STUDIES ON THE GENUS *MELILOTUS* (SWEETCLOVER) WITH SPECIAL REFERENCE TO INTERRELATIONSHIPS AMONG SPECIES FROM A CYTOLOGICAL POINT OF VIEW

By

FUMIJI KITA

Department of Agronomy, Faculty of Agriculture,
Hokkaido University
Sapporo, Hokkaido, Japan

Contents

[Journ. Facul. Agr. Hokkaido Univ., Sapporo, Vol. 54, Pt. 2, 1965]

I. Introduction

The genus *Melilotus* (sweetclover) is comprised of some twenty species (SCHULZ 1901, ISELY 1954). The chromosome number thus far examined is 2n=16 (DARLINGTON and JANAKI AMMAL 1945). Four species of *Melilotus*, *M. alba*, *M. officinalis*, *M. suaveolens*, and *M. indica*, have been grown commercially, but most of the economic varieties are derivatives of two species, *M. alba* and *M. officinalis*, which are widely grown in the United States and Canada for hay, pasture, and green manure crops.

The low-coumarin strains of sweetclover were produced by the interspecific hybridization between *M. alba* and *M. dentata*, which was reared by grafting on common varieties because of its inability to grow F_1 plants due to chlorophyll deficiency (SMITH 1943). Since then, the exploration of basic data on interspecific relationships of the genus *Melilotus* has attracted the interest of many workers, in the utilization of desirable germ plasm from related species in sweetclover breeding programs.

The studies on the relationships of interspecific cross compatibility has been conducted by several workers (STEVENSON and KIRK 1935, STEVENSON and WHITE 1940, JOHNSON 1942, SMITH 1954, WEBSTER 1950, GREENSHIELDS 1954, JARANOWSKI 1962). Regarding interspecific crosses among the subgenus *Eumelilotus* species, SMITH reported that the affective chlorophyll deficiency barrier against the interspecific transfer of genes existed between pairs of species belonging the subgenus *Eumelilotus* (SMITH 1943, 1954). It was also noted that the development of embryo was interrupted at an early stages in some interspecific crosses and resulted in aborted ovules or aborted seeds (GREENSHIELDS 1954).

Cytological studies of interspecific hybrids have also been undertaken by several workers (WEBSTER 1950; BRINGHURST 1951; SHASTRY, SMITH and COOPER 1960; JARANOWSKI 1961; KITA 1962, 1964). From the studies of meiotic chromosome behaviors of the interspecific F_1 hybrids, it was indicated that structural differences of chromosomes were in a role of speciation of the genus *Melilotus* in part.

In spite of the fact that a considerable amount of basic research on sweetclover has been accumulated, numerous points still largely remain to be clarified. For many years, the present author has been engaged in cytological studies on the genus *Melilotus*. These were mainly concentrated on the karyotype analysis of nineteen species, chromosome aberrations found in the course of meiosis of the interspecific hybrids, and the interspecific cross compatibility. Through

these studies, the author has attempted to present more detailed information on interspecific relationships of the genus *Melilotus* in relation to speciation of the genus.

The present work was initiated in 1957 and continued till 1959, at the Department of Genetics, University of Wisconsin, U. S. A. Thereafter the work was continued at the Plant Breeding Institute, Department of Agronomy, Faculty of Agriculture, Hokkaido University, Japan, since 1959.

Before going further, the author wishes to express his sincere gratitude to Dr. SEIJIN NAGAO, Professor of the Plant Breeding Institute, Department of Agronomy, Hokkaido University, Japan, for his guidance, suggestions and invaluable critisism in regard to the present work. Likewise, the author wishes to express his heartful gratitude to Dr. K. TAGUCHI, Professor of the Crop Science Institute, at the same University; and to Dr. S. HOSOKAWA, Professor of the Industrial Crops Laboratory of the same University, who gave guidance to the author during the course of this work. The author also wishes to express his thanks to Dr. M. TAKAHASHI, Associate Professor of the Plant Breeding Institute, at the same University, for his many helpful suggestions and encouragement in making this work possible.

The author must acknowledge the kindnesses of Dr. W. K. SMITH, Professor of Department of Genetics and Agronomy, University of Wisconsin, U. S. A., who not only directed the author during his stay in the United States of America but also, after his return to Japan supplied all species used and encouraged the author to carry out this work.

Expense of the present work was partly defrayed with grants in aid for fundamental scientific research from the Ministry of Education and the Rockefeller Foundation.

II. Observation of Morphological Characters of *Melilotus* Species

The keys for identification of the species of *Melilotus* were provided by SCHULZ (1901). The most recent identification keys were reported by ISELY (1954). The author employed nineteen species including twenty-seven strains, which were introduced. General external morphology such as flower structure, flowering habits, leaves, pods and seeds were examined to determine whether or not the introduced species or strains are given the proper name of classification. In this chapter, the author also presents general descriptions of morphological appearance of species and geographical distributions of species as an introduction for the following studies.

1. **Materials**

The species and strains used in this study are described in the experimental results.

2. **Experimental Results**

a. *M. alba* DESR.

M. alba var. *common* and *hubum*, the former is biennial and the latter is annual, were examined. In both varieties, petals are entirely white and the standard is longer than the wing and keel, which are about the same in length (table 1). The number of flowers per raceme is about the same in both

Fig. 1. *M. alba* var. *common*

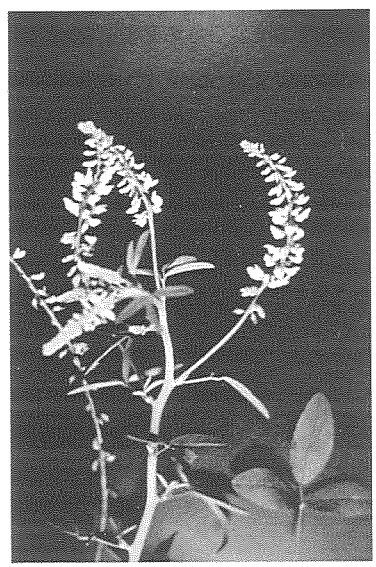


Fig. 2. *M. alba* var. *hubum*

varieties, but the raceme is more dense in *common* because of shorter raceme of *common* than that of *hubum*. Raceme exceeds the subtending leaf at the beginning of flowering and mature stage (table 2). Leaflets are rather long and narrow in *hubum* than in *common* (table 3). Margin of leaflets is toothed (fig. 1 and 2). Pods are globrous and irregular by reticulate-nerves. Color of pods is blackish brown when matured. The size of pods is about the same in both varieties (table 5). Seeds are yellowish brown in color and small in size (table 6). Pod setting is very low without insect or artificial tripping (table 7).

M. alba was originally distributed in considerably large areas of Eurasia; east to Tibet and China, south to Afghanistan and Arabia, but now is widely

grown in temperate agricultural areas throughout the world (ISELY 1954).

TABLE 1. Flower color and comparison of petals in length

species	color	flower						
		length of S.		length of W.		length of K.		comparison
		mean	s. d.	mean	s. d.	mean	s. d.	of S. W. K.
		mm		mm		mm		
alba var. *common*	white	5.98	0.02	5.00	0.00	5.00	0.00	S > W = K
" " *hubum*	"	5.45	0.15	4.18	0.06	4.18	0.00	S > W = K
altissima T 454 Ac 158	yellow	5.94	0.09	5.94	0.09	5.94	0.09	S = W = K
" T 455 B 130	"	5.87	0.17	5.87	0.17	5.87	0.17	S = W = K
dentata N 158	"	3.51	0.10	3.06	0.09	3.06	0.09	S > W = K
" N 338 Ac 91	"	3.16	0.14	2.93	0.12	2.93	0.12	S > W = K
hirsuta F 149	"	6.00	0.00	5.98	0.06	5.98	0.06	S = W = K
officinalis	"	7.02	0.06	6.62	0.15	6.15	0.28	S > W > K
polonica J 135 Ac 141	"	—	—	—	—	—	—	—
suaveolens N 348	"	4.06	0.09	4.06	0.09	4.06	0.09	S = W = K
" N 171	"	3.74	0.15	3.74	0.15	3.74	0.15	S = W = K
taurica PI 193951	white	7.00	0.00	6.87	0.17	6.02	0.06	S > W > K
wolgica K 443 Ac 163	"	4.45	0.16	4.04	0.29	3.45	0.16	S > W > K
elegans Ac 337	yellow	4.20	0.09	4.02	0.04	4.02	0.04	S > W = K
indica Ac 296	"	2.57	0.11	2.20	0.00	2.20	0.00	S > W = K
" Fc 30341	"	2.58	0.04	2.50	0.00	2.50	0.00	S > W = K
infesta Ac 335	"	7.46	0.28	5.47	0.19	7.41	0.26	S > K > W
italica V 285	"	8.04	0.09	8.04	0.09	7.04	0.09	S = W > K
macrocarpa Ac 336	"	8.10	0.21	5.95	0.50	7.49	0.10	S > K > W
messanensis C 21	"	5.28	0.04	4.92	0.11	5.28	0.04	S = K > W
neapolitana Ac 334	"	4.66	0.40	4.66	0.40	4.66	0.40	S = W = K
segetalis N 80	"	5.96	0.03	5.50	0.00	6.54	0.25	K > S > W
speciosa V 287	white	7.80	0.07	7.80	0.07	7.80	0.07	S = W = K
sulcata V 298	yellow	2.96	0.09	2.96	0.09	3.58	0.13	K > S = W
" S 379	"	3.08	0.11	2.88	0.09	3.80	0.00	K > S = W
" N 118	"	3.70	0.14	2.98	0.11	3.70	0.14	K = S > W

b. *M. altissima* THILL.

This species is biennial. *M. altissima* T 454 Ac 158 and T 455 B 130 were used for observation. Petals are yellow in color and standard, wing, and keel are about the same in length (table 1). The number of flowers per raceme is rather small and the raceme exceeds the subtending leaf at the beginning of

flowering and mature stage (table 2). Leaflets are elliptic and margin is sharply toothed (fig. 3). Pod is compressed and has a short beak at tip. Pods are blackish brown in color when matured, and the size is rather large (table 5). Color of seeds is yellowish brown and size is somewhat larger than that of *M. alba* (table 6). Remarkable differenc is observed in pod setting without insect or artificial tripping, namely *M. altissima* T 454 Ac 158 is very low in pod seeting while 70.7% of setting is observed in *M. altissima* T 455 B 130 (table 7).

This species is native primarily in central and northern Europe (ISELY 1954).

Fig. 3. *M. altissima*

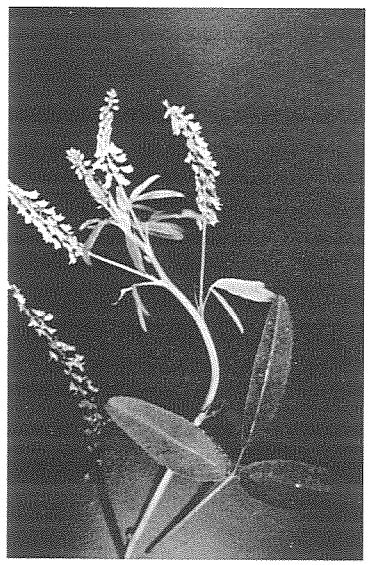


Fig. 4. *M. dentata*

c. *M. dentata* (W. K.) PERS.

Biennial and annual forms are reported in this species. Coumarin, which lowers palatability of sweetclover for forage, is low or almost free in this species, hence *M. dentata* is a promising source of germ plasm for transfering the low coumarin character to commercial varieties in sweetclover breeding programs. Two strains, *M. dentata* N 338 Ac 91 and N 158 are used in this experiment.

Petals are yellow in color and small in size which is comparable to that of *M. wolgica*. Standard is remarkably longer than wing and keel (table 1). The raceme is dense and about equal to or longer than the subtending leaf at the beginning of flowering but exceeds it at mature stage (table 2). Leaflets are mostly of a elongated elliptical shape with very prominent thin veins, which

are strongly dentate from base to apex of the leaflet. Teeth are distinctly needle-shaped (fig. 4). Pods are blackish brown at mature stage. Pod setting is very high without insect and artificial tripping (table 7).

This species is distributed in temperate Eurasia and east to Mongolia (ISELY 1954).

TABLE 2. Number of florets per a raceme, and length of raceme and subtending leaf at the beginning of flowering and mature stage

species	no. of floret		length at beginning of flowering		length at mature stage	
	mean	s. d.	raceme	leaf	raceme	leaf
			cm	cm	cm	cm
alba var. *common*	68.1	8.3	3.9	2.3	7.2	2.7
" var. *hubum*	68.7	7.8	4.8	4.4	8.4	3.0
altissima T 454 Ac 158	32.0	5.7	4.9	4.7	8.0	4.1
" T 455 B 130	32.7	4.1	2.5	2.8	5.8	2.9
dentata N 158	59.8	5.4	2.5	3.8	9.3	4.8
" N 338 Ac 91	52.0	7.5	3.6	4.2	9.0	4.5
hirsuta F 149	44.0	4.4	9.5	3.2	13.4	4.6
officinalis	54.1	2.6	7.9	3.0	13.2	4.3
polonica J 135 Ac 141	—	—	—	—	—	—
suaveolens N 348	34.1	4.2	4.8	2.5	8.7	2.8
" N 171	41.9	3.0	5.3	2.0	19.4	2.7
taurica PI 193951	64.6	4.0	10.6	4.1	18.2	4.5
wolgica K 443 Ac 163	51.0	4.9	6.1	2.5	15.1	3.5
elegans Ac 337	41.4	2.2	4.4	3.8	7.5	3.0
indica Ac 296	51.8	1.9	5.0	4.8	11.5	3.5
" Fc 30341	41.1	3.1	2.2	2.7	7.6	3.1
infesta Ac 335	33.3	6.3	4.9	4.6	12.3	4.5
italica V 285	30.2	2.9	3.8	3.6	17.2	4.1
macrocarpa Ac 336	38.1	1.9	6.6	5.2	13.0	5.2
messanensis C 21	10.8	1.5	1.8	6.5	2.9	6.4
neapolitana Ac 334	12.9	3.1	6.9	4.4	12.7	4.3
segetalis N 80	39.3	2.9	8.3	4.9	10.0	5.0
speciosa V 287	43.8	3.6	9.8	3.7	19.1	5.3
sulcata V 298	22.9	4.0	2.2	2.8	4.9	5.0
" S 379	11.1	1.1	2.0	3.7	4.9	4.1
" N 118	17.4	2.6	2.5	4.3	4.8	4.0

d. *M. hirsuta* LIPSKY.

Only one strain, F 149, of *M. hirsuta* was used for observation. Plant of this species is biennial.

Petals are yellow in color and standard is as long as wing and keel, which are about the same in length. The size of flowers is large as a whole (table 1). The raceme is longer than the subtending leaf at the beginning of flowering and mature stage (table 2). The margin of leaflets is usually not sharply toothed (fig. 5). Pods are elliptic-oblong, and yellowish brown in color at mature stage (table 5). Seeds are longer than those of the other species in *Eumelilotus* and yellowish brown in color (table 6). Pod setting is low without insect and artificial tripping (table 7).

This species is native in south western Russia (ISELY 1954).

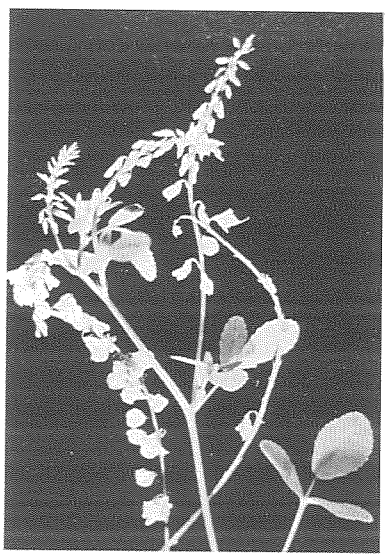


Fig. 5. *M. hirsuta*

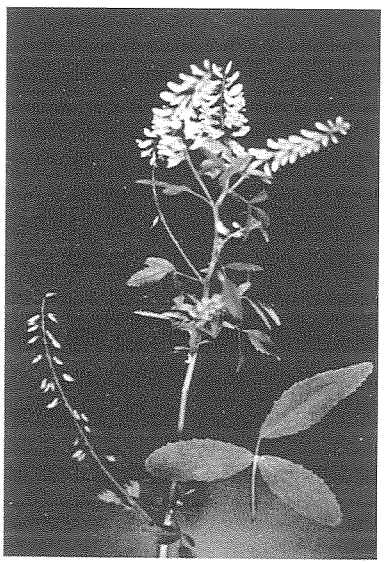


Fig. 6. *M. officinalis*

e. *M. officinalis* (L) DESR.

Plants of this species is biennial and used for actual cultivation covering large acreages.

Petals are yellow and size is large, which is comparable to that of *M. hirsuta*. Standard is longest, wing is intermediate, and keel is shortest in length (table 1). Raceme is longer than the subtending leaf at the beginning of flowering and mature stage (table 2). Leaflets are obovate and sharply toothed (fig. 6). Pods are glabrous and broad but rather small in size. Color of pods

are yellowish brown when matured (table 5). Seeds are small in size and yellowish brown in color (table 6). Pollen fertility is high but selfed seeds are seldom obtained even when insect or artificial tripping is applied (table 7). The high self incompatibility is the characteristic nature of this species.

The native area is Europe and adjacent Asia, but this species is introduced in temperate agricultural regions throughout the world (ISELY 1954).

f. *M. polonica* (L.) DESR.

M. polonica J 135 Ac 141 was studied for morphological observation. A plant of this species is biennial. Petals are yellow and large in size. Standard is longer than wing and keel, which are comparable to each other in length (table 1). Flower is attached on a long stalk. Raceme is rather short and possesses very few flowers. Raceme exceeds the subtending leaf at the beginning of flowering and mature stage (table 2). Leaflets are comparably small in size and cuneate. Margin of leaflets is toothed but not conspicuous (fig. 7). Pods are yellowish brown in color and elliptic-oblong as in *M. hirsuta*. Observations on seeds have not been made as yet, because the plants did not reach maturity in the introduced year.

This species has a limited distribution area, Urals to south-eastern Russia and adjacent Turkestan (ISELY 1954).

g. *M. suaveolens* LEDEB.

Biennial and annual forms are known in this species. *M. suaveolens*

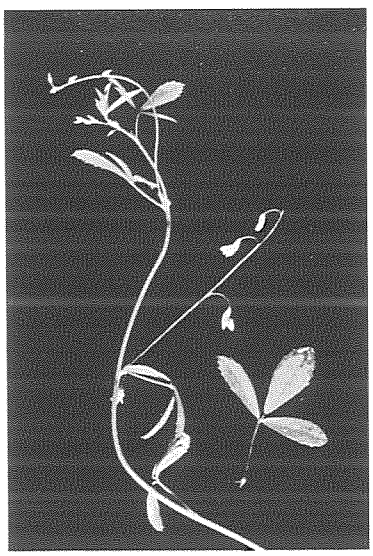


Fig. 7. *M. polonica*

Fig. 8. *M. suaveolens*

TABLE 3. Length and width of central leaflet and side leaflet

species	central leaflet				side leaflet			
	length		width		length		width	
	mean	s. d.	mean	s. d.	mean	s. d.	mean	s. d.
	cm		cm		cm		cm	
alba var. *common*	2.7	0.2	1.3	0.2	2.3	0.1	1.1	0.1
» var. *hubum*	3.1	0.2	1.1	0.2	2.7	0.2	1.0	0.1
altissima T 454 Ac 158	3.9	0.3	1.1	0.1	3.4	0.3	0.9	0.3
» T 455 B 130	3.7	0.2	1.3	0.1	3.2	0.2	1.1	0.1
dentata N 158	4.5	0.5	1.3	0.1	4.1	0.8	1.1	0.2
» N 338 Ac 91	4.7	0.2	1.4	0.0	4.3	0.2	1.3	0.1
hirsuta F 149	2.6	0.1	1.6	0.1	2.3	0.1	1.3	0.1
officinalis	3.0	0.2	1.3	0.1	2.5	0.1	1.1	0.1
polonica J 135 Ac 141	2.0	0.2	1.3	0.2	1.9	0.2	1.0	0.1
suaveolens N 348	2.2	0.2	0.7	0.1	1.9	0.2	0.6	0.1
» N 171	2.5	0.2	1.0	0.1	2.2	0.2	0.7	0.1
taurica PI 193951	2.1	0.1	1.6	0.1	1.9	0.1	1.4	0.1
wolgica K 443 Ac 163	3.2	0.3	1.1	0.1	2.8	0.2	1.0	0.1
elegans Ac 337	3.2	0.1	2.5	0.2	3.2	0.0	1.9	0.1
indica Ac 296	3.0	0.2	1.6	0.2	3.0	0.3	1.6	0.2
» Fc 30341	3.4	0.4	1.8	0.2	3.1	0.1	1.6	0.1
infesta Ac 335	2.4	0.1	1.4	0.2	2.1	0.1	1.1	0.1
italica V 285	4.9	0.3	4.0	0.2	4.5	0.2	3.6	0.1
macrocarpa Ac 336	3.1	0.2	2.5	0.2	2.8	0.3	1.9	0.3
messanensis C 21	2.8	0.1	1.8	0.1	2.7	0.2	1.6	0.1
neapolitana Ac 334	2.2	0.1	1.4	0.1	2.2	0.3	1.3	0.1
segetalis N 80	3.2	0.3	1.9	0.2	2.9	0.2	1.6	0.1
speciosa V 287	3.3	0.3	2.4	0.2	2.9	0.3	2.0	0.3
sulcata V 298	2.6	0.2	1.2	0.1	2.5	0.1	1.0	0.1
» S 379	2.0	0.3	0.7	0.0	1.8	0.2	0.5	0.1
» N 118	2.5	0.2	0.8	0.1	2.3	0.2	0.6	0.1

N 348 and N 171 used in this study are annual form.

Petals are yellow and somewhat smaller than those of *M. alba*. Standard, wing, and keel are about the same in length (table 1). Raceme is longer than the subtending leaf at the beginning of flowering and mature stage (table 2). Leaflets are toothed at margin (fig. 8). Color of pods changes to blackish brown at mature stage and pods are terminated by a sharp, straight beak. The size of pods is small (table 5). Seed color is blackish brown as in *M. indica* and

distinguishable from the other species. The size of seeds is small (table 6). Pod setting is low without insect or artificial tripping (table 7).

This species is native in the area from Siberia to Japan, especially common in temperate China (ISELY 1954).

h. *M. taurica* (M. B.) SER.

This species was introduced under the name of *M. italica* PI 193951, but identified with *M. taurica* based on ISELY's identification keys. Plants of this species are biennial.

Petals are white and large in size. Standard is longer than wing, which is longer than keel in length (table 1). Raceme is very long and exceeds the subtending leaf at the beginning of flowering and mature stage. Leaflets are small and characterized with clear transverse wrinkling in the shape of pronounced deep grooves. Color of pods is yellowish brown at mature stage. Seeds are yellowish brown and rather large in size among *Eumelilotus* species (table 6). Pod setting is low without visiting of insects and artificial tripping (table 7).

This species is called Crimean-sweetclover, an ancient endemic species in Crimea, which has a very limited distribution area which is largely confined to the southern sea board of the Crimea (SUVOROV 1950). Isely suggested that this species was distributed in the eastern half of the Black Sea, possibly south to Iraq (ISELY 1954).

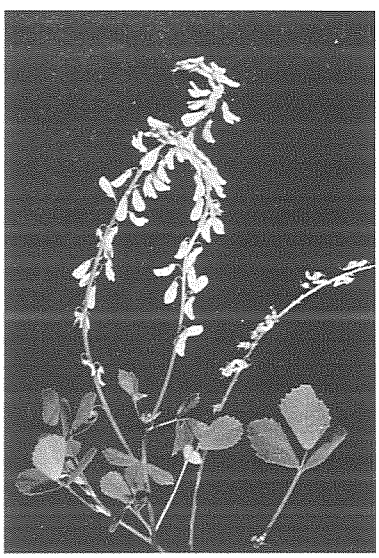


Fig. 9. *M. taurica*

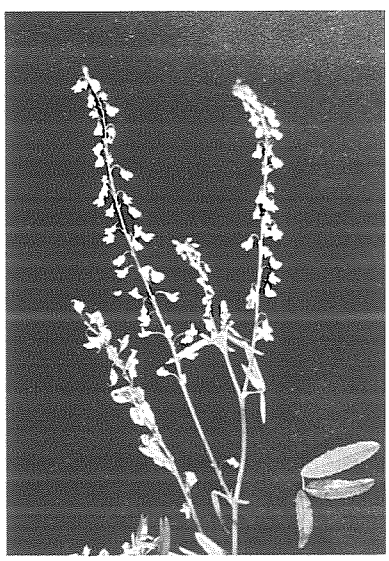


Fig. 10. *M. wolgica*

i. *M. wolgica* POIR.

This species is biennial. *M. wolgica* K 443 Ac 163 was used for this study. Petals are white in color and small in size. Standard is longer than wing and keel, which are about the same in length (table 1). Raceme has many flowers and exceeds the subtending leaf (table 2). Leaflet is obovate in shape with inconspicuous teeth (fig. 10). Pod stalk is very thin, threadlike, and long. Pods are elongated oval shape and somewhat flattened. Color of pods is

TABLE 4. Number of teeth observed at margin of leaflets

species	central leaflet		side leaflet	
	mean	s. d.	mean	s. d.
alba var. *common*	19.2	2.3	16.4	1.3
" var. *hubum*	22.8	1.7	21.2	1.4
altissima T 454 Ac 158	30.7	3.1	24.9	3.7
" T 455 B 130	21.5	1.6	19.5	0.8
dentata N 158	79.5	7.7	73.6	10.7
" N 338 Ac 91	67.2	4.4	60.9	4.0
hirsuta F 149	27.0	2.4	24.0	2.7
officinalis	34.5	1.6	28.0	2.9
polonica J 135 Ac 141	15.5	3.6	15.0	3.2
suaveolens N 348	13.7	4.7	10.4	3.4
" N 171	20.7	2.8	16.2	2.7
taurica PI 193951	19.7	1.6	17.2	1.8
wolgica K 443 Ac 163	28.8	3.4	25.7	3.6
elegans Ac 337	18.4	3.2	19.8	2.7
indica Ac 296	18.4	2.7	19.8	2.0
" Fc 30341	16.2	3.1	15.8	3.3
infesta Ac 335	46.6	2.8	40.2	2.5
italica V 285	—	—	—	—
macrocarpa Ac 336	22.4	5.0	21.0	3.2
messanensis C 21	32.0	2.1	28.6	2.5
neapolitana Ac 334	10.1	1.1	9.3	0.8
segetalis N 80	31.4	3.5	25.6	3.0
speciosa V 287	20.2	3.0	16.0	2.7
sulcata V 298	32.8	3.2	29.0	2.4
" S 379	19.1	3.3	17.7	2.7
" N 118	23.0	2.3	19.9	4.1

yellowish brown (table 5). Seeds are yellowish brown and small (table 6). Self-pollination is decidedly predominant in this species, in other words high percentage of pod setting is observed without insect or artificial tripping (table 7).

This species is distributed from southern Urals to south eastern Russia (ISELY 1954).

j. *M. elegans* SULZM.

This species is an annual. *M. elegans* Ac 337 was used for this study. Petals are yellow in color. Standard is longer than wing and keel, which are almost equal in length (table 1). Raceme is longer than the subtending leaf at the beginning of flowering and mature stage (table 2). Leaflets are obovate-cuneate and toothed primarily on upper portion of margin (fig. 11). Pods are yellowish brown in color and broad. Pod setting is high without insect or artificial tripping (table 7).

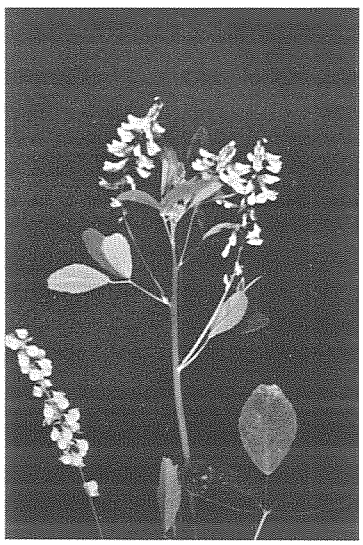


Fig. 11. *M. elegans*

ISELY (1954) described that the plant height of *M. elegans* is very small, but the species used in this study is very high, about 150–200 cm. Some question whether this species is *M. elegans* or not remains, but the author treated this species as *M. elegans*. This is because aside from the plant height all the characteristics agree with ISELY's identification key of *M. elegans*.

Distribution of *M. elegans* is Mediterranean Europe and Africa and Palestin (ISELY 1954).

k. *M. indica* (L.) ALL.

This species is annual. The striking characteristic of *M. indica* is very small flowers, which are smallest among all the species of *Melilotus*.

