, Newcombe, June 9, 1924 (WS); Hedley, T. M. Taylor No. 2087, June 3, 1949 (WS); Oak Bay, Macoun No. 5268, June 18, 1887 (CAN); Vernon, M. O. Malte, July 11, 1918 (CAN); Salmon Arm, M. O. Malte, Aug. 15, 1911 (CAN); Ymir Dist., W. Sandercock No. 89901, 1914 (CAN); Cascade, Macoun No. 63,758, June 25, 1902 (CAN); Smithers, V. C. Brink, Sept. 4, 1943 (UBC); Armstrong, Wilson No. 213, June 25, 1904 (UBC); Armstrong, J. Davidson, July 20, 1913 (UBC); Oak Bay, Eastham, June 6, 1938 (UBC); Stamp Falls, Alberni, Eastham, July 1, 1939 (UBC); Monte Creek,

Eastham, June 12, 1940 (UBC); between Cariboo Rd., and Barkerville, Eastham, July 14, 1945 (UBC); Princeton, A. H. Hutchinson, June 19, 1918 (UBC); Armstrong, C. Tice, July 17, 1939 (UBC); Knutsford, Kamloops, Kirk, July 19, 1916 (UBC); Vancouver E., W. Taylor, June 28, 1916 (UBC); Rykerts, Creston, Eastham, June 20, 1940 (UBC); Okanagan, Armstrong, E. Wilson, No. 427, Sept. 5, 1904 (UBC); Merritt, E. Tisdale, June 29, 1937 (UBC); Armstrong, J. Henry, July, 1911 (UBC); Oak Bay, McCalla, May 21, 1921 (ALTA); Victoria, W. Anderson, June 26, 1917 (V); seashore, J. Travis, July 11, 1928 (V); Chain Islands, J. Anderson No. 2917, May 30, 1897 (V); Nicola, J. Anderson No. 2919, June 29, 1904 (V); Armstrong, Thatcher and Anderson No. 2918, Aug. 6, 1898 (V); East Saanich, W. Newcombe No. 9263, July 17, 1932 (V); Trial Island, G. Hardy No. 7609, July 18, 1925 (V); Sulivan Valley, G. Copley, No. 6754, July 8, 1921 (V); Deep Cave, Vancouver Island, J. Macoun No. 2908, June 18, 1914 (V); Armstrong, Anderson and Garrett, June 27, 1907 (V); Rykerts, Eastham No. 12,992, June 20, 1940 (V); near Victoria, J. Macoun, July 22, 1893 (UWO); Esquimalt, Macoun, June 10, 1893 (CAN); Armstrong, J. Anderson, June 27, 1907 (WS); North Thompson River, J. Macoun No. 5270, June 12, 1889 (CAN); Kamloops, J. Macoun No. 5271, June 13, 1889 (CAN); near Lytton, Dawson No. 5269, July 5, 1890 (CAN); Osoyoos Lake, J. Macoun No. 70,433, June 2,

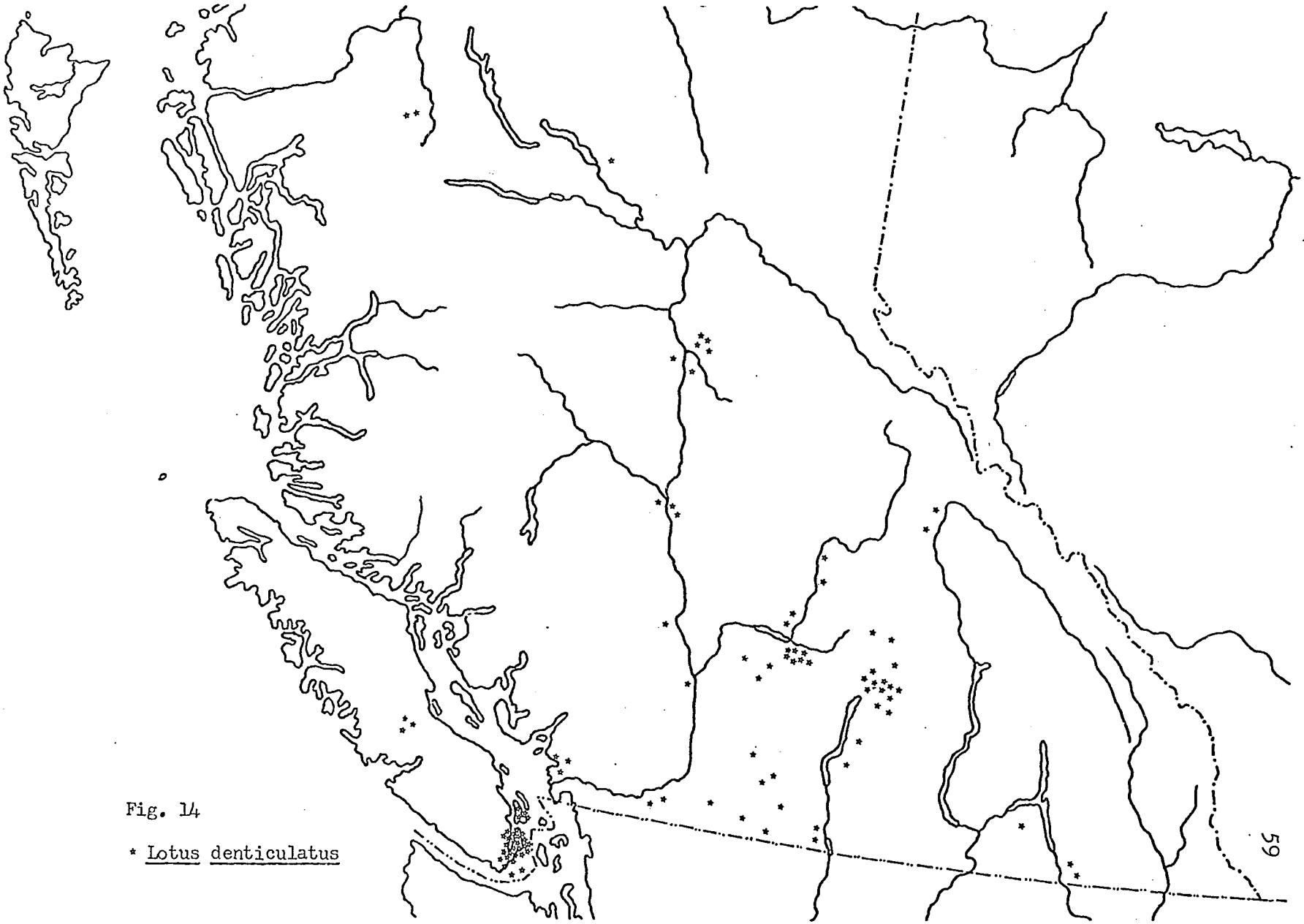


Fig. 14

* Lotus denticulatus

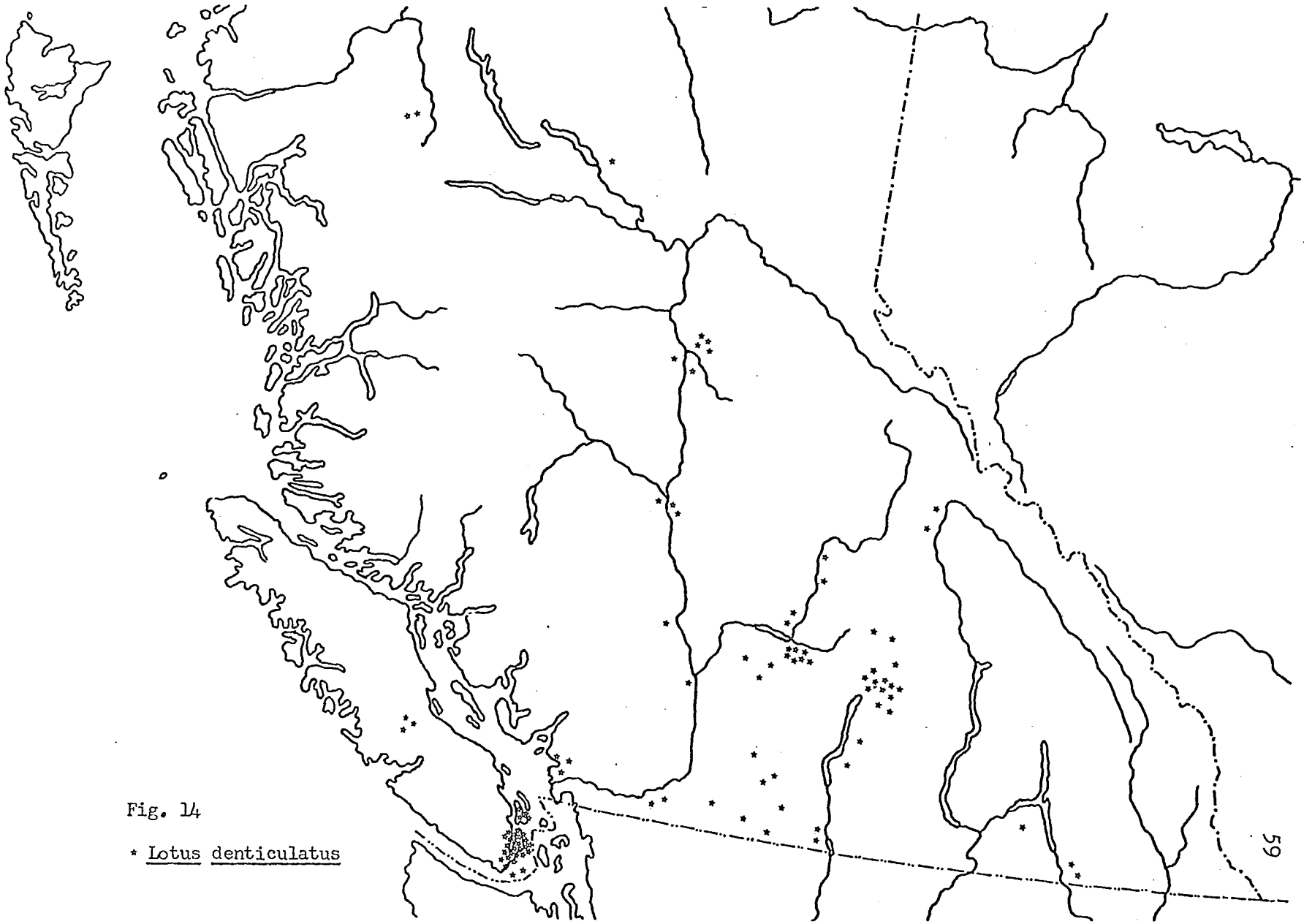


Fig. 14

* Lotus denticulatus

1905 (CAN); New Westminster, A. J. Hill, July, 1895 (UBC); Enderby, C. Tice, July 15, 1931 (UBC); Saltspring Island, T. R. Ashlee, June 17, 1956 (UBC); Island off Oak Bay, G. A. Hardy, June 3, 1953 (UBC); Pass Lake, Kamloops Dist., J. Davidson, July 6, 1939 (UBC); Hedley, Beamish and Gilmartin No. 7469, June 15, 1957 (UBC).

Lotus corniculatus L.

Lotus corniculatus was probably introduced into North America from Europe, where it enjoys widespread distribution. In Canada it is under cultivation as a forage crop in some areas and now is established as a wild species in other areas. The 84 collections seen range from the southern tip of Vancouver Island through mainland British Columbia, to the northeastern part of Nova Scotia (Figures 15 and 16). In Quebec and Ontario, it is found along the St. Lawrence River-Great Lakes waterway. Three collections come from southern Manitoba and Saskatchewan. As well as being found in southern Alberta and British Columbia, one collection was grown at Beaverlodge in northern Alberta and two at Dawson Creek in northern British Columbia. Only two specimens of L. corniculatus were among the material representing Washington State.

Representative material seen. NOVA SCOTIA: King's Co., Gaspereau, Erskine No. 52,060, June 22, 1952

(ACAD); King's Co., Kentville Exptl. Station, Roberts, July 3, 1956 (ACAD); Granville centre, D. S. McColl, Aug. 14, 1931 (DAO); King's Co., Gaspereau, J. S. Erskine No. 52,060, June 22, 1952 (DAO); King's Co., Kentville Exptl. Station, I. Hall, Sept. 11, 1952 (DAO); Antigonish Co., West River, J. E. Langille, June 20, 1966.

NEW BRUNSWICK: St. John, G. Hay, July, 1883 (MTMG); St. John, G. Hay No. 31, July 20, 1877 (ACAD); Charlotte Co., St. Andrews, G. Mears No. 6138, July 23, 1963 (NBM); St. John, G. Hay, Aug., 1881 (NBM).

QUEBEC: Chateauguay Co., Ormstown, Du Boulay No. 3615, July 26, 1964 (MTMG); Jacques Cartier Co., Montreal, Du Boulay No. 2493, June 18, 1962 (MTMG); Wolfe Co., Lake Aylmer, J. Bailey No. 1593, July 11, 1957 (CAN); Gatineau Park Hwy. near Fairy Lake, M. C. Stonor, Sept. 7, 1959 (CAN); Jacques Cartier Co., Macdonald College, L. Cinq-Mars, Aug. 20, 1947 (QFA); Compton Co., 4 mi. NW. of Scotstown, Bassett and Hamel No. 2236, July 23, 1951 (QFA); Portneuf Co., Neuville, R. Cayouette No. 57-92, June 18, 1957 (QFA); Lotbinière Co., Saint-Flavien, M. Ferron, Aug. 13, 1962 (QFA); Portneuf Co., Cap-Santé, Doyon and J. M. Deschêne, July 25, 1960 (QFA); Montmaguy Co., Ile-Aux-Gues, J. Cayouette No. 459, June 27, 1962 (QFA); Montmorency Co., Saint-Laurent I. O., P. Morisset, July 9, 1963 (QFA); St. Pierre, Rout du Cap, L. Gallo No. 409, Aug. 27, 1938 (DAO); Lunham Co., Missisquoi, L. Cinq-Mars,

July 17, 1957 (DAO); Deux Montagnes Co., La Trappe, P. Louis-Marie No. 782, June 18, 1958 (DAO); Richmond Co., Ulberton, Terrill No. 7820, July 18, 1956 (DAO); Compton Co., Lengwick, Doucet and Beaulieu, July 7, 1955 (RIN); Ste.-Anne-de-la-Pocatière, E. C., June 22 (ITA); Rivière du Loup Co., Brandy Pots Island, 5 mi. from Caeouma, Terrill, July 10, 1952 (DAO); Jacques Cartier Co., Macdonald College, W. Dore, Aug. 8, 1935 (DAO).

