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Karyotypes and Idiograms of Some Western North American Species of Lotus (Fabaceae)

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KARYOTYPES AND IDIOGRAMS OF SOME WESTERN NORTH AMERICAN SPECIES OF *LOTUS* (FABACEAE)

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ABSTRACT

Karyotypes and idiograms are presented for 12 species (*L. argophyllus* var. *argophyllus, L. crassifolius, L. dendroideus* var. *dendroideus, L. grandiflorus, L. humistratus, L. oblongifolius* var. *oblongifolius, L. stipularis, L. scoparius* var. *scoparius, L. salsuginosus* var. *salsuginosus, L. rigidus, L. wrangelianus, L. wrightii)* and 3 varieties (*L. argophyllus* var. *argenteus, L. nevadensis* var. *douglasii, L. scoparius* var. *brevialatus)* of *Lotus* L. (Fabaceae) belonging to four different groups of the genus (*Hosackia, Syrmatium, Microlotus,* and *Simpteria*). The chromosome number for *L. dendroideus* var. *dendroideus* ($2n = 14$) is reported for the first time. Tetraploid cells ($2n = 28$) were observed in a root tip of *L. grandiflorus* in an otherwise diploid plant. Chromosome number differences between the species in group V (*Microlotus*) conform to the taxonomic arrangement by Isely in 1981 separating the species into those with $n = 6$ and those with $n = 7$. No correlation was found for chromosome length between annuals and perennials. With the exception of *L. crassifolius,* the longest chromosome of the complement in the other taxa is clearly distinguished from the second longest chromosome by its greater length averaging a difference of 3.47%. In each taxa, two or more chromosomes have the same length making it impossible to recognize individual chromosomes. It is considered that chromosome morphology alone is not sufficient to separate the North American species into different taxonomic groupings.

Key words: chromosome numbers, Fabaceae, idiograms, karyotypes, *Lotus* species, western North America.

INTRODUCTION

Of the chromosome numbers reported for the 30 indigenous North American species and 12 varieties of *Lotus* (Grant 1995), karyotypes have been published for only five species (Zandstra and Grant 1968). Karyotype analyses and idiograms are presented here for an additional 12 species and 3 varieties. The somatic chromosome number is 14 for all the species with the exception of *L. humistratus* and *L. wrangelianus* for which the somatic chromosome number is 12.

MATERIALS AND METHODS

The taxa studied and their taxonomic groupings are listed in Table 1. The source of the taxa are given in Table 2. Herbarium specimens are deposited in the herbarium of the Canada Department of Agriculture Ottawa (DAO).

Several plants were raised from seeds for each accession. Root tips were pretreated with 0.002 M 8-hydroxyquinoline for 1 h (Tjio and Levan 1950) and fixed in 3:1 95% ethanol:glacial acetic acid. The chromosomes were stained employing the Feulgen method (Darlington and LaCour 1976). Root tips were prepared for maceration by immersion in 4% pectinase (to facilitate spreading of the cells) for 1.0 to 2.0 h and stored in 70% ethanol. Temporary slides were prepared by squashing the root-tip meristems in 45% acetic acid on a microscope slide and sealing the coverslips with clear fingernail polish or rubber cement. Temporary mounts could be kept for a minimum of two weeks by placing them on a wet filter paper in a covered petri dish, which was stored in a refrigerator to prevent dehydration. With the aid of a Zeiss drawing apparatus, karyotypes of the somatic chromosomes were prepared for each taxon.

For the construction of idiograms, measurement were made of the entire chromosome complements (Zandstra and Grant 1968). Measurements from the drawings of the chromosomes were made using calipers. The average length of each set (chromosome pair), percent chromosome and arm lengths and centromere characteristics, were calculated, and the idiograms drawn, by using the chromosome analysis package CHROMPAC III (Green et al. 1984). The chromosomes were drawn as percentage of total complement length (vertical axis) and are arranged in order by decreasing length.

RESULTS AND DISCUSSION

Measurements from the karyotypes for the different taxa are given in Table 3. Idiograms of the taxa are presented in Fig. 1–15. With the exception of *L. humistratus* and *L. wrangelianus* which have somatic chromosome numbers of 12, all of the other taxa are 2n =

Table 1. Taxa and their taxonomic groupings (after Isely 1981).

