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# CYTOLOGICAL STUDIES OF SEVERAL INTERSPECIFIC F<sub>1</sub> HYBRIDS IN THE SUBGENUS *EUMELILOTUS*

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## Introduction

The genus *Melilotus* (sweetclover) is comprised of some twenty species (SCHULZ 1901, ISELY 1954, STEVENSON 1969). Most of the cultivated varieties which have been used for forage and soil improvement for many years are derivatives of two species, *M. alba* and *M. officinalis*, belonging to the subgenus *Eumelilotus*.

Chlorophyll deficiency widely occurring in the interspecific hybrids within 9 species of the subgenus *Eumelilotus*, constitutes an effective barrier against the exchange of genes between the two species (SMITH 1954, KITA 1965).

Regarding cytological studies in the interspecific F<sub>1</sub> hybrids, several papers have been reported by several workers (WEBSTER 1950, 1955, BRINGHURST 1951, SHASTRY, SMITH and COOPER 1960, JARANOWSKI 1961, KITA 1962, 1965, KITA and NIIZEKI 1967). From the studies of meiotic chromosome behaviors of the interspecific F<sub>1</sub> hybrids, it appeared that structural differences of chromosomes were in a role of speciation of the genus *Melilotus* in part.

The purpose of this study is to make clear cytological relationships among species of the subgenus *Eumelilotus* systematically.

## Materials and Methods

Eight species belonging to the subgenus *Eumelilotus* were used in this study. These are described in the results.

Cross pollinations were made in the green house from 1970 to 1972. In making each cross, the petals were removed from recently opened flowers and pollens were removed by suction using a small vacuum pump.

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Pollen of the staminate parent was applied without delay to the stigma of the pistillate parent with a toothpic tip (SMITH 1954).

Seeds obtained were classified by size and plumpness and seed coats were scratched with a razor blade, and then germinated in a petry dish under favorable temperatures. Germinated seeds were transferred to flats filled with soil. Most of the interspecific hybrids were easily detected by chlorophyll deficiency in the seedling stage and/or morphological differences from the maternal and paternal parent species, as described in the results.

Young inflorescences of the desired stage of development 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, and maintained at low temperatures (ca. 2-4°C) for 24 hours. The anthers were removed and smeared in a drop of acetocarmine and the meiotic configurations were analysed.

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

## Results

### 1. Cytological observations in the parental species

All of the parental strains of each species of the subgenus *Eumelilotus* used in this study had high pollen fertility, having higher than 90% except *M. polonica* C 30. The parental strains at each of the following stages in microsporogenesis -diakinesis, metaphase 1, anaphase 1, and anaphase 2- were examined. In all of the parental strains without exception, meiosis was normal with eight bivalents (8<sub>II</sub>) regularly present at diakinesis and metaphase 1 (Fig. 1). Some abnormalities following irregular disjunctions such as a lagging chromosome were present in a very small proportion of the spore nuclei.

As reported by WEBSTER (1950), it is considered that high pollen sterility of *M. polonica* C 30 (ca. 0.0-21.4% of stainable pollen grains) was caused by some environmental factors such as high temperatures rather than chromosomal abnormalities. As far as examined microsporogenesis, meiosis was normal.

### 2. Cytological observations in the F<sub>1</sub> hybrids

#### a. *M. alba* × *M. suaveolens* F<sub>1</sub>

In the crosses between *M. alba* and *M. suaveolens*, five biennial strains of *M. alba*, Arctic, Bdn 928, Bdn 647, Cumino, and Denta, were used as pistillate parents, and three biennial strains of *M. suaveolens*,