ONTARIO: Glengarry Co., 2 mi. E. of Lancaster, R. Herman, July 1, 1961 (MTMG); Lincoln Co., 2 mi. S. of St. Catharines, B. Miller No. 737, Aug. 26, 1952 (HAM); between Courtland and Tillonsburg, A. Tamsalu, Aug. 1, 1956 (HAM); Waterloo Co., Paradise Lake, St. Clements, F. H. Montgomery No. 887, July 19, 1941 (HAM); Guelph, Ontario Agric. Coll., J. N. Hamilton (TRT); Niagara, W. J. Potter, 1908 (TRT); Grey Co., near Jackson, C. Heimburger No. 491, June 7, 1952 (TRT); Wellington Co., Guelph, J. Stroud, June 8, 1937 (TRT); Huron Co., 6 mi. NW. of Seaforth, Montgomery and Shumovich No. 51, June 10, 1952 (TRT); Lambton Co., Sarnia, Montgomery and Shumovich No. 795, July 20, 1953 (TRT); north Toronto, W. Baldwin, July 28, 1928 (TRT); Prince Edward Co., 2 mi. E. of Wellington, Montgomery and Shumovich No. 1095, July 19, 1954 (TRT); Kent Co., Raleigh Tp., L. Stock, July 23, 1953 (TRT); Haldimand Co., Dunn Tp., W. Judd No. 642, Aug. 26, 1961 (TRT); Ilfracombe, Muskoka, S. L. Thompson No. 1158, June 30, 1954 (TRT);

York Co., Forest Hill, T. N. Taylor No. 368, June 17, 1930 (TRT); York Co., Milliken, S. L. Thompson No. 414, Aug. 7, 1922 (TRT); Thunder Bay Dist., Neebing Tp., A. E. Allen No. 146953, Aug. 19, 1965 (TRT); Algoma Dist., Blind River, F. B. Sharp, July, 1962 (DAO); Wellington Co., Guelph Exptl. Plots, Davey, June, 1916 (OAC); Wellington Co., Guelph Exptl. Plots, J. E. Howitt, June 12, 1919 (OAC); Muskoka, Milford Bay, Riley No. 671, June 20, 1953 (OAC); Wellington Co., Guelph Exptl. Plots, Wright No. 2775, July 6, 1920 (OAC); Guelph, W. Sammou, June 13, 1933 (OAC); Grey Co., 2 mi. S. of Owen Sound, Shumovich No. 1087, July 10, 1954 (OAC); Lambton Co., 3 mi. E. of Reece's Corners, Gaiser No. 1307 RC, June 20, 1958 (OAC); Lambton Co., Bosanquet Tp. Gaiser No. 2066T, June 14, 1959 (OAC); Lambton Co., Plympton Tp., 3 mi. E. of Reece's Corners, Gaiser No. 1307RC, July 7, 1957 (OAC); Hamilton, Buchan No. 422 1/2 (DAO); Waterloo Co., Paradise Lake, St. Clements, F. H. Montgomery No. 887, July 19, 1941 (DAO); Elgin Co., Yarmouth Tp., W. Steward No. 1085, June 24, 1964 (DAO).

MANITOBA: Brandon, G. A. Stevenson No. 2313, June 27, 1961 (DAO). SASKATCHEWAN: Regina, Wagner and Ledingham No. 3418, July 17, 1962 (V); Regina, C. F. Ledingham, July 17, 1962 (NBM). ALBERTA: Lethbridge Exptl. Plots, Sexsmith No. 489, June 25, 1957 (TRT); Beaverlodge, Albright, Sept. 10, 1924 (DAO); Stavely, Russell No.S59238,

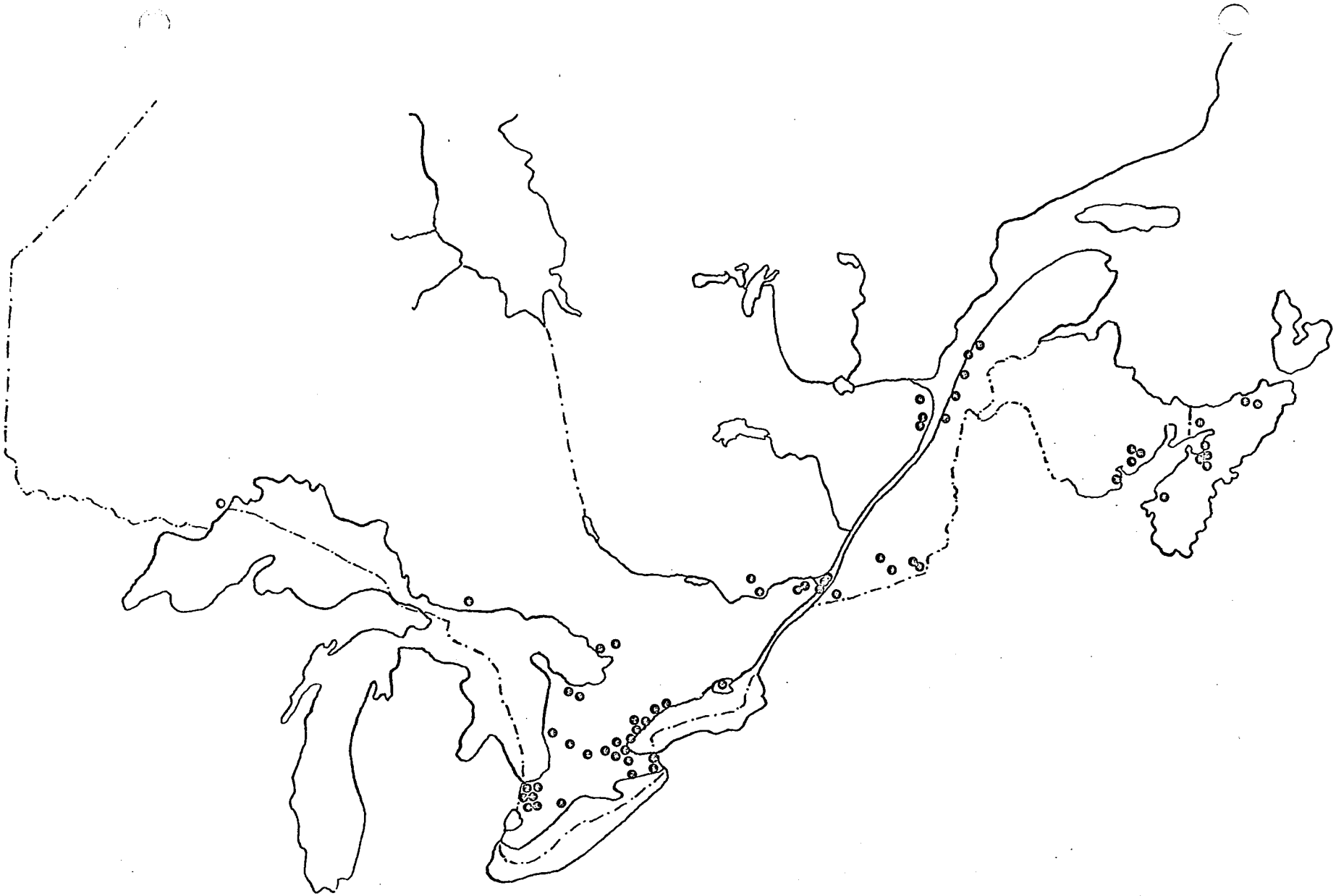


Fig. 15

o Lotus corniculatus

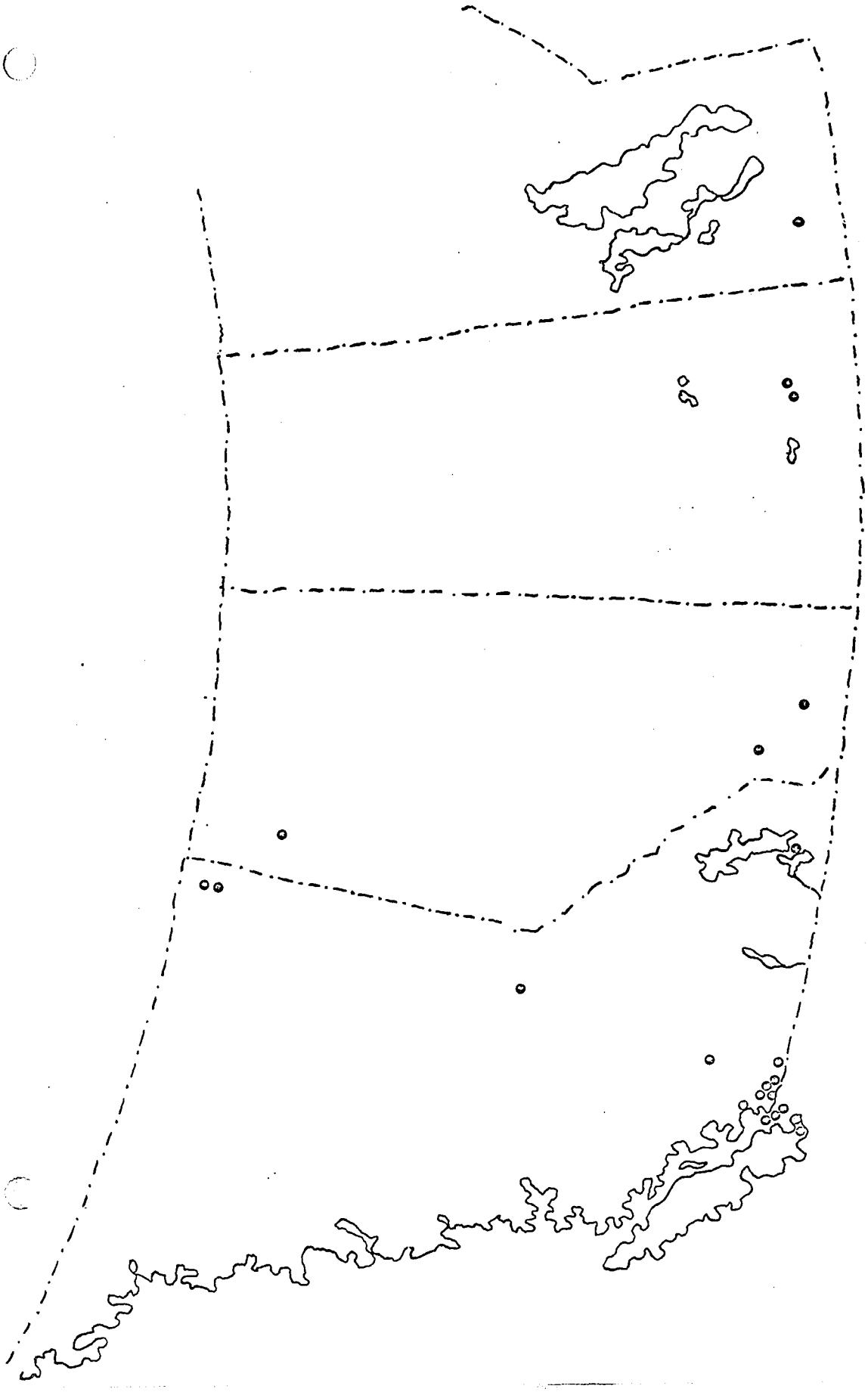


Fig. 16

o Lotus corniculatus

Aug. 2, 1959 (DAO). BRITISH COLUMBIA: Dawson Creek, W. Savale Jr. No. 227, July 20, 1958 (WTU); Prince George, S. Florian No. 90, July 19, 1953 (DAO); Dawson Creek, D. Calverby No. 227, July 20, 1958 (V); Victoria, Copley No. 499, July 26, 1925 (V); Prevost Island, Tice, June 6, 1938 (V); Prevost Island, Tice, June 16, 1933 (V); 9 mi. W. of Abbotsford, Lindsay and Woodbury No. 1167, June 22, 1955 (V); interior, Tice, July 2, 1936 (V); Crawford Bay, Eastham, July 27, 1939 (UBC); 2 mi. W. of Rosedale, D. Farris Jr. No. 74, June 3, 1954 (DAO); Matsqui, Thompson, Sept. 24, 1936 (UBC); Sumas, Barss, June 8, 1932 (UBC); Vancouver, Rogers No. 301, July 4, 1948 (UBC); Ganges, Saltspring Island, Ashlee, July 7, 1957 (UBC).

Lotus pedunculatus Cav.

Twenty-four sheets of L. pedunculatus were mapped (Figures 17 and 18). Except for one collection from each of Nova Scotia, Quebec and Ontario, all were from the southwestern part of mainland British Columbia. This species was not among the material from Washington State.

Representative material seen. NOVA SCOTIA: Halifax Co., Dartmouth, Dore, Judd and Gorham No. 45,1097, Aug. 28, 1945 (ACAD). QUEBEC: Exptl. Station, Ste-Anne-de-la-Pocatière, D. Doyon, Oct., 1951 (QFA). ONTARIO: Ottawa Exptl. Farm, P. Louis-Marie, Sept. 23, 1954 (RIM). BRITISH COLUMBIA: Elgin, J. K. Henry, Aug. 10, 1919 (WS);

3 mi. E. of Langley Prairie, Mulligan and Woodbury No. 1845, July 28, 1955 (V); Fry's Corner, Pacific Hwy., J. W. Eastham No. 11882, Sept. 7, 1939 (V); Queen's Park, New Westminster, J. W. Eastham No. 11882, Sept. 7, 1939 (V); New Westminster, J. W. Eastham, July 3, 1945 (WS); Fry's Corner, J. W. Eastham No. 13407, July 11, 1945 (WS); Fry's Corner, H. Groh No. 410, July 29, 1939 (DAO); Fry's Corner, J. W. Eastham, July 7, 1939 (DAO); Fry's Corner, J. W. Eastham No. DA 13,467, July 3, 1945 (DAO); New Westminster, J. W. Eastham No. DA 13,466, July 3, 1945 (DAO); 1 mi. N. of Durien, D. Farris Jr. No. 190, July 12, 1954 (DAO); 3 mi. E. of Langley Prairie, Mulligan and Woodbury No. 1845, July 28, 1955 (DAO); New Westminster, J. W. Eastham No. 18,717, July 3, 1945 (V); Pacific Hwy. W. of Fry's Corner, J. W. Eastham No. 18,717 A, July 3, 1945 (V); Queen's Park, New Westminster, J. W. Eastham, July 3, 1945 (UBC); Kensington, J. W. Eastham, June 29, 1942 (UBC); New Westminster, J. W. Eastham, July 7, 1939 (UBC); Dom. Exptl. Farm, Agassiz, J. W. Eastham, July 30, 1945 (UBC); Acc. No. 13,467b, UBC Herbarium (UBC); Fry's Corner, J. W. Eastham, July 7, 1939 (UBC); Fry's Corner, J. W. Eastham, July 3, 1945 (UBC).

Lotus tenuis Waldst. et Kit.

Sixteen collections of Lotus tenuis, a species related to L. corniculatus, were mapped (Figures 17 and 18).

These were found only in southern Ontario and southern British Columbia, both on the mainland and on Vancouver Island. This species was also not among the material from Washington State.

Representative material seen. ONTARIO: Bruce Co., Inverhuron, W. Stewart, Aug. 4, 1960 (UWO); York Co., near Wilcox Lake, L. T. C., Aug. 27, 1939 (TRT); York Co., Wilcox Lake, T. M. C. Taylor, Aug. 27, 1940 (TRT); near Goderich, F. McCully, June 27, 1959 (OAC); Waterloo Co., 1 mi. S. of Kitchener, F. H. Montgomery No. 632 (OAC); BRITISH COLUMBIA: Skinner Bottom, near Victoria, J. R. Anderson No. 546, Aug. 7, 1916 (WS); 5 mi. W. of Victoria, M. C. Melburn, July 9, 1959 (DAO); Esquimalt Dist., Victoria, W. R. Carter, June 26, 1923 (V); Cascade Range, near Lillooet, J. M. Macoun No. 91605, July 5, 1916 (CAN); Victoria, E. L. Greene No. 5570, July 18, 1890 (CAN); Victoria, G. V. Copley No. 7715, July 26, 1925 (V); U.B.C. Agronomy Garden, Vaartnau, Sept. 2, 1951 (UBC); Esquimalt, W. R. Carter No. 6396, June 26, 1922 (V); Dominion Exptl. Plots Agassiz, J. W. Eastham No. 1536, July 30, 1945 (UBC); Gorge, Victoria Dist., W. A. Newcombe, July 30, 1929 (V).

Lotus krylovii Schischk. and Serg.