III. *Hosackia*
L. crassifolius (Benth.) Greene
L. oblongifolius (Benth.) Greene var. *oblongifolius*
L. stipularis (Benth.) Greene
IV. *Syrmatium*
L. argophyllus (Gray) Greene var. *argenteus* Dunkle
L. argophyllus var. *argophyllus*
L. grandiflorus (Benth.) Greene
L. nevadensis (Wats.) Greene var. *douglasii* (Greene) Ottley
L. scoparius var. *brevialatus* Ottley
L. scoparius (Nutt.) Ottley var. *scoparius*
V. *Microlotus*
L. humistratus (Benth.) Greene
L. salsuginosus Greene var. *salsuginosus* Isely
L. wrangelianus Fisch. & Meyer (*L. subpinnatus* Lag.)
VI. *Simpteria*
L. dendroideus Greene var. *dendroideus*
L. rigidus (Benth.) Greene
L. wrightii (A. Gray) Greene

14 (Grant 1995). This is the first chromosome number report for *L. dendroideus* var. *dendroideus* ($2n = 14$).

In contrast to European species in which both diploid and tetraploid chromosome numbers are known, all of the North American species are diploid (Grant 1991). However, a single root tip of *L. grandiflorus* in an otherwise diploid plant had tetraploid cells ($2n = 28$) (Fig. 16). A similar observation was made in root tips of the European species *L. gebelia* Vent. (Grant 1965).

As a result of pretreatment of the root tips with 0.002 M 8-hydroxyquinoline (for ease in counting) the chromosomes were uniformly contracted so that the measurements for the total length of the somatic chromosomes is considered to be somewhat shorter than would have been the case without pretreatment (Tjio and Levan 1950).

Chromosome number differences between the species in group V (*Microlotus*) conform to the taxonomic arrangement by Isely (1981) separating the species into those with $n = 6$ and those with $n = 7$.

As may be seen from the idiograms, similarities in chromosome morphology exist among the chromosomes for all groups. The species in group III (*Hosackia*) on the average have the greatest total complement length (TCL averages 28.41 μm). The average TCL for the other groups averages 19.35 μm. No correlation was found for chromosome length between annuals and perennials. One species, *L. grandiflorus* (group IV, *Simpeteria*) has the greatest TCL of all the species. However, this species exceeds the TCL of *L.*

Table 2. Source of taxa. Herbarium specimens are deposited in the herbarium of the Canada Department of Agriculture and Agri-Food, Ottawa (DAO).

L. argophyllus var. *argenteus*: Botanic Garden Santa Ana Canyon, Orange Co., California, 1950, from plant No. 4188; Origin: from Santa Catalina Island, N.W. side of Isthmus Harbor in rocky dry clay; Los Angeles Co., June 24, 1941; Collector C. B. Wolf, No. 10888. Rancho Santa Ana No. 7365.
L. argophyllus var. *argophyllus*: Foothills of the Sierra Nevada, elevation between 1800 and 2500 feet in eastern central Fresno Co., California, near Auberry, collector R. Bacigalupi No. 192, 1955.
L. crassifolius: Botanic Garden Santa Ana Canyon, Orange Co., California, 1950, from plant No. 4188; Origin: from Santa Catalina Island, N.W. side of Isthmus Harbor in rocky dry clay; Los Angeles Co., June 24, 1941; Collector C. B. Wolf, No. 10888. Rancho Santa Ana No. 7365.
L. dendroideus var. *dendroideus*: Los Angeles Co., Santa Catalina Island, California, N.W. side of Isthmus Harbor; dry rocky, clay, sun; collector: C. B. Wolf, No. 10886, June 24, 1941; Ranch Santa Ana No. 7455.
L. grandiflorus: California; USDA Plant Introduction No. 13096.
L. humistratus: Foothills of the Sierra Nevada, at an elevation between 1800 and 2500 feet in eastern central Fresno Co., near Auberry, California, June 1955; collector Ella Carter; R. Bacigalupi No. 189.
L. nevadensis var. *douglasii*: Spokane, Washington; USDA Plant introduction No. 231451.
L. oblongifolius: Huntington Lake, California, damp place near the lake just above the Boy Scout boat landing at Lakeshore; collector R. Bacigalupi No. 204, August 30, 1955.
L. rigidus: Riverside Co., California, Palms to Pines Hywy., north base of Santa Rosa Mts., ca 2650 feet; Collector P.A. Munz No. 11581; Rancho Santa Ana Botanic Garden No. 6449, Oct. 1955.
L. scoparius var. *brevialatus*: Riverside Co., California; 9 miles east of Temecula on road to Aguanga; sunny disturbed slopes, decomposed granite; elevation 1100 feet; collector C.B. Wolf, No. 10927, June 25, 1941; Rancho Santa Ana Botanic Garden No. 4218.
L. scoparius var. *scoparius*: Orange Co., California, canyon, heavy clay loam, ca. 550 ft.; collector E.K. Balls, July 13, 1950; Rancho Santa Ana No. 7362.
L. stipularis: Amador Co., California, in foothills of the Sierra Nevada several miles south of lone; R. Gankin, July 5, 1956.
L. salsuginosus var. *salsuginosus*: California; seed from P.R. Henson, Forage and Range Section, Beltsville, C.F. 1059-09.
L. wrangelianus: Marin Co., California, 1.5 miles south of McClure's Beach, Pt. Reyes Peninsula; collector B. Crampton, June 8, 1960.
L. wrightii: Arizona; Soil Conservation Service, Pleasanton, California, No. A-11590.