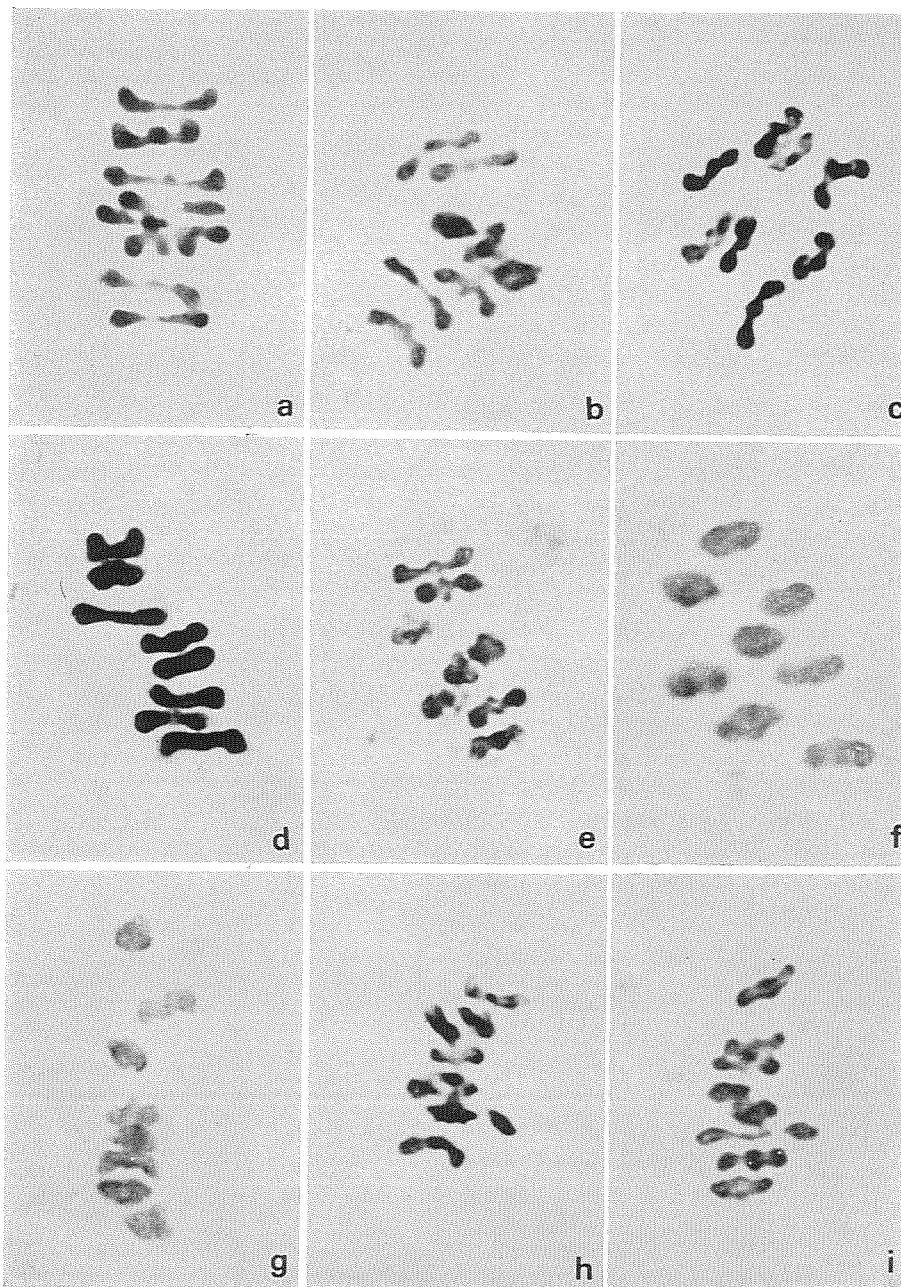


Fig. 1. Metaphase I of the parental species.  $\times 2100$ .

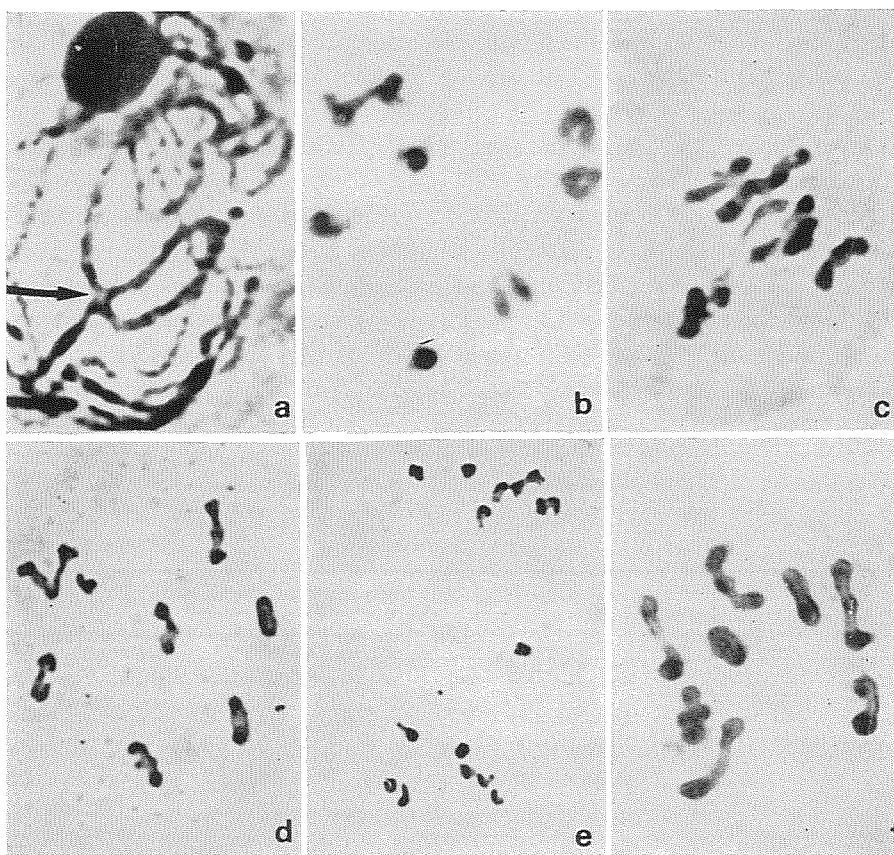
- a. *M. alba* Arctic    b. *M. suaveolens* Redfield Yellow    c. *M. officinalis* P.I. 178985  
 d. *M. officinalis* Gold Top    e. *M. hirsuta* P.I. 314096  
 f. *M. polonica* 3446    g. *M. taurica* 70    h. *M. dentata* Bdn 58-190  
 i. *M. wolgica* 58-254

TABLE 1. Pollen fertility of the interspecific F<sub>1</sub> hybrids examined

	cross	no. of plants	average of pollen fertility (%)
<i>M. alba</i> Arctic	× <i>M. suaveolens</i> 70	2	28.2
" "	× " 18-4	5	26.8
" "	× " Golden Annual	11	40.1
" Cumino	× " 70	2	40.3
" Bdn 928	× " Redfield Yellow	4	53.0
" Denta	× " Bdn 60-59	1	19.1
" Denta	× " Redfield Yellow	1	28.7
<i>M. officinalis</i> P.I. 178985	× <i>M. alba</i> Arctic	4	52.4
" "	" × " Cumino	2	57.1
" "	" × " Brandon Dwarf	4	63.8
" "	" × " Bdn 928	10	51.0
" "	" × " Bdn 647	1	67.1
" "	" × " Hubam	2	57.1
" "	" × <i>M. hirsuta</i> P.I. 314096	11	96.8
" "	" × " 58-4	2	86.8
" "	" × <i>M. polonica</i> 3446	4	98.0
<i>M. polonica</i> C 30	× <i>M. suaveolens</i> Redfield Yellow	4	20.4
<i>M. hirsuta</i> 58-4	× " Bdn 60-59	1	87.6
<i>M. alba</i> Arctic	× <i>M. hirsuta</i> P.I. 314096	11	67.6
" Bdn 928	× " "	4	66.8
" Bdn 647	× " "	3	59.7
<i>M. hirsuta</i> 58-4	× <i>M. alba</i> Bdn 647	2	42.2
" P.I. 314096	× " "	1	60.0
" "	" × " Hubam	2	50.4
<i>M. alba</i> Arctic	× <i>M. polonica</i> 3446	6	27.0
" Denta	× " "	12	56.1
" Bdn 928	× " "	5	60.0
" Hubam	× " "	2	56.3
<i>M. polonica</i> C 30	× <i>M. alba</i> Arctic	1	56.2
" "	× " Evergreen	2	34.8
" 3446	× " Arctic	3	44.9
" "	× " Hubam	1	59.9
<i>M. hirsuta</i> 58-4	× <i>M. polonica</i> 3446	8	96.4
<i>M. taurica</i> 70	× <i>M. alba</i> Cumino	6	25.0
" "	× " Denta	2	25.2
" P.I. 314094	× " Cumino	1	27.7
" 70	× <i>M. wolgica</i> 58-254	5	74.5
<i>M. dentata</i> Bdn 58-190	× <i>M. taurica</i> 70	2	41.7
" "	× " P.I. 314094	1	24.7
" P 783-2	× " 134-1	1	1.9

Redfield Yellow, 70, Bdn 60-59, and two annual strains of *M. suaveolens*, 18-4, Golden Annual, were used as pollen parents.