As mentioned previously, two collections of Lotus krylovii came from White Lake, Oliver, British Columbia

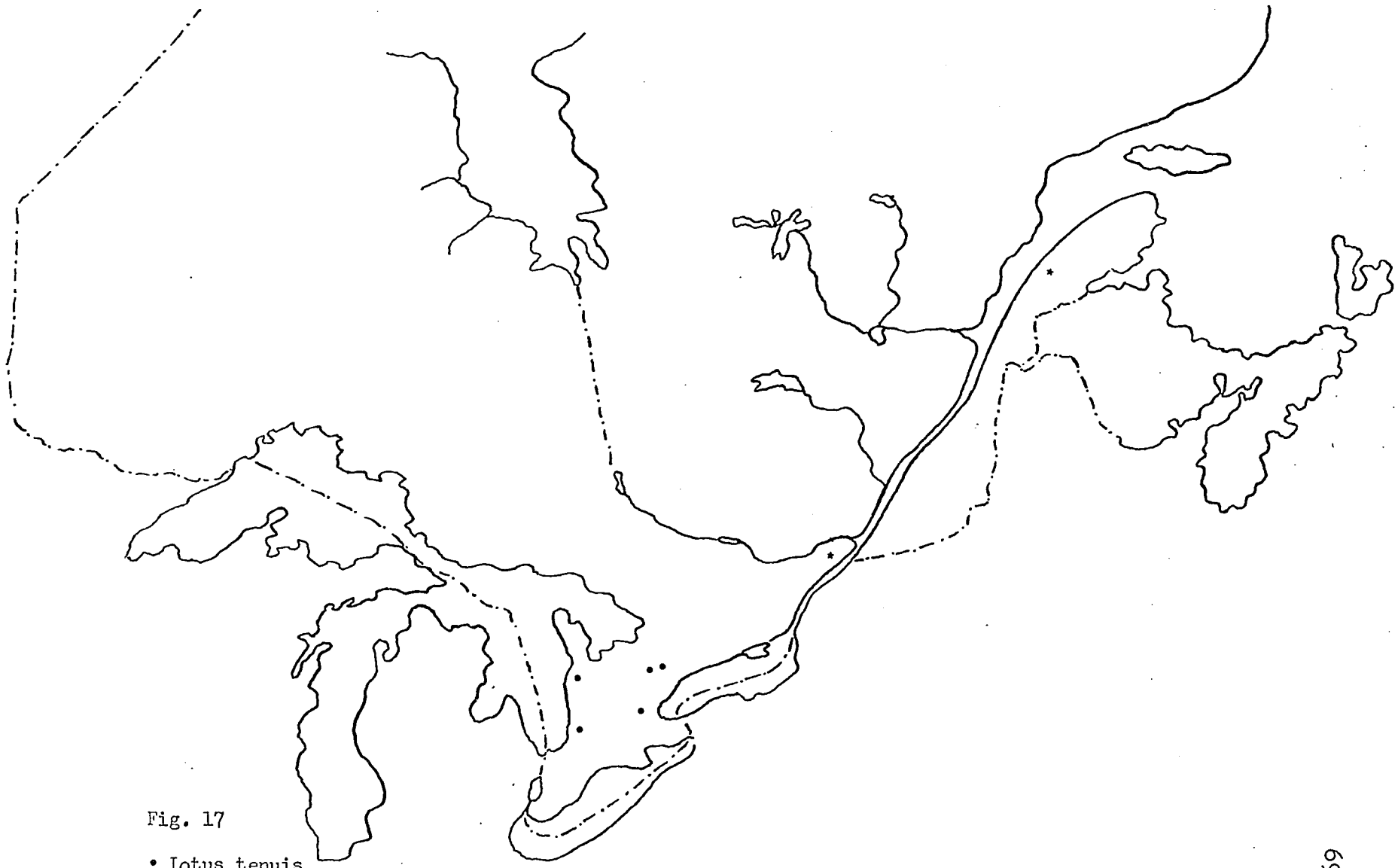


Fig. 17

• Lotus tenuis

* Lotus pedunculatus

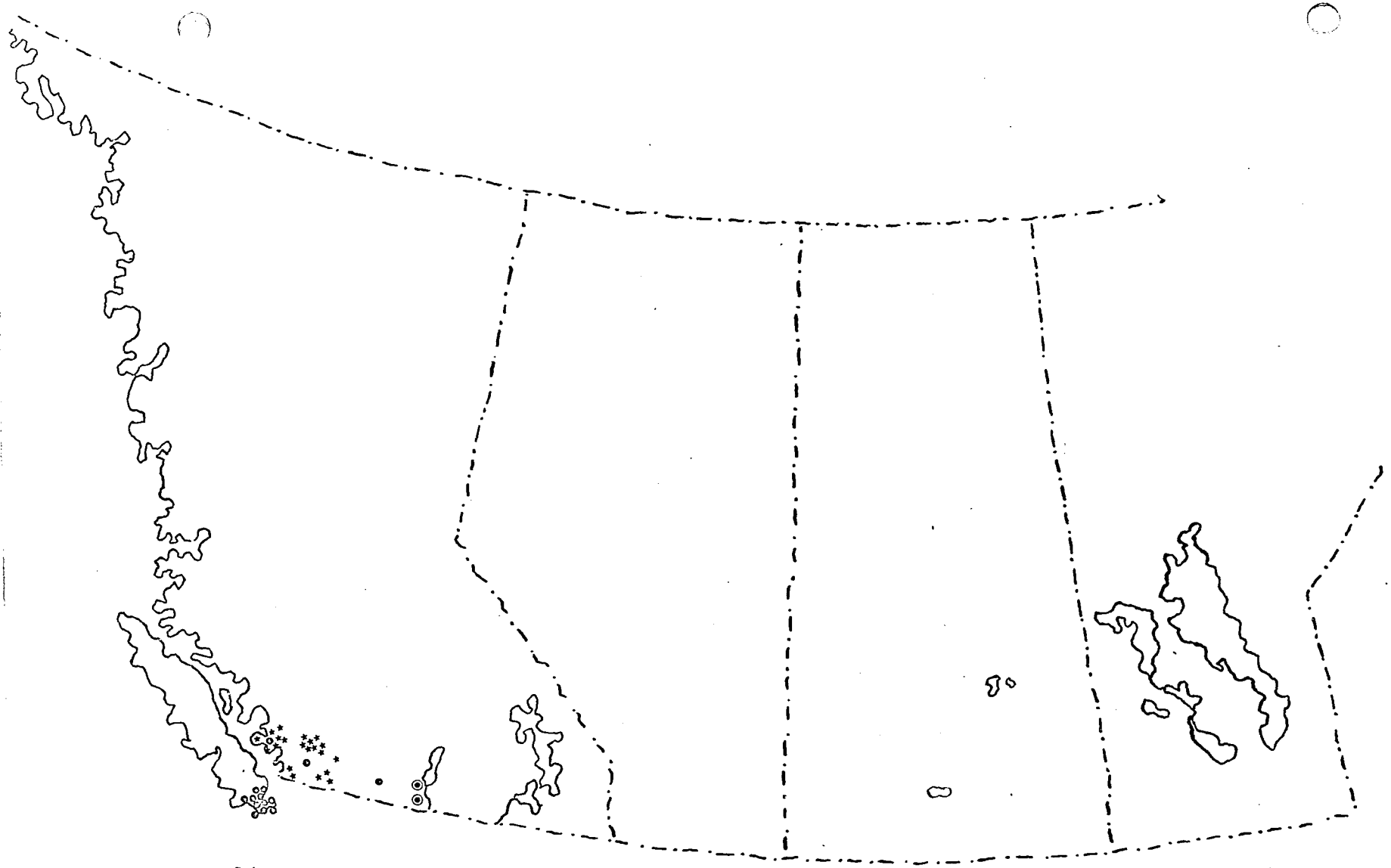


Fig. 18

∘ Lotus tenuis

* Lotus pedunculatus

⊙ Lotus krylovii

(Figure 18). There is no report of this material being found in Washington State.

Representative material seen. BRITISH COLUMBIA: Oliver, White Lake Rd., S. Okanagan, K. Beamish No. 610442, July 7, 1961 (UBC).

Three species not found in Canada were among the material from Washington State, namely, L. Douglassi Greene, a very common species there, L. crassifolius Greene, also fairly widespread, and L. aboriginum Jeps., confined to Mason County. It is not known why these species should not be found in Canada.

## 5. Karyotypes and idiograms

Drawings of the karyotypes of the nine species of Lotus found in Canada are shown in Figures 28 through 31 and photographs are shown in Figures 19 through 27. Idiograms of the species are represented in Figures 32 through 34. Results for any one species are all taken from the same accession number. The chromosomes are represented as per cent of length of the total complement, which is shown along the vertical axis. Per cent total complement length (TCL) was calculated by dividing the total length of each chromosome pair into the total length of the chromosome complement and multiplying the

quotient by 100 (Table 3). The ratio of the total length of a chromosome to the long arm of the same chromosome (T/L) was calculated by dividing the average length of the two long arms of a chromosome pair, into the average length of the two chromosomes.

The ratio of the long to short arm of a chromosome (L/S) was calculated by dividing the average of the two short arms of one pair into the average of the two long arms. Both T/L and L/S give a ratio which indicates the shape of the chromosome (metacentric or submetacentric) and from which the centromere position can be calculated (Table 3). Lengths of the chromosomes in microns were calculated from the actual measurements of the chromosomes (Table 3).

Lotus pinnatus Hook.

The somatic chromosome number for this species was $2n = 14$, and had not been reported previously. The length of the total chromosome complement ranged from 21.86 u to 30.90 u with an average of 24.94 u (Table 3). The variation in complement length can be due to the fact that not all the ten cells were at exactly the same stage of contraction, although only cells in metaphase were analyzed. Due to the difficulty of germinating seed of this species there was a scarcity of root tips and other than perfect cells sometimes had to be used. The average

length for the longest chromosome was 2.78 u and the shortest was 1.33 u (Table 3). The chromosomes of the complement were all submetacentric (Fig. 28B, 19 and 32). One long chromosome pair was very easily distinguished and the six other chromosome pairs showed a gradual decrease in size, although there was not much difference between any two neighbouring ones. No satellites were observed.

Lotus formosissimus Greene

The somatic chromosome number for this species was 2n = 14 and had not been reported previously. This species, which closely resembled L. pinnatus morphologically, did so also from a cytological point of view. Total complement length ranged from 21.24 u to 31.00 u with an average of 26.60 u which differed from that of L. pinnatus by 0.83 u (Table 3). The average of the longest chromosome was 3.00 u and the shortest, 1.39 u (Table 3). The relative chromosome lengths of L. formosissimus were also very similar to those of L. pinnatus, and, as well, L. formosissimus did not have any chromosomes bearing satellites (Figs. 28A, 20 and 32).

Lotus purshianus (Benth.) Clem. and Clem.

This species had a somatic chromosome number of 2n = 14. The total complement length varied from 21.94 u to 33.28 u with an average of 29.70 u for the ten cells

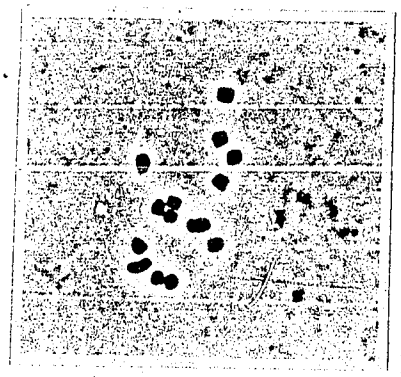


Fig. I9

Metaphase plate of somatic chromosomes of L. pinnatus. (X558)

Fig. 20

Metaphase plate of somatic chromosomes of L. formosissimus. (X690)

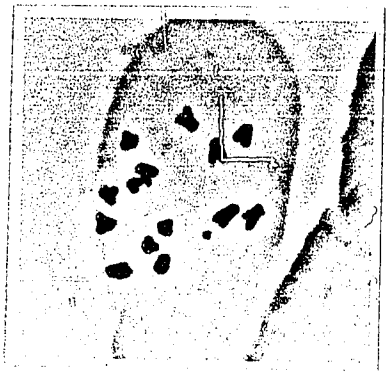


Fig. 2I

Metaphase plate of somatic chromosomes of L. micranthus. (X7I4)

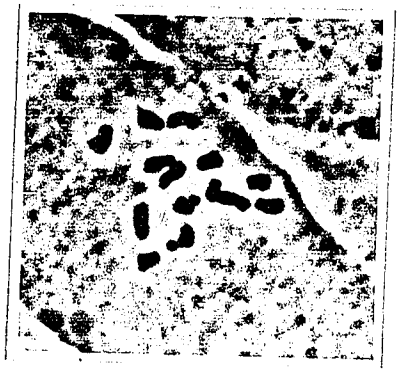


Fig. 22

Metaphase plate of somatic chromosomes of L. purshianus. (XI024)

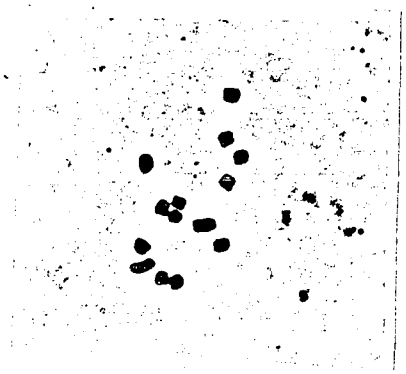


Fig. I9

Metaphase plate of somatic chromosomes of L. pinnatus. (X558)

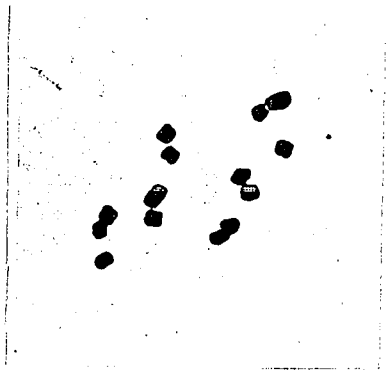


Fig. 20

Metaphase plate of somatic chromosomes of L. formosissimus. (X690)

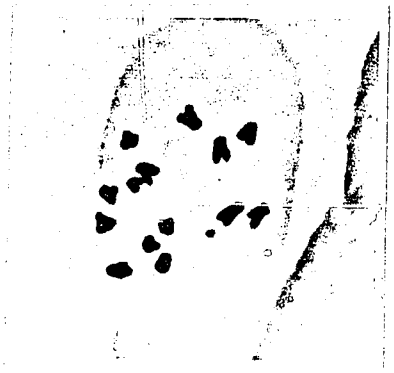


Fig. 2I

Metaphase plate of somatic chromosomes of L. micranthus. (X7I4)

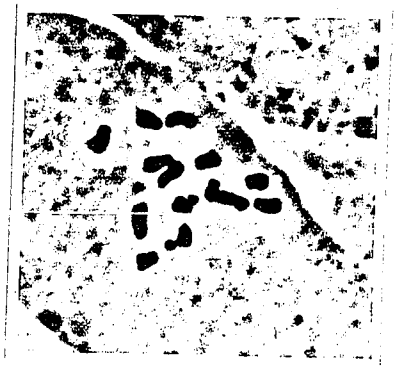


Fig. 22

Metaphase plate of somatic chromosomes of L. purshianus. (XI024)

Fig. 23

Metaphase plate of somatic chromosomes of L. denticulatus. (X526)

Fig. 24

Metaphase plate of somatic chromosomes of L. corniculatus. (X558)

Fig. 25

Metaphase plate of somatic chromosomes of L. tenuis. (X558)

Fig. 26

Metaphase plate of somatic chromosomes of L. pedunculatus. (X558)

Fig. 27

Metaphase plate of somatic chromosomes of L. krylovii.
(X507)