Petals are yellow in color. Standard is somewhat longer or equal to wing and keel, which are the same in length (table 1). Raceme is short and compact, and pedancle is short. Raceme is of equal length to the subtending leaf at the beginning of flowering but exceeds it at mature stage (table 2). Leaflet is cuneate and toothed (fig. 12 and 13). Pods are very small and of spherical shape, seeds are also very small and spherical (table 5 and 6). Color of seeds is blackish brown as in *M. suaveolens*. Pod setting is very high without insect or artificial tripping (table 7).

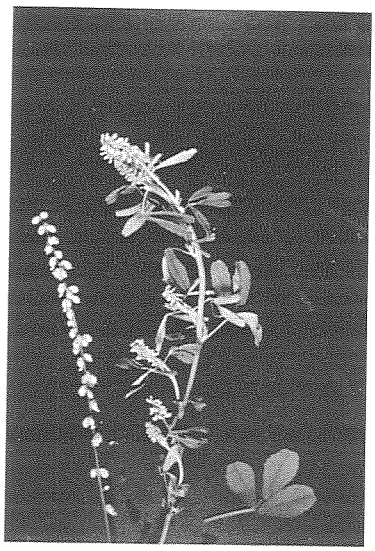


Fig. 12. *M. indica* Ac 296

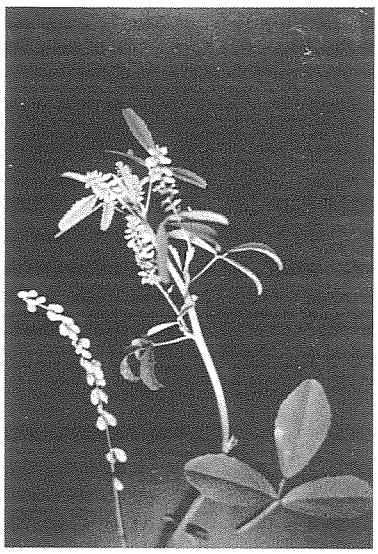


Fig. 13. *M. indica* Fc 30341

The strain which was introduced under the name of *M. neapolitana* Fc 30341 (fig. 13) showed the same morphological characteristics with *M. indica* Ac 296 (fig. 12) except for somewhat large size of pods and seeds (table 5 and 6). The author considered this strain should be included in *M. indica*.

The primary distribution center of this species is India, from where it migrated westward into the tropical and south tropical zones of the southern hemisphere (SUVOROV 1950).

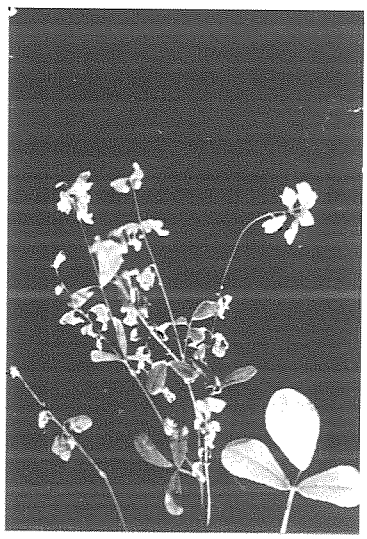


Fig. 14. *M. infesta*

1. *M. infesta* GUSS.

Plants of this species are annual. The strain of *M. infesta* Ac 335 was used for this study.

Petals are yellow and large in size. Standard and wing, which are about the same, are longer than keel in length (table 1). Raceme is equal to the subtending leaf at the beginning of flowering but exceeds it at mature stage (table 2). Leaflet is obovate and toothed inconspicuously (fig. 14). Pods are obtuse or inconspicuously apiculate. Size of pods is large and

coarse ribs are observed on the surface of pods (table 5). Seeds are brown in color and large in size (table 6). Pod setting is low without insect or artificial tripping.

This species is distributed in Mediterranean Europe and Africa, and Asia Minor (ISELY 1954).

TABLE 5. Color, length, and width of pod

species	color	length		width	
		mean	s. d.	mean	s. d.
		mm		mm	
alba var. *common*	blackish brown	3.8	0.4	2.3	0.2
" var. *hubum*	"	3.6	0.2	2.4	0.1
altissima T 454 Ac 158	"	4.5	0.2	3.0	0.1
" T 455 B 130	"	4.7	0.1	2.9	0.1
dentata N 158	"	—	—	—	—
" N 338 Ac 91	"	3.4	0.2	2.4	0.1
hirsuta F 149	yellowish brown	7.4	0.2	2.5	0.2
officinalis	"	3.8	0.2	2.2	0.1
polonica J 135 Ac 141	"	—	—	—	—
suaveolens N 348	blackish brown	3.2	0.1	2.1	0.2
" N 171	"	3.3	0.1	2.1	0.1
taurica PI 193951	yellowish brown	4.7	0.1	3.1	0.2
wolgica K 443 Ac 163	"	5.6	0.2	2.7	0.1
elegans Ac 337	"	4.0	0.3	2.7	0.3
indica Ac 296	"	2.0	0.1	1.5	0.1
" Fc 30341	"	3.7	0.3	2.6	0.2
infesta Ac 335	"	5.0	0.6	3.6	0.1
italica V 285	"	4.5	0.3	3.3	0.1
macrocarpa Ac 336	"	5.0	0.1	3.4	0.2
messanensis C 21	"	5.7	0.3	3.1	0.2
neapolitana Ac 334	"	3.4	0.2	2.5	0.2
segetalis N 80	"	5.2	0.2	3.9	0.6
speciosa V 287	"	6.3	0.2	3.8	0.1
sulcata V 298	"	3.3	0.1	2.3	0.1
" S 379	"	3.4	0.2	2.6	0.1
" N 118	"	2.8	0.1	2.2	0.1

m. *M. italica* (L.) LAM.

This species is annual. The strain, *M. italica* V 285, was used for observation.

Petals are yellow and large in size. Standard and wing, which are the same, are longer than keel (table 1). Raceme is relatively short and has the same length with the subtending leaf at the beginning of flowering but exceeds it remarkably at mature stage (table 2). Leaflets are very large, by which this species is easily distinguishable from the other species. Leaflets are of orbicular shape with entire or more rarely indistinctly toothed margin (fig. 15). Pods are yellowish brown and large and broadly oval in shape (table 6). Pod setting is very high without insect or artificial tripping (table 7).

This species is native in Mediterranean Europe and Asia Minor.

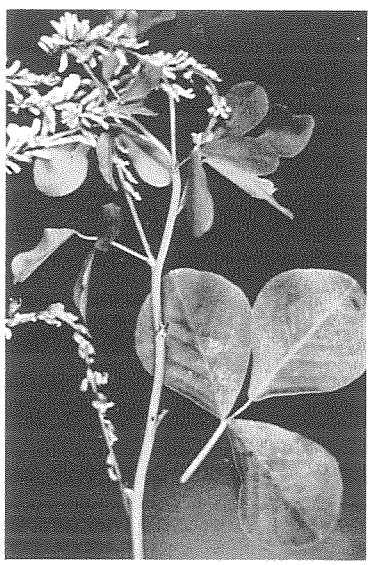


Fig. 15. *M. italica*

Fig. 16. *M. macrocarpa*

n. *M. macrocarpa* GOSS and DUR.

This species is annual. *M. macrocarpa* Ac 336 was used for this study.

Petals are yellow and very large in size. Standard and keel, which are the same in length, are longer than keel (table 1). Raceme is longer than wing (table 1.) Raceme is longer than the subtending leaf at the beginning of flowering and mature stage (table 2). Leaflet is nearly entire or irregularly toothed at margin (fig. 16). Color of pods is yellowish brown. Seeds are large and spherical. The pod setting is rather low without insect or artificial tripping

(table 7).

This species is native in Algeria and Morocco.

o. *M. messanensis* (L.) ALL.

This species is annual. The strain, *M. messanensis* C 21, was used for this study.

Petals are yellow in color. Standard and keel, which are the same in length, are longer than wing (table 1). Raceme is very short, which is characteristic of this species, and shorter than the subtending leaf at both the beginning of flowering and mature stage (table 2). Leaflets are cuneate and very small inconspicuous teeth are observed at margin (fig. 17). Pods are very sharply pointed at apex. Pods have distinctly concentric venation on surface. Seeds are large and brown in color (table 6). Pod setting is very high without insect or artificial tripping (table 6).

The native area of this species is Mediterranean Europe and Africa, and Asia Minor (ISELY 1954).

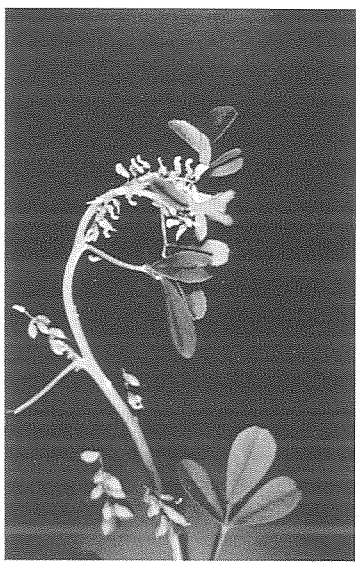


Fig. 17. *M. messanensis*

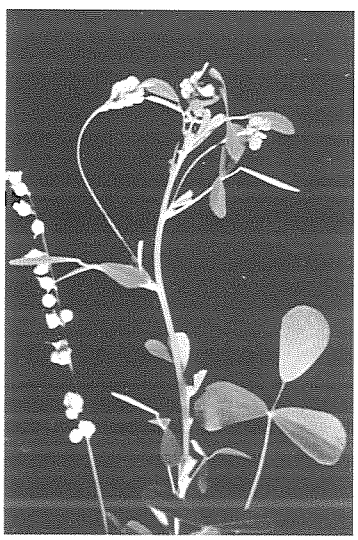


Fig. 18. *M. neapolitana*

p. *M. neapolitana* TEN.

Plants of this species are annual. *M. neapolitana* Ac 334 was used for this study.

Petals are yellow in color and medium size. Standard is as long as wing and keel in length (table 1). Raceme is longer than the subtending leaf at

the beginning of flowering and mature stage. The number of flowers per raceme is very few (table 2). Leaflets are obovate and usually conspicuously cuneate. The margin of leaflet is toothed primarily toward apex. Pods are globose and terminate in a sharp, straight beak. Seeds are round and yellowish brown in color. Pod setting is high without insect or artificial tripping (table 7).

This species is distributed in southern Europe and adjacent north Africa, and south Asia (ISELY 1954).

TABLE 6. Color, length, and width of seed

species	color	length		width	
		mean	s. d.	mean	s. d.
		mm		mm	
alba var. *common*	yellowish brown	2.1	0.1	1.6	0.1
» var. *hubum*	»	2.2	0.1	1.7	0.1
altissima T 454 Ac 158	»	2.3	0.1	1.8	0.1
» T 455 B 130	»	2.4	0.1	1.8	0.1
dentata N 158	»	—	—	—	—
» N 338 Ac 91	»	2.0	0.1	1.7	0.1
hirsuta F 149	»	3.1	0.1	1.7	0.1
officinalis	»	1.9	0.1	1.6	0.1
polonica J 135 Ac 141	»	—	—	—	—
suaveolens N 348	blackish brown	2.0	0.1	1.5	0.1
» N 171	»	2.0	0.1	1.5	0.1
taurica PI 193951	yellowish brown	2.8	0.1	2.0	0.1
wolgica K 443 Ac 163	»	2.9	0.1	1.7	0.1
elegans Ac 337	»	2.2	0.0	1.8	0.1
indica Ac 296	blackish brown	1.6	0.1	1.2	0.1
» Fc 30341	»	2.3	0.2	1.5	0.0
infesta Ac 335	yellowish brown	3.2	0.1	2.3	0.1
italica V 285	»	2.9	0.1	2.2	0.2
macrocarpa Ac 336	»	3.1	0.1	2.3	0.1
messanensis C 21	»	3.2	0.2	2.0	0.1
neapolitana Ac 334	»	2.0	0.1	1.6	0.1
segetalis N 80	»	3.6	0.1	2.2	0.1
speciosa V 287	»	3.9	0.2	2.7	0.2
sulcata V 298	»	2.4	0.1	1.8	0.0
» S 379	»	2.4	0.1	1.8	0.1
» N 118	»	2.2	0.1	1.5	0.1

q. *M. segetalis* (BROT.) SER.

A plant of this species is annual. *M. segetalis* N 80 is used for this observation.

Petals are yellow and large in size. Keel is longer than standard, which is longer than wing in length (table 1). Raceme is longer than the subtending leaf at the beginning of flowering and mature stage (table 2). Leaflets are relatively large and obovate in shape. Fine and conspicuous teeth are observed at margin (fig. 19). Pods are large and round shaped with numerous thin and concentrically arranged veins. Seeds are large. Pod setting is low without insect and artificial tripping (table 7).

This species is distributed along the northern and southern coast of the Mediterranean sea (ISELY 1954).

Fig. 19. *M. segetalis*

Fig. 20. *M. speciosa*

r. *M. speciosa* DUR.

This species is annual. *M. speciosa* V 287 is used for this study.

Petals are white and very large in size. Standard is as long as wing and keel, which are the same in length (table 1). Raceme is long and exceeds largely the subtending leaf at the beginning of flowering and mature stage (table 2). Leaflets are obovate and weakly toothed (fig. 20). Pods are broadly oval and strongly flattened with a curved, sharp terminal beak. The surface of pods is reticurated with predominantly transverse veins. Seeds are large in size and

flattened (table 6). Pod setting is low without insect or artificial tripping (table 7).

This species is distributed in west Africa, Algeria, and Morocco (ISELY 1654).

s. *M. sulcata* DESF.

Plants of this species are annual. Three strains; *M. sulcata* V 298, S 379,

TABLE 7. Growth habit, pollen fertility, and pod setting without insect or artificial tripping

species	growth habit	pollen fertility	pod setting
		%	%
alba var. *common*	biennial	98.8	10.7
» var. *hubum*	annual	92.2	15.3
altissima T 454 Ac 158	biennial	96.8	16.9
» T 445 B 130	»	99.4	70.7
dentata N 158	»	99.4	95.2
» N 338 Ac 91	»	98.9	95.0
hirsuta F 149	»	97.9	10.5
officinalis	»	96.3	0.0
polonica J 135 Ac 141	»	—	—
suaveolens N 348	annual	99.2	40.4
» N 171	»	99.5	52.7
taurica PI 193951	biennial	98.1	38.6
wolgica K 443 Ac 163	»	99.9	99.0
elegans Ac 337	annual	99.5	80.7
indica Ac 296	»	99.1	95.5
» Fc 30341	»	99.2	100.0
infesta Ac 335	»	98.5	15.9
italica V 285	»	96.6	99.3
macrocarpa Ac 336	»	96.8	30.8
messanensis C 21	»	99.4	96.3
neapolitana Ac 334	»	99.8	95.8
segetalis N 80	»	99.1	24.3
speciosa V 287	»	99.0	5.7
sulcata V 298	»	100.0	98.0
» S 379	»	98.7	97.2
» N 118	»	100.0	99.0

Fig. 21. *M. sulcata* V 298

Fig. 22. *M. sulcata* S 379

Fig. 23. *M. sulcata* N 118

and the strain which was introduced under the name of *M. segetalis* N 118; were used for this study.

Petals are yellow and small in size. Keel is longer than wing and standard, which are comparable to that of *M. segetalis* N 80 (table 1). Raceme is shorter than the subtending leaf at the beginning of flowering but about the same or longer in length at mature stage (table 2). Leaflets are rather variable in shape but obovate as a rule. Sparse teeth are observed at margin of leaflets (fig. 21, 22, and 23). Pods are rather small in size and round, somewhat flattened shape with numerous thin concentrically arranged veins on surface (table 5). Seeds are small and oval flattened shape (table 6). The strain which was introduced under the name of *M. segetalis* N 118 is smaller than the two other strains in size of pods and seeds, however, morphological appearance of the other characters are almost identical to the two other strains of *M. sulcata* and distinctly different from *M. segetalis* N 80. It should be, therefore, included in *M. sulcata*. Pod setting of three strains is very high without insect or artificial tripping (table 7).

Distribution of this species is the entire Mediterranean region; west Africa, south east Asia, Spain, Italy, Sordima, and Greece (SUVOROV 1950, ISELY 1954).

3. Discussion and Conclusion

SCHULZ (1901) described twenty-two species in the genus Melilotus. ISELY (1954) pointed out that *M. kotschyi* and *M. urbanii* named by SCHULZ should be included within the limits of the *M. alba* complex because SCHULZ's demarcation was based on limited and incomplete materials. ISELY, then, considered that the genus *Melilotus* comprises twenty species, and presented identification keys for *Mililotus* species. SUVOROV (1950) described that *M. kotschyi* Schulz often occurred in the steppe regions of the Ukraine and northern Caucasus and synonymous with *M. alba*. He also pointed out that *M. urbanii* Schulz was a synonym of *M. wolgica* of which the wide range of variability was obviously not available to Schulz. Further more, according to SUVOROV's classification it was pointed out that *M. infesta* and *M. macrocarpa* were synonyms of *M. sulcata*, and that *M. elegans* was synonymous with *M. neapolitana*. Regarding *M. infesta*, *M. segetalis*, and *M. sulcata*, ISELY (1954) noted that they appeared to form intergrading series, the very distinct *M. sulcata* and *M. infesta* constituting the extremes, and *M. segetalis*, a heterogenous series of intermediate. Then he suggested that for the present, the three principal units comprising this complex were maintained as separate species.

From the author's observation, *M. infesta* A c335, *M. macrocarpa* Ac 336, *M. segetalis* N 80, and *M. sulcata* V 298 and S 379 are clearly different from

each other, consequently they should be considered as independent species. Namely the results of the author accept the taxonomic concepts of the genus *Melilotus* provided by ISELY (1954). And therefore, identification of species for this experiment was principaly based on ISELY's identification keys.

From observational study, it was shown that the strain introduced as *M. neapolitana* Fc 30341 is synonymous with *M. indica*, although some minor differences are observed in pod and seed size. It is also pointed out that the strain introduced as *M. segetalis* N 118 is similar to *M. sulcata* rather than to *M. segetalis*. As mentioned by ISELY, the strain *M. segetalis* N 118 may be considered as a variant type of *M. segetalis*, however, karyotype of this strain is different from that of *M. segetalis* in a pair of satellited chromosomes and is more likely *M. sulcata*. Consequently, it should be considered that the strain *M. segetalis* N 118 is a synonym of *M. sulcata*.

Some questions remain in the species *M. elegans* Ac 337, namely plant height of this species is more than 150 cm, while ISELY described that *M. elegans* was only 20–40 cm in plant height. However, this species is clearly distinguishable from other species of the genus and confirmed by Dr. G. STEVENSON, Experimental Farm, Brandon, Man, Canada, who is working on the taxonomy of the genus *Melilotus*, as *M. elegans*. Therefore the author used this species as *M. elegans* for following studies.

III. Interspecific Cross Compatibility in the Genus *Melilotus*

This experiment was made in order to explore the general pattern of interspecific cross compatibility between available species of *Melilotus*. Possible paired cross combinations were made among eighteen species, including a fraction of cross combinations previously reported, and several new interspecific hybrids were obtained.

1. Materials

Eight species belonging to the subgenus *Eumelilotus* and ten species belonging to the subgenus *Micromelilotus* were used in this study. These are listed in table 8.

2. Methods

Cross pollinations were made in the green house from 1960 to 1964 at the Department of Agronomy, Faculty of Agriculture, Hokkaido University. In making each cross, the petals were removed from recently opened flowers and pollens were removed by suction using a small vacum pump. Pollen of

TABLE 8. Species and strains used

section	species	strain	form	chromosome number
				(2n)
Eumelilotus	*alba* (a)	common	biennial	16
"	" (b)	evergreen	"	"
"	" (c)	hubum	annual	"
"	*altissima* (a)	T 453 Ac 165	biennial	"
"	" (b)	T 454 Ac 158	"	"
"	" (c)	T 455 B130	"	"
"	*dentata* (a)	N 338 Ac 91	"	"
"	" (b)	N 158	"	"
"	*hirsuta*	F 149	"	"
"	*officinalis*		"	"
"	*suaveolens* (a)	N 348	annual	"
"	" (b)	N 171	"	"
"	*taurica*	PI 193951	biennial	"
"	*wolgica*	K 443 Ac 163	"	"
Micromelilotus	*elegans*	Ac 337	annual	"
"	*indica* (a)	Fc 30341	"	"
"	" (b)	Ac 296	"	"
"	*infesta*	Ac 335	"	"
"	*italica*	V 285	"	"
"	*macrocarpa*	Ac 336	"	"
"	*messanensis*	C 21	"	"
"	*neapolitana*	Ac 334	"	"
"	*segetalis*	N 80	"	"
"	*speciosa*	V 287	"	"
"	*sulcata* (a)	V 298	"	"
"	" (b)	S 379	"	"
"	" (c)	N 118	"	"

the staminate parent was applied without delay to the stigma of the pistillate parent with a toothpic tip (SMITH 1654).

In some species, in which the self pollination rate is high, emasculation was made one or two days before flower opening and pollens were applied twice one or two days after emasculation.

Seeds obtained were classified by size and plumpness and seed coats were scratched with a razor blade, and then germinated in petry dish under favarable

temperatures. Germinated seeds were planted in soil in a green house flat. Most of the interspecific hybrids were easily detected by chlorophyll deficiency in the seedling stage and/or morphological differences between parental specise.

3. **Experimental Results**

TABLE 9. Cross combination and number of flowers made cross-pollinations between pairs of species of *Eumelilotus*

	alba (a)	" (b)	" (c)	*altissima* (a)	" (b)	" (c)	*dentata* (a)	" (b)	*hirsuta*	*officinalis*	*suaveolens* (a)	" (b)	*taurica*	*wolgica*
alba (a)				11	166*	39*	189*	72*	86*	40	75*	65*	119*	17*
" (b)									8*					
" (c)							99*							
altissima (a)	11								8*			22	4	
" (b)	14						15			26		14	22	
" (c)	58*						49		103*			14	7	
dentata (a)														
" (b)														
hirsuta	55*	13	8*		14	10*	123*			50			98*	14*
officinalis	15								45				43	
suaveolens (a)	16				19	32	73	28	163				46	12
" (b)	42	18	14		35	59				38			54	
taurica	44*					53			84*	46				
wolgica														

a. Interspecific cross compatibility among the subgenus *Eumelilotus*.

Cross pollinations were made between pairs of eight species belonging to the subgenus *Eumelilotus*. The number of flowers used for crosses, and the interspecific cross combinations are indicated in table 9. Among them the crosses which gave the F_1 hybrid seedlings are marked by* in the table. For the interspecific crosses which gave seeds, the seeds obtained from the crosses were classified as plump, shrunken, much shrunken, small, and brown aborted seeds. The number of selfed seedlings and hybrid seedlings derived from each class of seeds are presented in table 10 and 11.

In crosses of *M. alba* × *M. altissima*, *M. alba* var. *common* was used as pistillate parent and three strains of *M. altissima* T 453 Ac 165, T 454 Ac 158, and T 455 B 130 were used as pollen parents. In cross, *M. alba* var. *common* × *M. altissima* T 453 Ac 165, no seeds were obtained because of the small number of crossed flowers. In the other two crosses, 26 seeds from the cross of *M. alba* var. *common* × *M. altissima* T 454 Ac 158 and 8 seeds from the cross of *M. alba* var. *common* × *M. altissima* T 455 B 130 were obtained. The seeds consisted of plump, shrunken, and small seeds in the former cross, and all plump seeds in the latter cross. From all classes of the above seeds hybrid seedlings were resulted (table 10). The hybrid seedlings showed a heavy chlorophyll deficiency but were slightly more pigmented than that of *M. alba* × *M. dentata* (fig. 24–a). All the seedlings died when the first true leaves sprouted.

In the reciprocal cross, *M. altissima* × *M. alba*, 21 seeds were obtained from the cross of *M. altissima* T 455 B 130 × *M. alba* var. *common* (table 11), although no hybrid seeds were obtained from crosses of *M. altissima* T 453 Ac 165 × *M. alba* var. *common* and *M. altissima* T 454 Ac 158 × *M. alba* var. *common*, which cross pollinations were made (table 9). Thirteen out of 21 seeds were plump and gave only one hybrid seedling with chlorophyll deficiency and died soon after the first true leaves began to emerge (fig. 24–b). The remaining seeds consisted of shrunken, much shrunken, and aborted brown seeds, and they did not give hybrid seedlings. The degree of chlorophyll deficiency is comparable to that of *M. alba* × *M. altissima* hybrids.

The F_1 hybrids of *M. alba* × *M. dentata* were obtained from all crosses, *M. alba* var. *common* × *M. dentata* Ac 91 N 338, *M. alba* var. *common* × *M. denta* N 158, and M. *alba* var. *hubum* × *M. dentata* Ac 91 N 338, which were examined in this test. As a whole, 67 seeds obtained consisted of plump, shrunken, much shrunken, small, and brown aborted seeds, from all classes of which, except brown aborted seeds, the F_1 hybrid seedlings were derived (table

TABLE 10. Plumpness of seeds and number of hybrid seedlings obtained from the crosses between pairs of species of *Eumeliotus*

crosses	no. of seeds	no. of seeds					seedlings	
		plump	shrunken	much shrunken	small	brown aborted	no. of selfs	no. of hybrids
alba (a) × *altissima* (b)	26	16	7		3		15 1 2	1 2 5
alba (a) × *altissima* (c)	8	8					3	5
alba (a) × *dentata* (a)	28	10	10		8		7 1 7	3 7 3
alba (a) × *dentata* (b)	26	13		4	6	3	13 3 2 0	0 3 2 0
alba (c) × *dentata* (a)	13	8		3		2	5 2 0	3 0 0
alba (a) × *hirsuta*	24	17			1	6	12 0 0	5 1 0
alba (b) × *hirsuta*	1	1					0	1
alba (a) × *suaveolens* (a)	19	9			10		9 2	0 8
alba (a) × *suaveolens* (b)	13	4			9		3 1	1 8
alba (a) × *taurica*	28	8	7	6		7	6 4 5 0	2 3 1 0
alba (a) × *wolgica*	6	5			1		4 0	1 1

10). The F_1 seedlings showed a distinct chlorophyll deficiency and could not live beyond the cotyledonal stage. (fig. 24–c).

It was inconvenient to use *M. dentata* as pistillate parent, since biennial *M. dentata* shadded pollen in the bud stage. Consequently, *M. dentata* were

not used as pistillate parents in crosses with any other species in this study.

In crosses of *M. alba* × *M. hirsuta*, three strains of *M. alba* var. *common*, var. *evergreen*, and var. *hubum*, were used for pistillate parents, and *M. hirsuta* F 149 was used for pollen parent. From the crosses of *M. alba* var. *common* × *M. hirsuta* F 149 and *M. alba* var. *hubum* × *M. hirsuta* F 149, 25 seeds in total were obtained (table 10). They consisted of plump, small, and brown aborted seeds. The F_1 hybrid seedlings which were obtained from plump and small class of seeds showed less chlorophyll deficiency and lived to maturity.

In the reciprocal cross, *M. hirsuta* × *M. alba*, using three strains of *M. alba* mentioned above as pollen parents, hybrid plants resulted from two crosses, *M. hirsuta* F 149 × *M. alba* var. *common* and *M. hirsuta* F 149 × *M. alba* var. hubum (table 11). The seeds consisted of plump, shrunken, and brown aborted seeds but the high percentage of hybrid plants resulted were from plump seeds as well as from the crosses of *M. alba* × *M. hirsuta*. The hybrid seedlings showed a slight chlorophyll deficiency which was comparable to that of *M. alba* × *M. hirsuta* hybrids (fig. 24–d).

Cross pollinations were made between *M. alba* and *M. suaveolens* by using *M. alba* var. *common* as pistillate parent and *M. suaveolens* N 348 and N 171 as pollen parents. In both crosses, the seeds obtained were classified into plump and small seeds. Most of the hybrid seedlings were derived from small seeds in high percentage (table 10). The seedlings were normal green in color, but the cotyledon of the hybrid failed to differentiate normaly so that all seedlings died at the stage when the cotyledon opens.

The reciprocal cross pollinations, *M. suaveolens* × *M. alba*, by using the same two strains of *M. suaveolens* as pistillate parents and three strains of *M. alba*, var. *common*, var. *evergreen*, and var. *hubum*, as pollen parents. No hybrids, however, were obtained from these crosses.

Twenty-eight seeds were obtained from the crosses of *M. alba* var. *common* × *M. taurica*. The seeds consisted of plump, shrunken, much shrunken, and brown aborted seeds. Six hybrid seedlings resulted from all classes of seeds except brown aborted seeds but showed heavy chlorophyll deficiency which was similar to that of *M. alba* × *M. altissima* hybrids (table 10, fig. 24–e). The seedlings were very weak and not able to survive more than 3–4 cm in plant height.

On the other hand, 10 seeds which consisted of plump and brown aborted seeds were obtained from the cross of *M. taurica* × *M. alba* var. *common*. Only one seedling had almost the same pigmentation in color with that of *M. alba* × *M. taurica* hybrids (fig. 24–f), but is continuing to grow to about

30 cm in plant height at the time of this writing.