From these crosses, many seeds which consisted of plump, small, and brown aborted seeds were obtained. All of the successful hybrid seedlings,



**Fig. 2.** Different stages in meiosis of the F<sub>1</sub> hybrids examined.

- a. *M. alba* Denta × *M. suaveolens* Bdn 60-59 F<sub>1</sub>. Pachytene showing a cross-shaped configuration. The breakage point is indicated by arrow. ×2450.
- b. *M. alba* Bdn 928 × *M. suaveolens* Redfield Yellow F<sub>1</sub>. Diakinesis with 6II plus a chain of 4 chromosomes. ×1800.
- c. The same F<sub>1</sub> as b. Metaphase 1 with 1IV+6II. ×2100.
- d. *M. officinalis* P.I. 178985 × *M. alba* Bdn 928 F<sub>1</sub>. Metaphase 1 with 1III+6II+1I. ×1500.
- e. The same F<sub>1</sub> as d. Anaphase 1 with a lagging chromosome. ×1300.
- f. *M. officinalis* Gold Top × *M. officinalis* P.I. 178985 F<sub>1</sub>. Metaphase 1 with 8II. ×2100.

in the most of cases, were derived from the small seeds and grew vigorously. Upon flowering, all plants produced pale yellow flowers indicating that they were hybrids since the maternal parent, *M. alba*, was white-flowered. On the other hand, when annual strains of *M. alba* such as Hubam, Bdn 631, and Bdn 633, were used as pistillate parents, the  $F_1$  hybrid seedlings showed moderate chlorophyll deficiency and were very weak. The seedlings were not able to survive beyond cotyledonary stage.

In the reciprocal crosses, *M. suaveolens*  $\times$  *M. alba*, by using the same strains of *M. alba* as pollen parents, a lot of aborted seeds were obtained, but no hybrid seedlings.

All of the hybrid plants obtained were highly sterile as shown in Table 1, and aberrant chromosome behaviors occurred during the course of meiosis. Usually, six bivalents and a chain of four chromosomes existed at diakinesis and metaphase 1 (Fig. 2 b, c). Six bivalents plus a trivalent and one univalent ( $6_{II} + 1_{III} + 1_I$ ), seven bivalents plus two univalents ( $7_{II} + 2_I$ ) and eight bivalents ( $8_{II}$ ) were present in the remaining nuclei. High fre-

TABLE 2. Chromosome configurations at diakinesis and metaphase 1 and their distribution in later stages of meiosis in the interspecific  $F_1$  hybrids, showing that they are heterozygous for a reciprocal translocation

cross	no. of cross combinations examined	stage	frequency of PMCs with					total	
			$1_{IV} + 6_{II}$	$1_{III} + 6_{II} + 1_I$	$8_{II}$	$7_{II} + 2_I$	nor. abnor.		
<i>M. alba</i> $\times$ <i>M. suaveolens</i>	5	diakinesis	272	49	49	17		388	
		metaphase 1	273	186	27	24		510	
		anaphase 1					156	89	245
		anaphase 2					181	51	232
<i>M. officinalis</i> $\times$ <i>M. alba</i>	4	diakinesis	268	40	40	29		377	
		metaphase 1	227	218	31	33		509	
		anaphase 1					202	103	305
		anaphase 2					239	73	311
<i>M. alba</i> $\times$ <i>M. hirsuta</i> and the reciprocal	4	diakinesis	193	27	21	19		260	
		metaphase 1	158	178	14	11		361	
		anaphase 1					127	94	221
		anaphase 2					185	78	263
<i>M. alba</i> $\times$ <i>M. polonica</i> and the reciprocal	3	diakinesis	67	11	12	4		94	
		metaphase 1	64	94	17	18		193	
		anaphase 1					55	26	79
		anaphase 2					77	23	100

quency of the chromosome association such as 6<sub>II</sub>+1<sub>III</sub>+1<sub>I</sub> at metaphase 1, presumably, indicates a precocious segregation of one member of the chain. Pachytene analysis was undertaken in order to determine whether the multivalent association of four chromosomes was the result of a reciprocal translocation. A cross-shaped configuration observed in pachytene stage clearly revealed that the multivalent association of four chromosomes was of the reciprocal translocation origin (Fig. 2 a). Some abnormalities such as 7-9 disjunction which could lead to the production of aneuploid gametes, and lagging chromosomes occurred at anaphase 1. Lagging chromosomes were also present at anaphase 2, and the frequency of the abnormalities was relatively high.

From the observations mentioned above, it is clear that the F<sub>1</sub> hybrids between *M. alba* and *M. suaveolens* in all cross combinations examined, are heterozygous for a reciprocal translocation, explaining partial pollen fertility. Consequently, this result is different from WEBSTER's result (1950) that the course of meiosis in the F<sub>1</sub> hybrid between *M. alba* and *M. suaveolens* was normal.

Chromosome configurations at diakinesis, metaphas 1, and distributions of chromosomes at anaphase 1 and anaphase 2 in several hybrids are presented in Table 2.

#### b. *M. officinalis* × *M. alba* F<sub>1</sub>

It has been generally considered that the two principal economic species, *M. alba* and *M. officinalis*, are completely isolated reproductively, since none of the varieties of either species has been known to hybridize with those of the other and produce a fully developed hybrid seed or mature F<sub>1</sub> plant without the use of embryo culture. However, it was reported that *M. officinalis* P.I. 178985, an introduction from Turkey, was crossed successfully with *M. alba* Spanish in natural conditions (LANG and GORZ 1960).