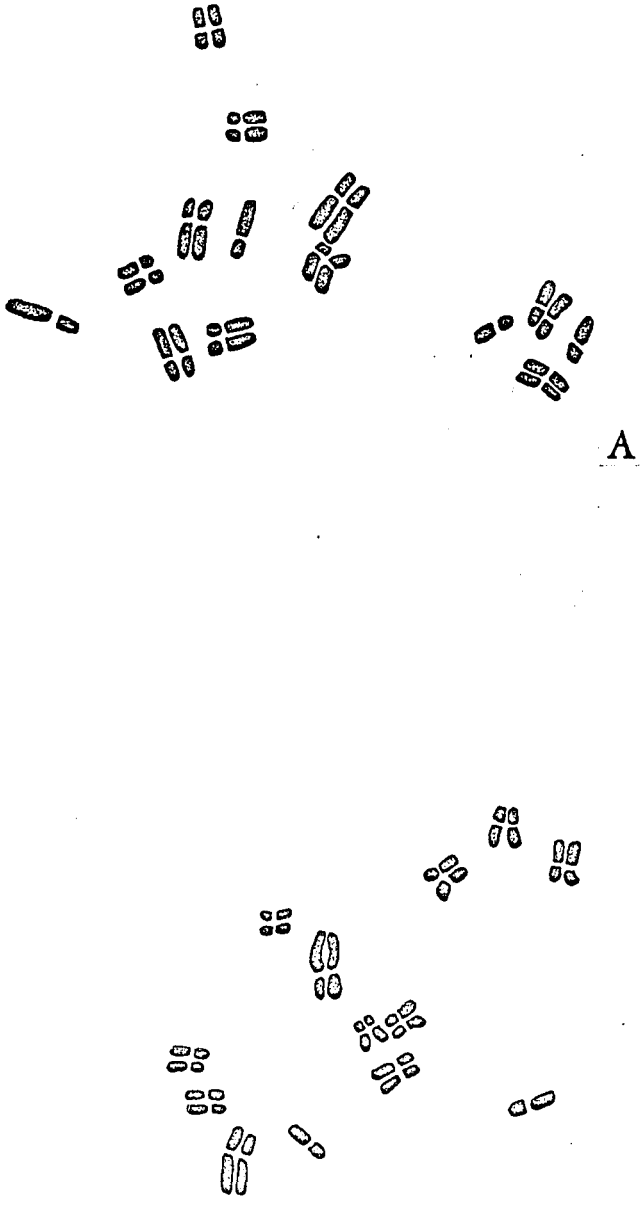
analyzed (Table 3).. The average length for the longest chromosome was 2.24 u and the shortest was 1.29 u (Table 3). Fairly prominent satellites were seen on the sixth pair (second shortest) of chromosomes (Figs. 29B, 22 and 33). The satellites were not included in the measurements of chromosome length. All of the chromosomes of the complement were submedian, the largest and the smallest chromosomes being more nearly median.

Lotus micranthus Benth.

This species also had a somatic chromosome number of 2n = 14, which had not been previously reported. The length for the total complement ranged from 19.08 u to 38.40 u with an average of 27.44 u (Table 3). The longest chromosome had an average length of 2.66 u and the shortest had an average length of 1.24 u (Table 3). All of the chromosomes were submetacentric, however, there was not much of a pairing problem since, as with L. purshianus, they were quite variable in size and arm ratio (Figs. 29C, 21 and 33). No satellites were seen in this species.

Lotus denticulatus (Drew) Greene

Lotus denticulatus had a somatic chromosome number of 2n = 12. Its total complement length ranged from 18.82 u to 24.18 u with an average of 21.88 u (Table 3). Thus, with a reduction in the number of chromosomes, there seemed also to be a reduction in the total amount of



A

B

Fig. 28.--A, Lotus formosissimus
B, Lotus pinnatus

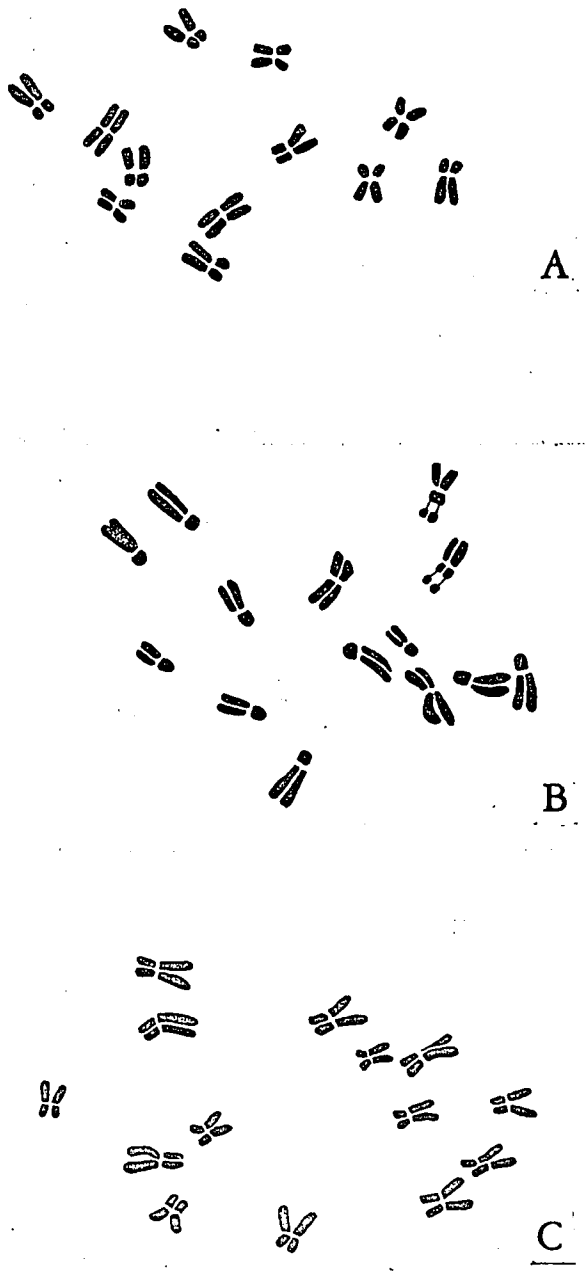



Fig. 29.--A, Lotus denticulatus
B, Lotus purshianus
C, Lotus micranthus

chromatin present. This, however, may also have been due to the greater contraction of the chromosomes of this species, although this did not seem as likely a possibility. The average length of the longest chromosome was 2.40 u and the shortest was 1.36 u (Table 3). The longest chromosomes (pair 1) and the second shortest (pair 5) were almost metacentric, having an L/S ratio of 1.15 and 1.20, respectively (Table 3). The other chromosomes were submetacentric (Figs. 29A, 23 and 33). No satellites were seen in the accession number (B-240) analysed here. However, satellites were observed on the two chromosomes of pair 5 of this species for another accession number (B-224).

Lotus tenuis Waldst. et Kit.

The somatic chromosome number for this species was $2n = 12$, as had been reported previously. The total complement length varied from 22.04 u to 37.06 u, the average being 28.66 u (Table 3). The longest chromosome had an average length of 3.72 u and the shortest, 1.56 u (Table 3). All the chromosomes were submetacentric, having fairly similar arm ratios (Figs. 30A, 25 and 34). No satellite chromosomes were observed for this species.

Lotus pedunculatus Cav.

As had been reported previously, this species was found to have a somatic chromosome number of $2n = 12$.

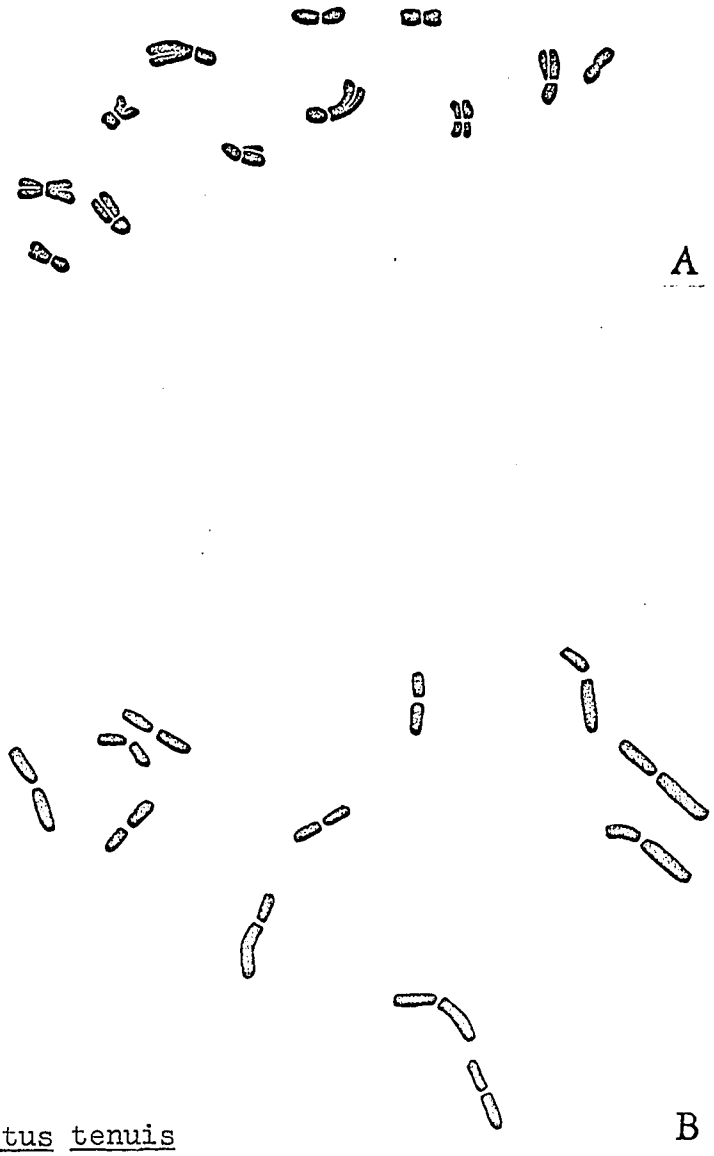
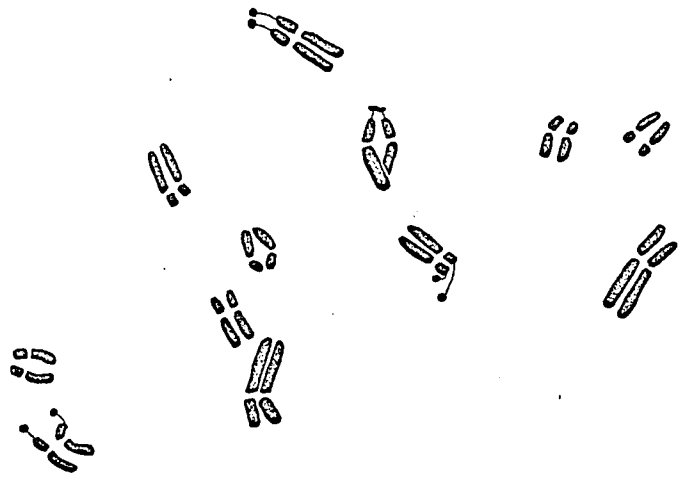



Fig. 30.--A. Lotus tenuis
B. Lotus krylovii

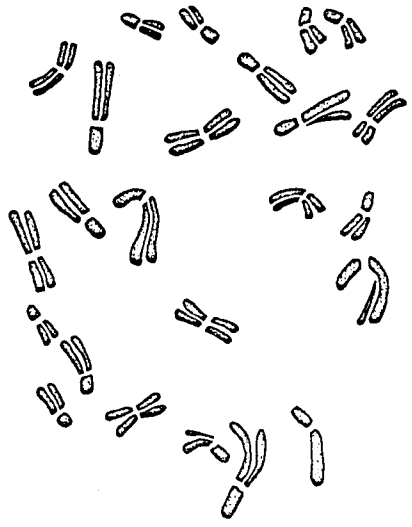
The lengths of the total complement varied from 22.48 u to 32.62 u with an average of 27.56 u (Table 3). The average for the longest chromosome was 3.61 u and the average for the shortest was 1.50 u (Table 3). All the chromosomes were submetacentric and not too different from those of L. tenuis except for the presence of two pairs of satellite chromosomes (Figs. 31A, 26 and 34). Pair 2 and pair 4 had satellites. These were not as prominent as the satellites of L. purshianus and were also not included in the measurements of the chromosome length.

Lotus krylovii Schischk. and Serg.

The somatic chromosome number for this species was $2n = 12$. The length of the total complement varied from 27.46 u to 41.14 u with an average of 32.56 u (Table 3). Of the eight diploid species found in Canada, this had the longest total chromosome length. The length of individual chromosomes ranged from 3.64 u for the longest, to 1.97 u for the shortest (Table 3). The chromosomes were all metacentric with pair 4 being nearly metacentric, having an L/S ratio equal to 1.17 (Table 3). The chromosomes varied considerably in lengths and arm ratio, and thus were easily distinguished from each other (Figs. 30B, 27 and 34). No satellite chromosomes were observed.



A



B

Fig. 31.--A, Lotus pedunculatus
B, Lotus corniculatus

TABLE 3.--Results of analysis of somatic chromosomes for eight Lotus species

| Chromosome pair | % TCL | Standard deviation | L/S | Standard deviation | T/L | Standard deviation | Length in microns (u) |
|---|---|---|---|---|---|---|---|
| L. pinnatus | | | | | | | |
| 1 | 22.33 | 1.68 | 2.01 | 0.51 | 1.52 | 0.09 | 2.78 |
| 2 | 15.33 | 0.40 | 1.76 | 0.26 | 1.58 | 0.10 | 1.91 |
| 3 | 13.95 | 0.40 | 1.83 | 0.36 | 1.57 | 0.11 | 1.74 |
| 4 | 13.27 | 0.53 | 1.63 | 0.32 | 1.64 | 0.14 | 1.66 |
| 5 | 12.52 | 0.65 | 1.55 | 0.33 | 1.68 | 0.15 | 1.56 |
| 6 | 11.97 | 0.48 | 1.75 | 0.15 | 1.57 | 0.05 | 1.49 |
| 7 | 10.65 | 0.60 | 1.61 | 0.28 | 1.64 | 0.09 | 1.33 |
| | | | | | | Total* = | 24.94 |
| L. formosissimus | | | | | | | |
| 1 | 22.47 | 1.04 | 1.92 | 0.19 | 1.53 | 0.05 | 3.00 |
| 2 | 15.66 | 1.43 | 1.81 | 0.46 | 1.60 | 0.23 | 2.02 |
| 3 | 14.15 | 0.47 | 1.65 | 0.25 | 1.62 | 0.96 | 1.89 |
| 4 | 13.21 | 0.34 | 1.78 | 0.38 | 1.59 | 0.13 | 1.77 |
| 5 | 12.59 | 0.65 | 1.71 | 0.33 | 1.61 | 0.12 | 1.68 |
| 6 | 11.52 | 0.78 | 1.63 | 0.46 | 1.65 | 0,14 | 1.54 |
| 7 | 10.41 | 0.70 | 1.59 | 0.22 | 1.64 | 0.09 | 1.39 |
| | | | | | | Total* = | 26.60 |

(table continued)