From 6 seeds produced from cross, *M. alba* var. *common* × *M. wolgica* K 443 Ac 163, 2 hybrid seedlings were obtained. The seedlings showed a distinct chlorophyll deficiency which was comparable to that of the hybrid,

TABLE 11. Plumpness of seeds and number of hybrid seedlings obtained from the crosses between pairs of species of *Eumelilotus*

crosses	no. of seeds	no. of seeds					seedlings	
		plump	shrunken	much shrunken	small	brown aborted	no. of selfs	no. of hybrids
altissima (a) × *hirsuta*	10	3		2		5	1 1 0	2 0 0
altissima (c) × *alba* (a)	21	13	4	1		3	12 4 1 0	1 0 0 0
altissima (c) × *hirsuta*	27	13	6			8	12 1 0	1 5 0
hirsuta × *alba* (a)	19	9	2			8	0 1 0	9 1 0
hirsuta × *alba* (c)	6	5	1				0 1	5 0
hirsuta × *altissima* (c)	2	2					1	1
hirsuta × *dentata* (a)	43	17	8			18	15 4 0	2 4 0
hirsuta × *taurica*	19	6	5			8	6 2 0	0 3 0
hirsuta × *wolgica*	10		4	2		4	2 2 0	1 0 0
taurica × *alba* (a)	10	8				2	7 0	1 0
taurica × *hirsuta*	30	19		1		10	12 1 0	7 0 0

M. alba × *M. dentata*, and did not survive beyond the cotyledonal stage (table 10, fig. 25–a).

In the crosses, *M. altissima* × *M. hirsuta*, 2 hybrid seedlings were obtained from the cross of *M. altissima* T 453 Ac 165 × *M. hirsuta* F 149 and 6 hybrid seedlings resulted from the cross of *M. altissima* T 455 B 130 × *M. hirsuta* F 149 (table 11). The seeds produced from each cross consisted of plump, shrunken, much shrunken and brown aborted seeds. All seedlings showed distinct chlorophyll deficiency and were not able to grow beyond the cotyledonal stage (fig. 25–b). In the case of the cross, *M. hirsuta* × *M. altissima*, only one hybrid seedling was obtained from the cross of *M. hirsuta* F 149 × *M. altissima* T 455 B 130, but showed distinct chlorophyll deficiency as seen in *M. altissima* × *M. hirsuta* hybrid and died soon after cotyledon completely opened (table 11).

In crosses, *M. hirsuta* × *M. dentata*, 43 seeds which were consisted of plump, shrunken, and brown aborted seeds were produced from the cross of *M. hirsuta* F 149 × *M. dentata* N 338 Ac 91. Six hybrid seedlings resulted from plump and shrunken seeds but showed distinct chlorophyll deficiency and could not live beyond the cotyledonal stage (table 11, fig. 25–c).

From the cross of *M. hirsuta* F 149 × *M. taurica* PI 193951, 19 seeds which consisted of plump, shrunken, and brown aborted seeds were produced. Three hybrid seedlings resulted from shrunken seeds (table 11). They showed distinct chlorophyll deficiency and did not develop beyond the cotyledonal stage (fig. 25–d).

The reciprocal cross, *M. taurica* PI 193951 × *M. hirsuta* F 149, was also attempted and 30 seeds which consisted of plump, much shrunken, and brown aborted seeds were obtained. Seven hybrid seedlings resulting from plump seeds also showed a distinct chlorophyll deficiency and died soon after cotyledon completely opened (table 11, fig. 25–e).

Cross pollination was made between *M. hirsuta* F 149 and M. *wolgica* K 443 Ac 163 using *M. hirsuta* as pistillate parent. Ten seeds, which consisted of plump and brown aborted seeds, were obtained. Only one hybrid seedling resulted from plump seeds (table 11). The seedlings showed distinct chlorophyll deficiency and did not live beyond the cotyledonal stage (fig. 25–f).

Numerous cross pollinations were made between pairs of species besides the interspecific crosses which gave F_1 hybrid seedlings as described above, but no hybrid seedlings resulted from these crosses thus far examined. Among them, it should be mentioned that the crosses between *M. officinalis* and each of the following species, *M. alba*, *M. altissima*, *M. hirsuta*, *M. taurica*, and *M. suaveolens* in using *M. officinalis* as pistillate parent or pollen parent did

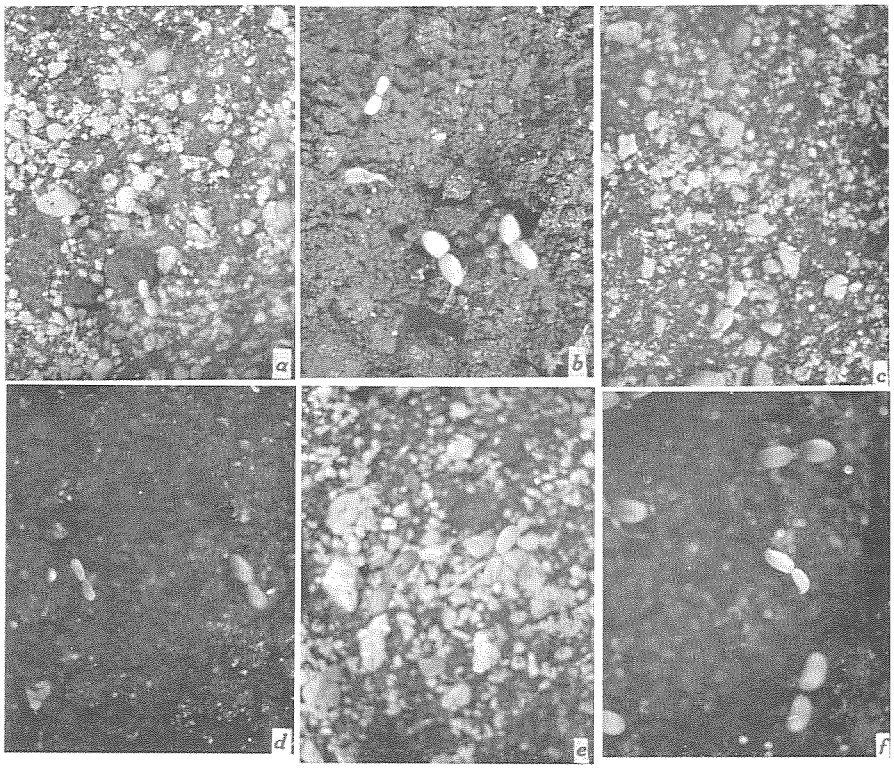


Fig. 24. The F_1 hybrid seedlings showing chlorophyll deficiency
a. *M. alba*×*M. altissima*
b. *M. altissima*×*M. alba*
c. *M. alba*×*M. dentata*
d. *M. alba*×*M. hirsuta*
e. *M. alba*×*M. taurica*
f. *M. taurica*×*M. alba*

not give any hybrid seedling (table 9). *M. officinalis* was the only species which did not give hybrids among crosses conducted in this test.

M. suaveolens also showed low cross compatibility in this study, giving hybrid seedlings only from the cross of *M. alba*× *M. suaveolens.*

b. Interspecific cross compatibility among *Micromelilotus* species.

Cross pollinations were also made among 10 species belonging to the subgenus *Micromelilotus*. *M. indica* was not used as pistillate parents in this subgenus, because the very small size of flowers made it difficult to emasculate pollens of *M. indica*. Considerable large numbers of interspecific crosses were made. The number of flowers pollinated in each cross are given in table 12.

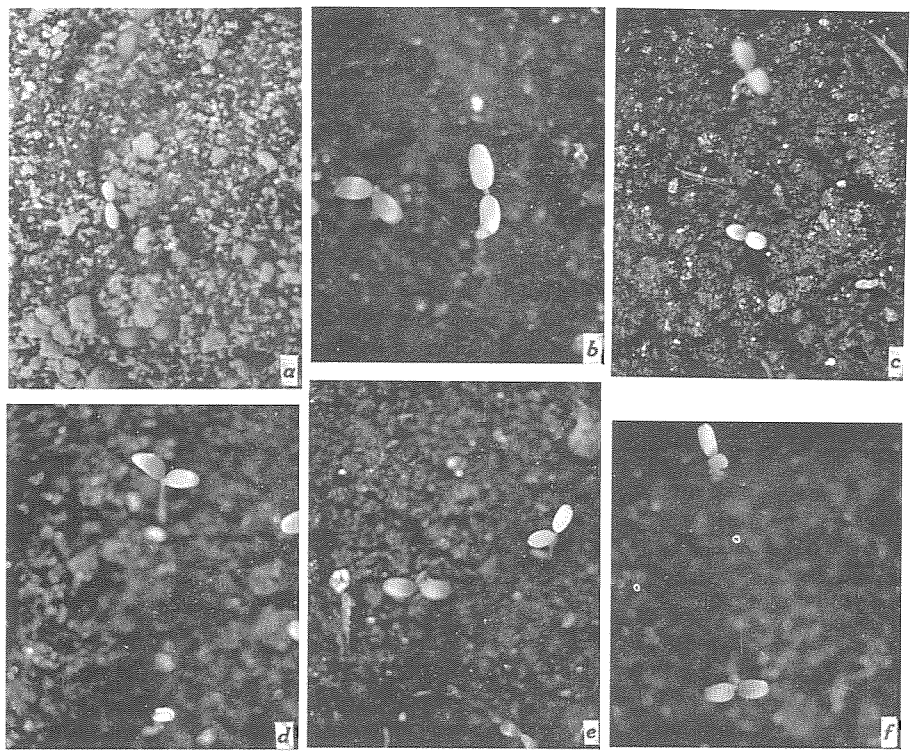


Fig. 25. The F_1 hybrid seedlings showing chlorophyll deficiency
a. *M. alba*×*M. wolgica*
b. *M. altissima*×*M. hirsuta*
c. *M. hirsuta*×*M. dentata*
d. *M. hirsuta*×*M. taurica*
e. *M. taurica*×*M. hirsuta*
f. *M. hirsuta*×*M. wolgica*

A few interspecific hybrid plants were obtained as indicated by the mark * in the table 12.

From the cross of *M. messanensis* C 21 × *M. segetalis* N 80, 12 seeds which consisted of 10 plump and 2 brown aborted seeds were obtained. Eight hybrid seedlings resulting from plump seeds were completely normal green in color and vigorously grew to maturity (table 13). Pod setting of *M. messanensis* is very high without insect or artificial tripping, therefore, cross pollinations were made by conducting emasculation one or two days before flowers opened in order to avoid selfing.

From the cross of *M. segetalis* N 80 × *M. messanensis* C 21, 10 seeds were obtained. They consisted of 8 plump and 2 brown aborted seeds and all 8

TABLE 12. Cross combination and number of flowers made cross-pollinations between pairs of species of *Micromelilotus*

	elegans	*indica* (a)	" (b)	*infesta*	*italica*	*macrocarpa*	*messanensis*	*neapolitana*	*segetalis*	*speciosa*	*sulcata* (a)	" (b)	" (c)
elegans		15		48	47	38	38	15	46	19		9	
indica (a)													
" (b)													
infesta						28	30	49		14		29	14	19
italica					38		45	42	53	53	98		18	16
macrocarpa				28	72	94		32	51	51	71	12	16	17
messanensis			12		24	42	30		10	52*	28	37		40
neapolitana	41	27	12	10	16	22			17	8	24	11	10
segetalis				23	46	87	64*	10*	51		123	191	5	68
speciosa	8	8		11	6	89	24	40	87		23		48
sulcata (a)							8		10				
" (b)													
" (c)			8	7*	28	12*	17		37	9			

plump seeds resulted in hybrid seedlings with complete normal green color and continued to grow to maturity (table 13). In this case, emasculation of *M. segetalis* was done one or two days before flowers opened.

Thirty-two seeds were produced from the cross of *M. segetalis* N 80 × *M. macrocarpa* Ac 336. They consisted of 29 plump, 1 much shrunken, and 2 brown aborted seeds. Three hybrid seedlings resulted from the plump seeds

and showed normal green color. They vigorously grew to maturity (table 13). The reciprocal cross, *M. macrocarpa* Ac 336 × *M. segetalis* N 80, was also attempted, but no hybrid was produced.

TABLE 13. Plumpness of seeds and number of hybrid seedlings obtained from the crosses between pairs of species of *Micromelilotus*

crosses	no. of seeds	no. of seeds					seedlings	
		plump	shrunken	much shrunken	small	brown aborted	no. of selfs	no. of hybrids
messanensis × *segetalis*	12	10				2	2 0	8 0
segetalis × *macrocarpa*	32	29		1		2	26 1 0	3 0 0
segetalis × *messanensis*	10	8				2	0 0	8 0
sulcata (c) × *infesta*	2	2					1	1
sulcata (c) × *macrocarpa*	4	4					2	2

From 2 mature seeds produced from the cross of *M. sulcata* × *M. infesta* Ac 335, only one hybrid seedling resulted (table 13). The hybrid showed light green in color but continued to grow to maturity. No hybrid was obtained from the reciprocal cross, *M. infesta* Ac 335 × *M. sulcata.*

Four mature seeds were obtained from the cross of *M. sulcata* × *M. macrocarpa* Ac 336. Two hybrids resulted from the plump seeds and showed completely normal green in color (table 13). The reciprocal cross, *M. macrocarpa* Ac 336 × *M. sulcata*, did not give any hybrids so far as examined.

M. sulcata which was used in crosses, *M. sulcata* × *M. infesta* and *M. sulcata* × *M. macrocarpa*, was the species introduced under the name of *M. segetalis* N 118. As mentioned previously, *M. segetalis* N 118 was shown to be a synonym of *M. sulcata.*

Numerous cross pollinations were made between pairs of other species of *Micromelilotus* (table 12), however, no other hybrid was obtained in this experiment.

c. Interspecific cross compatibility between species of *Eumelilotus* and *Micromelilotus*.

In the limited scale, cross pollinations between species of *Eumelilotus* and *Micromelilotus* were attempted (table 14). All crosses made in this test were conducted by using species belonging to the subgenus *Micromelilotus* as pistillate

TABLE 14. Cross combination and number of flowers made cross-pollinations between species of *Eumelilotus* and *Micromelilotus*

	alba (a)	" (b)	" (c)	*altissima* (a)	" (b)	" (c)	*dentata* (a)	" (b)	*hirsuta*	*officinalis*	*suaveolens* (a)	" (b)	*taurica*	*volgica*
elegans										57				
indica (a)														
" (b)														
infesta														
italica	10		13						26	30				
macrocarpa														
messanensis	13	41	9				57		38	37			84	
neapolitana														
segetalis	23								53					
speciosa			43											
sulcata (a)														
" (b)														
" (c)			21						19					

parents. So far as examined, no hybrids were obtained.

4. Discussion and Conclusion

The purpose of the present studies was to obtain informations on the general pattern of interspecific cross compatibility in relation to speciation of the genus *Melilotus*.

In regard to the interspecific cross compatibility of this genus, although a considerable number of studies have been reported during the past several decades (STEVENSON and KIRK 1935, STEVENSON and WHITE 1940, JOHNSON 1942, SMITH 1943 and 1954, WEBSTER 1950, GREENSHIELDS 1954, JARANOWSKI 1962), no experiments systematically planned have been undertaken except for one or two reports.

The relationship of cross compatibility among *Melilotus* species worked out by the author are summarised in table 15, together with the results reported by other workers mentioned above.

Cross pollinations among 13 species were made by MENDOZA (1946) and seeds resulting therefrom were planted by WEBSTER (1950). WEBSTER reported that normal green hybrids were obtained from the crosses of *M. alba* × *M. polonica* and *M. polonica* × *M. suaveolens*. He reported that the resulting hybrids from the cross between *M. suaveolens* and *M. wolgica* showed light green in color but that it developed to maturity; on the other hand, the hybrid seedlings from the cross, *M. alba* × *M. taurica*, was a weak hybrid with light green color and did not grow to maturity. Viable normal green hybrid from the cross between *M. alba* and *M. suaveolens* was also reported by STEVENSON and KIRK (1935). From the cross, *M. alba* × *M. dentata*, STEVENSON and WHITE (1940) mentioned that the resulting F_1 hybrid seedlings were deficient in chlorophyll and did not develop beyond the cotyledonal stage.

The first step of systematic cross pollinations were made by SMITH (1954) among eight species belonging to the subgenus *Eumelilotus*. SMITH classified 8 species into 3 groups (Group A, B, C) and explained that crosses between any member of Group A (*M. alba*, *M. suaveolens*, *M. polonica*) and a member of Group B (*M. altissima*, *M. dentata*, *M. taurica*, and *M. wolgica*) gave hybrid seedlings that were almost devoid of chlorophyll and did not live beyond the seedling stage. He mentioned that crosses between pairs within species of Group A produced normal green hybrids and crosses within species of Group B resulted in hybrids that showed more pigment than hybrids between Group A and Group B. *M. officinalis* was classified in Group C because no mature seeds were obtained from crosses between *M. officinalis* and anyone of the other species.

The results obtained by the author in the subgenus *Eumelilotus* largely support the description by SMITH and the other workers regarding the wide spread occurence of chlorophyll deficiency in the interspecific hybrids.

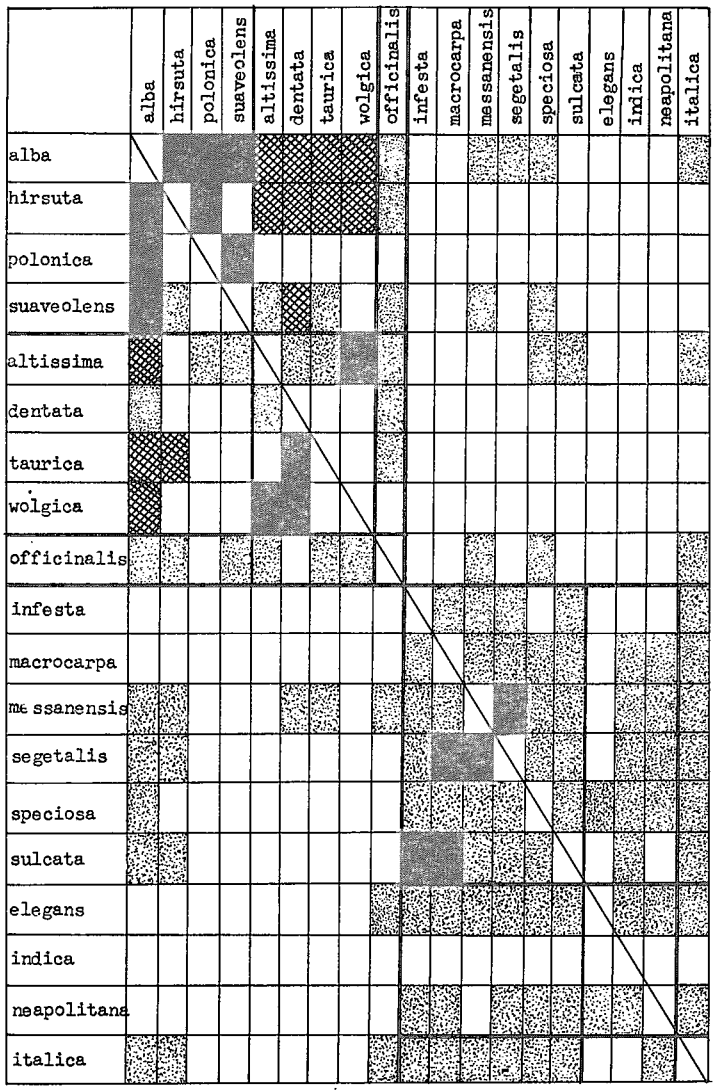
Several hybrids were obtained from the crosses between *M. hirsuta* and any single species of *Eumelilotus* made by the author, while GREENSHIELDS (1954) attempted partly but no hybrid was obtained. The hybrid between *M. hirsuta* and *M. alba* showed less green in color compared with normal parents but vigorously grew to maturity. In the case of crosses between *M. hirsuta* and *M. altissima*, *M. dentata*, *M. taurica*, and *M. wolgica*, however, the resulting hybrids from the crosses showed heavy chlorophyll deficiency and did not live beyond the seedling stage. Consequently, *M. hirsuta* is considered to be classified in Group A proposed by SMITH (1945). It should be, however, pointed out that the hybrid between *M. alba* and *M. hirsuta* was not complete normal green in color as mentioned in the cross between *M. alba* and *M. suaveolens* or *M. alba* and *M. polonica* reported by other workers.

Six hybrid seedlings resulted from the cross of *M. alba* × *M. taurica* were almost devoid of chlorophyll and did not live beyond seedling stage, while only one hybrid obtained from the reciprocal cross, *M. taurica* × *M. alba*, showed also same degree of chlorophyll deficiency. But this hybrid plant has attained about 30 cm height in green house at the time of this writing. SMITH (1954) mentioned that the F_1 hybrids of *M. taurica* × *M. alba* and the reciprocal showed a somewhat more green color than the *M. alba* × *M. dentata* hybrids but none survived to show true leaves. WEBSTER (1950), however, obtained light green F_1 hybrids from the cross of *M. alba* × *M. taurica*, which survived for two years and produced a few flowers. The F_1 hybrid of *M. taurica* × *M. alba* in this experiment is showing a response very much akin to the hybrid produced by WEBSTER. The fact indicates that there are some variations in degree of chlorophyll deficiency between strains of the same species or even in the same strain used.

Seven crosses between *M. officinalis* and other species of *Eumelilotus* were attempted but no viable hybrid seeds were obtained. This result completely agrees with the report that no mature seeds were obtained from crosses between *M. officinalis* and any single of the other species (SMITH 1954).

As far as the subgenus *Eumelilotus* is concerned, it may be concluded that a high interspecific compatibility exists between pairs of species except *M. officinalis*. Although some cross combinations have not been made as yet (table 15), it is possible to assume that the additional hybrid seedlings may be obtained from most of these remaining crosses if a large number of flowers are used for pollinations in an appropriate manner.

TABLE 15. Relationships of interspecific cross compatibility of nineteen species of the genus *Melilotus*



Viable hybrid was obtained.

Hybrid seeds were obtained but the F_1 seedlings were distinct chlorophyll deficiency and could not survive.

Pollination was made but hybrid seeds were not obtained.

Pollination has not been attempted.

Regarding crosses among ten species of the subgenus *Micromelilotus*, a few of interspecific hybrids have been reported (WEBSTER 1950; GREENSHIELDS 1954; SHASTRY, SMITH and COOPER 1960). WEBSTER (1954) made a cross between *M. italica* and *M. messanensis* and obtained 4 plants which were judged to be F_1 hybrids on the basis of a very highly aborted pollen. SHASTRY, SMITH and COOPER (1960) reported a cytogenetic study on the interspecific hybrid, *M. messanensis* × *M. segetalis*, which seeds were produced by H. J. GORZ, Lincoln, Nebraska. GREENSHIELDS (1954) made crosses between *M. sulcata* and *M. speciosa*, *M. italica* and *M. messanensis*, and *M. italica* and *M. speciosa*, but no hybrids were obtained.

The author made cross pollinations among ten species of this subgenus and obtained five different interspecific hybrids; *M. messanensis* × *M. segetalis*, *M. segetalis* × *M. messanensis*, *M, segetalis* × *M. macrocarpa*, *M. sulcata* × *M. infesta*, and *M. sulcata* × *M. macrocarpa*. The low frequency of occurrence of hybrids from crosses among *Micromelilotus* species is presumably due to flowering habits of *Micromelilotus* species in part, namely, selfing is very high in most of species in this subgenus without insect or artificial tripping.

The hybrid plant derived from the cross of *M. sulcata* × *M. infesta* showed light green in color but continued to grow to maturity. It is suggested that the chlorophyll deficiency series may exist in the interspecific hybrids among *Micromelilotus* species also.

Large numbers of seeds from cross pollinations between *M. italica* and *M. messanensis* were obtained but no morphological difference was observed. Consequently all plants derived from these seeds were judged to be selfed ones.

Through data of crosses among *Micromelilotus*, it may be suggested that a full understanding of the nature of flowering habits of each species are required for the success of interspecific hybridization in this subgenus.

Crosses between species of *Eumelilotus* and *Micromelilotus* have been attempted by some workers (GREENSHIELDS 1954, JARANOWSKI 1962), however no hybrids were obtained as yet. JARANOWSKI (1962) reported that fertilization did take place in such crosses as *M. italica* × *M. alba* var. *hubum*, *M. italica* × *M. alba* var. *evergreen*, and *M. messanensis* × *M. alba* var. *hubum*. Among them, he observed that the embryo, when *M. messanesis* was crossed with *M. alba* var. *hubum*, continued to develope to a few days before ripening but shrivelled and finally only the shrunken testa were left. Although no hybrids were obtained from crosses between species of these two subgenus, it is expected with certainty that hybrids may be secured by using embryoculture technique in order to clarify whether the genom of the two sections, *Eumelilotus* and *Micromelilotus*, are the same or not by cytological study.

IV. Studies on Morphology of the Somatic Chromosomes of the Genus *Melilotus*

From the experiments of interspecific cross compatibility, it was revealed that a high compatibility existed within the species of the subgenus *Eumelilotus* and between several pairs of species of the subgenus *Micromelilotus*.

It was also indicated that non-crossability observed between species of *Eumelilotus* and *Micromelilotus* and pairs of certain species within each subgenus, and inability of the interspecific F_1 seedlings to survive due to chlorophyll deficiency play at least in part a role in the isolation mechanism of species in the genus *Melilotus*.

It has been recognized by many workers that the study of karyomorphology is becoming increasingly important in estimating the phylogeny and differentiation of plant species. During the past several years, the author has been engaged in studies of karyotype analysis of nineteen species of the genus in order to find the precise picture of the interspecific relationship from a cytological view point. At the same time, the author has attempted to find correlations, if any, between interspecific cross compatibility and karyotypic differences of the nineteen species of the genus examined.

Though the present data are not sufficient to fully establish karyotypes of each species in detail, this paper presents some basic information on the phylogenetic relationships based on karyomorphology.

1. Materials

Nineteen species used in this experiment are listed in table 16.

2. Methods

Somatic chromosomes were examined in root tip cells. Root tips were cooled in a refrigerator (ca. 0–4°C) for 14 hours before fixing so that chromosomes would be shortened and scattered. Afterwards the materials were killed immediately in CARNOY's fixative, 3 parts absolute alcohol to 1 part glacial acetic acid for 1 hour. These were hydrolysed in 1 N HCl at 60°C for 6 to 7 minutes and then stained in leuco fuchsin for 1 to 2 hours. The stained root tips were transfered to a slide after a quick rinse in 45% glacial acetic acid and quickly crushed between cover slip and slide by applying uniform pressure to the cover slip.

All figures of somatic chromosomes were drawn with the aid of an Abbe's drawing apparatus using Olympus oil-immersion objective 100× with Olympus P 20× ocular at a magnification of about 3000 times at table level.

For observation of meiotic chromosome behaviors, the same method des-

TABLE 16. Materials used for karyotype analysis

section	species	chromosome number	from	pollen fertility
		(2n)		(%)
Eumelilotus	*alba* var. *evergreen*	16	biennial	98.8
"	" var. *hubum*	"	annual	92.2
"	*altissima* T 454 Ac 158	"	biennial	96.8
"	*dentata* N 338 Ac 91	"	"	98.9
"	*hirsuta* F 149	"	"	97.9
"	*officinalis*	"	"	96.3
"	*polonica* J 135 Ac 141	"	annual	—
"	*suaveolens* N 348	"	biennial	99.2
"	*taurica* PI 193951	"	"	98.1
"	*wolgica* K 443 Ac 163	"	annual	99.1
Micromelilotus	*elegans* Ac 337	"	"	99.5
"	*indica* Ac 296	"	"	99.1
"	*infesta* Ac 335	"	"	98.5
"	*italica* V 285	"	"	96.6
"	*macrocarpa* Ac 336	"	"	96.8
"	*messanensis* C 21	"	"	99.4
"	*neapolitana* Ac 334	"	"	99.8
"	*segetalis* N 80	"	"	99.1
"	*speciosa* V 287	"	"	99.0
"	*sulcata* V 298	"	"	100.0

cribed in the following chapter were used.

3. **Experimental Results**

a. *M. alba* var. *evergreen*:

Eight bivalents at metaphase-1 of meiosis are regularly occurred (fig. 26-b). The number of chromosomes is $2n=16$ in the somatic cell (fig. 26-a).

The measurements of eight pairs of somatic chromosomes for this strain in length are given in table 17. The complements of somatic chromosomes are idiogramed in figure 36-A. The chromosome sizes vary from 3.5 μ to 2.7 μ in length. Among the eight pairs of chromosomes, six pairs have submedian primary constriction and one pair has median primary constriction. The rest one pair with the submedian primary constriction possesses a secondary constriction situated subterminaly in the short arm cutting off a microsatellite.