In this experiment, reciprocal crossings between *M. officinalis* P.I. 178985 and each of many strains of *M. alba* were made. When *M. officinalis* P.I. 178985 was used as the pistillate parent a lot of F<sub>1</sub> hybrids were obtained but no hybrid plant resulted from the reciprocal. The hybrid seedlings continued to grow to maturity in spite of having slight chlorophyll deficiency.

All hybrids obtained had partial pollen sterility (Table 1). Six bivalents and a chain of four chromosomes, as shown in Table 2, frequently occurred at diakinesis and metaphase 1 of microsporogenesis in all cross combinations (Fig. 2 d.) Cross-shaped configurations characteristics of a

reciprocal translocation were present at pachytene, indicating that one of the parent is homozygous for an interchange of relatively large section between two unhomologous chromosomes as well as in the case of the hybrid of *M. alba* × *M. suaveolens*. At anaphase 1 and anaphase 2, irregularities in chromosome disjunction such as lagging chromosomes existed to some extent (Fig. 2 e).

The observations described above are different from the result reported by SHASTRY *et al* (1960) that meiotic chromosome behaviors in the F<sub>1</sub> hybrid between *M. alba* and *M. officinalis*, which was reared by using embryo culture (WEBSTER 1955), were regular.

In order to resolve the controversial result, it became necessary that the intraspecific F<sub>1</sub> hybrids between P.I. 178985 and the other strains of *M. officinalis* should be cytologically examined. Since no chromosomal difference apparently existed among the strains of *M. alba*, if the both results were true, the chromosomal difference might be observed in the interstrain F<sub>1</sub> hybrids between P.I. 178985 and the other strains of *M. officinalis*.

**c. The intraspecific F<sub>1</sub> hybrids between P.I. 178985 and the other strains of *M. officinalis*.**

The reciprocal crossings between P.I. 178985 and the other eight strains of *M. officinalis*, Bdn 62-1, Goldtop, Madrid, Bdn 62-13, Erector, P 197-1, P 198-1 and P 200-2, were made. The F<sub>1</sub> plants from the crosses in which P.I. 178985 was used as pollen parent, were normally green and produced large pods characteristic of P.I. 178985, showing that they were F<sub>1</sub> hybrids. Although the F<sub>1</sub> plants resulted from the reciprocal crosses using P.I. 178985 as pistillate parents could not be identified with the certainty by morphological characteristics whether they were F<sub>1</sub> hybrids

TABLE 3. Chromosome configurations at diakinesis and metaphase 1 and their distribution in later stages of meiosis in the intraspecific F<sub>1</sub> hybrids of *M. officinalis*, showing that meiotic chromosome behaviors were regular

no. of cross combinations examined	stage	frequency of PMCs with				total
		8II	7II+2I	normal	abnormal	
6	diakinesis	192	10			202
	metaphase 1	209	39			248
	anaphase 1			153	3	156
	anaphase 2			77	2	79

or not, the most of crossed plants were also considered as the successful hybrids. The reasons for this are that crossability was very high in the crosses in spite of the very high degree of self incompatibility in all strains of *M. officinalis*. The intraspecific F<sub>1</sub> plants made good growth as well as the parental strains.

The pollen fertility of the F<sub>1</sub> hybrids was higher than 90%. And the chromosomal behaviors at meiosis were remarkably regular in all cross combinations of F<sub>1</sub> hybrids, namely, the chromosome association such as 8<sub>II</sub> occurred constantly at diakinesis and metaphase 1 (Fig. 2 f); and normal disjunction was present in most of microspore nuclei at anaphase 1 and anaphase 2 (Table 3). As far as examined, it was considered that there was no chromosomal difference among these strains of *M. officinalis*. Henceforth, it is reasonably concluded that structural differences of chromosomes by a reciprocal translocation exist between *M. alba* and *M. officinalis*.

**d. *M. officinalis* P.I. 178985 × *M. hirsuta* F<sub>1</sub>**

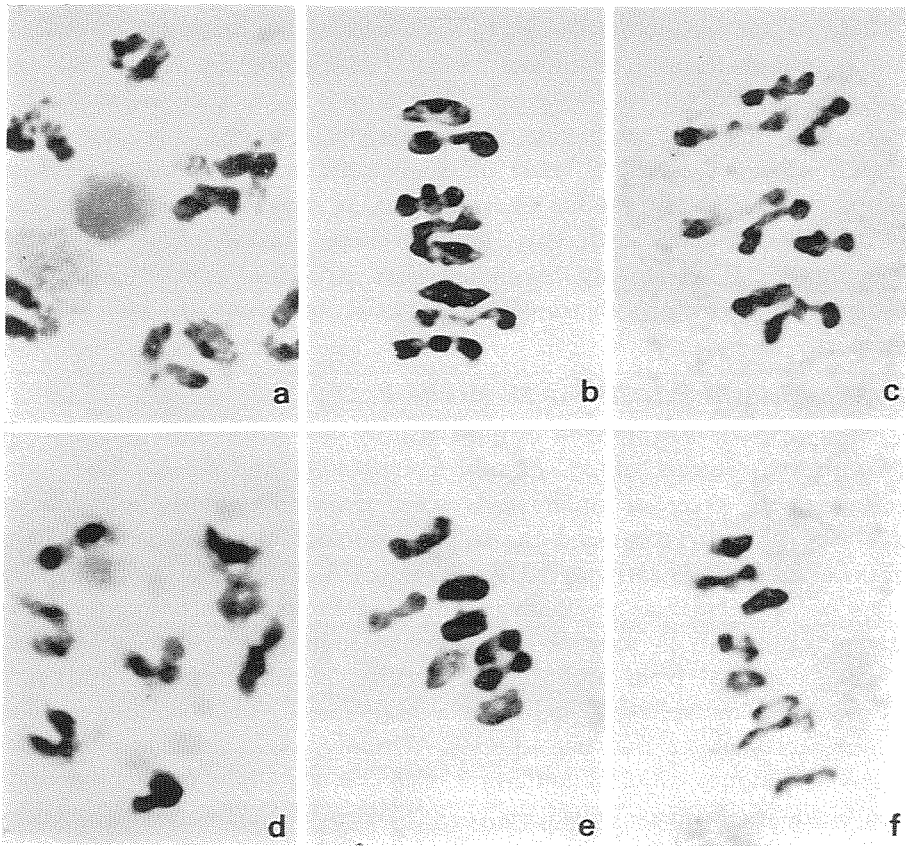
*M. officinalis* P.I. 178985 crossed readily not only with *M. alba* but also with two strains of *M. hirsuta* P.I. 314096 and 58-4. The hybrid seedlings from greenish small seeds were distinctly chlorophyll deficient, but as the growth progressed the leaves gradually increased green. In the reciprocal crosses, *M. hirsuta* × *M. officinalis* P.I. 178985, by using the same strains of *M. hirsuta* as pistillate parents, numerous cross pollinations were made in vain. All F<sub>1</sub> hybrids obtained had high pollen fertility (Table 1). As being expected from high pollen fertility, chromosome associations at diakinesis and metaphase 1 indicated a high degree of homology between the chromosomes of the two species. Usually, eight bivalents (8<sub>II</sub>) were present at diakinesis and metaphase 1 (Fig. 3 a, b), while in the remainder, seven bivalents plus two univalents (7<sub>II</sub>+2<sub>I</sub>) occurred. Abnormal disjunctions in later stages of meiosis were quite rare. Chromosome configurations at diakinesis and metaphase 1, and their distributions in later stages of meiosis are given in Table 4.