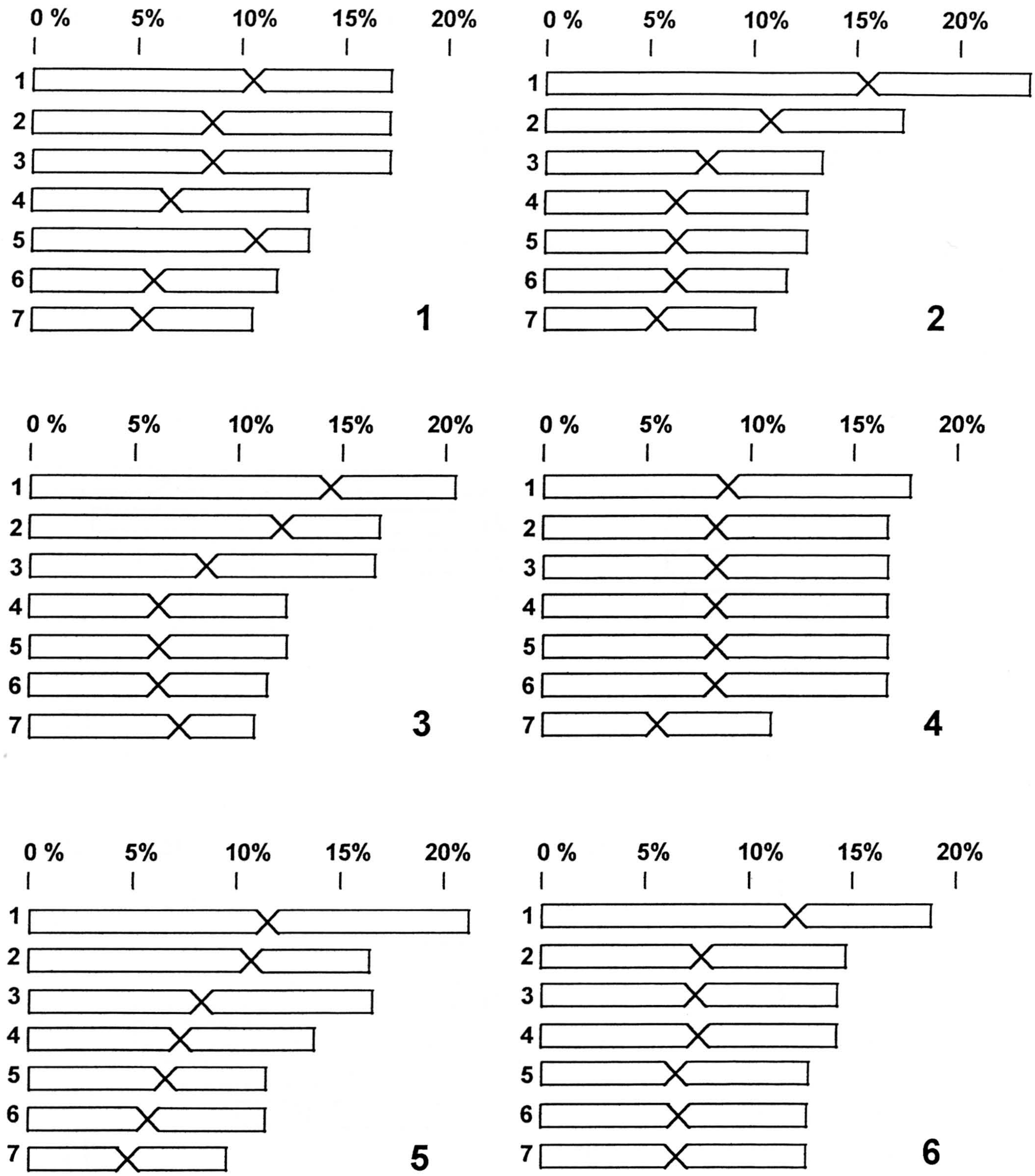


Fig. 1–6. Idiograms of western North American taxa of *Lotus*.—1–3. Group *Hosackia*.—1. *L. crassifolius*.—2. *L. oblongifolius*.—3. *L. stipularis*.—4–6. Group *Syrmatium*.—4. *L. argophyllus* var. *argenteus*.—5. *L. argophyllus* var. *argophyllus*.—6. *L. grandiflorus*. The chromosomes are drawn as percentage of total complement length (vertical axis) for each set (chromosome pair) and are arranged in order by decreasing length.

stipularis (group III, *Hosackia*) by only 0.29 μm. With the exception of *L. crassifolius* in which there is no difference in length between the longest chromosome (No. 1) and the second longest chromosome (No. 2), the longest chromosome in each species can be clearly distinguished from the second longest chromosome. On average chromosome No. 1 is 3.47% longer than chromosome No. 2, with the difference varying be-