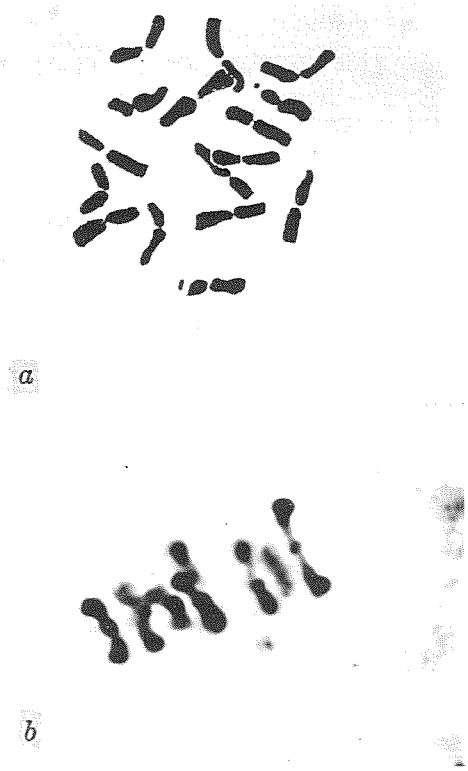


Fig. 26. *M. alba* var. *evergreen*
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8II. × 2500.

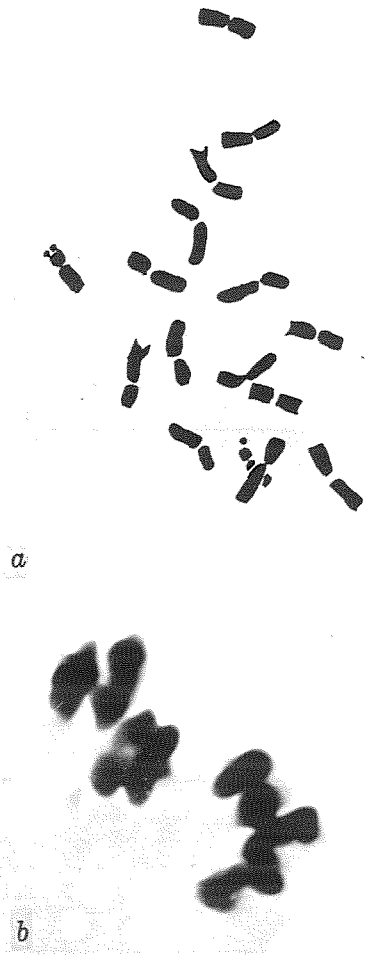


Fig. 27. *M. alba* var. *hubum*
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8II. × 2500.

TABLE 17. Measurements of somatic chromosomes of *M. alba* var. *evergreen*

chromosomes	length in (μ)	relative length	constriction
1-2	3.5=1.9+1.6	100	sm
3-4	3.4=2.1+1.3	97	sm
5-6	3.4=1.8+1.6	97	sm
7-8	3.4=1.7+1.7	97	m
9-10	3.2=1.9+1.3	91	sm
11-12	3.2=1.9+1.3	91	sm
13-14	3.1=1.6+1.5	89	sm
15-16	2.7=1.6+0.9+0.2	77	sm

TABLE 18. Measurements of somatic chromosomes of *M. alba* var. *hubum*

chromosomes	length in (μ)	relative length	constriction
1-2	3.5=2.1+1.4	100	sm
3-4	3.4=2.1+1.3	97	sm
5-6	3.4=1.9+1.5	97	sm
7-8	3.1=1.8+1.3	89	sm
9-10	3.0=1.5+1.5	86	m
11-12	3.0=1.7+1.3	86	sm
13-14	2.5=1.3+1.2	71	sm
15-16	2.3=1.5+0.6+0.2	66	sm

var. *hubum*:

The meiotic chromosome behaviors of this annual form of strain, *M. alba* var. *hubum*, is regular (fig. 27-b), and the pollen fertility is very high (table 16).

The chromosome number, $2n=16$, is observed at metaphase of somatic cell (fig. 27-a). Chromosome measurements of somatic chromosomes for this strain in length are presented in table 18. The size range of the eight pairs of chromosomes is from 3.5 μ to 2.3 μ in length. The idiogram of the eight pairs of chromosomes is given in figure 36-B.

The karyotype of this strain is very similar to that of *evergreen*. Six pairs of chromosomes have submedian primary constriction. The smallest pair of chromosomes with the submedian primary constriction has a secondary constriction in the short arm cutting off a microsatellite.

b. *M. altissima*

M. altissima T 454 Ac 158 used for karyotype analysis is normal in meiotic chromosome behaviors as indicated in fig. 28–b. The pollen fertility is also very high (table 16). The somatic metaphase, with $2n=16$, is shown in figure 28–a.

TABLE 19. Measurements of somatic chromosomes of *M. altissima*

chromosomes	length in (μ)	relative length	constriction
1–2	4.4=2.3+2.1	100	sm
3–4	3.9=2.3+1.6	89	sm
5–6	3.9=2.2+1.7	89	sm
7–8	3.8=2.3+1.5	86	sm
9–10	3.7=1.9+1.8	84	sm
11–12	3.4=1.8+1.1+0.5	77	sm
13–14	3.3=2.0+1.3	75	sm
15–16	3.3=1.7+1.6	75	sm

The measurements in length of eight pairs of somatic chromosomes for this species are given in table 19. The complements of eight pairs of chromosomes are idiogramed in figure 36–C. The size range of chromosomes is from 4.4 μ to 3.3 μ in length. Five pairs among eight pairs of chromosomes have primary constriction in the submedian position, and in two pairs the primary constriction is located close to the median position. The remaining single pair of chromosomes with a submedian primary constriction has a secondary constriction situated in the short arm cutting off a microsatellite.

c. *M. dentata*

Biennial form of *M. dentata* N 338 Ac 91 was used for this study. Meiosis is regular (fig. 29–b), and pollen fertility is very high (table 16).

The somatic metaphase which has $2n=16$ in somatic chromosome number is presented in figure 29–a. The measurements of eight pairs of somatic chromosomes for this species in length are shown in table 20. The eight pairs of chromosomes are idiogramed in figure 36–D. The size range of chromosomes is from 4.2 μ to 2.7 μ in length. An analysis of the idiogram reveals that six pairs of chromosome have submedian primary constriction and one pair possesses primary constriction in median position. The remaining single pair of chromosomes with median primary constriction has a secondary con-

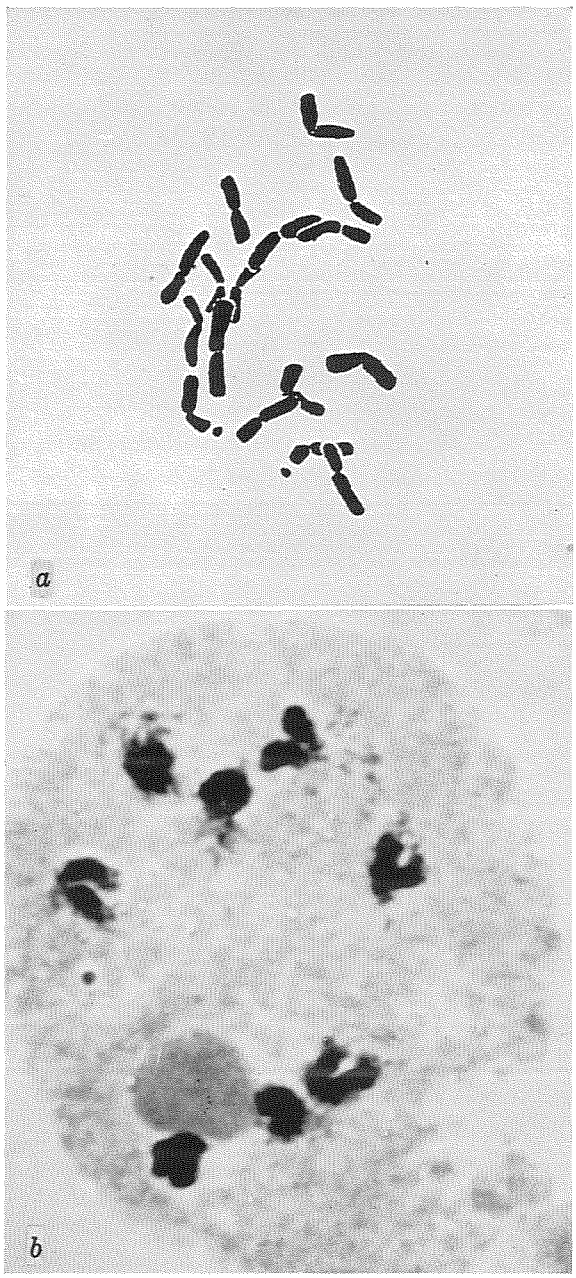


Fig. 28. *M. altissima* T 454 Ac 158
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic diakinesis with 8II. × 2500.

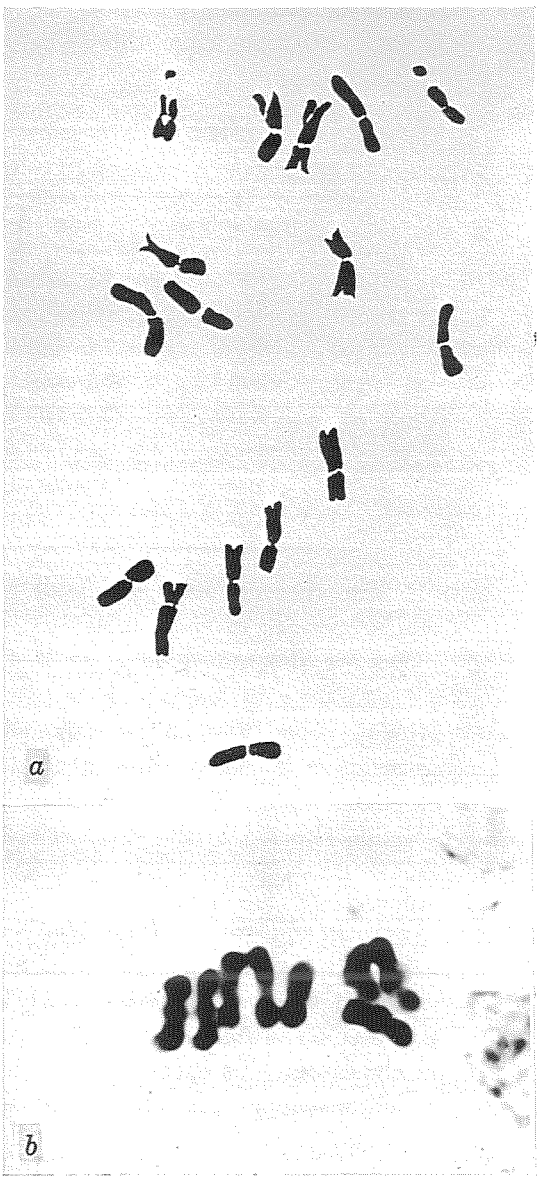


Fig. 29. *M. dentata* N 338 Ac 91
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8II. × 2500.

TABLE 20. Measurements of somatic chromosomes of *M. dentata*

chromosomes	length in (μ)	relative length	constriction
1-2	4.2=2.4+1.8	100	sm
3-4	3.7=2.4+1.3	88	sm
5-6	3.5=1.8+1.7	83	sm
7-8	3.4=1.7+1.7	81	m
9-10	3.3=2.0+1.3	79	sm
11-12	3.3=2.0+1.3	79	sm
13-14	3.3=1.8+1.5	79	sm
15-16	2.7=1.1+1.1+0.5	64	m

striction situated in one arm cutting off a microsatellite. This could be one of the characteristic differences in karyotype of this species as compared with those of other species, in other words, a microsatellite is attached to one arm of the chromosome with median primary constriction in *M. dentata*, while a microsatellite is attached to the short arm of the chromosome with submedian primary constriction in most of the other species.

d. *hirsuta*

M. hirsuta F 149 was used for this study. Eight bivalents are regularly formed at metaphase-1 (fig. 30-b), indicating that the meiosis of this species is regular. The pollen fertility is also very high (table 16).

2n=16 chromosomes at metaphase of somatic cell is given in figure 30-a. The measurements in length of eight pairs of somatic chromosomes for this

TABLE 21. Measurements of somatic chromosomes of *M. hirsuta*

chromosomes	length in (μ)	relative length	constriction
1-2	3.1=1.9+1.2	100	sm
3-4	2.6=1.6+1.0	84	sm
5-6	2.5=1.0+0.8+0.7	81	sm
7-8	2.4=1.4+1.0	77	sm
9-10	2.4=1.3+1.1	77	sm
11-12	2.4=1.3+1.1	77	sm
13-14	2.4=1.3+1.1	77	sm
15-16	2.3=1.2+1.1	84	sm

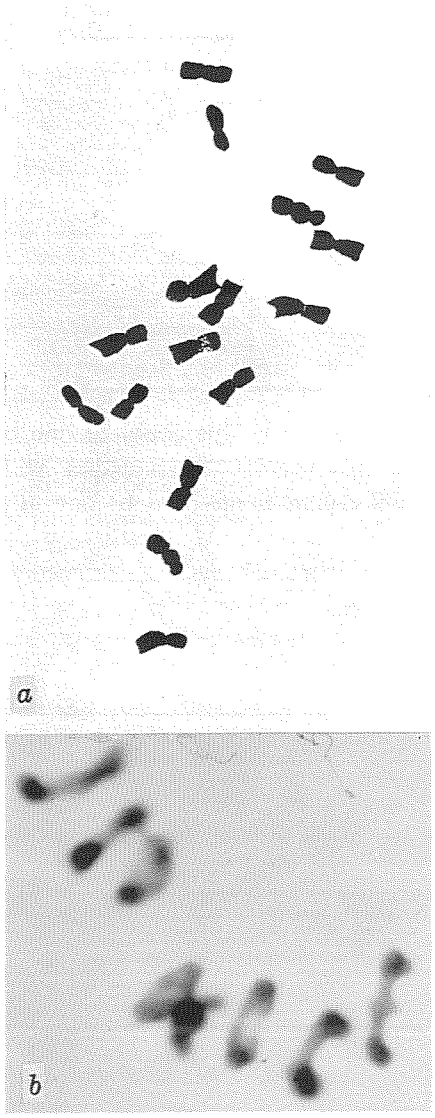


Fig. 30. *M. hirsuta* F 149
a. Mitotic metaphase with 16 chromosomes × 2700.
b. Meiotic metaphase-1 with 8 II. × 2500.

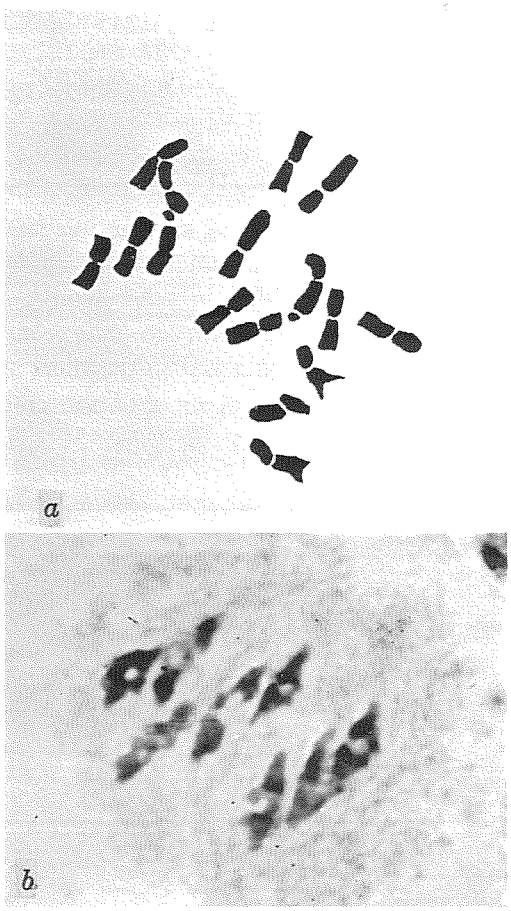


Fig. 31. *M. officinalis*
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8 II. × 2500

species is shown in table 21. The length of chromosomes vary from 3.1 μ to 2.3 μ. The eight pairs of somatic chromosomes are idiogramed in figure 36-E arranged from the longest chromosomes to the shortest ones. Seven pairs of chromosomes possess submedian primary constriction. The remaining single pair of chromosomes with submedian primary constriction has a secondary constriction situated in the submedian position in the short arm cutting off the large distal segment, which should be noted as a characteristic feature in the karyotype of this species.

e. *M. officinalis*

Eight bivalents are regularly formed at metaphase-1 of this species used here (fig. 31-b). The pollen fertility is very high (table 16).

TABLE 22. Measurements of somatic chromosomes of *M. officinalis*

chromosomes	length in (μ)	relative length	constriction
1-2	3.5=2.2+1.3	100	sm
3-4	3.2=1.7+1.5	91	sm
5-6	3.1=1.6+1.5	89	sm
7-8	3.0=1.7+1.3	86	sm
9-10	3.0=1.4+1.1+0.5	86	sm
11-12	2.9=1.6+1.3	83	sm
13-14	2.7=1.5+1.2	77	sm
15-16	2.7=1.4+1.3	77	sm

At metaphase of somatic cell, 2n=16 in chromosome number is observed (fig. 31-a). The length of the somatic chromosomes of this species ranges from 3.5 μ to 2.7 μ as shown in table 22. All eight pairs of chromosomes are idiogramed in figure 36-F. Seven pairs of chromosomes among eight pairs have submedian primary constriction, in two pairs of which the primary constriction is located close to the median position. The remaining single pair of chromosomes with submedian primary constriction possess a subterminal secondary constriction in the short arm cutting off a microsatellite.

f. *M. polonica*

M. polonica J 135 Ac 141 used in this study is regular in meiotic chromosome behaviors (fig. 32-b). The percentage of pollen fertility was not determined, since the plants did not reach flowering in the introduced year.

TABLE 23. Measurements of somatic chromosomes of *M. polonica*

chromosomes	length in (μ)	relative length	constriction
1-2	3.3=2.1+1.2	100	sm
3-4	3.3=1.8+1.5	100	sm
5-6	3.0=1.8+1.2	91	sm
7-8	3.0=1.6+1.4	91	sm
9-10	2.9=1.7+1.2	88	sm
11-12	2.7=1.0+0.8+0.9	82	sm
13-14	2.6=1.4+1.2	79	sm
15-16	2.6=1.3+1.3	79	m

At the meiotic metaphase, $2n=16$ in chromosome number is observed (fig. 32-a). The measurements of eight pairs of somatic chromosomes for this species in length are presented in table 23. The chromosome complements of this species is idiogramed in figure 36-G. The size range of chromosomes is from 3.3 μ to 2.6 μ in length. Six pairs of chromosomes have submedian primary constriction, and one pair has median primary constriction. A characteristic difference noted in karyotype is observed in the remaining single pair of chromosomes with submedian primary constriction, namely the secondary constriction is located in the submedian position of the one arm cutting off the large distal segment.

g. *M. suaveolens*

An annual form of *M. suaveolens* N 348 was used for this analysis. The

TABLE 24. Measurements of somatic chromosomes of *M. suaveolens*

chromosomes	length in (μ)	relative length	constriction
1-2	3.4=2.2+1.2	100	sm
3-4	3.2=2.0+1.2	94	sm
5-6	3.1=1.9+1.2	91	sm
7-8	3.0=1.6+1.4	88	sm
9-10	2.8=1.8+1.0	82	sm
11-12	2.6=1.4+1.2	76	sm
13-14	2.3=1.4+0.9	68	sm
15-16	2.2=1.2+0.7+0.3	65	sm

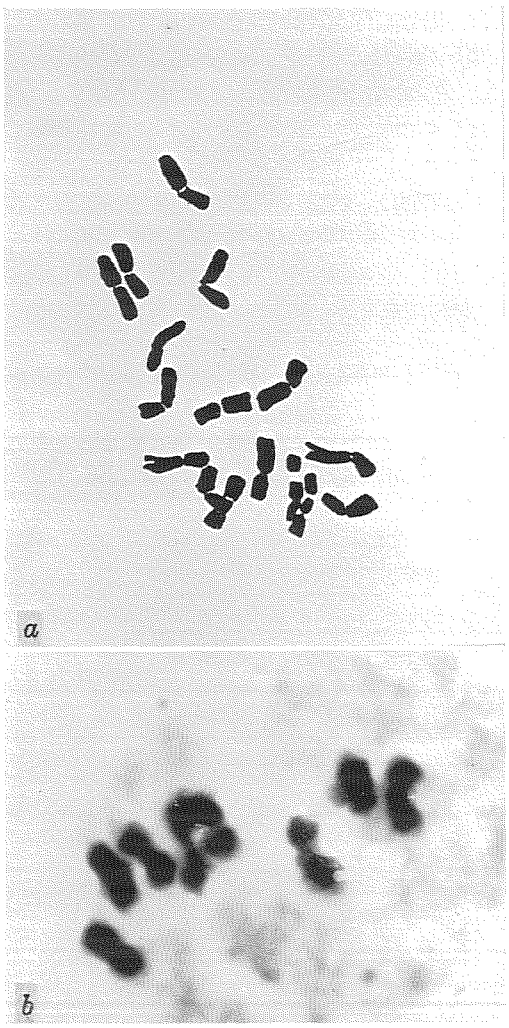


Fig. 32. *M. polonica* J 135 Ac 141
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8 II. × 2500.

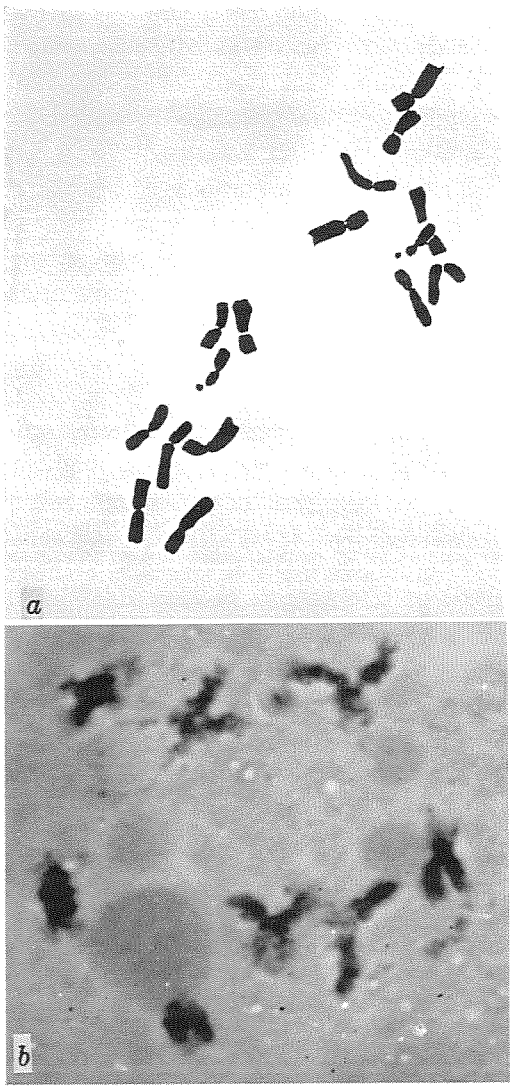


Fig. 33. *M. suaveolens* N 348
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic diakinesis with 8 II. × 2500.

regular occurrence of eight bivalents at diakinesis indicates that the course of meiosis of this species is normal (fig. 33–b). The pollen fertility is also very high (table 16).

The somatic metaphase reveals that the chromosome number of this species is $2n=16$ (fig. 33-a). The measurements in length of eight pairs of somatic chromosomes of this species is given in table 24, and the idiogram of eight pairs of chromosomes is presented in figure 36-H. The size range of chromosomes is from 3.4 μ to 2.2 μ in length. Among eight pairs, seven pairs of chromosomes have submedian primary constriction, in two pairs of which the primary constriction is located close to the median position. The remaining single pair of chromosomes with submedian primary constriction has a secondary constriction situated in the subterminal position of the short arm cutting off a microsatellite.

h. *M. taurica*

Eight bivalents are regularly formed at diakinesis (fig. 34-b). The pollen fertility of this species is very high (table 16).

At metaphase of the somatic cell, $2n=16$ in chromosome number is always observed (fig. 34-a). The measurements in length of eight pairs of somatic chromosomes for this species are shown in table 25, and the idiogram of eight

TABLE 25. Measurements of somatic chromosomes of *M. taurica*

chromosomes	length in (μ)	relative length	constriction
1-2	3.6=2.1+1.5	100	sm
3-4	3.3=2.0+1.3	92	sm
5-6	3.2=1.9+1.3	89	sm
7-8	2.9=1.6+1.3	81	sm
9-10	2.8=1.4+1.4	78	m
11-12	2.8=1.6+1.2	78	sm
13-14	2.7=1.4+1.3	75	sm
15-16	2.6=1.2+0.9+0.5	72	sm

pairs of chromosomes is presented in figure 36-I. The length of eight pairs of chromosomes ranges from 3.6 μ to 2.6 μ from the longest chromosomes to the shortest ones. Six pairs of chromosomes among eight pairs have submedian primary constriction, in one pair of which the primary constriction is located close to the median position. One pair of chromosomes has a median primary constriction. The remaining single pair of chromosomes with submedian primary constriction possess a secondary constriction situated in the subterminal position of the short arm cutting off a microsatellite.

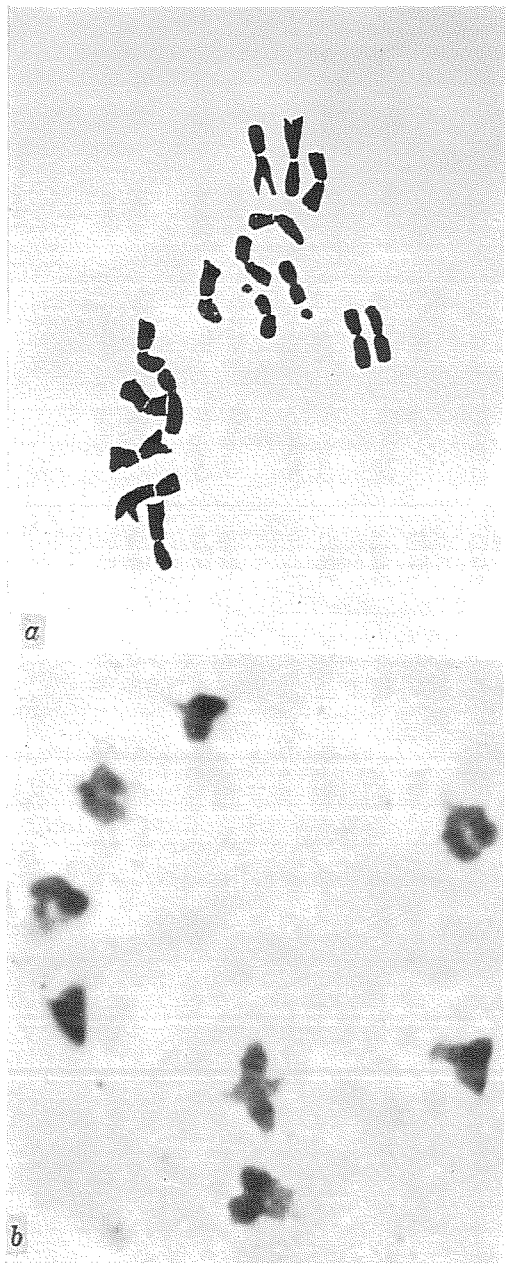


Fig. 34. *M. taurica* PI 193951
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic diakinesis with 8 II. × 2500.

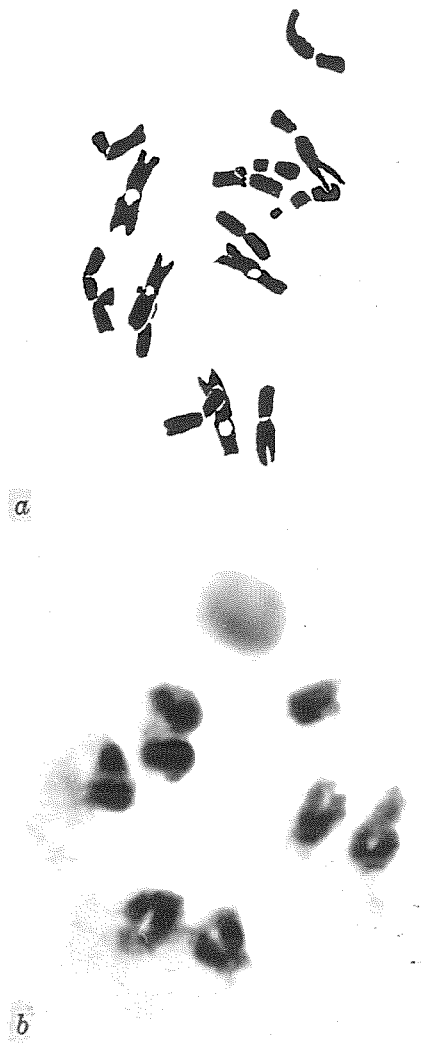


Fig. 35. *M. wolgica* K 443 Ac 163
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic diakinesis with 8 II. × 2500.