**e. *M. officinalis* P.I. 178985 × *M. polonica* F<sub>1</sub>**

*M. officinalis* P.I. 178985 was also able to cross readily with *M. polonica* 3446, when *M. officinalis* P.I. 178985 was used as the pistillate parent in the cross, and many small F<sub>1</sub> seeds were obtained. All seedlings germinated from these seeds did not show chlorophyll deficiency at all and were vigorous, being almost as strong as the parental species. Margin of their leaflets was sharply dentate and the plants produced lax racemes

showing characteristic of *M. polonica*. The reciprocal cross, *M. polonica* 3446  $\times$  *M. officinalis* P.I. 178985, was also attempted but no hybrid seedling was obtained, though many flowers were cross pollinated.

The  $F_1$  hybrids had high pollen fertility, having about 97.9% of



**Fig. 3.** Different stages in meiosis of the  $F_1$  hybrids examined.  $\times 2100$ .

- a. *M. officinalis* P.I. 178985  $\times$  *M. hirsuta* 58-4  $F_1$ . Diakinesis with 8 $_{II}$ .
- b. *M. officinalis* P.I. 178985  $\times$  *M. hirsuta* P.I. 314096  $F_1$ . Metaphase 1 with 8 $_{II}$ .
- c. *M. officinalis* P.I. 178985  $\times$  *M. polonica* 3446  $F_1$ . Metaphase 1 with 8 $_{II}$ .
- d. *M. polonica* C 30  $\times$  *M. suaveolens* Redfield Yellow  $F_1$ . Diakinesis with 8 $_{II}$ .
- e. The same  $F_1$  as d. Metaphase 1 with 8 $_{II}$ .
- f. *M. hirsuta* 58-4  $\times$  *M. suaveolens* Bdn 60-59  $F_1$ . Metaphase 1 with 8 $_{II}$ .

stainable pollen grains. As well as the hybrids between *M. officinalis* and *M. hirsuta*, the course of microsporogenesis in the hybrids, *M. officinalis* P.I. 178985 × *M. polonica* 3446, was regular (Fig. 3 c and Table 4).

f. *M. polonica* × *M. suaveolens* F<sub>1</sub>

TABLE 4. Chromosome configurations at diakinesis and metaphase 1 and their distribution in later stages of meiosis in the interspecific F<sub>1</sub> hybrids, showing meiotic chromosome behaviors were regular

cross	no. of cross combinations examined	stage	frequency of PMCs with				total
			8II	7II+2I	normal	abnormal	
<i>M. officinalis</i> × <i>M. hirsuta</i>	2	diakinesis	204	21			225
		metaphase 1	183	23			206
		anaphase 1			120	8	128
		anaphase 2			137	1	138
<i>M. officinalis</i> × <i>M. polonica</i>	1	diakinesis	66	4			70
		metaphase 1	83	19			101
		anaphase 1			56		56
		anaphase 2			41	1	42
<i>M. polonica</i> × <i>M. suaveolens</i>	1	diakinesis	70	1			72
		metaphase 1	84	90			103
		anaphase 1			53	2	55
		anaphase 2			19	1	20
<i>M. hirsuta</i> × <i>M. suaveolens</i>	1	diakinesis	93	10			103
		metaphase 1	97	11			108
		anaphase 1			64		64
		anaphase 2			92	9	101
<i>M. hirsuta</i> × <i>M. polonica</i>	1	diakinesis	41	2			43
		metaphase 1	97	12			109
		anaphase 1			54	5	59
		anaphase 2			42	3	45
<i>M. taurica</i> × <i>M. wolgica</i>	1	diakinesis	125	3			128
		metaphase 1	83	22			105
		anaphase 1			89		89
		anaphase 2			70	2	72
<i>M. dentata</i> × <i>M. taurica</i>	3	diakinesis	198	22			220
		metaphase 1	139	52			191
		anaphase 1			201	19	220
		anaphase 2			107	13	120

In the reciprocal crossings between *M. polonica* and *M. suaveolens*, two strains of *M. polonica* C 30 and 3446 and three strains of *M. suaveolens*, Redfield Yellow, 18-4, and Annu were used. Mature hybrid seeds were obtained at low frequency from only the crosses, *M. polonica* × *M. suaveolens*. Although the hybrid seedlings showed no chlorophyll deficiency at all, they were very weak and several of them died before flowering. Consequently, two F<sub>1</sub> plants from the cross of *M. polonica* C 30 × *M. suaveolens* Redfield Yellow were able to live to maturity. In comparison with the maternal parent, the hybrid plants produced relatively dense racemes.

The pollen of the hybrids were highly sterile (ca. about 20.4% of stainable pollen grains). In the F<sub>1</sub> hybrids, eight bivalents occurred constantly at diakinesis and metaphase I (Fig. 3 d, e) and abnormal disjunctions were present in a very low frequency at both anaphase I and anaphase 2 (Table 4). Therefore, chromosomal behaviors of meiosis in the hybrids were normal. The pollen sterility in the hybrids, *M. polonica* C 30 × *M. suaveolens* Redfield Yellow, was apparently caused not by chromosome aberrations but by genic factors of one of the parental strains, because of very high pollen sterility of *M. polonica* with the regularity of chromosome behaviors in the course of meiosis, as mentioned previously.