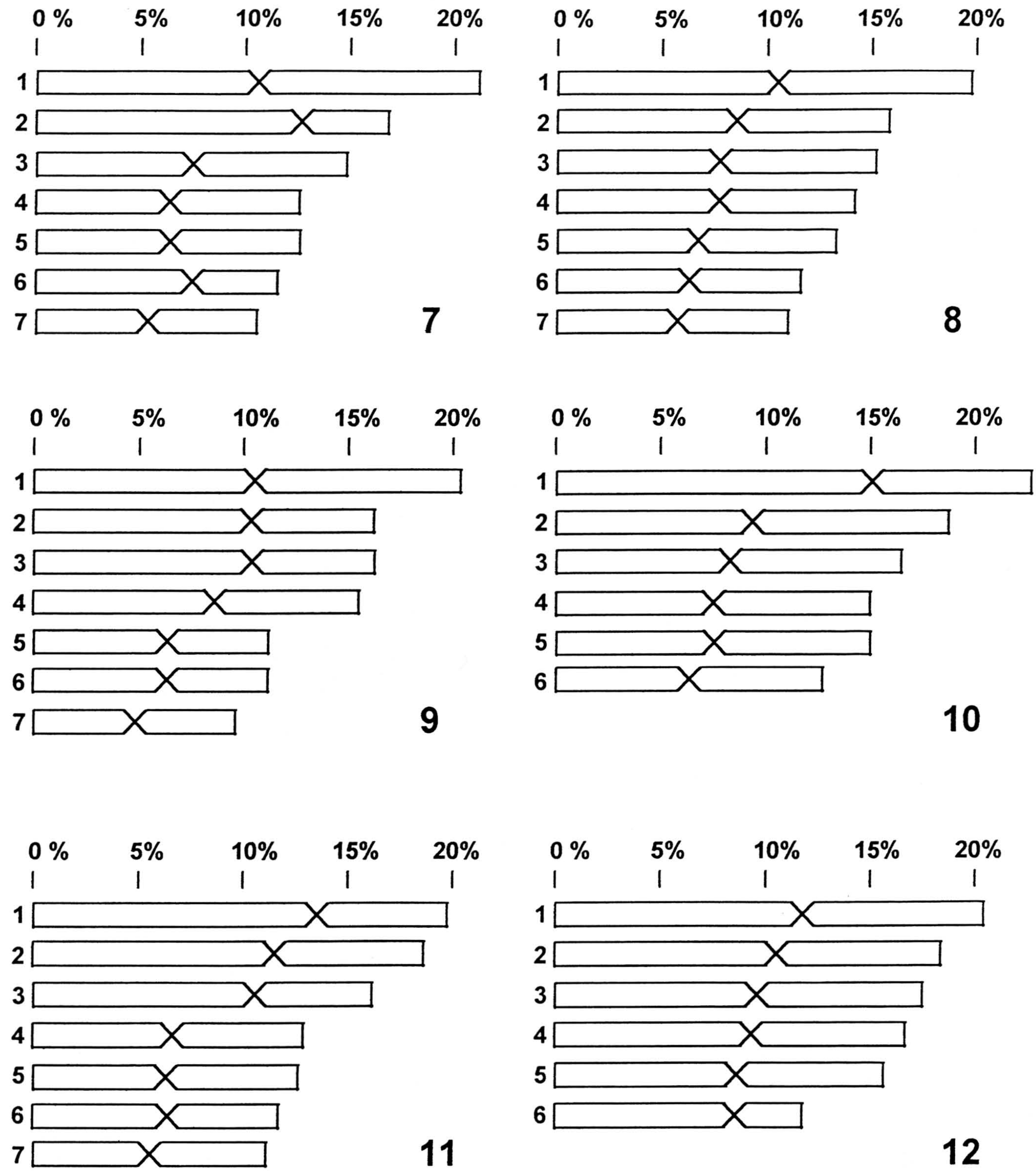


Fig. 7–12. Idiograms of western North American taxa of *Lotus.*—7–9. Group *Syrmatium* continued.—7. *L. nevadensis* var. *douglasii.*—8. *L. scoparius* ssp. *brevialatus.*—9. *L. scoparius* var. *scoparius.*—10–12. Group *Microlotus.*—10. *L. humistratus.*—11. *L. salsuginosus* var. *salsuginosus.*—12. *L. wranglianus.* Arrangement of chromosomes as described in Fig. 1–6.

tween 0.9% and 6.2%. The average percentage length difference between the first and second chromosome is similar for each taxonomic group.

For several species, the chromosome length is the same for two or more chromosomes in the same species and morphologically the chromosomes can not be told apart. Such chromosomes may be distinguished by chromosome banding. In *L. uliginosus* (*L. pedunculatus*), the two shortest chromosomes of the complement are almost identical morphologically but were

Table 3. Karyotype measurements of western North American *Lotus* species.