Fig. 36. Idiograms of the complements of somatic chromosomes of the subgenus *Eumelilotus*

A. *M. alba* var. *evergreen.*
B. *M. alba* var. *hubum.*
C. *M. altissima* T 454 Ac 158.
D. *M. dentata* N 338 Ac 91.
E. *M. hirsuta* F 149.
F. *M. officinalis.*
G. *M. polonica* J 135 Ac 141.
H. *M. suaveolens* N 348.
I. *M. taurica* PI 193951.
J. *M. wolgica* K 443 Ac 163.

i. *M. wolgica*

Eight bivalents are regularly observed at diakinesis of *M. wolgica* K 443 Ac 163 used for this study (fig. 35–b). The pollen fertility is also very high (table 16).

The chromosome number, $2n=16$, is constantly observed in somatic metaphase of this species (fig. 35–a). The idiogram of eight pairs of somatic chromosomes for this species is given in figure 36–J, and the measurements of eight pairs in length are indicated in table 26. The length of eight pairs of

TABLE 26. Measurements of somatic chromosomes of *M. wolgica*

chromosomes	length in (μ)	relative length	constriction
1–2	4.1=2.8+1.3	100	sm
3–4	3.9=2.4+1.5	95	sm
5–6	3.7=1.9+1.8	90	sm
7–8	3.5=1.9+1.6	85	sm
9–10	3.1=1.9+1.2	76	sm
11–12	3.1=1.6+1.5	76	sm
13–14	2.5=1.3+0.8+0.4	61	sm
15–16	2.4=1.4+1.0	59	sm

chromosomes ranges from 4.1 μ to 2.4 μ. The primary constriction is situated in the submedian position in seven pairs of chromosomes, in two pairs of which the primary constriction is located close to the median position. The remaining single pair of chromosomes with submedian primary constriction has a secondaly constriction situated in the subterminal position of the short arm cutting off a microsatellite.

j. *M. elegans*

At metaphase-1 of meiosis, eight bivalents are always observed in *M. elegans* Ac 337 used for this study (fig. 37–b). The pollen fertility is very high (table 16).

$2n=16$ in somatic chromosome number is observed at metaphase of mitosis (fig. 37–a). The idiogram of eight pairs of somatic chromosomes for this species is presented in figure 47–A, and the measurements in length of the eight pairs of chromosomes is shown in table 27. The size of chromosomes is somewhat smaller than that of the species belonging to the subgenus *Eumelilotus* and ranges from 3.1 μ to 1.7 μ in length. Seven pairs of chromosomes among eight

TABLE 27. Measurements of somatic chromosomes of *M. elegans*

chromosomes	length in (μ)	relative length	constriction
1-2	3.1=2.0+1.1	100	sm
3-4	3.0=1.8+1.2	97	sm
5-6	3.0=1.7+1.3	97	sm
7-8	2.9=1.5+1.4	94	sm
9-10	2.4=1.3+1.1	77	sm
11-12	2.0=1.1+0.9	65	sm
13-14	2.0=1.0+0.6+0.4	65	sm
15-16	1.7=0.9+0.8	55	sm

pairs have submedian primary constriction, in two pairs of which the primary constriction is situated close to the median position. The remaining single pair of chromosomes with submedian primary constriction possess a secondray constriction situated in the subterminal position of the short arm cutting off a microsatellite.

k. *M. indica*

M. indica Ac 296 was used for this study. At metaphase-1 of meiosis, eight bivalents are regularly formed (fig. 38-b) and the pollen fertillity is very high (table 16).

The observation of somatic metaphase reveals that the chromosome number is $2n=16$ in somatic cell (fig. 38-a). The measurements in length of the eight pairs of somatic chromosomes for this species are given in table 28, and the

TABLE 28. Measurements of somatic chromosomes of *M. indica*

chromosomes	length in (μ)	relative length	constriction
1-2	3.5=1.5+0.7+1.3	100	sm
3-4	3.4=2.1+1.3	97	sm
5-6	3.0=1.5+1.5	86	m
7-8	2.8=1.6+1.2	80	sm
9-10	2.8=1.6+1.2	80	sm
11-12	2.7=1.8+0.9	77	sm
13-14	2.4=1.3+1.1	69	sm
15-16	1.7=1.0+0.4+0.3	49	sm

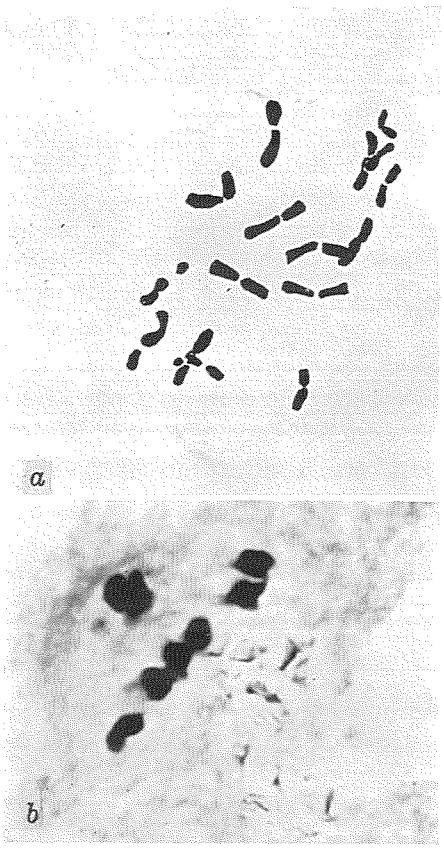


Fig. 37. *M. elegans* Ac 337
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8II. × 2500.

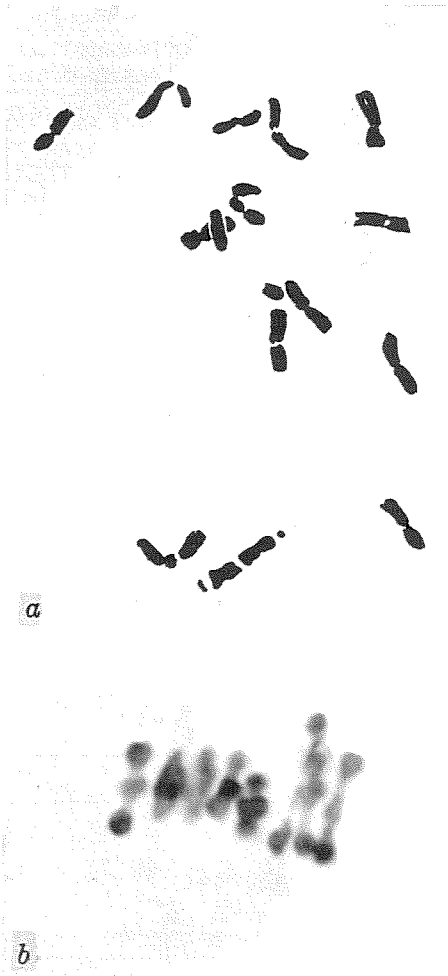


Fig. 38. *M. indica* Ac 296
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8II. × 2500.

idiogram of eight pairs of chromosomes is shown in figure 47–B. The size range of chromosomes is from 3.4 μ to 1.7 μ in length, which is smaller than that of the species of the subgenus *Eumelilotus* and comparable to that of *M. elegans* and *M. neapolitana*. Among the eight pairs of chromosomes, four pairs of chromosomes have submedian primary constriction, and one pair has a median primary constriction. In the remaining two pairs of chromosomes,

the longest pair of chromosomes with submedian primary constriction possess a secondary constriction situated in the long arm cutting off a large distal segment, and the shortest pair of chromosomes with submedian primary constriction has also a secondary constriction situated in the short arm cutting off a microsatellite.

l. *M. infesta.*

M. infesta Ac 335 was used for this study. At metaphase-1 of meiosis, eight bivalents regularly occurred (fig. 39–b) and the pollen fertility is very high (table 16).

At the mitotic metaphase, $2n=16$ in somatic chromosome number was observed (fig. 39–a). The eight pairs of somatic chromosomes for this species are idiogramed in figure 47–C. The size of chromosomes is clearly smaller than that of the species belonging to the subgenus *Eumelilotus* and four species, *M. elegans*, *M. indica*, *M. neapolitana*, and *M. italica*, which belong to the subgenus *Micromelilotus*. The size range of chromosomes is from 2.6 μ to 1.1 μ in length, and it was difficult to analyse the karyotype of this species accurately. Satellited chromosomes have not been found in this species so far.

m. *M. italica*

Eight bivalents regularly occurred at metaphase-1 of meiosis of *M. italica* V 298 used for this study (fig. 40–b). The percentage of pollen fertility is very high (table 16).

At metaphase of the somatic cell, $2n=16$ in the somatic chromosome number was observed (fig. 40–a). The measurements in length of eight pairs of somatic chromosomes for this species are given in table 29. The eight pairs

TABLE 29. Measurements of somatic chromosomes of M. *italica*

chromosomes	length in (μ)	relative length	constriction
1–2	4.5=2.4+2.1	100	sm
3–4	4.3=2.7+1.6	96	sm
5–6	4.2=2.4+1.8	93	sm
7–8	3.8=2.0+1.8	84	sm
9–10	3.6=2.1+1.5	80	sm
11–12	3.3=1.8+1.5	73	sm
13–14	3.2=1.8+1.4	71	sm
15–16	3.0=2.3+0.7	67	st

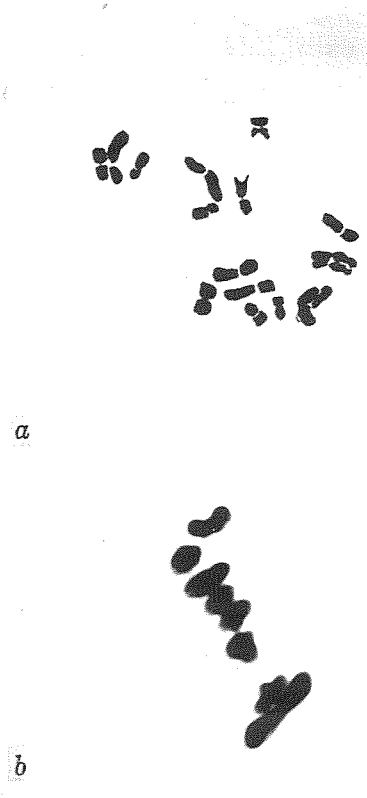


Fig. 39. *M. infesta* Ac 335
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8 II. × 2500.

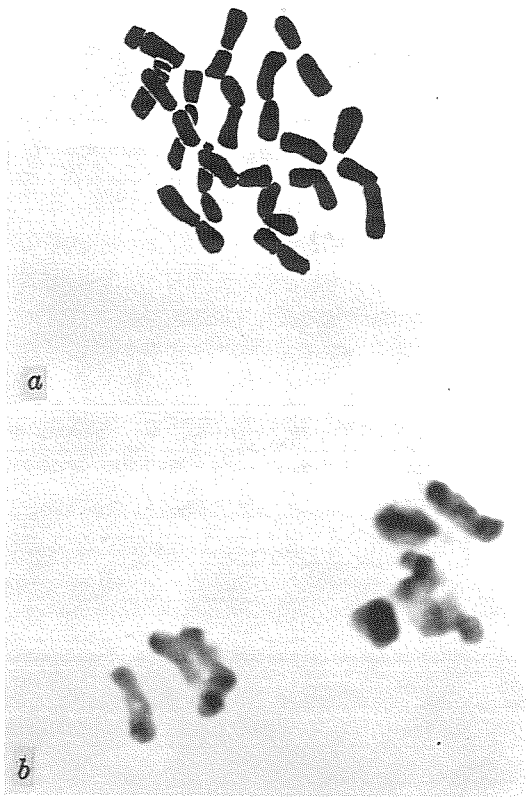


Fig. 40. *M. italica* V 285.
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8 II. × 2500.

of chromosomes are idiogramed in figure 47–D from the longest chromosomes to the shortest ones. The size range of chromosomes is from 4.3 μ to 3.0 μ, which indicates that the size of chromosomes in this species is conspicuously larger than that of any other species belonging to the subgenus *Micromelilotus* and is comparable to or rather larger than that of the species belonging to the subgenus *Eumelilotus*. Seven pairs among eight pairs of chromosomes have submedian primary constriction. The remaining single pair of chromosomes has subterminal primary constriction, which is the striking characteristic of this

species. Satellited chromosomes have not been observed so far.

n. *M. macrocarpa*

M. macrocarpa Ac 336 was used for this study. At metaphase-1 of meiosis, eight bivalents are regularly formed (fig. 41-b), and the percentage of pollen fertility is very high (table 16).

The chromosome number is $2n=16$ in somatic cells (fig. 41-a). The eight pairs of somatic chromosomes are idiogramed in fugure 47-E. The size

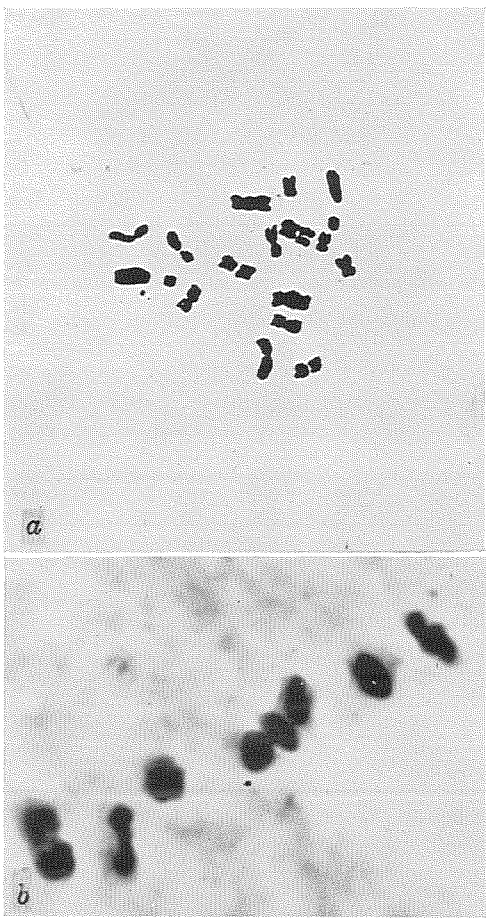


Fig. 41. *M. macrocarpa* Ac 336
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8II. × 2500.

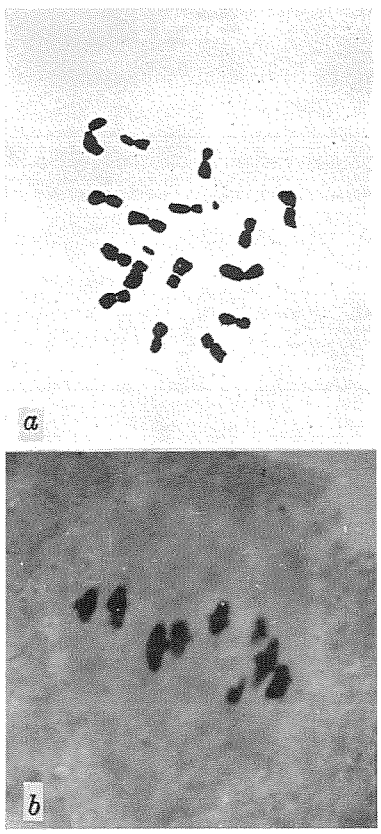


Fig. 42. *M. messanensis* C 21
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8II. × 2500.

range of the chromosomes is from 2.3 μ to 1.0 μ in length. The size of chromosomes is conspicuously small and almost similar to that of *M. infesta*, *M. messanensis*, *M. segetalis*, *M. speciosa*, and *M. sulcata*. This makes it difficult to analyse the karyotype of this species in detail. Satellited chromosomes have not been found so far. In the longest pair of chromosomes, however, the primary constriction is situated in a subterminal position which is a characteristic difference in the karyotype of this species.

o. *M. messanensis*

M. messanensis C 21 was used for this study. Eight bivalents are regularly observed at metaphase-1 of meiosis (fig. 42–b). The percentage of pollen fertility of this species is very high (table 16).

Observation of somatic metaphase reveals that the chromosome number is $2n=16$ in somatic cells (fig. 42–a). The eight pairs of somatic chromosomes for this species are idiogramed in figure 47–F. The size of chromosomes ranges from 2.1 μ to 1.5 μ, which is almost identical in size with that of *M. infesta*, *M. macrocarpa*, *M. segetalis*, *M. speciosa*, and *M. sulcata*. It is difficult to analyse the karyotype of this species in detail. However, one pair of chromosomes with a submedian primary constriction has secondary constriction in the short arm cutting off a microsatellite.

p. *M. neapolitana*

M. neapolitana Ac 334 was used for this study. Eight bivalents are constantly observed at metaphase-1 of meiosis (fig. 43–b). The percentage of pollen fertility is very high (table 16).

The somatic chromosome number is $2n=16$ in somatic cell (fig. 43–a). The measurements in length of the eight pairs of somatic chromosomes for

TABLE 40. Measurements of somatic chromosomes of *M. neapolitana*

chromosomes	length in (μ)	relative length	constriction
1–2	2.7=1.4+1.3	100	sm
3–4	2.6=1.6+1.0	96	sm
5–6	2.5=1.4+1.1	93	sm
7–8	2.4=1.3+1.1	89	sm
9–10	2.4=1.3+1.1	89	sm
11–12	2.2=1.2+1.0	81	sm
13–14	2.1=1.1+1.0	74	sm
15–16	1.5=0.8+0.5+0.2	56	sm

this species are presented in table 30, and the idiogram of eight pairs is given in figure 47-G. The size of chromosomes is somewhat smaller than that of the species belonging to the subgenus *Eumelilotus* and *M. italica* belonging to the subgenus *Micromeliotus* and comparable to that of *M. elegans* and *M. indica* which belong to the subgenus *Micromelilotus*. The eight pairs of

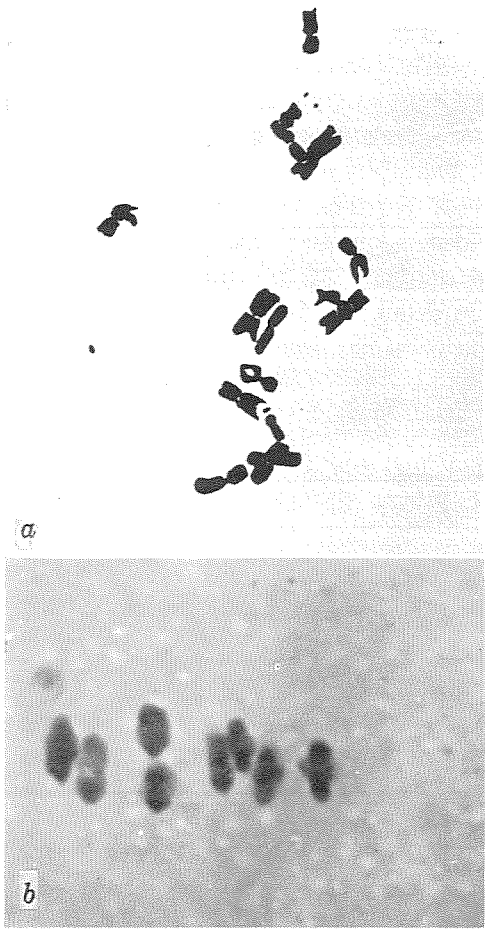


Fig. 43. *M. neapolitana* Ac 334
a. Mitoitc meaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8II. × 2500.

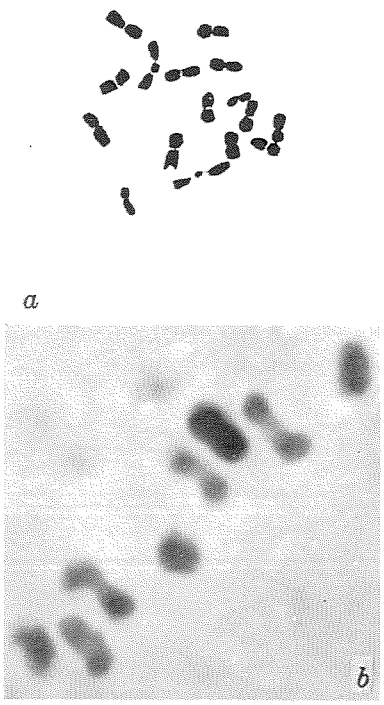


Fig. 44. *M. segetalis* N 80
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8II. × 2500.

chromosomes range from 2.7 μ to 1.5 μ in length. Seven pairs among eight pairs of somatic chromosomes have submedian primary constriction. The remaining single pair of chromosomes with a submedian primary constriction has a secondary constriction situated submedianly in the short arm cutting off a microsatellite.

q. *M. segetalis*

M. segetalis N 80 was used for this study. Eight bivalents are regularly formed at metaphase-1 of meiosis (fig. 44–b). The pollen fertility is also very high (table 16).

The somatic chromosome number is $2n=16$ (fig. 44–a). The eight pairs of somatic chromosomes for this species are idiogramed in figure 47–H. The size of chromosomes is as small as that of *M. infesta*, *M. macrocarpa*, *M. messanensis*, *M. speciosa* and *M. sulcata*. The size range of chromosomes is from 2.4 μ to 1.3 μ. It is difficult to analyse the karyotype of this species in detail. In the longest pair of chromosomes with a submedian primary constriction, however, the secondary constriction is situated in the long arm cutting off a large distal segment. The size of the segment lying between two constrictions is small and can be said to be the intercalary travant as mentioned by DARLINGTON (1932).

r. *M. speciosa*

M. speciosa V 287 was used for this study. Eight bivalents are regularly formed at metaphase-1 of meiosis (fig. 45–b) and the percentage of pollen fertility is very high (table 16).

Observation of somatic metaphase reveals that the chromosome number is $2n=16$ (fig. 45–a). The eight pairs of somatic chromosomes are idiogramed in figure 47–I. The size range of chromosomes is from 3.3 μ

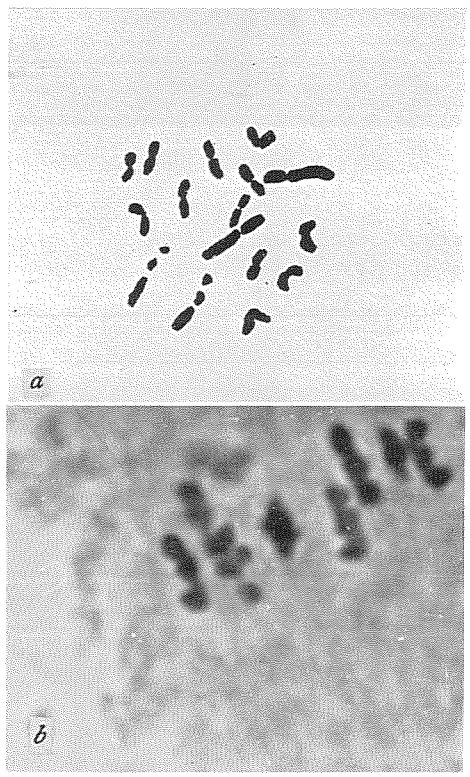


Fig. 45. *M. speciosa* V 287
a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic metaphase-1 with 8II. × 2500.

to 1.6 μ. Since the size of chromosomes is as small as that of *M. infesta*, *M. macrocarpa*, *M. messanensis M. segetalis* and *M. sulcata*, the detailed anaysis of the karyotype of this species is rather difficult. However, the following facts may be pointed out, i. e. the longest pair of chromosomes has a submedian primary constriction, and the second longest pair of chromosomes with a submedian primary constriction possesses a secondary constriction situated in

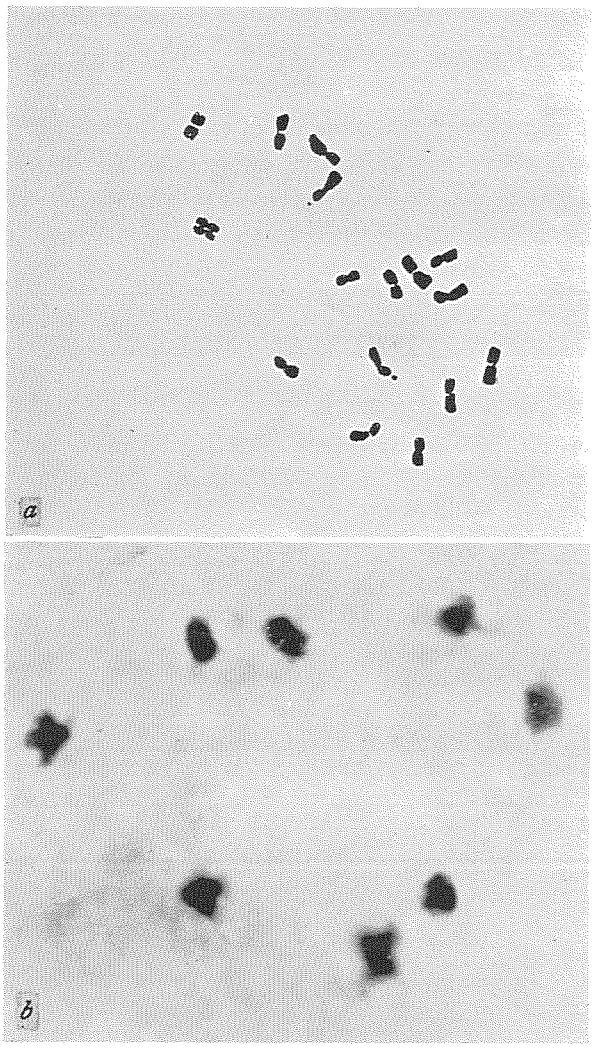


Fig. 46. *M. sulcata* V 298

a. Mitotic metaphase with 16 chromosomes. × 2700.
b. Meiotic diakinesis with 8 II. × 2500.

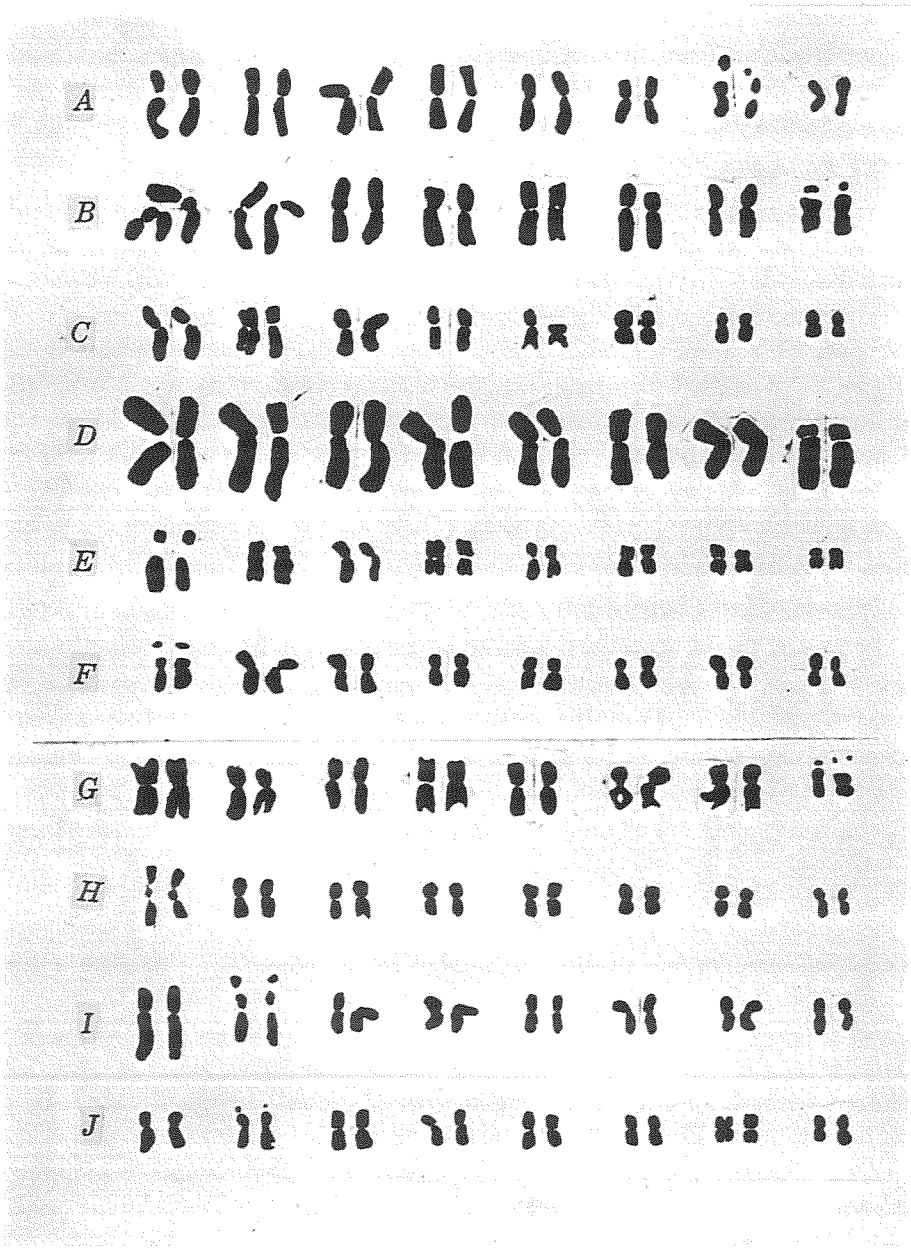


Fig. 47. Idiograms of the complements of somatic chromosomes of the subgenus *Micromelilotus*

A. *M. elegans* Ac 337.
B. *M. indica* Ac 296.
C. *M. infesta* Ac 335.
D. *M. italica* V 285..
E. *M. macrocarpa* Ac 336.
F. *M. messanensis* C 21.
G. *M. neapolitana* Ac 334.
H. *M. segetalis* N 80.
I. *M. speciosa* V 287.
J. *M. sulcata* V 298.

the submedian position of the short arm cutting off a microsatellite. The size of these two pairs of chromosomes are conspicuously larger than that of the other pairs of chromosomes and is distinguishable from the other pairs of chromosomes under a microscope.

s. *M. sulcata*

M. sulcata V 298 was used for this study. Eight bivalents were constantly observed at diakinesis (fig. 46–b). The percentage of pollen fertility is very high (table 16).