TABLE 3 (continued)

| Chromosome pair | % TCL | Standard deviation | L/S | Standard deviation | T/L | Standard deviation | Length in microns (u) |
|---|---|---|---|---|---|---|---|
| *L. micranthus* | | | | | | | |
| 1 | 19.34 | 1.29 | 2.71 | 0.61 | 1.38 | 0.07 | 2.66 |
| 2 | 16.99 | 0.52 | 2.76 | 0.44 | 1.37 | 0.05 | 2.34 |
| 3 | 15.65 | 0.90 | 2.62 | 0.46 | 1.39 | 0.07 | 2.15 |
| 4 | 14.61 | 0.63 | 2.48 | 0.40 | 1.41 | 0.07 | 2.01 |
| 5 | 13.17 | 0.59 | 2.27 | 0.54 | 1.46 | 0.11 | 1.80 |
| 6 | 11.08 | 1.00 | 2.04 | 0.44 | 1.51 | 0.11 | 1.52 |
| 7 | 9.16 | 0.81 | 1.76 | 0.24 | 1.58 | 0.08 | 1.24 |
| | | | | | | Total* = | 27.44 |
| *L. purshianus* | | | | | | | |
| 1 | 17.84 | 0.79 | 1.55 | 0.32 | 1.67 | 0.11 | 2.32 |
| 2 | 16.55 | 0.80 | 2.59 | 0.48 | 1.40 | 0.09 | 2.16 |
| 3 | 15.50 | 0.40 | 2.23 | 0.43 | 1.46 | 0.08 | 2.02 |
| 4 | 14.68 | 0.41 | 2.29 | 0.40 | 1.45 | 0.07 | 1.91 |
| 5 | 13.23 | 0.76 | 2.07 | 0.39 | 1.50 | 0.10 | 1.73 |
| 6 | 11.91 | 1.02 | 1.78 | 0.30 | 1.58 | 0.12 | 1.53 |
| 7 | 10.31 | 1.12 | 1.54 | 0.18 | 1.66 | 0.08 | 1.34 |
| | | | | | | Total* = | 25.92 |

(table continued)

TABLE 3 (continued)

| Chromosome pair | % TCL | Standard deviation | L/S | Standard deviation | T/L | Standard deviation | Length in microns (u) |
|---|---|---|---|---|---|---|---|
| L. denticulatus | | | | | | | |
| 1 | 21.69 | 1.41 | 1.15 | 0.08 | 1.88 | 0.06 | 2.40 |
| 2 | 19.10 | 1.74 | 1.91 | 0.40 | 1.54 | 0.10 | 2.14 |
| 3 | 16.55 | 0.66 | 1.99 | 0.31 | 1.52 | 0.08 | 1.80 |
| 4 | 15.34 | 0.90 | 1.89 | 0.30 | 1.54 | 0.08 | 1.69 |
| 5 | 14.10 | 0.72 | 1.21 | 0.15 | 1.84 | 0.09 | 1.55 |
| 6 | 12.32 | 0.61 | 1.62 | 0.18 | 1.62 | 0.07 | 1.36 |
| | | | | | | Total* = | 21.88 |
| L. krylovii | | | | | | | |
| 1 | 22.27 | 1.09 | 1.45 | 0.14 | 1.70 | 0.07 | 3.64 |
| 2 | 19.77 | 0.47 | 1.24 | 0.10 | 1.81 | 0.06 | 3.22 |
| 3 | 17.03 | 0.49 | 1.94 | 0.29 | 1.53 | 0.08 | 2.77 |
| 4 | 15.58 | 0.70 | 1.18 | 0.06 | 1.85 | 0.05 | 2.53 |
| 5 | 13.26 | 0.61 | 1.38 | 0.19 | 1.74 | 0.11 | 2.15 |
| 6 | 12.11 | 0.44 | 1.30 | 0.16 | 1.78 | 0.08 | 1.97 |
| | | | | | | Total* = | 32.56 |

(table continued)

TABLE 3 (continued)

| Chromosome pair | % TCL | Standard deviation | L/S | Standard deviation | T/L | Standard deviation | Length in microns (u) |
|---|---|---|---|---|---|---|---|
| L. tenuis | | | | | | | |
| 1 | 25.84 | 1.90 | 1.76 | 0.23 | 1.58 | 0.07 | 3.72 |
| 2 | 18.88 | 1.49 | 1.51 | 0.34 | 1.69 | 0.14 | 2.68 |
| 3 | 16.80 | 1.11 | 1.28 | 0.15 | 1.79 | 0.09 | 2.41 |
| 4 | 14.74 | 0.96 | 1.29 | 0.25 | 1.80 | 0.13 | 2.11 |
| 5 | 12.87 | 0.59 | 1.34 | 0.16 | 1.76 | 0.10 | 1.85 |
| 6 | 10.87 | 0.98 | 1.26 | 0.18 | 1.81 | 0.11 | 1.56 |
| | | | | | | Total* = | 28.66 |
| L. pedunculatus | | | | | | | |
| 1 | 26.34 | 1.00 | 2.06 | 0.31 | 1.49 | 0.07 | 3.61 |
| 2 | 19.60 | 0.81 | 2.00 | 0.33 | 1.51 | 0.08 | 2.69 |
| 3 | 15.60 | 1.11 | 1.96 | 0.34 | 1.52 | 0.08 | 2.20 |
| 4 | 14.93 | 1.07 | 1.90 | 0.30 | 1.54 | 0.08 | 2.05 |
| 5 | 12.63 | 0.63 | 1.99 | 0.26 | 1.51 | 0.07 | 1.73 |
| 6 | 10.91 | 0.64 | 1.49 | 0.25 | 1.69 | 0.11 | 1.50 |
| | | | | | | Total* = | 27.56 |

*Total length of chromosome complement in microns is given X2.

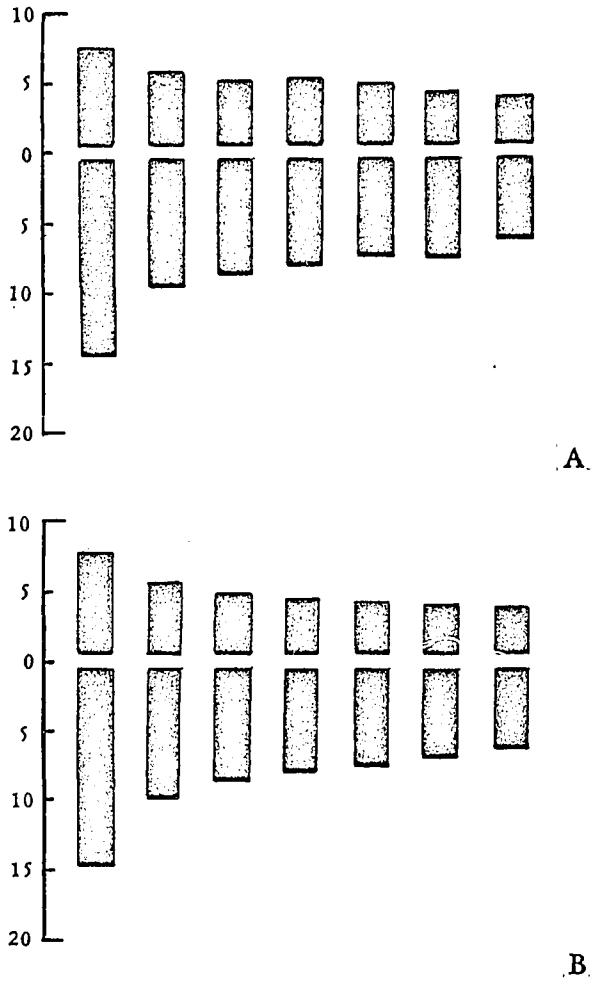



Fig. 32.--A, Lotus pinnatus
B, Lotus formosissimus

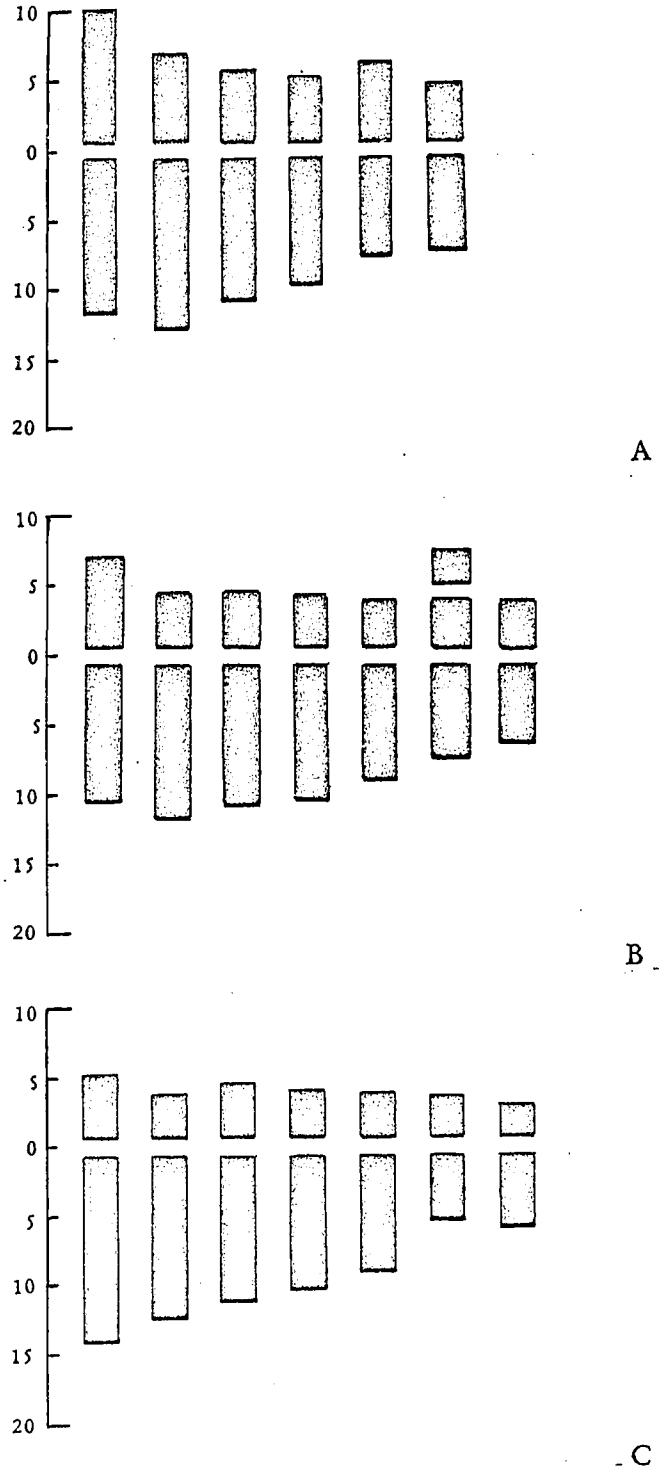



Fig. 33.--A, Lotus denticulatus
B, Lotus purshianus
C, Lotus micranthus

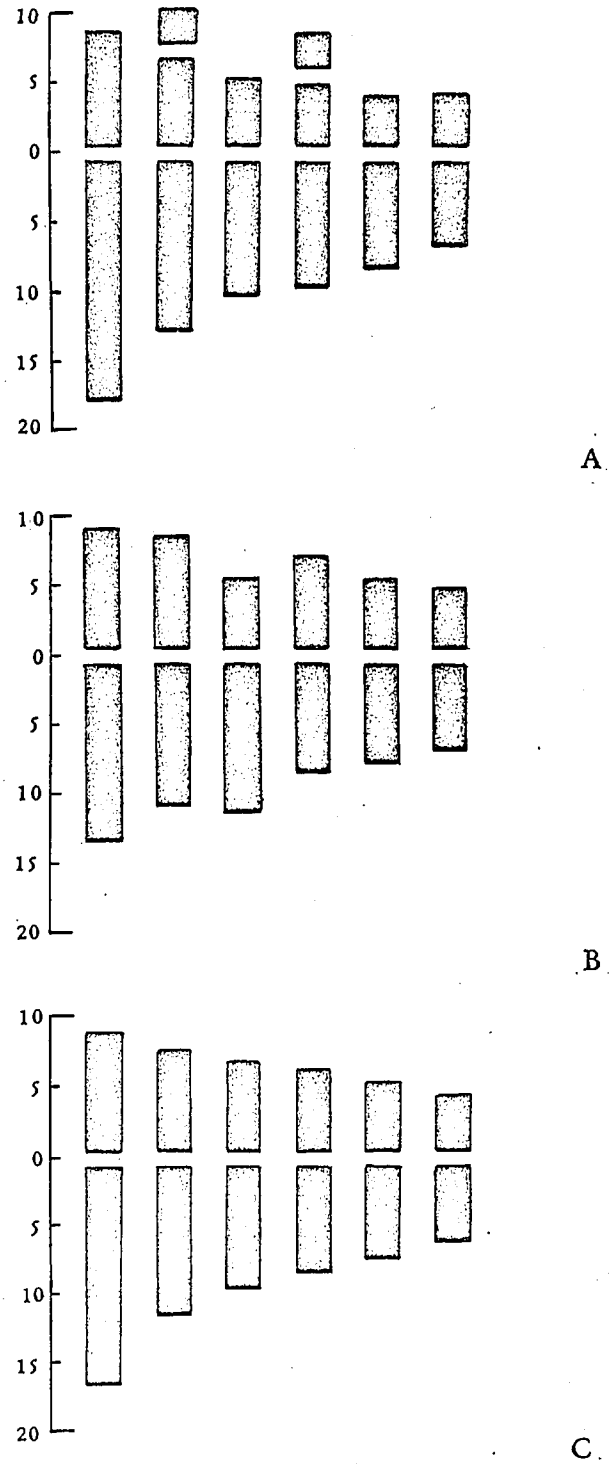



Fig. 34.--A, Lotus pedunculatus
B, Lotus krylovii
C, Lotus tenuis

Lotus corniculatus L.

An idiogram was not prepared for this species. However, it can be seen from the drawing (Fig. 31B) that although most of the chromosomes were submetacentric, some approached metacentricity. No satellites were seen (Figs. 31B and 24).

## 6. Hybridization studies

A total of 241 artificial interspecific hybridizations were attempted during the late fall and winter, 1965, through June, 1966 (see Table 4). It was hoped that a study of interspecific hybrids between the species growing in Canada would supply some information on relationships between these species and that a study of meiosis of these hybrids would supply information on chromosome homologies. A successful cross of a species with a basic number of $n = 6$ with one that has a basic number of $n = 7$ could have supplied important information on the evolution of an aneuploid series.

Several difficulties were encountered in carrying on the programme of hybridization. Plants growing in the greenhouse flowered very poorly during the winter, even though they were on a photoperiod of seventeen hours daylight with the aid of artificial lighting from four in the afternoon until midnight. However, the light

intensity during the winter months was not strong enough to give profuse blooming. As an alternative, it was not possible to keep sufficient plants in the growth chamber due to lack of space.

Seeds of L. pinnatus and L. formosissimus were very difficult to germinate. Repeatedly, seeds of these species were scarified and sown in pots and placed in the growth chamber. When several seedlings did appear, these were very weak, did not produce many leaves, and died. An attempt was made to germinate seed of these two species by sowing them on moist filter paper in a Petri dish. Then, when the first true leaves appeared they were transferred to soil. The mortality rate of the seedlings was high, but by the spring of 1966 three or four plants of each of the species were established by keeping them under exceedingly wet conditions. However, by the time the first flowers appeared in July, it was too late to include them in the hybridization programme.

A cause of further difficulty in studying the Canadian material was due to the fact that the three other native species, L. micranthus, L. denticulatus and L. purshianus are annuals and produce small, solitary flowers. These species are autogamous and, as such, do not produce an abundance of pollen.

At the time that the programme of interspecific

hybridization was started, it was not certain how many and what species were growing in Canada. Therefore, L. subpinnatus and L. angustissimus were among the species being crossed, although it was revealed later that they are not found in Canada.

The crossing of L. denticulatus with L. purshianus and L. angustissimus was unsuccessful. However, from the cross of L. denticulatus with L. subpinnatus, a closely related species which is sometimes confused with L. denticulatus, one legume was produced which contained one embryo (Table 4). At 18 days after pollination when it was cultured on artificial media, it had already started to degenerate, probably due to some incompatibility between embryo and endosperm or within the chromosome complement of the embryo itself. It did not survive. The reciprocal cross, with L. subpinnatus as the female parent, resulted in the production of two legumes. One of these dropped off at fifteen days, the other contained one apparently mature embryo which was cultured at 24 days. This was believed to be a hybrid for the following reason. Four days later, when the cotyledons had opened, one cotyledon was a dark green, the other was a pale yellow colour. Unfortunately, the seedling did not survive due to fungal contamination. L. subpinnatus, as the female parent, was also crossed,

unsuccessfully, to L. purshianus, L. tenuis, L. corniculatus, L. angustissimus and L. micranthus. The latter attempt did produce legumes although they soon dried up.