Species	Set	Average length (mm)	% length	% length long arm	% length short arm	Centromere* Ratio	Centromere* Type	Centromere* Index
Group III. *Hosackia***								
L. crassifolius	1	13.00	17.10	10.52	6.58	1.60	M	0.38
	2	13.00	17.10	8.55	8.55	1.00	M	0.50
	3	13.00	17.10	8.55	8.55	1.00	M	0.50
	4	10.00	13.16	6.58	6.58	1.00	M	0.50
	5	10.00	13.16	10.53	2.63	4.00	ST	0.20
	6	9.00	11.84	5.92	5.92	1.00	M	0.50
	7	8.00	10.52	5.26	5.26	1.00	M	0.50
Total length of somatic chromosomes = 28.95 μm								
L. oblongifolius	1	15.00	23.26	15.51	7.75	2.00	SM	0.33
	2	11.00	17.06	10.86	6.20	1.75	SM	0.36
	3	8.50	13.18	7.76	5.42	1.43	M	0.41
	4	8.00	12.40	6.20	6.20	1.00	M	0.50
	5	8.00	12.40	6.20	6.20	1.00	M	0.50
	6	7.50	11.62	6.19	5.43	1.14	M	0.47
	7	6.50	10.08	5.43	4.65	1.17	M	0.48
Total length of somatic chromosomes = 24.57 μm								
L. stipularis	1	17.00	20.42	14.41	6.01	2.40	SM	0.29
	2	14.00	16.82	12.01	4.81	2.50	SM	0.29
	3	13.75	16.52	8.42	8.10	1.04	M	0.49
	4	10.00	12.02	6.01	6.01	1.00	M	0.50
	5	10.00	12.02	6.01	6.01	1.00	M	0.50
	6	9.50	11.42	6.01	5.41	1.11	M	0.47
	7	9.00	10.82	7.21	3.61	2.00	SM	0.33
Total length of somatic chromosomes = 31.71 μm								
Group IV. *Syrmatium*								
L. argophyllus var. *argophyllus*	1	13.00	21.14	11.40	9.74	1.17	M	0.46
	2	10.00	16.26	9.76	6.50	1.50	M	0.40
	3	10.00	16.26	8.13	8.13	1.00	M	0.50
	4	8.50	13.82	7.33	6.49	1.13	M	0.47
	5	7.00	11.38	6.50	4.88	1.33	M	0.43
	6	7.00	11.38	5.69	5.69	1.00	M	0.50
	7	6.00	9.76	4.88	4.88	1.00	M	0.50
Total length of somatic chromosomes = 23.43 μm								
L. argophyllus var. *argenteus*	1	6.50	17.80	8.90	8.90	1.00	M	0.50
	2	6.00	16.44	8.22	8.22	1.00	M	0.50
	3	5.00	13.70	6.85	6.85	1.00	M	0.50
	4	5.00	13.70	6.85	6.85	1.00	M	0.50
	5	5.00	13.70	6.85	6.85	1.00	M	0.50
	6	5.00	13.70	6.85	6.85	1.00	M	0.50
	7	4.00	10.96	5.48	5.48	1.00	M	0.50
Total length of somatic chromosomes = 13.9 μm								
L. dendroideus	1	7.00	18.76	12.06	6.70	1.80	SM	0.36
	2	5.50	14.74	7.70	7.04	1.10	M	0.48
	3	5.25	14.07	7.17	6.90	1.04	M	0.49
	4	5.25	14.07	7.35	6.72	1.10	M	0.48
	5	4.80	12.86	6.43	6.43	1.00	M	0.50
	6	4.75	12.73	6.49	6.24	1.04	M	0.49
	7	4.75	12.73	6.36	6.37	1.00	M	0.50
Total length of somatic chromosomes = 14.21 μm								
L. nevadensis	1	10.00	21.06	10.53	10.53	1.00	M	0.50
	2	8.00	16.84	12.63	4.21	3.00	ST	0.25
	3	7.00	14.74	7.37	7.37	1.00	M	0.50
	4	6.00	12.64	6.32	6.32	1.00	M	0.50
	5	6.00	12.64	6.32	6.32	1.00	M	0.50
	6	5.50	11.58	7.37	4.21	1.75	SM	0.36
	7	5.00	10.52	5.26	5.26	1.00	M	0.50
Total length of somatic chromosomes = 18.10 μm								

Table 3. Continued.