The chromosome number is $2n=16$ in the somatic cells (fig. 46–a). The eight pairs of somatic chromosomes in this species is shown idiogramatically in figure 47–J. The size range of the chromosomes is from 1.8 μ to 1.3 μ. The size of chromosomes is as small as that of *M. infesta*, *M. macrocarpa*, *M. messanensis*, *M. segetalis*, and *M. speciosa*. This makes it difficult to analyse the karyotype of this species in detail, however, it is clear that one pair of chromosomes with a submedian primary constriction has a secondary constriction in the short arm cutting off a microsatellite.

4. Discussion and Conclusion

In regard to the somatic chromosome numbers, it has been roughly recognized that all nineteen species have sixteen chromosomes in somatic cells (DARLINGTON 1945). However, with regard to detailed information on karyotypes covering species of this genus, no systematic efforts have been initiated.

As far as the author has examined, the meiotic chromosome behaviors of nineteen species are regular and the percentage of pollen fertility is very high. Consequently, the species used in this study are considered to be stable cytologically speaking.

In general, a considerable range of variation in choromosome sizes exists among nineteen species of the genus *Melilotus* (table 31). In nine species, *M. alba*, *M. altissima*, *M. dentata*, *M. hirsuta*, *M. officinalis*, *M. polonica*, *M. suaveolens*, *M. taurica*, and *M. wolgica*, which belong to the subgenus *Eumelilotus*, the chromosome sizes are almost the same and are very large compared with the species belonging to the subgenus *Micromelilotus* with the exception of *M. italica*. The size of chromosomes of *M. italica* is the same or larger than that of the species of the subgenus *Eumelilotus* but differ in karyotype. Therefore, the nine species of the subgenus *Eumelilotus* can be grouped into one group, provisionally termed as Type A by the author (table 32). In nine species, *M. elegans*, *M. indica*, *M. infesta*, *M. macrocarpa*, *M. messanensis*, *M. neapolitana*, *M. segetalis*, *M. speciosa*, and *M. sulcata*, which belong to the subgenus *Micromelilotus*, their chromosome sizes are obviously

TABLE 31. Karyotype in *Melilotus* species

species	no. of chromosomes	centromere			longest chromosome	shortest chromosome	no. of secondary constricted chromosomes
		median	submedian	subterminal			
	(2n)				(μ)	(μ)	
alba	16	2	12	—	3.5	2.7	2
altissima	"	—	16	—	4.4	3.3	2
dentata	"	2	14	—	4.2	2.7	2
hirsuta	"	—	16	—	3.1	2.3	2
officinalis	"	—	16	—	3.5	2.7	2
polonica	"	2	14	—	3.3	2.6	2
suaveolens	"	—	16	—	3.4	2.2	2
taurica	"	—	16	—	3.6	2.6	2
wolgica	"	—	16	—	4.1	2.4	2
elegans	"	2	14	—	3.1	1.7	2
indica	"	2	14	—	3.5	1.7	4
neapolitana	"	—	16	—	2.7	1.5	2
infesta	"				2.6	1.1	—
macrocarpa	"				2.3	1.0	—
messanensis	"				2.1	1.5	2
segetalis	"				2.4	1.3	2
speciosa	"				3.3	1.6	2
sulcata	"				1.7	1.3	2
italica	"	—	14	2	4.5	3.0	—

smaller than that of the species of the subgenus *Eumelilotus*. Three species among these nine, *M. elegans*, *M. indica*, and *M. neapolitata* have about the same size of chromosomes. But their chromosomes are larger than that of the six species, *M. infesta*, *M. macrocarpa*, *M. messanensis*, *M. segetalis*, *M. speciosa*, and *M. sulcata*. Therefore, these nine species are grouped as Type B. And further, based on the facts mentioned above, they may be subdivided into Type B–1 for *M. elegans*, *M. indica*, and *M. neapolitana* and Type B–2 for *M. infesta*, *M. macrocarpa*, *M. messanensis*, *M. segetalis*, *M. speciosa*, and *M. sulcata* (table 32). Here, it should be pointed out that *M. italica* which belongs to the subgenus *Micromelilotus* has distinctly larger chromosomes among the species of the subgenus *Micromelilotus*. Therefore, this species should be grouped under Type C (table 32).

In connection with the above, the following results presented briefly in table 15 should be recalled. Here, the relationship of interspecific cross

TABLE 32. Classification of the genus *Melilotus* based on morphology of the somatic chromosomes

Type A		*M. alba* *M. altissima* *M. dentata* *M. hirsuta* *M. officinalis* *M. polonica* *M. suaveolens* *M. taurica* *M. wolgica*
Type B	B-1 ?	*M. elegans* *M. indica* *M. neapolitana*
	B-2	*M. infesta* *M. macrocarpa* *M. messanensis* *M. segetalis* *M. speciosa* *M. sulcata*
Type C		*M. italica*

compatibility of the genus *Melilotus* was diagramatically illustrated. In regard to the interspecific cross compatibility in relation to the differences of chromosome size, a high compatibility is observed between pairs of species within the species of Type A and Type B–2. No interspecific hybrid was obtained from the crosses between the species of Type A and Type B, Type B and Type C, and Type A and Type C. As far as examined, interspecific hybrids have not been obtained within pairs of species belonging to Type B–1 and between the species of Type B–1 and Type B–2. Thus it can be said that a close relationship may exist between the size of chromosomes and the tendency of interspecific cross compatibility. This is true, at least, in the interspecific crosses between pairs of the species belonging to Type A and Type B–2.

Regarding the karyotypes of species within each type, it may be mentioned that a rough similarlity of karyotype can be noted among nine species of the subgenus *Eumelilotus* which are grouped under Type A. These species are characterised by symmetrical karyotype, that is, all eight pairs of chromosomes

are with median and submedian primary constrictions. Without exception, all species have a pair of chromosomes with secondary constrictions, it should be pointed out that the pairs of chromosomes with secondary constrictions of *M. hirsuta* and *M. polonica* are obviously different from that of the other species. Namely, the secondary constrictions cut off the large distal segments in *M. hirsuta* and *M. polonica*, while cutting off the microsatellites in the other species. The pair of satellited chromosomes of *M. dentata* may be a characteristic karyotype, in other words, a microsatellite is attached to the one arm of the median chromosome, while a microsatellite is attached to the submedian chromosome in the other species.

The three species, *M. elegans*, *M. indica*, and *M. neapolitana*, which belong to Type B–1 are also characterized by symmetrical karyotypes. In *M. indica*, two pairs of chromosomes with secondary constrictions were observed. One pair had a secondary constriction cutting off the large distal segment and the other pair of chromosomes had a secondary constriction cutting off a microsatellite.

The six species, *M. infesta*, *M. macrocarpa*, *M. messanensis*, *M. segetalis*, *M. speciosa*, and *M. sulcata*, which belong to Type B–2 are also characterized by a symmetrical karyotype, that is to say, the predominance of chromosomes with median and submedian constrictions to those with subterminal ones. The size of chromosomes is very small compared with that of species in Type A and Type C. The chromosome size is also somewhat smaller than that of the species in Type B–1. This makes it difficult to analyse karyotypes in detail except for a few pairs of chromosomes in each species. It should be mentioned here, however, that a pair of chromosomes with intercalary travants in *M. segetalis* and two longest pairs of chromosomes in *M. speciosa* are characteristic differences of karyotypes when karyotypes of six species are compared with each other. It is also pointed out that no satellite chromosomes have been detected in *M. infesta* and *M. macrocarpa*. This may partly due to the small size of chromosomes, and it requires further observations to determine whether the satellited chromosomes exist or not in two species.

M. italica grouped under Type C is also characterized by a symmetrical karyotype, that is to say, the chromosomes with median and submedian constrictions are predominant over those of subterminal ones. A pair of subterminal chromosomes is characteristic among eight pairs of chromosomes in this species. As a whole, this species is distinguishable from the other species by specific karyotype. As far as examined, no satellited chromosomes have been found.

In regard to the karyotypic differences observed within each type as

mentioned above, it is possible to assume that the structural differentiation of chromosomes exist among species within each type.

V. Cytogenetics of Interspecific Hybrids

The studies of karyotype analysis by the author present several interesting problems to be solved in relation to speciation of the genus *Melilotus*. First of all, does grouping species in Type A, B, and C agree with genomic differences or not? Secondary, what is the nature of the structural changes of chromosomes among species within each type which are assumed to exist from the results of karyotype analysis? Finally, what is the picture of the complex series of the structural changes of chromosomes throughout the whole species of this genus?

In this genus, however, the isolation mechanism such as non-crossability among certain species and inability of the F_1 hybrids to survive due to chlorophyll deficiency make it difficult to secure the desirable interspecific hybrids in a stable condition which can be used for such cytological studies.

As mentioned in the chapter III, several interspecific hybrids were obtained by the author, all of which were hybrids between pairs of species within Type A and Type B–2.

Through studies of chromosome behaviors during the course of meiosis of these interspecific hybrids, the author obtained some new informations which might clarify the existence of structural changes of chromosomes among species and their natures.

1. Materials

Materials which were used in this experiments are described in the experimental results.

2. Methods

Buds containing anthers wherein the microsporocyte were undergoing meiosis were fixed in a mixture of 3 parts absolute alcohol and 1 part propionic acid saturated with ferric acetate for the hybrid, *M. alba* × *M. hirsuta*, and maintained at a low temperature for 24 hours. For the hybrids, *M. segetalis* × *M. messanensis*, *M. segetalis* × *M. macrocarpa*, *M. sulcata* × *M. macrocarpa*, and *M. sulcata* × *M. infesta*, the buds were fixed in a mixture of 3 parts absolute alcohol and 1 part glacial acetic acid to which was added a few drops of 45% glacial acetic acid saturated with ferric chloride.

There were then transferred to 70% alcohol. Anthers were smeared in a drop of propionic-carmine for the hybrid, *M. alba* × *M. hirsuta*, or aceto-

carmine for the other hybrids and the meiotic configurations were analysed.

Pollen grains from mature anthers were placed in a drop of aceto-carmine in order to determine the percentage of stainable pollen grains.

Photomicrographs were taken with the aid of an Olympus oil-immersion objective 100× and an Olympus P 15× ocular for the meiotic chromosomes.

3. **Experimental Results**

a. The interspecific hybrid, *M. alba*×*M. hirsuta*

Cross pollinations were made in the green house at the Department of Agronomy, University of Wisconsin, U. S. A., during October, 1957 using *M. alba* as the pistillate parent. The seeds obtained were germinated in petry dishes and planted in soil in a green house flat. Twenty-four of the 65 seedlings were light green in color, indicating they were hybrids. The 24 light green plants were transplanted to 6″ pots and allowed to continue growth. Upon flowering, all plants produced pale yellow flowers indicating that they were hybrids since the maternal parent was white-flowered.

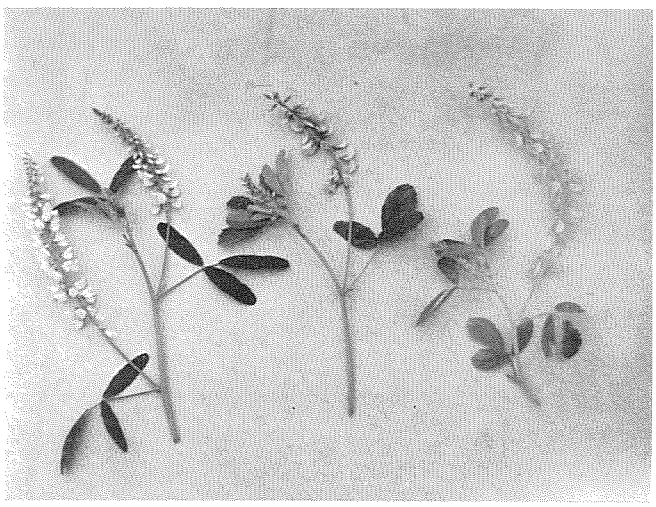


Fig. 48. Inflorescences and leaves
left: *M. alba.*
center: F_1 hybrid, *M. alba*×*M. hirsuta.*
right: *M. hirsuta.*

Morphological differences observed in the F_1 hybrid compared with both parents are indicated in table 33.

The pollen of both *M. alba* and *M. hirsuta* is highly fertile (ca. 99%). Both parental species have 8 pairs of chromosomes at metaphase-1 (fig. 49–a, b).

TABLE 33. Comparison of the F_1 hybrid, *M. alba*×*M. hirsuta*, with the two parents

morphological characters	*M. alba*	*M. hirsuta*	*M. alba* × *M. hirsuta* F_1
leaflet			
margin	toothed (25–20teeth)	toothed (15–20teeth)	toothed (15–20teeth)
shape	obovate	obovate	obovate
color	dark green	green	light green
raceme	exceed subtending leaf	exceed subtending leaf	exceed subtending leaf
flower			
color	white	yellow	pale yellow
standard	longer than keel	equal to keel	longer than keel
pod	blackish brown	yellowish brown	yellowish brown
seed	small	larger	larger

The course of meiosis is regular, producing four functional spores.

The F_1 hybrids are highly sterile (ca. 56% of aborted pollen grains). Aberrant chromosome behaviors occur during the course of meiosis. Usually six bivalent and a chain of four chromosomes exist at diakinesis (fig. 49–e). Such a chain was present in 130 of 142 figures examined. Six bivalents plus a trivalent and one univalent (6II+1III+1I), seven bivalents plus two univalents (7II+2I), and eight bivalents were noted in the remaining nuclei. The bivalents are either rod- or ring-shaped. The chiasmata are usually terminal but occasionally there was evidence of an interstitial chiasma.

The two types of chromosome associations (6II+1IV and 6II+1III+1I) occur at about equal frequency at metaphase-1 (fig. 50–a, b, c, d). Presumably the presence of a chain of three chromosomes and a univalent results from a precocious segregation of one member of the chain. If so, two types are of the same origin.

The mode of distribution of the chromosomes in the chain occur at metaphase-1. Alternate chromosomes may pass to the same pole (fig. 50–c), the terminal members may disjoin from the median members (fig. 51–a), or two disjunctional chromosomes may move to each pole (fig. 51–d). The latter two modes of distribution which occured in 58% of the figures would lead to duplication and deficiency, the end result being pollen sterility. The exact mode of distribution of the chromosomes involved in the 6II+1III+1I type of association could not be determined since it was impossible to recognize this particular univalent in metaphase configurations.

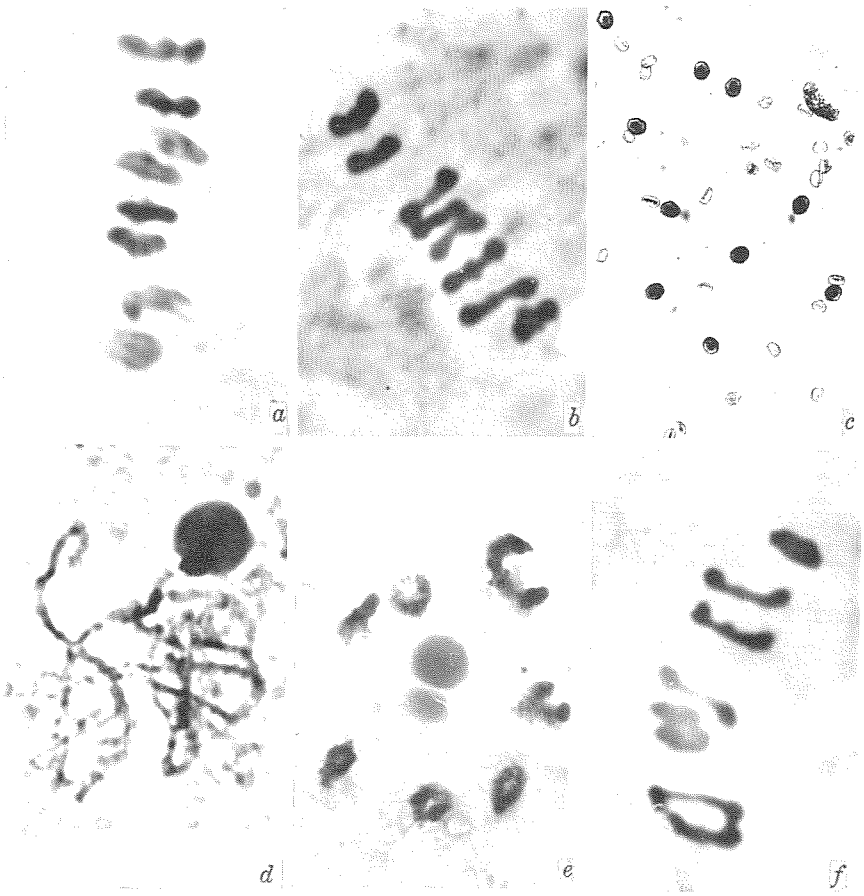


Fig. 49. a. *M. hirsuta.* Metaphase-1 with 8II. × 2240.
b. *M. alba.* Metaphase-1 with 8II. × 2240.
c. *M. alba*×*M. hirsuta* F_1. Normal and aborted pollen grains.
d. *M. alba*×*M. hirsuta* F_1. Pachytene with a cross-shaped configuration. × 1600.
e. *M. alba*×*M. hirsuta* F_1. Diakinesis with 6II+1IV. × 1960.
f. *M. alba*×*M. hirsuta* F_1. Metaphase with 6II plus a ring of 4 chromosomes. × 2240.

Although the quadrivalent usually occured as a chain at both diakinesis and metaphase-1, a closed ring of four chromosomes were presented in four instances at the latter stage (fig. 49–f), providing evidence for the possibility that both chain and ring are the end results of a reciprocal translocation between non-homologous chromosomes. Aside from the metaphase-1 configurations with six bivalents and a chain or ring of four chromosomes, occasionally seven

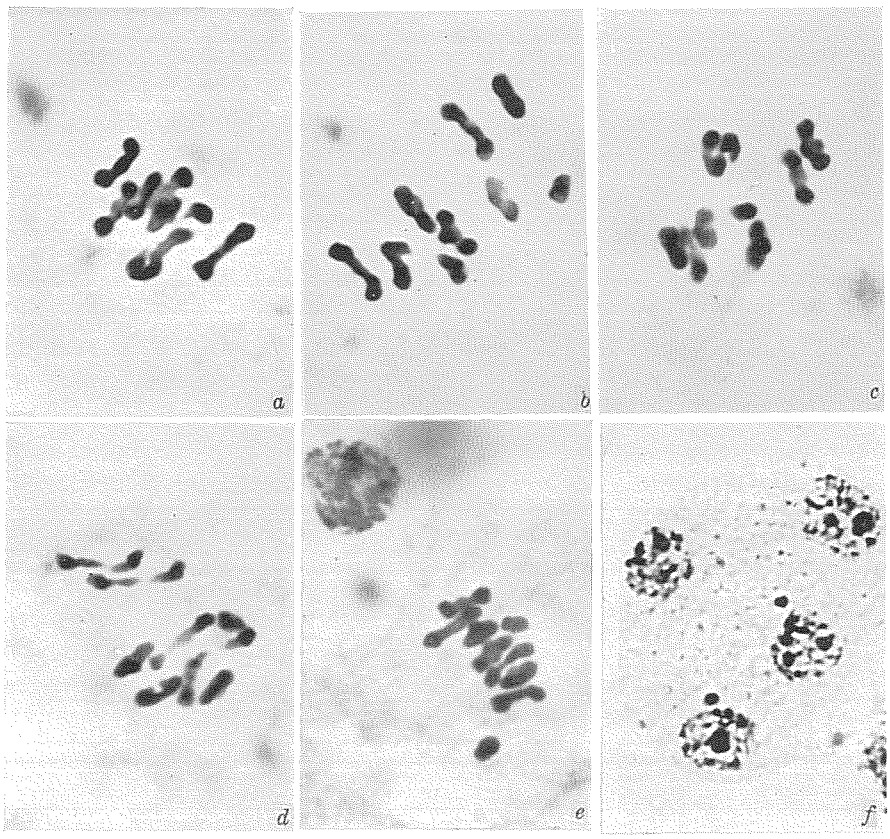


Fig. 50. *M. alba*×*M. hirsuta* F_1

a. Metaphase-1 showing terminal members disjoining from the median members of the chain. × 2240.
b. Metaphase-1 showing 7II+2I configuration. × 2240.
c. Metaphase-1 showing alternate separation of members of the chain. × 2240.
d. Metaphase-1 showing nondisjunctional chromosomes of the chain moving to each pole. × 2240.
e. Metaphase-1 showing 7II+2I configuration. × 2240.
f. Telophase-2 with micronuclei. × 1500.

bivalents plus two univalents (fig. 50–e) and eight bivalents occur.

Pachyteen analysis was undertaken in order to determine whether the multivalent association of four chromosomes was the result of a reciprocal translocation or a simple translocation. Suitable technique for smearing the P. M. C. nuclei made it possible to spread the pachyteen configuration sufficiently so that they could be analysed. A reciprocal translocation was immediately

evident. A crossshaped configuration regularly occurred. Synapsis was incomplete in one arm of these configurations and chiasmata were seldom noted in that arm (fig. 49-d). The chain of four chromosomes results from a complete segregation of the loosely associated chromatids of this arm during the further course of meiosis.

Normal 8-8 disjunction of the chromosomes at anaphase-1 occurs in 52% of the P. M. C. s, while in the remainder, abnormalities in chromosome number such as 7-9 disjunction which lead to the production of aneuploid spores occur. The frequency of lagging chromosomes is relatively high (39 out of 135 cells). Occasionally more than one laggard and also chromatid segregation are evident at anaphase-1.

Some abnormalities may occur at metaphase-2 as evidenced by the lagging chromosomes at anaphase-2. The number of lagging chromosomes is variable from cell to cell, but one or two laggards are most common. Sometimes a few univalents or micronuclei are present in the cytoplasm at late anaphase-2 or early telophase (fig. 50-f). Tetrads are usually formed following meiosis, there being no evidence of dyads, triads or pentads.

Chromosome configurations at diakinesis, metaphase-1, and distribution of chromosomes at anaphase-1 and anaphase-2 in this hybrid are presented in table 34.

TABLE 34. Chromosome configurations at diakinesis and metaphase-1 and their distribution in later stages of meiosis in the F_1 hybrid, *M. alba* × *M. hirsuta*

stage of meiosis	frequency of PMCs with										
	8 II	7 II+2 I	6 II +1 III +1 I	6 II+1 IV	8-8	8-7	9-7	7-7	normal PMCs	abnormal PMCs	total
diakinesis	8	2	2	130							142
metaphase-1	7	8	46	43							104
anaphase-1					70	39	25	1			135
anaphase-2									70	37	107

b. The interspecific hybrid, *M. segetalis* × *M. messanensis*

The F_1 hybrid, *M. segetalis* × *M. messanensis*, was secured easily without any interruption such as by chlorophyll deficiency and seed abortion at an early stage of embryo development in 1960. Eight bybrid plants resulting from the cross were grown in the green house in the same manner mentioned previously.

The comparisons of morphological characters of F_1 hybrids with parental species are given in table 35 and figure 51.

TABLE 35. Comparison of the F_1 hybrid, *M. segetalis*× *M. messanensis* with the two parents

morphological characters	*M. segetalis*	*M. messanensis*	*M. segetalis* × *M. messanensis* F_1
leaflet			
margin	sharply toothed	unequally toothed	sharply toothed
shape	obovate	cuneate	obovate
raceme	longer than subtending leaf	shorter than subtending leaf	equal or longer than subtending leaf
flower			
standard	shorter than keel	equal to keel	intermediate
size	6.5 mm	5.2 mm	6.2 mm
pod	round	acute	acute

Fig. 51. Inflorescences and leaves
a. *M. segetalis.*
b. *M. segetalis*×*M. messanensis* F_1.
c. *M. messanensis.*

The percentage of stainable pollen grains of the F_1 plants was very low, being about 17.8% (fig. 52-c), while the parents were high, about 95% in both species.

The chromosome behaviors at both diakinesis and metaphase-1 are regular, producing functional spores in the parents, *M. segetalis* and *messanensis* (fig. 52-a, b).

In the F_1 hybrid plants, aberrant chromosome behaviors occur during the

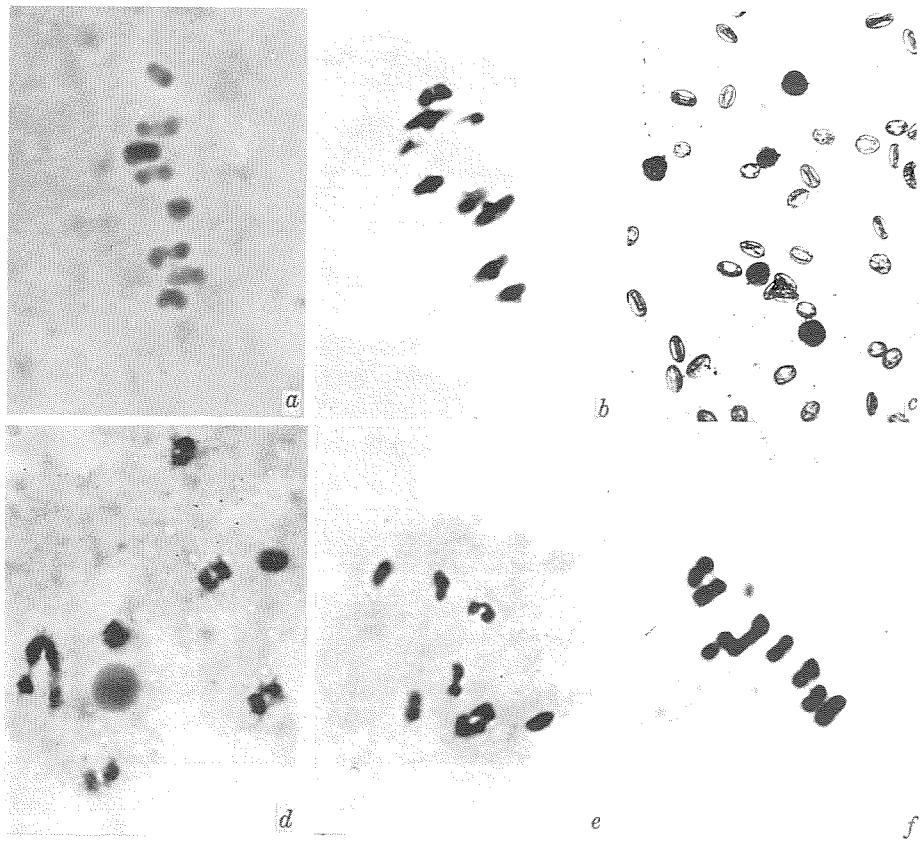


Fig. 52. a. *M. segetalis.* Metaphase-1 with 8II. × 2500.
b. *M. messanensis.* Metaphase-1 with 8II. × 2500.
c. *M. segetalis*×*M. messanensis* F_1. Normal and abnormal pollen grains.
d. *M. segetalis*×*M. messanensis* F_1. Diakinesis with 6II plus a chain of 4 chromosomes. × 1900.
e. *M. segetalis*×*M. messanensis* F_1. Diakinesis with 6II plus a ring of 4 chromosomes. × 1900.
f. *M. segetalis*×*M. messanensis* F_1. Metaphase-1 with 6II plus a chain of 4 chromosomes which shows alternate separation.

course of meiosis. A ring or chain of four chromosomes and six bivalents (1IV+6II) are observed at diakinesis (fig. 52–d, e,). Six bivalents plus a trivalent and one univalent (6II+1III+1I) were noted in the remaining nuclei.