When L. purshianus was used as the female parent with L. krylovii and L. denticulatus as pollen parents, legumes were produced. These legumes proved to be empty except in one instance where an ovule was produced, but which later turned out to be a self. Crosses of L. purshianus with L. angustissimus, L. subpinnatus and L. corniculatus were unsuccessful.

Lotus micranthus did not flower very well. It was also very difficult to emasculate the flowers without injuring them due to their extremely small size. The flowers self before they are fully open. In studying the pollen from fully open flowers of this species, it was found that the pollen had begun to germinate, for many pollen tubes were seen in the preparation. As well, each flower of L. micranthus was observed to yield only a small amount of pollen. Attempts to cross this species with L. purshianus, L. subpinnatus and L. denticulatus were unsuccessful.

Hybridization attempts in which introduced species were used as the female parents, were no more successful than those using native Canadian species. The crosses of L. corniculatus with L. tenuis, L. pedunculatus and

L. krylovii produced the occasional legume which dried up and fell off early. When L. corniculatus was crossed to L. angustissimus, a tetraploid with the same chromosome number ($2n = 24$) as L. corniculatus, one of the five legumes which resulted from the cross was found to contain three empty ovules and one degenerating embryo which was not cultured.

An attempt was made to cross L. tenuis with L. corniculatus, L. angustissimus, L. krylovii, L. pedunculatus and L. subpinnatus; but all were unsuccessful.

The crosses of L. krylovii with L. tenuis, L. angustissimus, L. corniculatus and L. pedunculatus produced no results except that in the crosses with the latter two species, several legumes were produced. However, these soon dried up.

Legumes were produced in the crosses of L. pedunculatus with L. corniculatus and L. tenuis. However, these fell off quite early.

Lotus angustissimus, a tetraploid which sets self seed spontaneously, produced legumes in half of the crosses which were attempted with L. krylovii, L. tenuis, L. pedunculatus and L. corniculatus. These legumes soon dried up, however. The cross of this species with L. purshianus produced no results.

TABLE 4.--Results of hybridization studies carried on during Winter-Spring, 1965-1966

| Female parent | | Male parent | | No. of florets | Results |
|---|---|---|---|---|---|
| L. denticulatus | B-240 | L. purshianus | B-489 | 4 | Flowers wilted |
| | B-240 | L. angustissimus | B-146 (4x) | 1 | Flower wilted |
| | B-240 | L. subpinnatus | B-160 | 3 | 2 flowers wilted<br>1 legume--slightly degenerating ovule cultured on Nitsch's media 18 days after pollination; dead |
| | B-224 | L. subpinnatus | B-160 | 2 | Flowers wilted |
| L. subpinnatus | B-160 | L. purshianus | B-489 | 3 | Flowers wilted |
| | B-160 | L. denticulatus | B-240 | 10 | 8 flowers wilted<br>2 legumes--1 legume dropped off at 15 days; 1 legume contained 1 ovule which was cultured at 24 days on Randolph-Cox media. Did not survive due to fungal contamination |
| | B-160 | L. denticulatus | B-224 | 5 | Flowers wilted |
| | B-160 | L. tenuis | B-309 | 1 | Flower wilted |
| | B-160 | L. micranthus | B-329 | 2 | 2 legumes produced; dried up |
| | B-160 | L. corniculatus | B-221 | 2 | Flowers wilted |
| | B-160 | L. angustissimus | B-146 (4x) | 2 | Flowers wilted |

TABLE 4 (continued)

| Female parent | | Male parent | | No. of florets | Results |
|---|---|---|---|---|---|
| L. micranthus | B-329 | L. purshianus | B-65 | 3 | Flowers wilted |
| | B-388 | L. subpinnatus | B-160 | 1 | Flower wilted |
| | B-388 | L. denticulatus | B-240 | 1 | Flower wilted |
| L. purshianus | B-318 | L. krylovii | B-226 | 3 | 2 flowers wilted<br>1 legume produced;<br>1 embryo cultured at 25 days on Randolph-Cox media; was a self |
| | B-489 | L. krylovii | B-226 | 2 | 2 legumes; empty |
| | B-489 | L. angustissimus | B-146 (4x) | 2 | Flowers wilted |
| | B-318 | L. denticulatus | B-224 | 1 | 1 legume; dried up |
| | B-489 | L. denticulatus | B-240 | 1 | 1 legume; dried up |
| | B-318 | L. subpinnatus | B-160 | 3 | Flowers wilted |
| | B-489 | L. sp. | B-181 | 1 | Flower wilted |
| L. corniculatus | B-221 | L. krylovii | B-226 | 7 | 5 florets wilted<br>2 legumes produced; dropped off early |
| | B-221 | L. tenuis | B-309 | 6 | 6 florets wilted |
| | B-221 | L. angustissimus | B-146 (4x) | 5 | 5 legumes produced; 4 dried up; at 24 days 1 legume contained 3 empty ovules and 1 degenerating embryo |

TABLE 4 (continued)

| Female parent | | Male parent | | No. of florets | Results |
|---|---|---|---|---|---|
| L. corniculatus | B-221 | L. pedunculatus | B-201 | 3 | 3 legumes produced; dried up |
| L. sp. | B-181 | L. pedunculatus | B-201 | 4 | Florets wilted |
| | B-181 | L. angustissimus | B-146 (4x) | 4 | Florets wilted |
| | B-181 | L. tenuis | B-309 | 13 | 12 florets wilted<br>1 legume produced; dried up |
| L. tenuis | B-309 | L. sp. | B-181 | 11 | Florets wilted |
| | B-309 | L. corniculatus | B-221 | 9 | Florets wilted |
| | B-309 | L. angustissimus | B-146 (4x) | 12 | Florets wilted |
| | B-309 | L. krylovii | B-226 | 8 | Florets wilted |
| | B-309 | L. pedunculatus | B-201 | 12 | Florets wilted |
| | B-309 | L. subpinnatus | B-160 | 8 | Florets wilted |
| L. krylovii | B-226 | L. corniculatus | B-221 | 7 | Florets wilted |
| | B-226 | L. sp. | B-181 | 3 | Florets wilted |
| | B-198 | L. corniculatus | B-221 | 4 | 1 floret wilted;<br>3 legumes produced; dried up |
| | B-226 | L. tenuis | B-309 | 4 | Florets wilted |

TABLE 4 (continued)

| Female parent | | Male parent | | No. of florets | Results |
|---|---|---|---|---|---|
| L. krylovii | B-226 | L. angustissimus | B-146 (4x) | 5 | Florets wilted |
| | B-226 | L. pedunculatus | B-201 | 8 | 7 florets dried up; 1 legume produced; dried up |
| L. angustissimus | B-146 (4x) | L. krylovii | B-226 | 5 | 3 florets wilted 2 legumes produced; dried up |
| | B-146 | L. tenuis | B-309 | 18 | 11 florets wilted 7 legumes produced; dried up |
| | B-146 | L. pedunculatus | B-201 | 6 | 3 florets wilted 3 legumes produced; dried up |
| | B-146 | L. purshianus | B-489 | 4 | Florets wilted |
| | B-146 | L. sp. | B-181 | 5 | 3 florets wilted 2 legumes produced; dried up |
| L. pedunculatus | B-201 | L. sp. | B-181 | 9 | 6 florets wilted 3 legumes produced; dried up |
| | | L. tenuis | B-309 | 8 | 6 florets wilted 2 legumes produced; dried up |

In the majority of the crosses, the flower merely wilted after pollination, producing no tangible results (see Table 4).

## 7. Hydrogen cyanide tests

All of the five native Canadian Lotus species were found to have a negative reaction when tested for the presence of hydrogen cyanide. Wherever possible, without damaging the specimens, a leaf was removed from each herbarium specimen so that it could be tested. If two or more plants were mounted on the same sheet, a leaf from each plant was taken. Leaves from plants grown from seed were also tested for the presence of this compound.

In all, 60 plants of L. purshianus, 121 plants of L. micranthus, 116 plants of L. denticulatus, 15 plants of L. pinnatus, 11 plants of L. formosissimus and 53 plants of L. pedunculatus were tested. All proved to be negative (Table 5).

Of the 28 plants tested belonging to L. tenuis, 15 were negative and 13 gave a weakly positive reaction (Table 5). Of the 20 plants belonging to the species L. krylovii which were tested, 15 gave a negative reaction and 5 gave a very weakly positive reaction (Table 5). Lotus corniculatus was the only species

TABLE 5.--Chromosome number, reproductive characteristics, life form, and HCN reaction of Canadian *Lotus* species

| Species | Chromosome number | Annual or Perennial | Self or Outcross | Style length (mm.) | Pollen size (in relative units) | Seed size (mm. in length) | HCN |
|---|---|---|---|---|---|---|---|
| *L. denticulatus* | 12 | A | S | 2.0 | 13.4 | 2.95 | - |
| *L. micranthus* | 14 | A | S | 0.7 | 11.1 x 8.5 | 2.17 | - |
| *L. purshianus* | 14 | A | S | 2.5 | 12.5 | 2.99 | - |
| *L. pinnatus* | 14 | P | O | 2.3 | 13.3 | 2.31 | - |
| *L. formosissimus* | 14 | P | O | 2.3 | 13.1 | 1.59 | - |
| *L. corniculatus* | 24 | P | O | 5.2 | 10.5 x 7.6 | 1.30 | -;±;+ |
| *L. tenuis* | 12 | P | O | 5.5 | 8.7 x 7.0 | 1.29 | -;± |
| *L. pedunculatus* | 12 | P | O | 5.6 | 8.13 x 6.7 | 0.93 | - |
| *L. krylovii* | 12 | A | S | 4.0 | 9.6 x 2.3 | 1.42 | -;± |

which gave a strong, positive reaction. One hundred and seven plants were tested; 72 proved to be negative, 25 weakly positive and 10 very strongly positive (Table 5).

## 8. Thin-layer chromatography

### (a) Fresh material

Representative phenolic patterns of unhydrolysed leaf extracts of the nine species of Lotus growing in Canada are shown in Figure 35. All the material used was picked in the evening, weighed, and immediately placed in a stoppered vial containing 1% HCL in methanol. The chromatograms were run the next day. Although the exact procedure was followed each time, the finished plates showed slight variations from day to day and from plate to plate. This variation consisted of a change in the $R_f$ value of the spots. Therefore, species which were to be compared were run on the same plate, if possible, to ensure that what was being interpreted as a different phenolic pattern was not due to some extrinsic factor, such as temperature.

The patterns illustrated for L. pinnatus, L. formosissimus, L. purshianus and L. micranthus were copied from a single plate (Fig. 35, A-D respectively, and Fig. 36). The L. denticulatus leaf extract was run on another plate. In the figure, three spots at $R_f$ 0.2

and three spots between $R_f$ 0.3 and 0.4 for this species Fig. 35, E and Fig. 37), were slightly adjusted for easier comparison with the corresponding positions of the four species mentioned above.

The patterns illustrated for L. pedunculatus, L. tenuis, L. corniculatus and L. krylovii (Fig. 35, F-I respectively, and Fig. 38), the four introduced species, were also developed on a single plate and drawn from this plate.

Of all the variation, the least was shown in the pink spots. Except for the lowest pink spot, these travelled with the first solvent, cyclohexane-ethylacetate, to nearly the exact same spot each time. Therefore, any difference found in these pink spots was a reliable one. However, these pink spots showed the least specific variation. In four of the five native Canadian species, the only difference found in these pink spots was in their intensity and size. The differences must therefore, be quantitative, not qualitative, ones. Lotus denticulatus, the only native Canadian Lotus species with a basic chromosome number of $n = 6$ had two differences in its pink spots (Fig. 35, E, and Fig. 37). The faint pink spot which was third from the top ($R_f$ 0.8) in the other four species was missing in L. denticulatus and the faint pink spot found fourth from the top ($R_f$ 0.6) was an extra one.

With the exception of a very faint pink spot, third from the top ($R_f$ 0.7) in L. corniculatus (Fig. 35, H, and Fig. 38), there was also no difference in the pink spots which ran to the top half of the plate ($R_f$ 0.5-0.10), in the introduced species found in Canada. As mentioned before, these pink spots were fairly stable from species to species and it was not surprising that they were identical in the three diploid species closely related to the tetraploid L. corniculatus. The pink spots of the native species, did, however, differ from the pink spots of the introduced species.

Most of the variability in the phenolic patterns was found in the variously coloured spots close to the origin. These were the ones carried by the second solvent, methanol-chloroform, which was allowed to develop half-way up the plate to $R_f$ 0.5.

The morphological similarity of L. pinnatus and L. formosissimus was reflected in their phenolic pattern (Fig. 35, A and B, and Fig. 36). Only three differences could be noted. The green-brown spot near the origin ($R_f$ 0.05) was traversed by a light yellow band in L. formosissimus (Fig. 35, B, and Fig. 36) and not in L. pinnatus (Fig. 35, A, and Fig. 36). Spots 10 and 11 from the origin ($R_f$ 0.25 and 0.32) of L. pinnatus were missing in L. formosissimus.

The basic pattern of the spots near the origin in L. purshianus (Fig. 35, C, and Fig. 36) and L. micranthus (Fig. 35, D, and Fig. 36) did not differ greatly. Both of these patterns did, however, differ from the phenolic pattern of L. pinnatus and L. formosissimus. Spots 10 and 11 from the origin ($R_f$ 0.25 and 0.32), yellow and very faint blue, respectively, of L. purshianus were missing in L. micranthus. Any other differences found occurred in the colour of the spots rather than in the number of spots.

Lotus denticulatus (Fig. 35, E, and Fig. 37) differed in two ways from the two species just mentioned. Between the yellow and the bright blue band was a faint blue-grey spot ($R_f$ 0.1). Above the bright blue (which may also be yellow--see discussion on colour differences) ($R_f$ 0.12) there was a bright yellow band ($R_f$ 0.13) in L. purshianus (Fig. 35, C, and Fig. 36) and L. micranthus (Fig. 35, D, and Fig. 36) which was missing in L. denticulatus.

The phenolic patterns for the introduced species were quite different from those of the native species. However, too close a comparison of spots for these two groups was not advisable since the leaf extracts were run on separate plates. Therefore, one did not know whether the very faint blue and two faint pink (spots 12, 13 and

14; $R_f$ 0.26 to 0.3) group of spots of L. pedunculatus (Fig. 35, F, and Fig. 38) was comparable to the two faint-blue and pink orange (spots 11, 12 and 13; $R_f$ 0.3 to 0.4) group of spots of L. pinnatus (Fig. 35, A and Fig. 36). It could also be questioned whether the one bright pink spot found slightly below $R_f$ 0.2 in the native species and slightly above $R_f$ 0.2 in the introduced species was the same in all species. This pink spot was thought to be the same in all species of the native group and the same in all species of the introduced group, but it was not known if it was the identical spot in these two groups.

The most striking differences in L. pedunculatus (Fig. 35, F, and Fig. 38) from the three other introduced species (Fig. 35, G-I, and Fig. 38), were the presence of a blue-white spot just below the pink spot at $R_f$ 0.2 and the absence of the large yellow-orange spot between $R_f$ 0.1 and 0.2. Differences in the chromatograms of L. tenuis (Fig. 35, G, and Fig. 38), L. corniculatus (Fig. 35, H, and Fig. 38) and L. krylovii (Fig. 35, I, and Fig. 38) were slight, the main differences being in the intensity and colour of the spots. L. tenuis and L. krylovii both had a bright yellow spot ($R_f$ 0.2) just below the pink spot, whereas L. corniculatus (Fig. 35, H, and Fig. 38) showed only a faint yellow one. The large

spot ($R_f$ 0.15) immediately below this yellow one ($R_f$ 0.2) was faint orange-pink in L. corniculatus and was yellow-orange in both L. tenuis and L. krylovii. Other slight differences close to the origin can be seen in the illustration (Fig. 35, F-I, and Fig. 38).