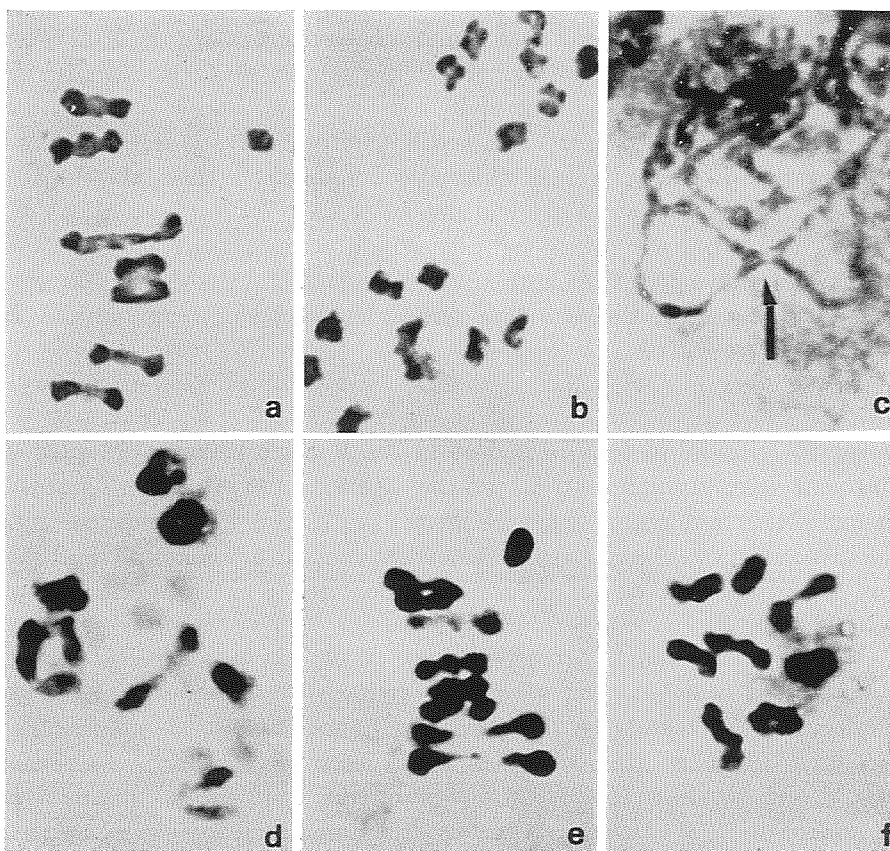
**g. *M. hirsuta* × *M. suaveolens* F<sub>1</sub>**

Three hybrid seedlings obtained at low frequency from the only cross, *M. hirsuta* 58-4 × *M. suaveolens* Bdn 60-59, though crossings were made reciprocally by using several strains of the two species. These seedlings showed heavy chlorophyll deficiency and two of them died soon. But the remaining one had leaflets with sectors of normal green tissue and fortunately a normal green branch arose on the plant. The F<sub>1</sub> hybrid became vigorous and came to flowering. Thus, the hybrid permitted the following observations of meiotic chromosome behaviors.

The percentage of stainable pollen grains of the hybrid was very high, being about 87.6%. At diakinesis and metaphase I, the chromosome associations such as 8<sub>II</sub> were regularly present (Fig. 3 f and Table 4). Normal disjunctions of the chromosomes occurred at anaphase I and anaphase 2 in almost spore nuclei examined. These observations indicate that the course of meiosis in the F<sub>1</sub> hybrid was normal.

**h. The F<sub>1</sub> hybrids among three species, *M. alba*,  
*M. hirsuta*, and *M. polonica***

Cytological relationship among three species, *M. alba*, *M. hirsuta*, and *M. polonica*, has been investigated by using several strains of each species.



**Fig. 4.** Different stages in meiosis of the F<sub>1</sub> hybrids examined.

- a. *M. alba* Arctic × *M. hirsuta* P.I. 314096 F<sub>1</sub>. Metaphase 1 showing 1III+6II+1I. ×2100.
- b. *M. alba* Bdn 647 × *M. hirsuta* P.I. 314096 F<sub>1</sub>. Anaphase 1 showing 7-9 disjunction. ×2100.
- c. *M. polonica* C 30 × *M. alba* Arctic F<sub>1</sub>. Pachytene showing a cross-shaped configuration. The breakage point is indicated by arrow. ×2450.
- d. The same F<sub>1</sub> as c. Diakinesis showing 6II plus a ring of 4 chromosomes configuration. ×2100.
- e. The same F<sub>1</sub> as c. Metaphase 1 showing 1III+6II+1I configuration. ×2100.
- f. *M. hirsuta* 58-4 × *M. polonica* 3446 F<sub>1</sub>. Metaphase 1 showing 8II configuration. ×2100.

Cross pollinations were made in order to ascertain cytological relationship among them from observations of meiotic behaviors in the hybrids within several strains used for this study. In reciprocal crossings between *M. alba* and *M. hirsuta*, a great number of F<sub>1</sub> hybrids were readily obtained using several strains of the species. The seedlings from the crossing, *M. alba* × *M. hirsuta*, showed much less chlorophyll deficiency than those of the reciprocal. Reciprocal cross pollinations between *M. alba* and *M. polonica* also gave many normal green hybrids which were almost more vigorous than the parental species, though the seedlings from the crosses in which *M. alba* was used for the pistillate parent showed slight chlorophyll deficiency in young leaves.

All of these interspecific F<sub>1</sub> hybrids had partial pollen sterility as shown in Table 1. From cytological observations of two cross combinations of F<sub>1</sub> hybrids, meiotic behaviors in the hybrids between *M. alba* and *M. hirsuta* were similar to those of the hybrids between *M. alba* and *M. polonica*. Aberrant chromosome behaviors were observed during the course of meiosis, namely, the quadrivalents were present at diakinesis and metaphase I, and cross-shaped configurations occurred at pachytene (Fig. 4 a, b, c, d and Table 2).