Similarly, a ring or chain of four chromosomes plus six bivalents (1IV+6II) was present in 84 of the 95 figures at metaphase-1. Two types of chromosome

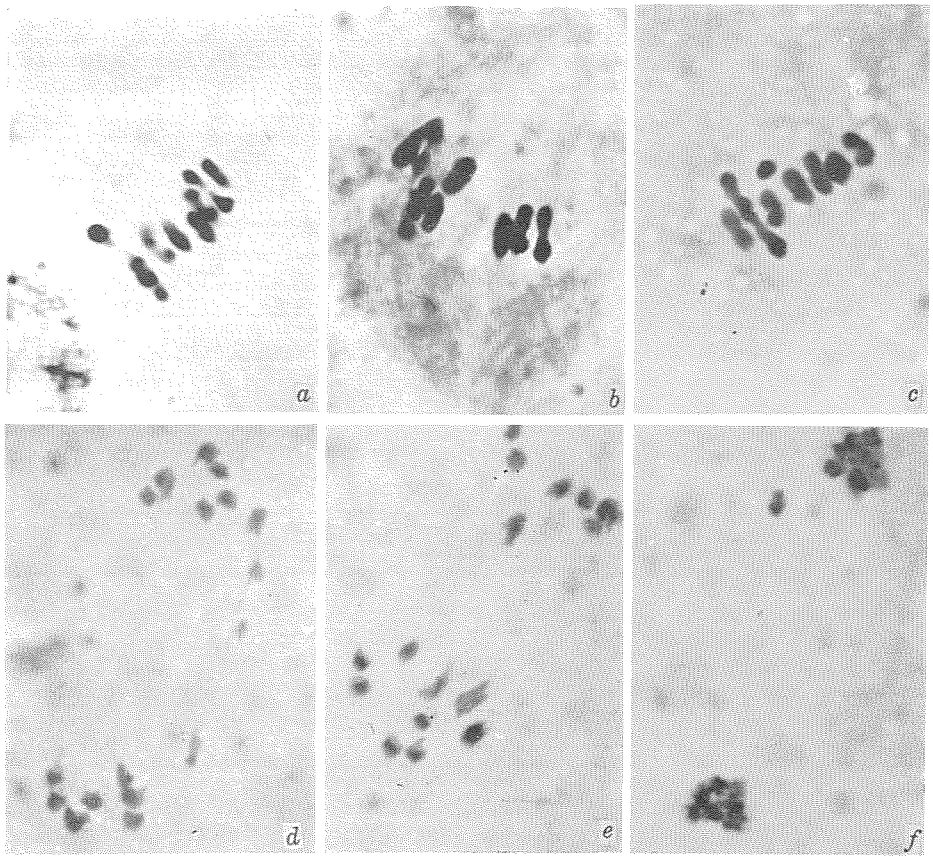


Fig. 53. *M. segetalis*×*M. messanensis* F_1

a. Metaphase-1. Nondisjunctional chromosomes of the chain moving to each pole. × 2500.
b. Metaphase-1. Terminal members disjoining from the median members of the chain. × 2500.
c. Metaphase-1. 1III+6II+1I configuration. × 2500.
d. Anaphase-1 Chromatid bridge and acentric fragment. × 2000.
e. Anaphase-1. 7–9 disjunction. × 2000.
f. Late anaphase-1. A lagging chromosome. × 2000.

associations, 6II+1III+1I and 8II, were observed in the remaining nuclei. Presumably the presence of a chain of three chromosomes and a univalent results from a precocious segregation of one member of the ring or chain of four chromosomes, if so, the two types are of the same origin. Namely, the presence of a ring or chain of four chromosomes in most of configurations at diakinesis and metaphase-1 is the evidence that the hybrid is heterozygous

for reciprocal translocation.

Normal 8−8 disjunction of the chromosomes at anaphase-1 occurred in 34 out of the 55 figures examined. In the remainder, abnormalities such as 7−9 disjunction and lagging chromosomes occur to some extent. Dicentric chromatid bridge with or without fragment was present in 12 out of the 55 figures, indicating that the hybrid plants are heterozygous for inversion (fig. 53-d, e, f).

Lagging chromosomes are observed at anaphase-2, and the number of laggards is different cell to cell but one or two lagging chromosomes are most common.

By the facts mentioned above, it is clearly evidenced that this F_1 hybrid is heterozygous for reciprocal translocation and inversion, explaining very high pollen sterility.

Chromosome configurations at diakinesis, metaphase-1, and distribution of chromosomes at anaphase-1 and anaphase-2 in this hybrid are presented in table 36.

TABLE 36. Chromosome configurations at diakinesis and metaphase-1 and their distribution in later stages of meiosis in the F_1 hybrid, *M. segetalis* × *M. messanensis*

stage of meiosis	frequency of PMCs with								
	8II	1III+6II+1I	1IV+6II	8−8	8−8 +bridge	9−7	normal	abnormal	total
diakinesis		12	54						66
metaphase-1	3	8	84						95
anaphase-1				34	12	9			55
anaphase-2							37	10	47

c. The interspecific hybrid, *M. segetalis* × *M. macrocarpa*

The interspecific cross pollinations between *M. segetalis* and *M. macrocarpa* were made in 1963. Three seedlings derived from 32 seeds obtained from the cross were somewhat different in morphological appearance from *M. segetalis*, a pistillate parent, indicating that they were hybrids. These three plants were transplanted to 6″ pots and allowed to continue growth.

M. segetalis and *M. macrocarpa* differ in several morphological characters. The hybrids resembled one or the other parent as far as some characters were concerned, and were intermediate for the other characters. The morphological differences of the F_1 plants are indicated in table 37 and figure 54.

In the parental species, the chromosome behaviors at diakinesis and metaphase-1 are regular and produce functional pollen spores. The percentage of

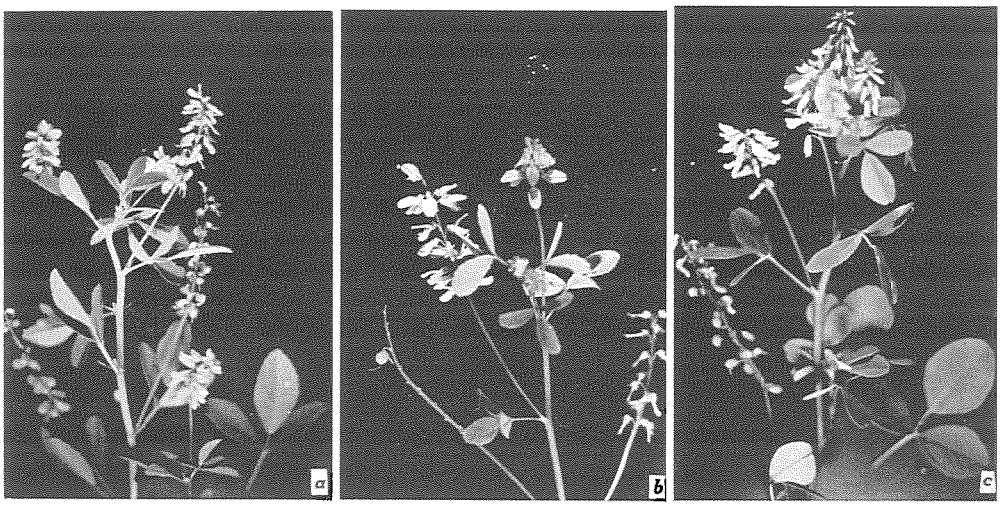


Fig. 54. Inflorescences and leaves
a. *M. segetalis.*
b. *M. segetalis*×*M. macrocarpa* F_1.
c. *M. macrocarpa.*

TABLE 37. Comparison of the F_1 hybrid, *M. segetalis*× *M. macrocarpa*, with the two parents

morphological characters	*M. segetalis*	*M. macrocarpa*	*M. segetalis* × *M. macrocarpa* F_1
leaflet			
margin	sharply toothed	inconspicuously toothed	sharply toothed
shape	elliptic or obovate	orbiculate or obovate	elliptic or obovate
raceme	longer than subtending leaf	longer than subtending leaf	longer than subtending leaf
flower			
standard	shorter than keel	equal to keel	equal to keel
size	6.5 mm	8.1 mm	7.5 mm
pod	corse surface with many vein	thin surface with less vein	corse surface with many vein

stainable pollen grains is high in both parents.

The F_1 hybrids are highly sterile, having about 73.7% of aborted pollen grains (fig. 55–a, b). There is little dificulty in classifying the pollen grains as either normal or aborted pollen, however, two types of aborted pollens are observed. Namely, normal or viable pollen grains are large and plump, which

take a deep stain, whereas abnormal or non-viable pollen grains are shrunken, taking little stain in most of cases but in some instances moderately plump, taking stain partially but the size is smaller than normal.

The chromosomal irregularities occur in the course of microsporogenesis in the F_1 hybrids. These first become clearly evident at diakinesis where most of configurations possess six bivalents and a ring or chain of four chromosomes

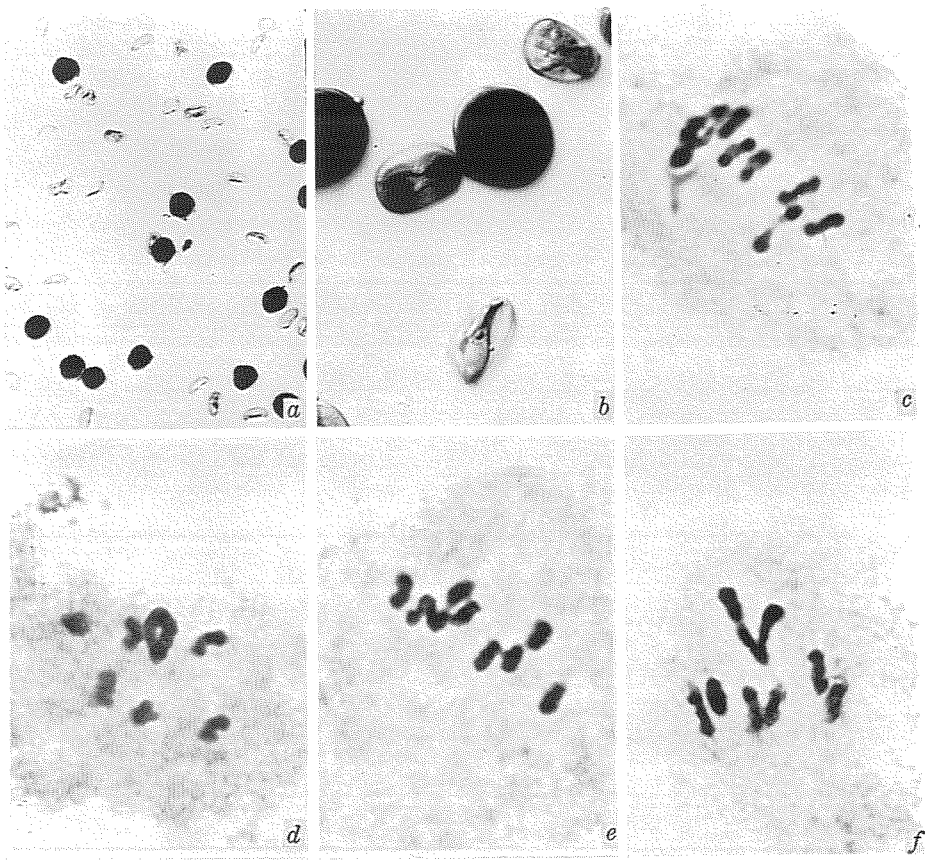


Fig. 55. *M. segetalis*×*M. macrocarpa* F_1

a. Normal and aborted pollen grains.
b. The same as a.
c. Metaphase-1 with 6II plus a ring of 4 chromosomes. × 2000.
d. The same as c.
e. Metaphase-1 with 6II plus a chain of 4 chromosomes which show alternate separation. × 2000.
f. Metaphase-1. 6II plus a chain of 4 chromosomes which show terminal members disjoining from the median members. × 2000.

(6II+1IV). In a few cases, seven bivalents and two univalents (7II+2I), and six bivalents plus one trivalent and one univalent (6II+1III+1I) are present. Occasionally eight bivalents are observed in a very low frequency.

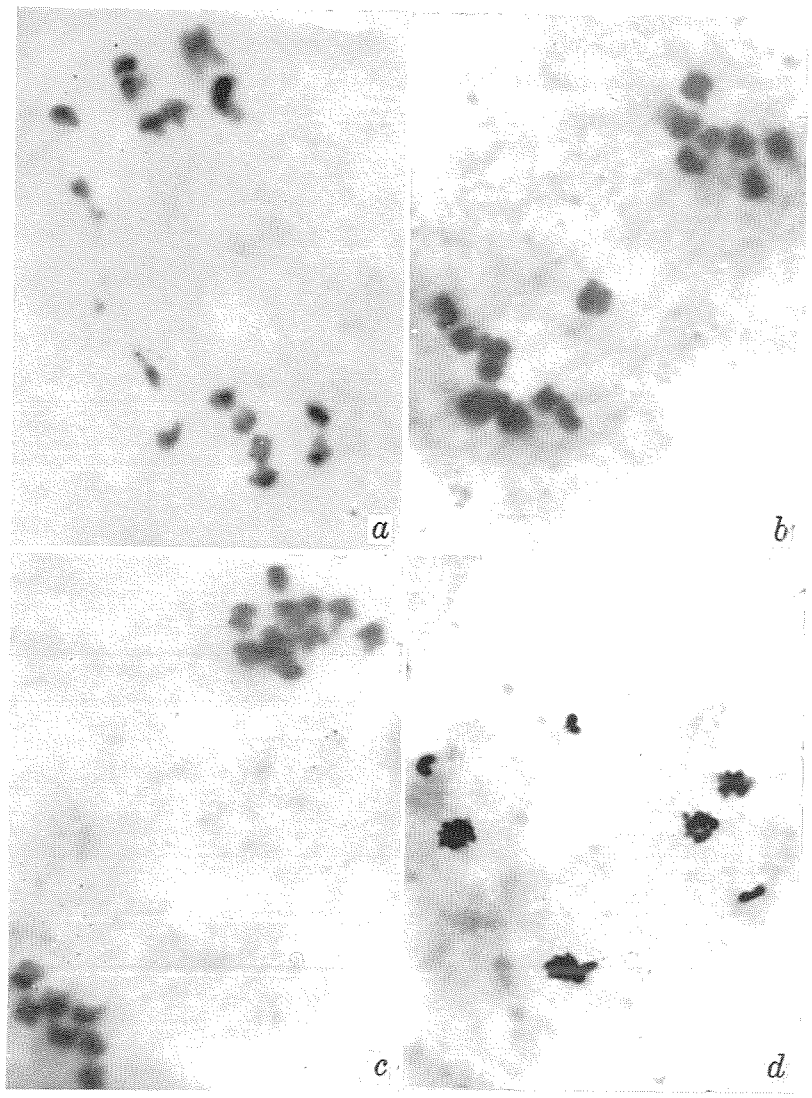


Fig. 56. *M. segetalis*×*M. macrocarpa* F_1

a. Anaphase-1 with chromatid bridge and acentric fragment. × 2000.
b. Anaphase-1 with a lagging chromosome. × 2000.
c. Anaphase-1. 7−9 disjunction. × 2000.
b. Anaphase-2 with lagging chromosomes. × 1500.

TABLE 38. Chromosome configurations at diakinesis and metaphase-1 and their distribution in later stages of meiosis in the F_1 hybrid, *M. segetalis*×*M. macrocarpa*

stage of meiosis	frequency of PMCs with										
	8II	7II+2I	1III+6II+1I	1IV+6II	8–8	8–8 + fragment & bridge	8–8 + fragment	7–9	normal	abnormal or lagger	total
diakinesis			1	52							53
metaphase-1	3	3	2	102							110
anaphase-1					21	7	8	8		17	61
anaphase-2									35	42	77

The closed ring or chain of four chromosomes become oriented on the metaphase plate in such a manner that either adjacent or alternate chromosomes move to the same pole at metaphase-1 (fig. 55–c, d, e, f). On the closed ring of four chromosomes, it is difficult to determine what first appears to be segregated closely associated homologous chromosomes without any markers. In the figures of a chain of four chromosomes, however, 30 out of 59 configurations showed alternate chromosome segregation resulting in pollen fertile.

The occurrence of a ring or chain of four chromosomes at diakinesis and metaphase-1 provide evidence for the possibility that the four chromosome association is the end result of a reciprocal translocation between two non-homologous chromosomes.

At anaphase-1, normal 8—8 disjunction of the chromosomes occurs in 21 of the 61 nuclei examined.

Lagging chromosomes at anaphase-1 exist in some instances (fig. 56–b). Dicentric chromatid bridges with an acentric fragment were observed in 7 of the 61 configurations (fig. 56–a) and acentric fragment without bridge was present in 8 of the 61 configurations, indicating that the F_1 hybrid is heterozygous for inversion. In the remainder, abnormalities such as 7—9 disjunction are present to some extent (fig. 56–c).

Lagging chromosomes were also observed at anaphase-2 (fig. 56–d) and number of lagging chromosomes is different cell to cell. Micronuclei at the late anaphase-2 and early telophase were present in several of the configurations examined.

Chromosome configurations at diakinesis, metaphase-1, and distribution of chromosomes at anaphase-1 and anaphase-2 in this hybrid are indicated in table 38.

d. The interspecific hybrid, *M. sulcata* × *M. macrocarpa*

Cross pollinations were made between *M. sulcata* and *M. macrocapa* in 1963. *M. sulcata* used as pistillate parent was the species which was introduced under the name of *M. segetalis* N 118 but was identified as *M. sulcata*. Two plants resulting from four mature seeds from the cross were judged as hybrid plants based on their morphological appearance.

Regarding the main traits of F_1 hybrid plants, the morphological characters are shown in table 39 in comparison with their parents.

Both parental species produce an abundance of fertile pollen, and the course of meiosis is regular (fig. 58–a, b).

The F_1 hybrid plants are highly sterile with about 48% of aborted pollen grains (fig. 58–e, f). There were briefly three kinds of pollen in the pollen

Fig 57. Inflorescences and leaves
a. *M. sulcata.*
b. *M. sulcata*×*M. macrocarpa* F_1.
c. *M. macrocarpa.*

TABLE 39. Comparison of the F_1 hybrid *M. sulcata*× *M. macrocarpa*, with the two parents

morphological characters	*M. sulcata*	*M. macrocarpa*	*M. sulcata* × *M. macrocarpa* F_1
leaflet			
margin	conspicuously toothed	inconspicuously toothed	inconspicuously toothed
shape	elliptic	orbiculate or obovate	elliptic
raceme	equal to subtending leaf	longer than subtending leaf	longer than subtending leaf
flower			
standard	shorter than keel	equal to keel	shorter than keel
size	3.7 mm	8.1 mm	6.2 mm
seed			
length	2.1 mm	3.5 mm	2.9 mm
width	1.3 mm	2.5 mm	2.0 mm

grains of the F_1 plants, i.e. plump and taking deep stain, smaller and taking partially stain, shrunken and taking no stain. The former is counted as normal and the latter two groups are estimated to be sterile pollen grains (fig. 58–f).

The course of meiosis of F_1 hybrid is aberrant. At diakinesis and

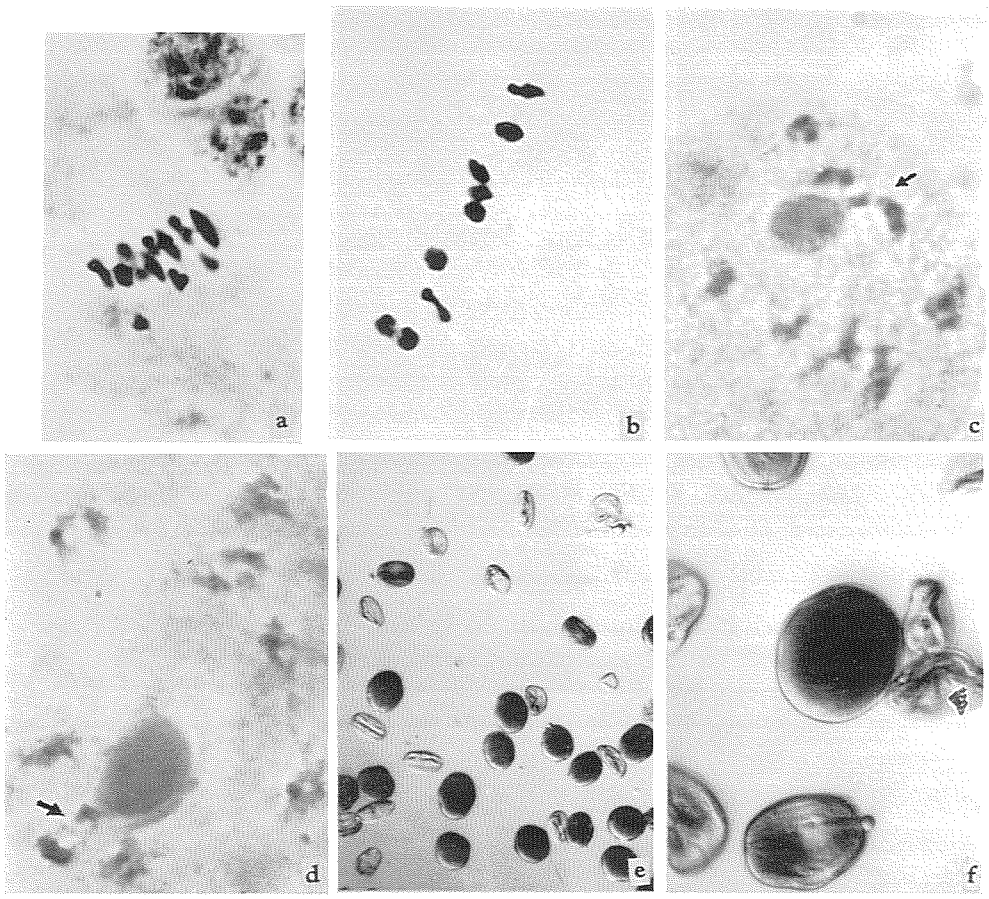


Fig. 58. a. *M. sulcata.* Metaphase-1 with 8II. × 2500.
b. *M. macrocarpa.* Metaphase-1 with 8II. × 2500.
c. *M. sulcata*×*M. macrocarpa* F_1. Diakinesis with 8II. × 2500. The rod shape of bivalent attached to nucleolus show unequal size of synapsis.
d. *M. sulcata*×*M. macrocarpa* F_1. Diakinesis with 8II. The ring shape of bivalent attached to nucleolus show unequal size of synapsis. × 2500.
e. *M. sulcata*×*M. macrocarpa* F_1. Normal and aborted pollen grains.
f. The same as e.

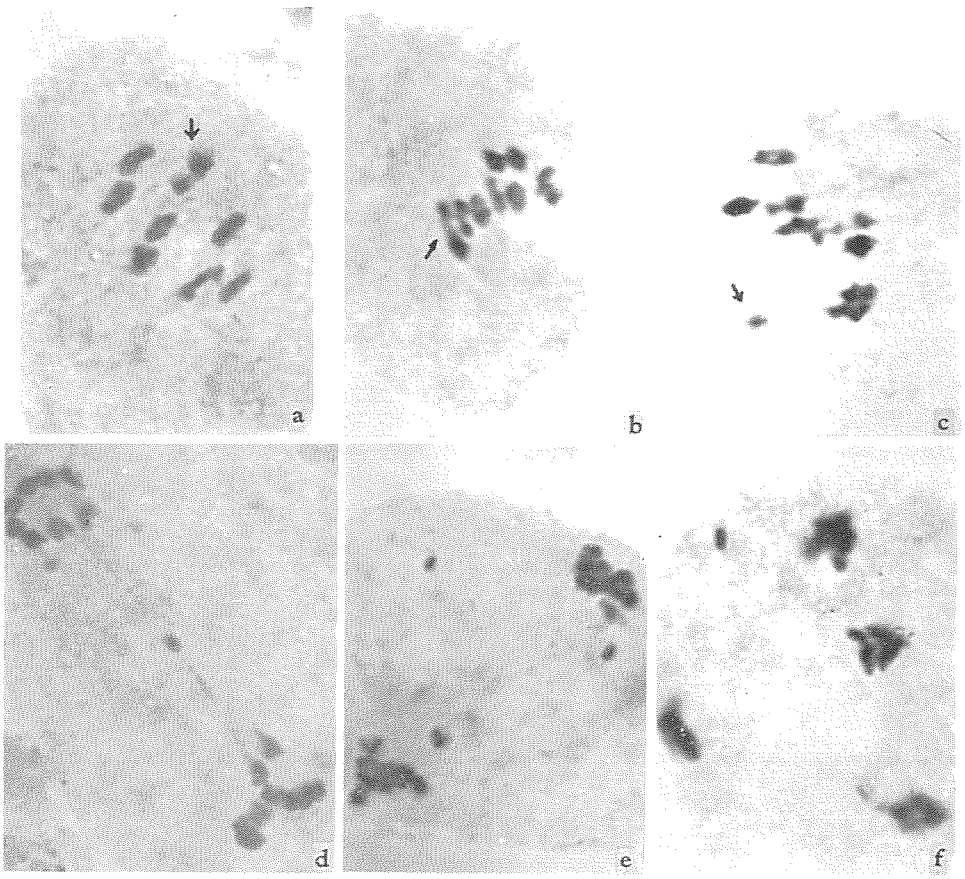


Fig. 59. *M. sulcata*×*M. macrocarpa* F_1

a. Metaphase-1 with 8II. The unequal size of bivalent is indicated by arrow. × 2500.
b. Metaphase-1 with 8II. The unequal size of bivalent is indicated by arrow. × 2500.
c. Metaphase-1 with 8II. The unequal size of univalent separated precociously. × 2500.
d. Anaphase-1 with chromatid bridge and acentric fragment. × 2500.
e. Anaphase-1 with lagging chromosomes. × 2500.
f. Anaphase-2 with lagging chromosome. × 2000.

metaphase-1, eight bivalents seemed to occur regularly. The bivalent which is in contact with the nucleolus, however, shows a kind of unequal size of synapsis. In the rod or ring of the bivalent, the difference in size of two chromosomes is clearly demonstrated at diakinesis configurations (fig. 58–c, d).

The characteristic synapsis of this bivalent is also observed at metaphase-1

TABLE 40. Chromosome configurations at diakinesis and metaphase-1 and their distribution in later stages of meiosis in the F_1 hybrid, *M. sulcata*×*M. macrocarpa*

stage of meiosis	frequency of PMCs with											
	8II	7II+2I	no frag. & brid.	a frag. & brid.	a frag.	a brid.	two frag.	a frag. & lagger	lagger	normal	abnormal	total
diakinesis	58											58
metaphase-1	153	3										156
anaphase-1			93	16	49	2	6	6	9			181
anaphase-2										31	44	75

(fig. 59–a, b). In some cases, the smaller chromosome of this bivalent segregate precociously and move to pole (fig. 59–c). Causal mechanism of this unusual bivalent has not been solved in this study.

At anaphase-1, normal 8—8 disjunction occur in 93 out of 181 figures examined. Abnormalities such as 7—9 disjunction and lagging chromosomes are observed in low frequency. The irreguralities which cause high pollen sterility became clearly evident at anaphase-1 when a high percentage of configurations possess dicentric chromatid bridge with or without acentric fragments (fig. 59–d). As a result it may be concluded that the hybrid is heterozygous for inversion.

Observations were made in order to determine whether the chromosomes possessing inversion were the same chromosomes which showed the unusual synapsis of bivalent at diakinesis and metaphase-1 or not. In the configuration of anaphase-1, conspicuously large sized chromosome is sometime observed in either pole. This chromosome which is the larger sized member of the bivalent is not associated with the chromosomes showing a bridge. Therefore, it is probable that the chromosomes with inversion may not be the same chromosomes which show unusual synapsis of bivalent.

Some abnormalities such as lagging chromosomes at anaphase-2, micronuclei at early telophase were also observed.