(b) Colour differences

It was found that the colours (fluorescent) of the chromatogram faded very quickly when placed under ultra-violet light. A photograph was taken immediately and a drawing of the spots onto paper was made, however, the latter took 15 to 20 minutes. By that time, some of the spots near the origin had begun to change colour. The pink spots, however, did not fade or change colours. The bright blue spots faded to a yellow colour quite quickly. Bright green colours soon lessened in intensity and became more yellow. Therefore, when drawings of the phenolic patterns were compared with the coloured slides (Kodak high speed Ektachrome film) the colour of the spots was taken from the slide and where necessary, the colour indicated on the chromatogram when drawn was adjusted.

(c) Seasonal variation

The phenolic patterns shown were of leaf extracts taken from the plants in early July. They also were an example of the most intense chromatograms obtained

throughout the entire study which extended from February through July. Perhaps the intenseness of the spots could be attributed to the health and vigorous growth of the plants, which were kept outside in a cold frame at this time.

Leaves for chromatographic analyses during the winter months were taken from plants growing in the greenhouse. These plants did not grow as fast as the ones kept outside during the summer. Furthermore, frequent spraying and fumigation was necessary in the greenhouse to keep the aphid and red spider mite populations under control. Although the leaves used were always healthy and uninjured, the chromatograms obtained were fainter and thus it was sometimes more difficult to make out as many spots.

(d) Variation within species

The extent of plant to plant variation within one accession number was tested for several of the species. Leaf extracts from several plants were run on the same plate and the phenolic patterns were found to be identical. Using several species, variation between different accession numbers of the same species were also looked for. Three accession numbers of L. micranthus, two accession numbers of L. purshianus, two accession numbers of L. tenuis, and two of L. corniculatus were tested and

the phenolic pattern of leaf extracts was found to be identical.

(e) Variation with physiological condition

One experiment was run in which leaf extracts of L. corniculatus were taken from a plant which was in flower and from another plant which was not in flower. There appeared to be no difference in the two chromatograms.

There also was found to be no difference between young and old leaves of the same healthy plant for any species. One test in which a very old plant of L. micranthus, an annual, was used, showed fainter and fewer spots than from a healthy young plant. This old plant had already produced mature seed and was not growing any more.

(f) Dried material

It would be quite an advantage to be able to use leaves which have been dried. These are easier to store and then the chromatograms can all be run within several days. Fresh leaves were picked, weighed and left in paper envelopes for three to nine months. The leaves were placed in the extracting solution the evening before the chromatogram was to be run.

The dried material always had much more intensely

coloured spots than did the fresh material (Figs. 39 and 40). The pink spots especially were larger and darker and there was considerable streaking. However, differences were found in the pattern of spots of the fresh and dried material. The black spots which were found close to the origin in all of the chromatograms from fresh leaves were usually absent in the dried material.

However, changes in the phenolic patterns of the leaf extracts after being dried were observed. One very noticeable difference was the absence of the lowermost pink spot ($R_f$ 0.5) in L. krylovii (Fig. 35, I) in the dried material (Fig. 39). This pink spot, however, was still present in the other four introduced species in chromatograms of dried material. It was thought that perhaps the increased intensity of some of the spots obscured some of the fainter spots, thus changing the appearance of the chromatogram. In another experiment, fresh leaves of L. krylovii were dried in the oven at 100° C. The resulting chromatogram had even brighter pink spots than the air-dried material and the lowermost pink spot ($R_f$ 0.5), absent in the air-dried material, was present.

A few leaves were carefully removed from several herbarium specimens to see how these would differ in their phenolic pattern. In every instance, the chromatogram

was different from the fresh material and from the dried material studied. There was also found to be some variation in the phenolic patterns for different herbarium specimens of the same species (Fig. 40). Therefore, it appeared that the phenolic content of the leaves changed with the different treatments accorded the leaves. There was also a possibility that the mounting media used for mounting the specimens may have caused some intraspecific variation, but this was not tested.

Figure 35.--Phenolic patterns and spot colours of chromatograms of unhydrolysed leaf extracts.

A. Lotus pinnatus

B. Lotus formosissimus

C. Lotus purshianus

D. Lotus micranthus

E. Lotus denticulatus

F. Lotus pedunculatus

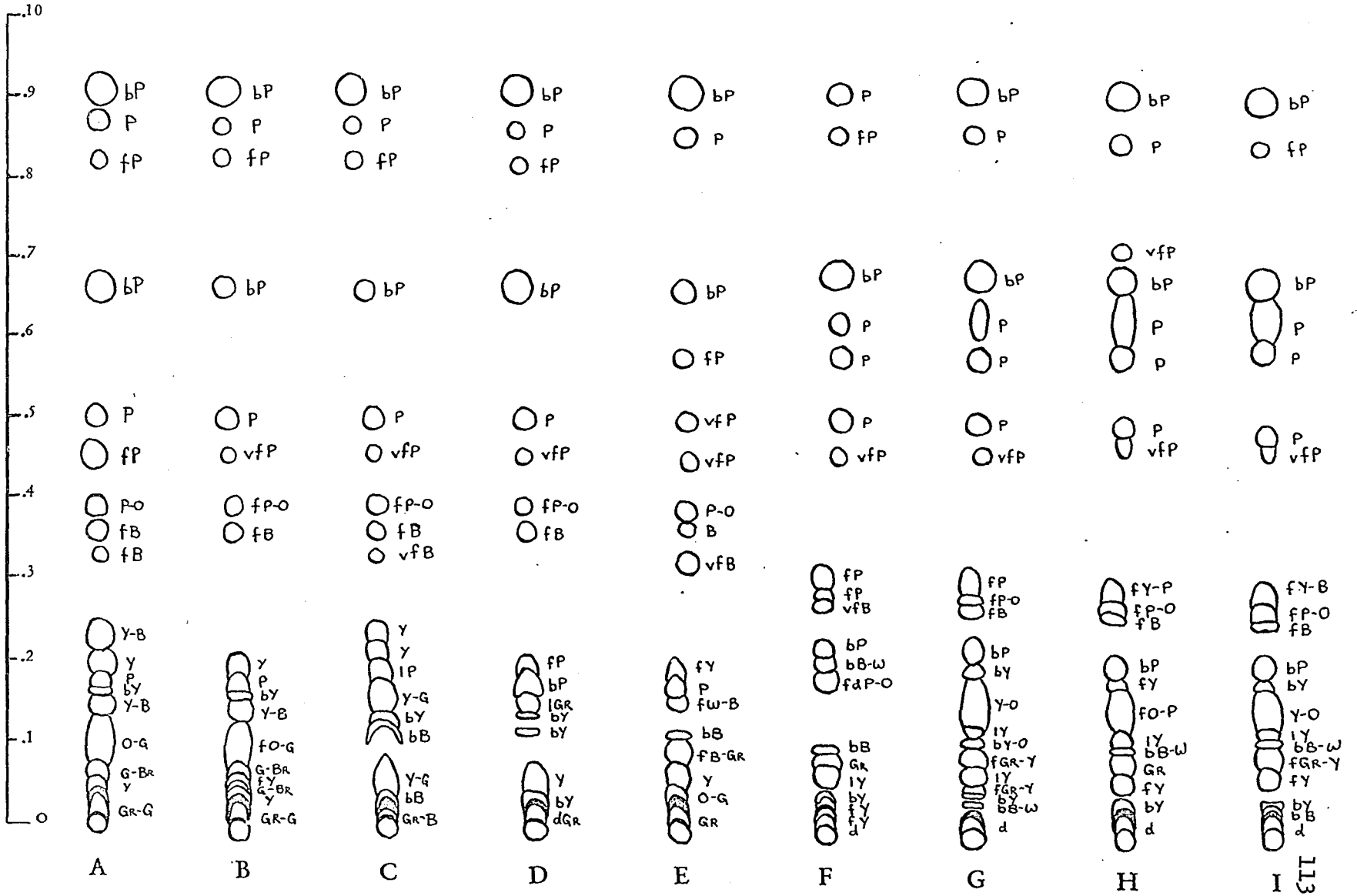
G. Lotus tenuis

H. Lotus corniculatus

I. Lotus krylovii

Spot colours:

| | | | |
|---|---|---|---|
| P, | pink | b, | bright |
| B, | blue | l, | light |
| Y, | yellow | f, | faint |
| O, | orange | d, | dark |
| G, | green | v, | very |
| Gr, | grey | | |
| Br, | brown | | |
| = | black | | |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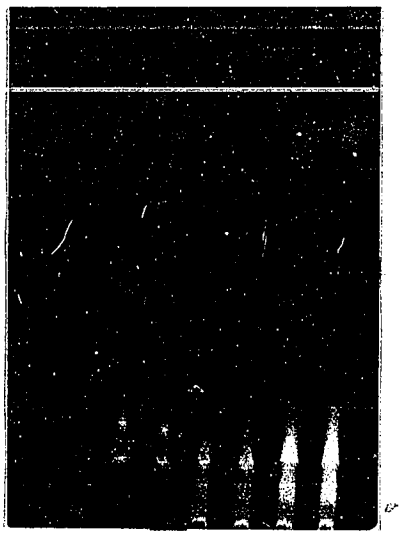

Fig. 36

Chromatogram showing phenolic pattern for (l. to r.) L. micranthus, L. purshianus, L. formosissimus and L. pinnatus.

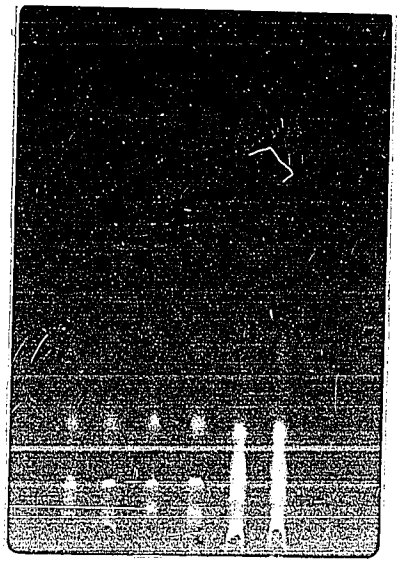

Fig. 37

Chromatogram showing phenolic pattern for (l. to r.) L. subpinnatus, L. denticulatus and L. humistratus.

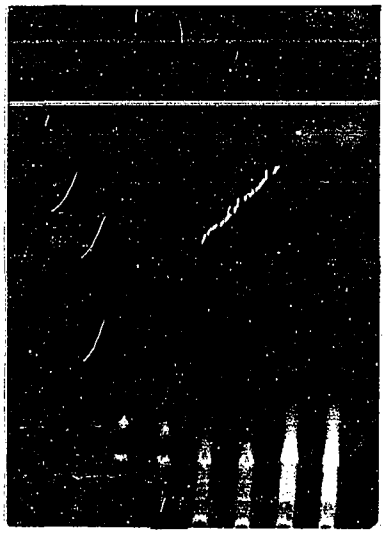

Fig. 36

Chromatogram showing phenolic pattern for (l. to r.) L. micranthus, L. purshianus, L. formosissimus and L. pinnatus.

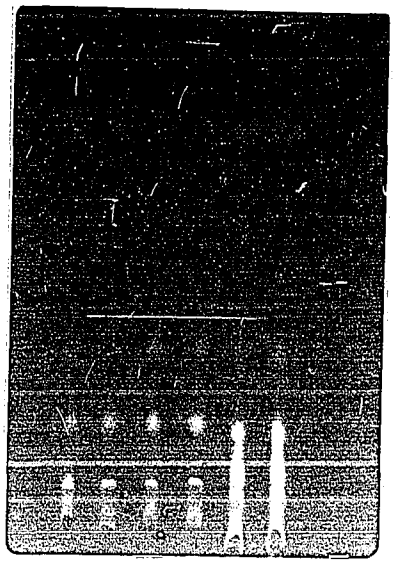

Fig. 37

Chromatogram showing phenolic pattern for (l. to r.) L. subpinnatus, L. denticulatus and L. humistratus.

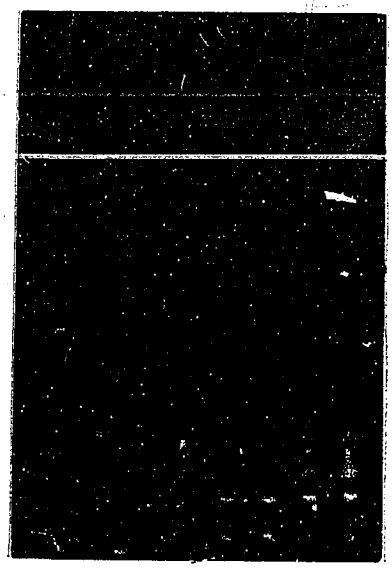

Fig. 38

Chromatogram showing phenolic pattern for (l. to r.) L. pedunculatus, L. tenuis, L. corniculatus and L. krylovii.

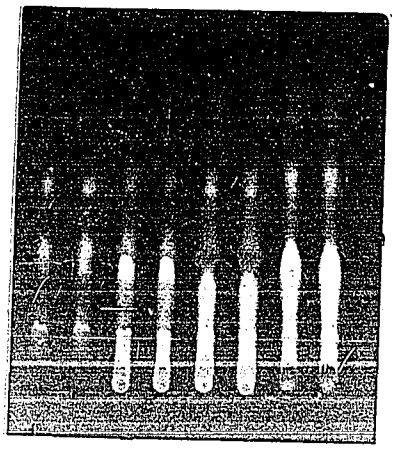

Fig. 39

Chromatogram showing phenolic pattern for (l. to r.) L. pedunculatus, L. tenuis, L. corniculatus and L. krylovii; extracts from dried material, compare with Fig. 38.

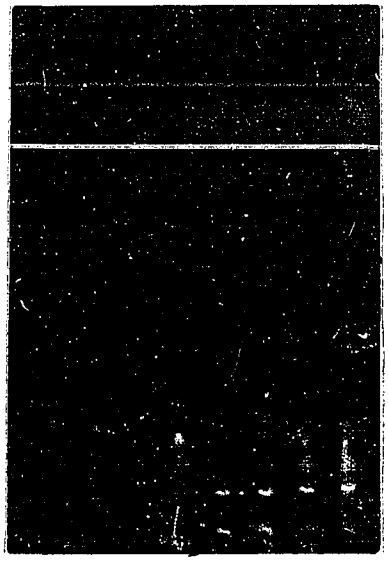

Fig. 38

Chromatogram showing phenolic pattern for (l. to r.) L. pedunculatus, L. tenuis, L. corniculatus and L. krylovii.

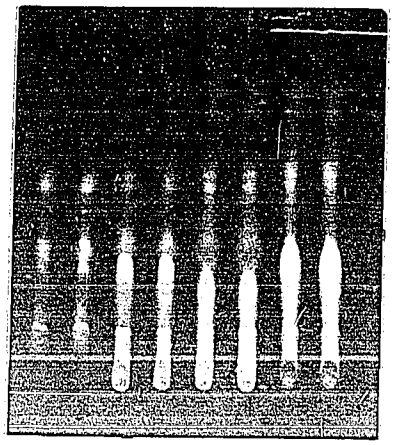

Fig. 39

Chromatogram showing phenolic pattern for (l. to r.) L. pedunculatus, L. tenuis, L. corniculatus and L. krylovii; extracts from dried material, compare with Fig. 38.