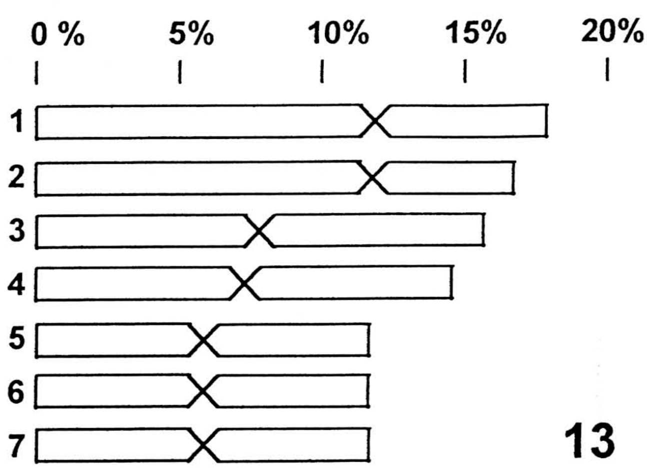
Species	Set	Average length (mm)	% length	% length long arm	% length short arm	Centromere* Ratio	Type	Index
L. scoparius v. *brevialatus*	1	9.50	19.80	10.42	9.38	1.11	M	0.47
	2	7.50	15.82	8.32	7.30	1.14	M	0.47
	3	7.25	15.10	7.81	7.29	1.07	M	0.48
	4	6.75	14.06	7.81	6.25	1.25	M	0.44
	5	6.25	13.02	6.76	6.26	1.08	M	0.48
	6	5.50	11.46	6.25	5.21	1.20	M	0.45
	7	5.25	10.94	5.73	5.21	1.10	M	0.48
Total length of somatic chromosomes = 18.29 μm								
L. scoparius var. *scoparius*	1	8.25	20.38	10.49	9.89	1.06	M	0.49
	2	6.50	16.05	10.26	5.79	1.77	SM	0.36
	3	6.50	16.05	10.26	5.79	1.77	SM	0.36
	4	6.25	15.44	8.64	6.80	1.27	M	0.44
	5	4.50	11.12	6.18	4.94	1.25	M	0.44
	6	4.50	11.12	6.18	4.94	1.25	M	0.44
	7	4.00	9.88	4.94	4.94	1.00	M	0.50
Total length of somatic chromosomes = 15.43 μm								
Group V. *Microlotus*								
L. humistratus	1	9.00	22.50	15.00	7.50	2.00	SM	0.33
	2	7.50	18.76	9.38	9.38	1.00	M	0.50
	3	6.50	16.26	8.13	8.13	1.00	M	0.50
	4	6.00	15.00	7.50	7.50	1.00	M	0.50
	5	6.00	15.00	7.50	7.50	1.00	M	0.50
	6	5.00	12.50	6.25	6.25	1.00	M	0.50
Total length of somatic chromosomes = 15.24 μm								
L. salsuginosus	1	11.00	19.56	13.33	6.23	2.14	SM	0.32
	2	10.50	18.66	11.56	7.10	1.63	M	0.38