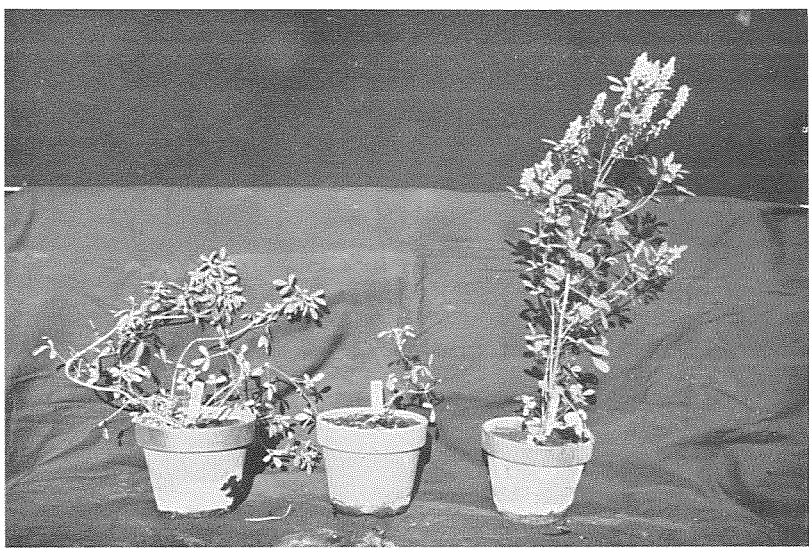


Fig. 60. left: *M. sulcata.*
center: *M. sulcata*×*M. infesta* F_1.
right: *M. infesta.*

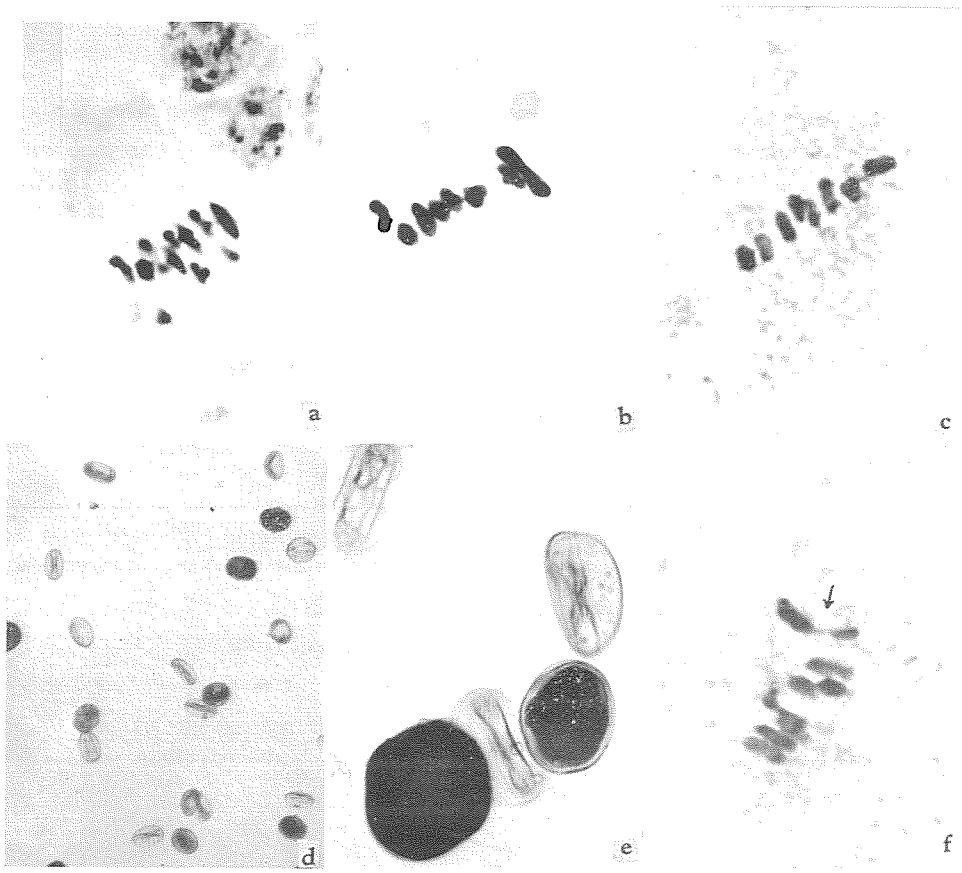


Fig. 61. a. *M. sulcata*. Metaphase with 8 II. × 2500.
b. *M. infesta*. Metaphase-1 with 8 II. × 2500.
c. *M. sulcata* × *M. infesta* F_1. Metaphase-1 with 8 II. × 2500.
d. *M. sulcata* × *M. infesta* F_1. Normal and aborted pollen grains.
e. The same as d.
f. *M. sulcata* × *M. infesta* F_1. A bivalent among eight bivalents show unequal size of synapsis. × 2500.

Chromosome configurations at diakinesis, metaphase-1, and distribution of chromosomes at anaphase-1 and anaphase-2 in this hybrid are given in table 40.

e. The interspecific hybrid, *M. sulcata* × *M. infesta*

Only one hybrid plant was derived from the cross, *M. sulcata* × *M. infesta*. The hybrid plant showed light green in color and never reached a height of more than 20 cm. But a few flowers were finaly obtained (fig. 60). The pistillate

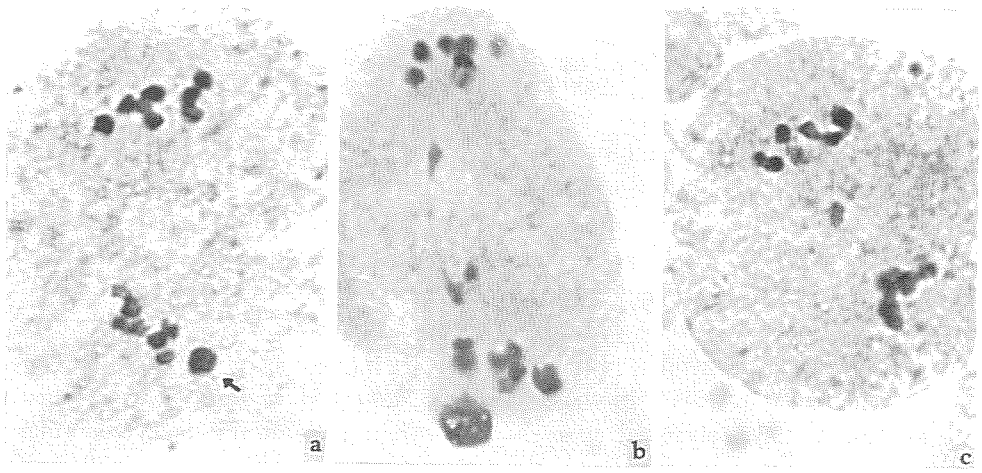


Fig. 62. *M. sulcata*×*M. infesta* F_1

a. Anaphase-1. A larger size of chromosome from the unusual bivalent is located in one pole. × 2500.
b. Anaphase-1. Chromatid bridge with an acentric fragment. × 2500.
c. Anaphase-1 with a lagging chromosome. × 2500.

parent, *M. sulcata*, is the same strain which was used in the cross of *M. sulcata* × *M. macrocarpa*.

Cytological observations were attempted by employing weak hybrid plant, sufficient materials for detailed analysis of meiotic chromosomes behaviors were not obtained.

In both parents, the percentage of fertile pollen was very high and the course of their meiosis are regular (fig. 61–a, b).

The pollen of the F_1 hybrid plant is highly sterile and three kinds of pollens, plump and taking deep stain, smaller size and taking a partial stain, shrunken and taking no stain, were observed. The plump and taking deep stain pollen grains were counted as fertile pollen (fig. 61–d, e). Thus, the percentage of fertile pollen was about 23.4%.

During the course of their meiosis aberrant chromosome behaviors occur. At metaphase-1, eight bivalents are regularly formed but one bivalent among eight shows unusual synapsis, that is to say, the size of chromosomes is different from each other in this bivalent (fig. 61–f). One larger chromosome separated from this bivalent is sometimes observed at either pole of anaphase-1 configurations which show 8—8 normal segregation (fig. 62–a). Dicentric chromatid bridge with an acentric fragment is also observed at anaphase-1 (fig. 62–b). It is clearly shown that the F_1 hybrid is heterozygous for inversion.

4. Discussion and Conclusion

Chromosome behaviors during the course of meiosis in the interspecific hybrids are one of the most revealing criteria available to the cytologist for an interpretation of the evolutional changes within a group of related species.

Regarding cytological studies of the interspecific hybrids between pairs of species within Type A, several papers have been reported by other workers. WEBSTER examined meiosis of three interspecific hybrids, *M. polonica* × *M. suaveolens*, *M. alba* × *M. polonica* (1950), and *M. alba* × *M. officinalis* (1955), and concluded that meiosis in these F_1 hybrids appeared to be as normal as those of the parental plants, although a high percentage of pollen abortion was observed in these hybrids. In the hybrid, (*M. alba* × *M. dentata*) × *M. dentata*, BRINGHURST (1950) observed cross-shaped configuration at the pachyteen stage indicating reciprocal translocation. SHASTRY, SMITH and COOPER (1960) reexamined the interspecific hybrid of *M. officinalis* × *M. alba* and arrived at the result that the course of meiosis was regular as mentioned by WEBSTER (1955). JARANOWSKI (1961) revealed that the hybrid of *M. polonica* × *M. alba* was heterozygous for reciprocal translocation, and refuted WEBSTER's conclusion (1950) that the chromosome behaviors of the reciprocal hybrid of *M. alba* × *M. polonica* was regular.

The studies by the author present further information on the cytological relationship of the group of species within Type A.

In the hybrid of *M. alba* × *M. hirsuta*, regular occurrences of 1) quadrivalent as a chain or ring of four chromosomes at diakinesis and methphase-1 and 2) a cross-shaped configuration observed at pachyteen indicate that the hybrid is heterozygous for reciprocal translocation. From the results of karyotype analysis, it was clearly demonstrated that the karyotype of a pair of chromosomes with secondary constriction in *M. hirsuta* was different from that of *M. alba*, that is to say, secondary constriction cut off a large distal segment in *M. hirsuta* while it cut off a microsatellite in *M. alba*. It is concluded, therefore, that the F_1 hybrid of *M. alba* × *M. hirsuta* is heterozygous for reciprocal translocation which took place between the pair of chromosomes with secondary constriction and any one of the other pair of chromosomes.

It should be also mentioned here that the karyotypic differences of a pair of chromosomes with secondary constriction in *M. dentata* and *M. polonica* are correlated with chromosome aberrations observed in the interspecific F_1 hybrids. A microsatellite is attached to the median chromosome in *M. dentata* while the submedian chromosome in *M. alba*, indicating that the pair of chromosomes with secondary constriction is associated with reciprocal transloca-

tion found in the hybrid of (*M. alba* × *M. dentata*) × *M. dentata* by BRINGHURST (1950). Also, it was detected by the author that karyotype of a pair of chromosomes in *M. polonica* was different from that of *M. alba*, that is to say, secondary constriction cut off a large distal segment in *M. polonica* while it cut off a microsatellite in *M. alba*. This supports the existence of reciprocal translocation found in the F_1 hybrid of *M. polonica* × *M. alba* by JARANOWSKI (1961), and it is possible to assume that the pair of chromosomes with secondary constriction is correlated with the reciprocal translocation.

In regard to the cytological study of the interspecific hybrids between pairs of species within Type B, only one paper has been reported on the F_1 hybrid of *M. messanensis* × *M. segetalis* (SHASTRY, SMITH and COOPER 1960).

In the study of the reciprocal hybrid, *M. segetalis* × *M. messanensis*, worked out by the author, a ring or chain of four chromosomes regularly presents at diakinesis and metaphase-1 and further a chromatid bridge with or without acentric fragment is observed at anaphase-1. These findings clearly explain that the F_1 hybrid is heterozygous for reciprocal translocation and inversion, agreeing with the result reported by SHASTRY, SMITH and COOPER (1960). It was pointed out that the karyotype of a pair of chromosomes with intercalary travants in *M. segetalis* was distinctly different from that of *M. messanensis*. Therefore, it can be said that a pair of chromosomes with secondary constriction is possibly correlated with either one of reciprocal translocation and inversion found in this hybrid.

The F_1 hybrid of *M. segetalis* × *M. macrocarpa* is also revealed to be heterozygous for reciprocal translocation and inversion, by the facts that a ring or chain of four chromosomes regularly found at diakinesis and metaphase-1 configurations and dicentric chromatid bridge with an acentric fragment exists at anaphase-1 configurations. The pistillate parent, *M. segetalis*, used in this cross is the same one as the species used in the cross of *M. segetalis* × *M. messanensis*. A pair of chromosomes with secondary constriction has not been detected by karyotype analysis made by the author in *M. macrocarpa*, however, it can be pointed out that karyotypic differences exist between a pair of chromosomes with secondary constriction in *M. segetalis* and a pair of chromosomes in *M. macrocarpa* which is homologous with the pair of chromosomes with secondary constriction in *M. segetalis*. Here again, it is possible to assume that such karyotypic difference is associated with translocation or inversion found in this hybrid.

Occurrence of dicentric chromatid bridge with or without an acentric fragment at anaphase-1 of the F_1 hybrid of *M. sulcata* × *M. macrocarpa* clearly indicates that this hybrid is heterozygous for inversion. At diakinesis and

metaphase-1, eight bivalents are regularly formed but one of the eight bivalents showed an unusual synapsis in unequal size. Unequal size of separation of this bivalent at anaphase-1 is also observed. The causal mechanism of this unequal synapsis is still an open question, however, it is, at least, clear that this bivalent is not associated with the bivalent causing inversion.

Almost identical data are obtained in the hybrid of *M. sulcata* × *M. infesta*. The limited materials make it impossible to reveal its details, but there is little doubt that the hybrid is heterozygous for inversion because of the presence of dicentric chromatid bridge with or without an acentric fragment at anaphase-1. At metaphase-1, eight bivalents constantly occurred but the unequal size of bivalent synapsis was noted in this hybrid, too.

As far as karyomorphology of somatic chromosomes were examined, no satellited chromosomes have been detected in *M. infesta* and *M. macrocarpa*. This may partly be due to the small size of chromosomes. Thus we are unable to point out which chromosome is associated with the chromosome aberration found in these two F_1 hybrids.

Based on the occurrences of the chromosome aberrations throughout the interspecific hybrids examined by the author, it is concluded that the structural differentiation of chromosomes such as reciprocal translocation and inversion play a significant role during the process of speciation among the group of species within types.

VI. General Discussion and Conclusion

Throughout the experiments by the author, considerable results have been accumulated with regard to the speciation of the genus *Melilotus*.

Concerning the data obtained from the studies on the relationship of interspecific cross compatibility, karyotype analysis, and cytological relationship of chromosome aberrations found in the interspecific F_1 hybrids, it is pointed out that several factors or mechanisms affect the differentiation of the species during the process of evolution of this genus.

The following factors could be mentioned from the results of the study on the relationship of interspecific cross compatibility.

1) Non-crossability between pairs of certain species; such as between species of the subgenus *Eumelilotus* and the subgenus *Micromelilotus*, between *M. officinalis* and the other species of the subgenus *Eumelilotus*, and probably between *M. italica* and the other species of the subgenus *Micromelilotus*; plays a significant role as the isolation mechanism of species from each other.

2) Chlorophyll deficiency widely occurring in the interspecific F_1 hybrids

renders the hybrid incapable to grow beyond the seedling stage. This is considered as another factor which isolates species within a group of species which are able to produce viable interspecific F_1 hybrid seeds.

From the experiments of karyotype analysis, the following factors which present more accurate information concerning speciation of this genus should be pointed out.

1) Differences in absolute size of chromosomes exist among the *Melilotus* species, by which the nineteen species examined are grouped into three types, Type A, Type B and Type C. Type B is further divided into Type B–1 and Type B–2. There is no evident reason why the size of chromosomes is different between each type, however, it is suggested that the demarcation of groups mentioned above has to do with the process of evolution of this genus in a more essential sense. And therefore, it can be said that the species within each type are in close relationship, agreeing with the results obtained from the study of interspecific cross compatibility as far as examined.

2) Differences of karyotypes of certain pairs of chromosomes are clearly demonstrated among the species within each type which seem to have the same size of chromosomes. This indicates that the structural differentiation of chromosomes took place during the process of evolution.

The cytological study of the interspecific F_1 hybrids clearly confirm that chromosome aberrations such as reciprocal translocation and inversion exist in the F_1 hybrids between pairs of some species within Type A and Type B–2. These results reveal that structural differentiation of chromosomes assumed from the data of karyotype analysis is undoubtedly associated with reciprocal translocation and inversion. Now it is natural to conclude that the structural differentiation of chromosomes mentioned above play an important role for speciation within closely related species.

The study of karyotype analysis together with the cytological study of the interspecific F_1 hybrids strongly indicates that a complex series of structural differentiation of chromosomes may exist throughout the *Melilotus* species. As mentioned previously, non-crossability between certain species and chlorophyll deficiency in the interspecific F_1 hybrids are the main barriers standing in the way of obtaining desirable interspecific hybrids by which to solve this problem.

Here, it should be suggested that grafting methods were successfully used to rear the distinct chlorophyll deficient hybrid of *M. alba* × *M. dentata* by SMITH (1943). And also, embryo culture was employed for obtaining the hybrid between *M. officinalis* and *M. alba* which showed embryo abortion at an early stage of development (WEBSTER 1955). These two methods, if successful, will provide a more precise picture of a complex series of structural differentiation

of chromosomes seemingly involved in this genus.

VII. Summary

I. Observations of Morphological Characters.

1. Nineteen species including twenty-seven strains which were introduced are, first of all, examined whether they possess proper name of classification or not.

2. The species introduced as *M. italica* PI 193951 is identified as synonymous with *M. taurica*, based on ISELY's identification keys. Therefore, this species is used as *M. taurica* PI 193951 for the following studies.

3. The species introduced as *M. neapolitana* Fc 30341 is a synonym of *M. indica*, although some minor differences were observed in pod and seed size. This species is used as *M. indica* Fc 30341 for the following studies.

4. The species introduced as *M. segetalis* N 118 is likely to be *M. sulcata* rather than *M. segetalis*. This species is used as *M. sulcata* N 118 for the following studies.

5. *M. elegans* Ac 337 confirmed by Dr. G. STEVENSON is different in plant height from the description of ISELY's keys, but is used as *M. elegans* with some reservations.

II. Interspecific Cross Compatibility.

1. Eight species belonging to the subgenus *Eumelilotus* (*M. alba*, *M. altissima*, *M. dentata*, *M. hirsuta*, *M. officinalis*, *M. suaveolens*, *M. taurica*, and *M. wolgica*), and ten species belonging to the subgenus *Micromelilotus* (*M. elegans*, *M. indica*, *M. infesta*, *M. italica*, *M. macrocarpa*, *M. messanensis*, *M. neapolitana*, *M. segetalis*, *M. speciosa*, and *M. sulcata*) were intercrossed within each subgenus. Cross pollinations were also attempted between species of the subgenus *Eumelilotus* and *Micromelilotus*.

2. In the *Eumelilotus*, hybrid seedlings were obtained from the following combinations; *M. alba* × *M. altissima*, *M. alba* × *M. dentata*, *M. alba* × *M. hirsuta*, *M. alba* × *M. suaveolens*, *M. alba* × *M. taurica*, *M. alba* × *M. wolgica*, *M. altissima* × *M. alba*, *M. altissima* × *M. hirsuta*, *M. hirsuta* × *M. alba*, *M. hirsuta* × *M. altissima*, *M. hirsuta* × *M. dentata*, *M. hirsuta* × *M. taurica*, *M. hirsuta* × *M. wolgica*, *M. taurica* × *M. alba*, and *M. taurica* × *M. hirsuta*.

3. Chlorophyll deficiency was widely observed in the hybrid seedlings resulting from the crosses mentioned above. The hybrid between *M. hirsuta* and *M. alba* was slightly less green in color than the parental species, but the hybrids between *M. hirsuta* and the other species were almost devoid of

chlorophyll. Thus, *M. hirsuta* is considered to be included in SMITH's "group A" (SMITH 1954).

4. No hybrid was obtained from the crosses between *M. officinalis* and any other species of the subgenus *Eumelilotus*.

5. In the subgenus *Micromelilotus*, hybrid plants were produced from the crosses, *M. messanensis* × *M. segetalis*, *M. segetalis* × *M. messanensis*, *M. segetalis* × *M. macrocarpa*, *M. sulcata* × *M. macrocarpa*, *M. sulcata* × *M. infesta*.

6. The derived hybrid plants from the above crosses showed complete normal green in color except for *M. sulcata* × *M. infesta*. The hybrid plant, *M. sulcata* × *M. infesta*, is slightly less green than the parental species.

7. No hybrid was obtained from the crosses between pairs of species belonging to the subgenus *Eumelilotus* and *Micromelilotus* as far as examined.

8. From these results, it is pointed out that non-crossability between certain species and chlorophyll deficiency occurring in the hybrid seedlings are in a role of isolation mechanism of species in this genus.

III. Studies on the Morphology of the Somatic Chromosomes of the genus *Melilotus*.

1. Karyotypes of nineteen species were analysed.

2. On the basis of chromosome size, nineteen species are grouped into four types, Type A (*M. alba*, *M. altissima*, *M. dentata*, *M. hirsuta*, *M. officinalis*, *M. polonica*, *M. suaveolens*, *M. taurica*, and *M. wolgica*), Type B, and Type C (*M. italica*). The species included in Type B are subdivided into Type B–1 (*M. elegans*, *M. indica*, and *M. neapolitana*) and Type B–2 (*M. infesta*, *M. macrocarpa*, *M. messanensis*, *M. segetalis*, *M. speciosa*, and *M. sulcata*).

3. The chromosome size of the species in Type A is large. The chromosome size of the species in Type B–1 is somewhat larger than that of species in Type B–2, but that of species included in both types is distinctly smaller than that of species in Type A. The size of chromosomes of the species in Type C is similar or larger than that of the species in Type A but is clearly distinguishable by its specific karyotype.

4. High interspecific cross compatibility is found in the crosses between pairs among species within Type A and within Type B–2. In contrast to this, no hybrid has been obtained from the interspecific crosses between these two types. Namely, grouping of types based on karyotype was correlated with interspecific cross compatibility as far as examined.

5. The pair of satellited chromosomes of *M. hirsuta*, *M. polonica*, and

M. dentata are distinguishable in karyotype from that of the other species. This indicates that these karyotypic differences are connected with reciprocal translocations found in the F_1 hybrids, *M. alba* × *M. hirsuta*, *M. polonica* × *M. alba*, and (*M. alba* × *M. dentata*) × *M. dentata*.

6. The pair of satellited chromosomes of *M. segetalis* is characteristic in its karyotype, which is correlated with reciprocal translocation or inversion found in the F_1 hybrids between *M. segetalis* and *M. messanensis* and between *M. segetalis* and *M. macrocarpa*.

IV. Cytogenetics of Interspecific Hybrids.

1. The F_1 hybrid, *M. alba* × *M. hirsuta*, shows irregularlities during the course of meiosis. The occurrence of a quadrivalent as a chain or ring of four chromosomes at diakinesis and metaphase-1 and a cross-shaped configuration at pachyteen indicates that the hybrid is heterozygous for reciprocal translocation.

2. In the F_1 hybrid, *M. segetalis* × *M. messanensis*, a ring or chain of four chromosomes is regularly formed at diakinesis and metaphase-1 of meiosis. In addition, a chromatid dicentric bridge with or without an acentric fragment is observed at anaphase-1. These findings reveal that the F_1 hybrid is heterozygous for reciprocal translocation and inversion.

3. The hybrid, *M. segetalis* × *M. macrocarpa*, is also heterozygous for reciprocal translocation and inversion by the facts that a ring or chain of four chromosomes is regularly formed at diakinesis and metaphase-1 and a dicentric chromatid bridge with an acentric fragment exists at anaphase-1.

4. A dicentric chromatid bridge with or without an acentric fragment is observed at anaphase-1 of meiosis of the F_1 hybrid plants from *M. sulcata* × *M. macrocarpa*. This indicates that the F_1 hybrid is heterozygous for inversion.

5. In the F_1 hybrid of *M. sulcata* × *M. infesta*, a dicentric chromatid bridge with or without an acentric fragment is also observed at anaphase-1 of meiosis. Consequently, the F_1 hybrid is heterozygous for inversion.

6. In the above two hybrids, *M. sulcata* × *M. macrocarpa* and *M. sulcata* × *M. infesta*, a bivalent among eight at diakinesis and metaphase-1 show an unequal size of synapsis. The causal mechanism of this unusual bivalent is not determined.

7. From the chromosome aberrations found in the several interspecific hybrids mentioned above, it is concluded that the structural differentiation of chromosomes such as reciprocal translocation and inversion play a significant role during the process of speciation of this genus.

Literature Cited

ADATI, S. 1958. Studies on the genus *Miscanthus* with special reference to the Japanese species suitable for breeding purpose as fodder crops. Bull. Fac. Agr. Mie Univ. 17 : 1–112.

————, and I. SHIOTANI. 1962. The cytotaxonomy of the genus *Miscanthus* and its phylogenetic status. Bull. Fac. Agr. Mie Univ. 25 : 514–535.

ARMSTRONG J. M. and J. E. R. GREENSHIELDS. 1958. Cytogenetics of forage crops. Proc. Gen. Soci. Canada. 3 : 30–34.

BRINGHURST, R. S. 1951. Genetic analysis of chlorophyll deficiency in *Melilotus alba* × *M. dentata* hybrids with some observations on meiotic irregularlities. Summaries of Doctoral Dissertations, Univ. Wis. 11 : 96–97.

COOPER, D. C. 1933. Macrosporogenesis and embryology of *Melilotus*. Bot. Gaz. 95 : 143–155.

DARLINGTON, C. D. 1932. Recent advances in cytology. London.

————————, and E. K. JANAKI AMMAL. 1945. Chromosome atlas of cultivated plants. London.

DAS, B. C. 1953. Cytological and morphological basis for sterility in autotetraploid sweetclover, *Melilotus alba* Desr. Iowa State College Jour. Soci. 27 : 537–561.

ELDERS, A. T. 1926. Some pollinations and cytological studies of sweetclover. Amer. Soci. Agr. 6 : 360–365.

Fryer, J. R. 1930. Cytological studies in *Medicago, Melilotus,* and *Trigonella.* Canadian Jour. Res. 3 : 3–50.

GOPLEN, B. P., J. E. R. GREENSHIELDS, and W. J. WHITE. 1956. Selection techniques in screening for coumarindeficient sweetclover plants. Can. J. Bot. 34 : 711–719.

————————, ————————, and H. BAENZIGER. 1957. The inheritance of coumarin in sweetclover. Can. Jour. Res. 3 : 3–50.

ISELY, D. 1954. Keys to sweet clovers (*Melilotus*). Iowa Acad. Scien. 61 : 119–131.

JARANOWSKI, J. K. 1961. Semisterility in the interspecific hybrid, *Melilotus polonica* × *M. alba.* Amer. Jour. Bot. 48 : 28–35.

————————. 1962. Chlorophyll deficiency and variegation as a result of interspecific crosses in *Melilotns.* Genetica Polonica. 3 : 1–18.

————————. 1962. Development of embryos and seeds in certain species and species crosses in *Melilotus.* Genetica Polonica. 3 : 45–59.

————————. 1963. Cytological studies in the F_1 sterility of the interspecific hybrid *Melilotus alba* × *M. polonica.* Genetica Polonica 4 : 53–63.

JOHNSON, I. J. 1942. Cross fertility relationships of "Golden Annual" sweet clover with common species of *Melilotus.* Jour. Amer. Soci. Agron. 34 : 259–262.

KIRK, L. E. 1929. Abnormal seed development in sweet clover species crosses. A new technique for emasculating sweet clover flowers. Sci. Ag. 10 : 321–327.

————. 1931. Inheritance of dwarf branching habit in a new variety of sweet clover and its potential economic value in breeding. Sci. Agr. 11 : 315–325.

KITA, F., M. L. MAGOON, and D. C. COOPER 1959 Simple smear techniques for the

study of chromosomes of *Melilotus*. Phyton 12:35–38.

———. 1962. Fundamental studies of interspecific hybrids in the genus *Melilotus* from the plant breeding standpoint. I. Cytological studies of the interspecific hybrid, *Melilotus alba*×*M. hirsuta*. Mem. Fac. Agr. Hokkaido Univ. 4 : 67–74.

———. 1964. Fundamental studies of interspecific hybrids in the genus *Melilotus* from the plant breeding standpoint. II. Cytological studies of the interspecific hybrid, *Melilotus segetalis*×*M. messanensis*. Jour. Agr. Exp. Farm, Hokkaido Univ. 12 : 74–82.

MENDOZA, A. B. 1946. A survey of self-fertility and crosscompatibility between species of *Melilotus*. M. S. thesis, Iowa State College Library.

MORIYA, A. 1959. Cytological basis of the sugarcane breeding in the Far East, especially in Taiwan. Special Bull. Coll. Agr. Utzunomiya Univ. 6 : 3–60.

SASAKI, M. 1961. Thremmatological studies on amphidiploids, and chromosome and nucleus substitution lines in wheat-rye hybrids. Special publication Fac. Agr. Tottori Univ.

SCHULZ, O. E. 1901. Monographie der Gattung *Melilotus*. Bot. Jahrb. 29 : 660–735.

SHARMA, A. K. and A. SHARMA. 1959. Chromosomal alterations in relation to speciation. Bot. Review. 25 : 514–535.

SHASTRY, S. V. S., W. K. SMITH, and D. C. COOPER. 1960. Chromosome differentiation in several species of *Melilotus*. Amer. Jour. Bot. 47 : 613–621.

SINSKAYA, E. V. 1961. Flora of cultivated plants of the USSR. XIII. perennial leguminous plant. Part 1. Medic, Sweetclover, Fennugreek.

SMITH, W. K. 1943. Propagation of chlorophyll-deficient sweetclover hybrids as grafts. Jour. Heredity 34 : 135–140.

———. 1954. Viability of interspecific hybrids in *Melilotus*. Genetics 39 : 266–279.

STEVENSON, T. M. and L. E. KIRK. 1935. Studies in interspecific crossing with *Melilotus* and intergeneric crossing with *Melilotus, Medicago* and *Trigonella*. Sci. Agr. 15 : 580–589.

———, and W. J. WHITE. 1940. Investigations concerning the coumarin content of sweetclover. I. The breeding of a low-coumarin line of sweet clover-*Melilotus alba*. Sci. Agr. 21 : 18–28.

WEBSTER, G. T. 1950. Fertility relationships and meiosis of interspecific hybrids in *Melilotus*. Agron. Jour. 42 : 315–322.

———. 1955. Interspecific hybridization of *Melilotus alba*×*M. officinalis* using embryo culture. Agron. Jour. 47 : 138–142.