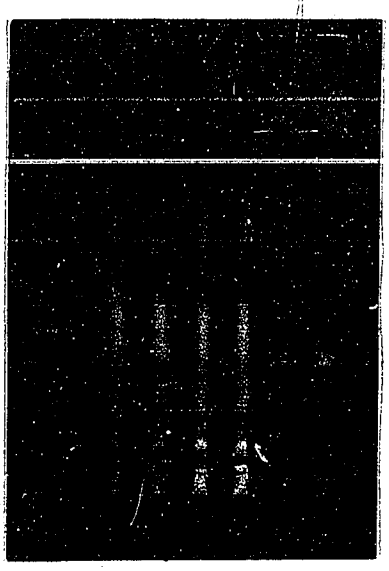

Fig. 40

Chromatogram showing phenolic pattern for Lotus denticulatus; right, fresh material; three patterns on left from three different herbarium specimens (Dried material).

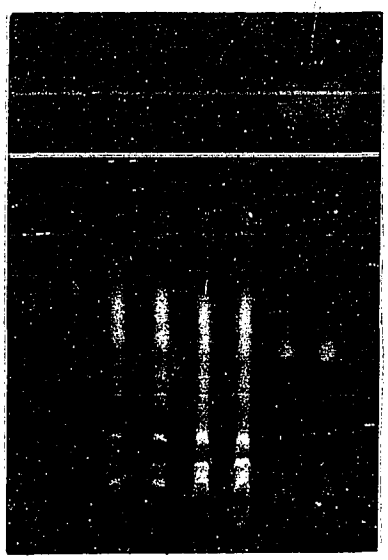

Fig. 40

Chromatogram showing phenolic pattern for Lotus denticulatus; right, fresh material; three patterns on left from three different herbarium specimens (Dried material).

## V. DISCUSSION

Nine species of Lotus were found in Canada, five native and four introduced species. Most of the interest centres around the native species, L. pinnatus, L. formosissimus, L. purshianus, L. micranthus and L. denticulatus, and their relationship to each other. It is difficult to say too much about relatedness of these species found in Canada without having studied in detail the material of the United States, since most of these species are far-ranging down the Pacific coast. However, some similarities and differences can be pointed out.

Lotus pinnatus and L. formosissimus must be very closely related, if morphological, cytological and chromatographic characteristics are true indicators of relatedness. Morphologically, the species can be separated reliably only by the absence of a bract in L. pinnatus and by flower colour. As well, seeds of L. formosissimus are about one-half the size of those of L. pinnatus. Other differences between the species are quantitative and are not as reliable because of overlap

of characters. Both species have a chromosome number of $2n = 14$ with chromosomes of similar shape, as shown in the idiograms (Fig. 32, A and B). There is only a slight difference in the sizes of the complements, that of L. formosissimus being 1.66 u larger, on the average, although it has not been shown that the difference is statistically significant. The relative size of each chromosome of the complement is nearly identical in both species (Table 3). It is of course important to realize that if two karyotypes are similar, it does not necessarily follow that their genetic make-up is similar. Gene mutation, small translocations, inversions, and even large, reciprocal translocations are not detected by this method.

Of the native species, L. pinnatus and L. formosissimus are the only two perennial, outcrossing ones, with large flowers borne on long-pedunculate umbels (Table 5). The author thinks it justifiable in placing them in the subgenus Hosackia according to Ottley (1923). Callen's (1959) subgenus Edentolotus which contains all species with a simple, erect style and in which all the Canadian species, including those from the Old and New World, are placed, does not seem natural to the author. Style character is certainly important, but not at the expense of all other distinguishing characteristics.

Lotus micranthus, L. purshianus and L. denticulatus are quite different from the two species just discussed. As well, they are not so obviously closely related to each other as L. pinnatus and L. formosissimus are. These three species are all annuals, self-fertilizing and have small, solitary flowers (Table 5). Lotus micranthus and L. purshianus are similar in that the flowers are borne on short, bracted peduncles. The species are easily distinguished, however, by flower size, number of leaflets per bract, length of calyx teeth and leaf number. Lotus purshianus, with three leaflets per leaf, is the only native Canadian species with a constant number of leaflets per leaf (Table 2).. This prompted Ottley (1944) to suggest that a species such as L. purshianus, with three leaflets and glandular stipules, may be an ancestral type. In the Old World, such a type could have given rise to species with a constant number of leaflets, i.e., five, and in the New World to species with leaflets which vary in number and position. It is unlikely that L. purshianus itself is the ancestral type since it is an annual and autogamous, which is indicative of a more advanced status. However, an ancestor of L. purshianus may indeed have given rise to the various Old and New World species.

Lotus denticulatus differs from L. micranthus and

L. purshianus mainly in that it has subsessile flowers, lacking a bract. Lotus denticulatus is also distinguished from all other native Canadian species in having a somatic chromosome number 2n = 12, L. micranthus and L. purshianus having 2n = 14.

Idiograms, which are diagrammatic representations of the karyotype, show fairly extensive differences in shape and relative sizes of the chromosomes of L. purshianus, L. micranthus and L. denticulatus (Fig. 33, A-C). Differences in karyotype are greater between these three species than between L. pinnatus and L. formosissimus. This would be expected due to their greater morphological variability. The most common chromosome type is a submetacentric one, although in the chromosomes of L. denticulatus the centromere tends more towards the middle of the chromosome (Fig. 33, A).

The annual habit usually stems from a perennial one (Stebbins, 1950) and a reproductive system which is self-fertile from one which is predominantly outcrossing (Stebbins, 1957). This argument puts L. pinnatus and L. formosissimus as more primitive species than L. purshianus, L. micranthus and L. denticulatus. The hypothesis that plants which self have flowers of a smaller size (Grant, 1956) is substantiated in the case of these five species. Autogamous plants have no need for large, showy flowers so it would seem natural that,

along with a reduction in flower size, one sees a reduction in length of the peduncle. This would place L. denticulatus as the most highly evolved of the native Canadian species, since its flowers are subsessile.

In an aneuploid series, it is more usual that the smaller chromosome number evolved from a larger one (Stebbins, 1950). This seems to be the case in Lotus, as already suggested by Larsen and Zertova (1965). Two basic chromosome numbers are found, $n = 6$ and $n = 7$. Only one native species, L. denticulatus, has a basic number $n = 6$. This species has a total complement length shorter by 2.82 to 5.56 microns than any of the four other species (Table 3). This would seem to be in support of the hypothesis that $n = 6$ was derived from $n = 7$, since loss of chromatin is more likely than gain, except in the case of polyploidy.

Of the introduced species, L. corniculatus was found to be the most variable in morphology and it was sometimes difficult to distinguish it from L. pedunculatus or L. tenuis. Distinguishing characteristics are a higher leaf index in L. tenuis (Table 2), i.e., narrower leaflets, and a higher number of florets per inflorescence (Table 2), as well as divergent calyx teeth in L. pedunculatus. The writer did not find a significant difference in the length of calyx teeth between L. pedunculatus and

L. corniculatus, as has been suggested by Boivin (1960). Calyx index for both species averaged to 1.82 (Table 2), therefore, not a reliable character by which to separate them.

Lotus krylovii, although belonging to the L. corniculatus group, differs considerably morphologically from the three other introduced species. It is an annual, is self-fertile and has solitary flowers which are borne on shorter peduncles (Tables 2 and 5). As well as differing morphologically, L. krylovii has a chromosome complement which differs considerably in shape and relative size of chromosomes from those of both L. pedunculatus and L. tenuis (Fig. 30, B). The total complement of L. krylovii is larger by 5 u when compared with that of L. pedunculatus and by 3.9 u when compared with L. tenuis (Table 3).

All the introduced species have a basic chromosome number $n = 6$, with L. corniculatus being a tetraploid. Since polyploidy is essentially irreversible (Stebbins, 1950), Lotus corniculatus is taken to be the youngest species (Larsen and Zertova, 1965).

When the idiograms of the New and Old World species are compared, similarities stand out more than differences. Although a survey of more species is necessary, it seems justified, on the basis of this as well as morphological

evidence, to place members of both groups in the same genus. This is in agreement with the findings of Larsen (1956).

Since no interspecific hybrids were produced (Table 4), the only conclusion that can be drawn is that the native species, L. purshianus, L. micranthus and L. denticulatus, have effective reproductive isolating barriers, although more emasculations and cross pollinations, utilizing new techniques should be tried. Putative hybrids were obtained in the reciprocal crosses of L. denticulatus and L. subpinnatus (Table 4). These two species seem to be very closely related and are sometimes difficult to distinguish morphologically, especially from herbarium material. However, L. subpinnatus is not found in Canada. Legumes were produced in several of the other crosses attempted (see Table 4), however, it was not always possible to ascertain whether fertilization had taken place since the legumes dried up early. Lotus purshianus was often stimulated to produce legumes, although these were always empty. Hybrids have been reported between species of the L. corniculatus group (Grant, 1965), although the author did not succeed in producing any.

It would be of great interest to attempt to hybridize L. pinnatus and L. formosissimus. Their similar

morphology and karyotypes suggest a certain amount of chromosome homology making it more likely that inter-specific hybridization would be successful. As can be seen from the distribution map (Fig. 11), these species do not have overlapping distribution, making it likely that, in Canada at least, they have not developed an effective isolating barrier.

Thin-layer chromatography was found to be a useful technique in the biosystematic study of Lotus. Paper chromatographic techniques have been used before in the study of this genus (Harney and Grant, 1965). However, advantages of thin-layer over paper chromatography are that it takes less time, less space and less material (only seven ul). A disadvantage is that the system is extremely sensitive to factors such as temperature and tank saturation, which greatly affect $R_f$ values and spot separation.

It was not within the scope of the present study to identify the separated compounds which fluoresced in ultraviolet light. Identification of the phenols is essential in further studies to make full use of the technique and for reliable comparisons between species.

Examination of the chromatograms showed that different species could be identified by their phenolic pattern (Fig. 35). The chromatographic pattern shown for

each species is a start at presenting a "biochemical profile" which was suggested by Alston and Turner (1959) for the identification of species. However, when pattern differences on the chromatograms are studied and relationships suggested from them, it is important to keep in mind that the spots are chemical compounds, not just "spots or patterns of spots on chromatograms" (Hagen, 1961).

The chromatograms of L. pinnatus and L. formosissimus bear out the earlier conclusion that these species are closely related. Only three differences are seen on their chromatograms (Fig. 35, A-B, and Fig. 36). This indicates the close biochemical similarity, at least in the secondary phenolic compounds, of these two species. Of the three other native species, the chromatograms of L. purshianus and L. micranthus are more similar to each other than to that of L. denticulatus (Fig. 35, C-E, Figs. 36 and 37). This is in keeping with the earlier conclusion that L. purshianus and L. micranthus are more closely related to each other than to L. denticulatus. This conclusion is based on the hypothesis that morphological similarities are the result of underlying physiological, and therefore, biochemical, similarities (excluding parallel evolution).

The phenolic patterns for the introduced Canadian

species did not differ extensively from one another (Fig. 35, F-I, and Fig. 38). Perhaps this is because the species are closely related, all belonging to the L. corniculatus group. Greatest difference was found in the chromatogram of L. pedunculatus (Fig. 35, F, and Fig. 38). The chromatograms of L. tenuis and L. krylovii were very similar, and the chromatogram of L. corniculatus differed only slightly from these (Fig. 35, G-I, and Fig. 38). This is contrary to the findings of Harney and Grant (1965) who found that the phenolic residues of L. tenuis were quite different from those of the other species in the group. However, this may be due to the different technique used.

The pattern of pink spots in the upper half of the chromatogram shows the least variation. Differences in these spots are evident, however, between the native and the introduced species (Fig. 35). Lotus denticulatus has some of the pink spots in common with both groups (Fig. 35, E), perhaps because it has characteristics common to both groups. It is a native North American species but it has a basic chromosome number $n = 6$. More phenolic patterns must be studied, and their chemistry known, before it can be certain which spots, or compounds, are characteristic of a certain condition, whether it is morphological, cytological or a matter of geographic distribution.

For the results of this chromotographic technique to be reliable, care must be taken in seeing that the leaf samples are fresh and are treated identically, since changes were seen to occur on drying (Figs. 39 and 40).

This study was conducted on Canadian Lotus species, and as such, did not include many related species growing in the United States. While this investigation is a start, a similar study of American Lotus is necessary to be able to draw firmer conclusions about relatedness. Only then, can the true position of Canadian Lotus in this world-wide genus be known.

## VI. SUMMARY AND CONCLUSIONS

1. Herbarium specimens of the genus Lotus were borrowed from eighteen Canadian herbaria and from two American herbaria.

2. Each of the 367 Canadian specimens was identified and at least three measurements of each character studied, were taken wherever possible. Each of the 349 herbarium specimens from Washington State was identified.

3. Five native species of Lotus were found in Canada, L. pinnatus Hook., L. formosissimus Greene, L. purshianus (Benth.) Clem. and Clem., L. micranthus Benth. and L. denticulatus (Drew) Greene. As well, four introduced species, L. corniculatus L., L. tenuis Waldst. et Kit., L. pedunculatus Cav. and L. krylovii Schischk. and Serg. were found to grow in Canada.

4. Studies were made on style and pollen size and shape, seed size, life form and manner of reproduction, as well as on vegetative and reproductive characters.

5. A key was prepared for easier identification of the nine species, as well as a description of each

species, based on observations of fresh and dried material.

6. A distribution map was prepared for each species. Lotus pinnatus and L. formosissimus were found only on Vancouver Island, British Columbia and their areas of distribution did not overlap. Lotus micranthus was limited mainly to Vancouver Island and surrounding islands, although a few collections were from Vancouver, on the mainland. Lotus denticulatus was found to be more widespread, ranging throughout British Columbia. Lotus purshianus was found on Vancouver Island and in Southern Saskatchewan and Manitoba. Of the introduced species, L. corniculatus ranged throughout Canada and L. tenuis and L. pedunculatus were found in eastern and western Canada. Lotus krylovii was found to be distributed in a limited area of British Columbia.

7. The following somatic chromosome numbers were reported: L. pinnatus, $2n = 14$; L. formosissimus, $2n = 14$; L. purshianus, $2n = 14$; L. micranthus, $2n = 14$; L. denticulatus, $2n = 12$; L. corniculatus, $2n = 24$; L. tenuis, $2n = 12$; L. pedunculatus, $2n = 12$; L. krylovii, $2n = 12$. Chromosome numbers for L. pinnatus, L. formosissimus and L. micranthus were reported for the first time. Chromosome numbers for the other species were in agreement with previous reports.

8. Drawings of the karyotype of each of the nine species are presented. Measurements were made of the chromosome complement of ten cells for each of the eight species (excluding L. corniculatus). Standard deviations for percentage TCL, L/S and T/L were performed on a 1620 IBM computer and idiograms, based on the results, were prepared.

9. Interspecific hybridizations were attempted between the species. Although several putative hybrid embryos were obtained (L. denticulatus x L. subpinnatus), the author was unsuccessful in obtaining a mature hybrid plant.

10. Results of HCN tests were negative for the five native species, as well as for L. pedunculatus, an introduced species. Lotus corniculatus, L. tenuis and L. krylovii had some plants which responded positively and some plants which responded negatively.

11. Thin-layer chromatographic patterns of secondary phenolic compounds of unhydrolysed leaf extracts are presented. Specific differences were found in the patterns, making this a useful tool in a biosystematic study.

12. Chromatograms of dried material were found not to be suitable for identification of taxa since changes take place in the phenols upon drying. Therefore,

for consistent results, fresh leaf material should be used. If dried material is to be used it must all be treated in an identical manner.

13. The relationships of the Canadian Lotus species to each other, and the place of the native Canadian species in this world-wide genus was discussed. It was concluded that the native Canadian species belong to Lotus not to Hosackia as had been claimed by several earlier authors.

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