On the other hand, crossings were made reciprocally between *M. hirsuta* and *M. polonica*, but several F<sub>1</sub> hybrids were obtained from only the crosses, *M. hirsuta* × *M. polonica*. And these F<sub>1</sub> hybrids showed slight chlorophyll deficiency but reached to the flowering vigorously. As reported by KITA and NIIZEKI (1967), the pollen of the hybrids was highly fertile with about 96.4% of stainable pollen grains, and the chromosome behaviors of meiosis were normal (Fig. 4 f and Table 4).

The observations mentioned above indicate that there are chromosomal differences by one reciprocal translocation both between *M. alba* and *M. hirsuta* and between *M. alba* and *M. polonica*, explaining partial pollen sterility. Moreover, these chromosomal changes are of the same origin, as discussed later, since the course of meiosis in the hybrid, *M. hirsuta* × *M. polonica*, which had high pollen fertility, was regular.

#### i. *M. taurica* × *M. alba* F<sub>1</sub>

Generally, the F<sub>1</sub> hybrids between *M. taurica* and *M. alba* showed distinct chlorophyll deficiency and none survived to the flowering stage. In this experiment, unexpectedly, F<sub>1</sub> hybrids from crossings by using two strains of *M. alba*, Cumino and Denta, derived from the crosses of (*M. alba* × *M. dentata*) × *M. alba*, as pollen parents, showed somewhat more green color than those from the use of the other strains of *M. alba* and

continued to grow to flowering. The reciprocal crossings, *M. alba* × *M. taurica*, gave distinct chlorophyll deficient hybrids and they were not able to survive beyond the unifoliolate stage.

The F<sub>1</sub> hybrids which continued to live to the flowering stage had high pollen sterility (Table 1). The chromosomal irregularities occurred during the course of microsporogenesis in the hybrids. These first became clearly evident at diakinesis where most of configurations possessed six bivalents and a ring or chain of four chromosomes (6<sub>II</sub>+1<sub>IV</sub>). In a few cases, five types of configurations such as 6<sub>II</sub>+1<sub>III</sub>+1<sub>I</sub>, 5<sub>II</sub>+1<sub>III</sub>+3<sub>I</sub>, 8<sub>II</sub>, 7<sub>II</sub>+2<sub>I</sub> and 6<sub>II</sub>+4<sub>I</sub>, were present. Aside from the six types of configurations at diakinesis, five bivalents plus one quadrivalent and two univalents (5<sub>II</sub>+1<sub>IV</sub>+2<sub>I</sub>) occurred at metaphase 1 in a very low frequency (Fig. 5 b, c). Presumably the presence of the configurations such as 5<sub>II</sub>+1<sub>IV</sub>+2<sub>I</sub> and 5<sub>II</sub>+1<sub>III</sub>+3<sub>I</sub> resulted from a precocious segregation of one member of not only a chain of four chromosomes but also one bivalent. At anaphase 1, abnormalities such as 7-9 disjunction and lagging chromosomes occurred frequently (Table 5).

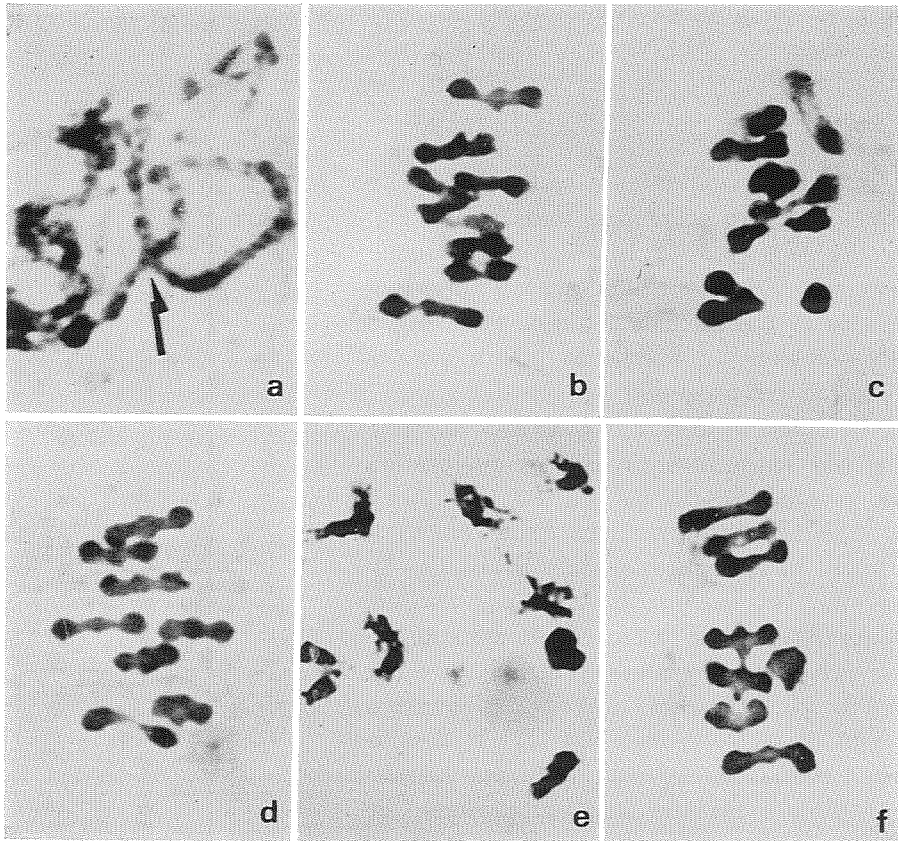
These observations showed the evidence that the hybrids were heterozygous for a reciprocal translocation. And expectedly, cross-shaped configurations were present at pachytene (Fig. 5 a).