	3	9.00	16.00	10.67	5.33	2.00	SM	0.33
	4	7.25	12.88	6.66	6.22	1.07	M	0.48
	5	7.00	12.44	6.22	6.22	1.00	M	0.50
	6	6.50	11.56	6.23	5.33	1.17	M	0.46
	7	5.00	8.88	4.44	4.44	1.00	M	0.50
Total length of somatic chromosomes = 21.43 μm								
L. wrangelianus	1	7.50	20.40	11.69	8.71	1.34	M	0.43
	2	6.75	18.36	10.57	7.79	1.36	M	0.42
	3	6.38	17.34	9.57	7.77	1.23	M	0.45
	4	6.13	16.66	9.24	7.42	1.25	M	0.45
	5	5.63	15.30	8.41	6.89	1.22	M	0.45
	6	4.38	11.90	8.58	3.32	2.59	SM	0.28
Total length of somatic chromosomes = 19.10 μm								
Group VI. *Simpteria*								
L. grandiflorus	1	15.00	17.86	11.91	5.95	2.00	SM	0.33
	2	14.00	16.66	11.90	4.76	2.50	SM	0.29
	3	13.00	15.48	7.74	7.74	1.00	M	0.50
	4	12.00	14.28	7.14	7.14	1.00	M	0.50
	5	10.00	11.90	5.95	5.95	1.00	M	0.50
	6	10.00	11.90	5.95	5.95	1.00	M	0.50
	7	10.00	11.90	5.95	5.95	1.00	M	0.50
Total length of somatic chromosomes = 32.00 μm								
L. rigidus	1	9.50	18.82	9.90	8.92	1.11	M	0.47
	2	8.00	15.84	7.92	7.92	1.00	M	0.50
	3	7.50	14.86	7.92	6.94	1.14	M	0.47
	4	7.00	13.86	7.91	5.95	1.33	M	0.43
	5	7.00	13.86	7.91	5.95	1.33	M	0.43
	6	6.00	11.88	5.94	5.94	1.00	M	0.50
	7	5.50	10.90	5.95	4.95	1.20	M	0.45
Total length of somatic chromosomes = 19.24 μm								

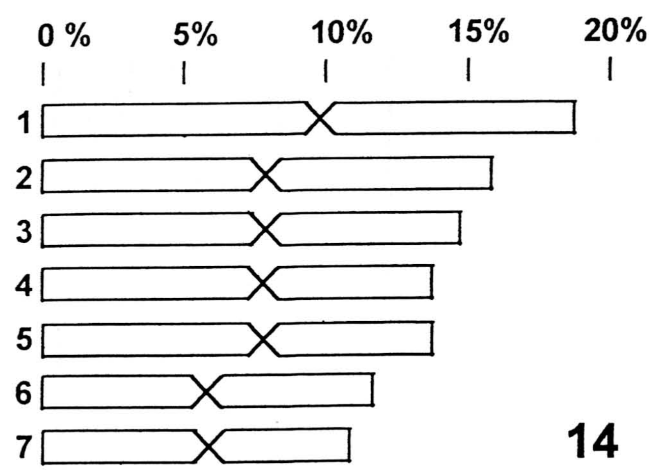
Table 3. Continued.

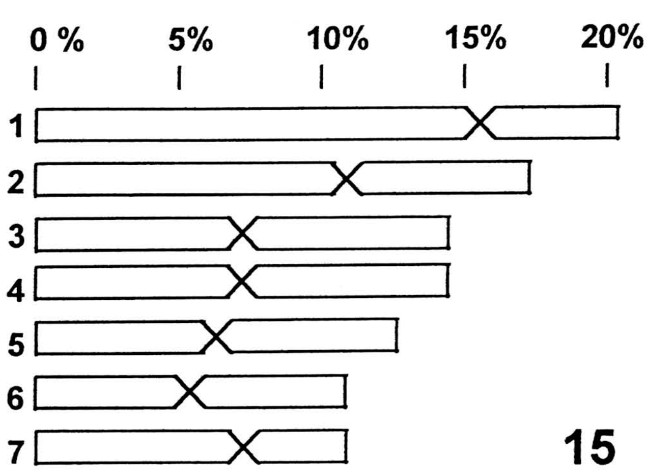
Species	Set	Average length (mm)	% length	% length long arm	% length short arm	Centromere* Ratio	Type	Index
L. wrightii	1	13.00	20.32	15.63	4.69	3.33	ST	0.23
	2	11.00	17.18	10.93	6.25	1.75	SM	0.36
	3	9.00	14.06	7.03	7.03	1.00	M	0.50
	4	9.00	14.06	7.03	7.03	1.00	M	0.50
	5	8.00	12.50	6.25	6.25	1.00	M	0.50
	6	7.00	10.94	5.47	5.47	1.00	M	0.50
	7	7.00	10.94	7.81	3.13	2.50	SM	0.29
Total length of somatic chromosomes = 24.38 μm								

* Ratio: Long arm divided by short arm; Type: M, chromosome with centromere in median position; SM, submedian; ST, subterminal; Index: p/p+q (length of short arm divided by the entire chromosome length)

** Taxonomic groupings follow that of Isely (1981).







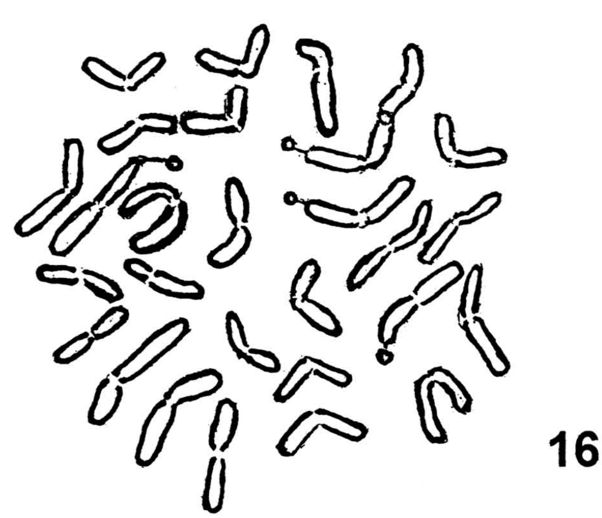


Fig. 13–15. Idiograms of western North American taxa of *Lotus*.—13–15. Group *Simpteria*.—13. *L. dendroideus* var. *dendroideus*.—14. *L. rigidus*.—15. *L. wrightii*. Arrangement of chromosomes as described in Fig. 1–6.—16. Karyotype of a 4*x* cell of *L. grandiflorus* showing four satellited chromosomes. Magnification × ca. 5250.

clearly distinguished by chromosome banding (Shankland and Grant 1976).

A comparison of the idiograms for the five western North American species of *Lotus* studied by Zandstra and Grant (1968) [group III, L. *pinnatus* Hook., *L. formosissimus* Greene; group IV, *L. micranthus* Benth., *L. purshianus* (now *L. unifoliatus* (Hook.) Benth., *L. denticulatus* (E. Drew) Greene] with those taxa in this study show that the overall chromosome morphology is very similar. Thus, chromosome morphology alone is not sufficient to separate the North American species into different taxonomic groupings.

Preliminary studies using chromatography showed that *L. pinnatus* and *L. formosissimus* (group III) were more closely related to each other than to the other three taxa in group V, and that *L. denticulatus* (group V), the only species studied with a chromosome number of $n = 6$, showed lower coefficients of association with the $n = 7$ species (Grant and Zandstra (1968). Thus, other experimental methods must be used in addition to cytology to aid in resolving taxonomic affinities in *Lotus*.

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