**j. *M. taurica* × *M. wolgica* F<sub>1</sub>**

Several chlorophyll deficient hybrids were obtained in a low frequency from the crosses, *M. wolgica* × *M. taurica*, but the seedlings were very weak and not able to develop beyond the unifoliolate stage. In the cross, *M. taurica* 70 × *M. wolgica* Bdn 58-254,

TABLE 5. Chromosome configurations at diakinesis and metaphase 1 and their distribution in later stages of meiosis in the interspecific F<sub>1</sub> hybrids, *M. taurica* × *M. alba*

no. of cross combinations examined	stage	frequency of PMCs with						total	
		1 <sub>IV</sub> +6 <sub>II</sub>	1 <sub>IV</sub> +5 <sub>II</sub> +2 <sub>I</sub>	1 <sub>III</sub> +6 <sub>II</sub> +1 <sub>I</sub>	1 <sub>III</sub> +5 <sub>II</sub> +3 <sub>I</sub>	8 <sub>II</sub>	7 <sub>II</sub> +2 <sub>I</sub>		6 <sub>II</sub> +4 <sub>I</sub>
	diakinesis	191		18	4	42	14	2	273
	metaphase 1	91	10	169	3	24	38	8	343
3	anaphase 1							182	106
	anaphase 2							155	90



**Fig. 5.** Different stages in meiosis of the  $F_1$  hybrids examined.

- a. *M. taurica* 70 × *M. alba* Cumino  $F_1$ . Pachytene with a cross-shaped configuration. The breakage point is indicated by arrow. ×2800.
- b. The same as a. Metaphase 1 with  $1_{IV}+6_{II}$  which show alternate disjunction. ×2100.
- c. The same  $F_1$  as a. Metaphase 1 with  $1_{III}+6_{II}+1_I$ . ×2100.
- d. *M. taurica* 70 × *M. wolgica* 58-254  $F_1$ . Metaphase 1 with  $8_{II}$ . ×2100.
- e. *M. dentata* Bdn 58-190 × *M. taurica* 70  $F_1$ . Diakinesis with  $8_{II}$ . ×2100.
- f. The same  $F_1$  as e. Metaphase 1 with  $8_{II}$ . ×2100.

however, a great number of hybrid seedlings were more vigorous than those from the reciprocal. When the plants reached the flowering stage, the color of the older leaves was approximately normal green.

The pollen of  $F_1$  hybrids were partially sterile, having about 25.5% of aborted pollen grains. In spite of low pollen fertility, chromosomal behaviors of meiosis in the  $F_1$  hybrids were regular. The configurations

such as 8<sub>II</sub> at diakinesis and metaphase 1, and normal disjunction at anaphase 1 and anaphase 2 were present in almost spore nuclei (Fig. 5 d and Table 4).

**k. *M. dentata* × *M. taurica* F<sub>1</sub>**

The crossings between *M. dentata* and *M. taurica*, by using three strains of *M. taurica*, 70, 134-1, and P.I. 314094, as pistillate parents, and two strains of *M. dentata*, Bdn 58-190 and P 783-2, as pollen parents, gave only much aborted seeds except for plump seeds resulting from accidental self-pollination of the maternal parent. From the reciprocal crosses, *M. dentata* × *M. taurica*, by using the same strains of two species, several hybrid seedlings were obtained and showed distinct chlorophyll deficiency. But the plants gradually became vigorous and produced pale yellow flowers showing that they were F<sub>1</sub> hybrids since the maternal parent was yellow-flowered.

Although the hybrids had a high percentage of aborted pollen grains (Table 1), the configuration with eight bivalents (Fig. 5 e, f) occurred regularly at diakinesis and metaphase 1 in all cross combinations examined, as well as the hybrid of *M. taurica* × *M. wolgica*. Occasionally, seven bivalents plus two univalents (7<sub>II</sub>+2<sub>I</sub>) were present. Abnormalities such as lagging chromosomes at anaphase 1 and anaphase 2 seldom occurred (Table 4). Hence, it is reasonably pointed out that no structural differences in chromosomes exist between *M. dentata* and *M. taurica*.

### Discussion

In regard to cytological studies of the interspecific hybrids in *Eumelilotus* of the genus *Melilotus*, several papers have been reported by the other workers. WEBSTER (1950, 1955) examined meiosis of three interspecific F<sub>1</sub> hybrids, *M. polonica* × *M. svaueolens*, *M. alba* × *M. polonica* and *M. officinalis* × *M. alba*, and pointed out that meiosis in these F<sub>1</sub> hybrids appeared to be as normal as those of the parental species. In the hybrid (*M. alba* × *M. dentata*) × *M. alba*, BRINGHURST (1951) observed cross-shaped configurations at pachytene indicating reciprocal translocation. SHASTRY *et al.* (1960) reexamined the hybrid of *M. officinalis* × *M. alba* reared by embryo culture and arrived at the same result as WEBSTER's. On the other hand, it was revealed that two F<sub>1</sub> hybrids, *M. polonica* × *M. alba* (JARANOWSKI 1961) and *M. alba* × *M. hirsuta* (KITA 1962), were heterozygous for a reciprocal translocation. Accordingly, JARANOWSKI refuted WEBSTER's conclusion (1950) that the chromosome behaviors of the hybrid,

*M. alba* × *M. polonica*, were regular.

In this report, it became clear that the five  $F_1$  hybrids between *M. alba* and each of the following species, *M. hirsuta*, *M. officinalis*, *M. polonica*, *M. suaveolens* and *M. taurica*, were heterozygous for a reciprocal translocation. Moreover, as far as examined, there is no evidence that intraspecific differences in chromosomal changes exist in any species. This might be also clearly pointed out from the observations that the intraspecific  $F_1$  hybrids in several species had high pollen fertility. It is important to know whether chromosomal changes by one reciprocal translocation between *M. alba* and the other four species are of the same origin or not. The courses of meiosis in the five hybrids, *M. hirsuta* × *M. polonica*, *M. hirsuta* × *M. suaveolens*, *M. officinalis* × *M. hirsuta*, *M. officinalis* × *M. polonica* and *M. polonica* × *M. suaveolens*, were normal. Then, it is reasonably estimated that the course of meiosis in the  $F_1$  hybrid between *M. officinalis* and *M. suaveolens* is normal. These results suggest that the interchange of chromosomes among two pairs of nonhomologous chromosomes had taken place in either *M. alba* or *M. hirsuta*, *M. officinalis*, *M. polonica* and *M. suaveolens* in the process of speciation. In the former case, the reciprocal translocation found in the four interspecific  $F_1$  hybrids are obviously the same origin. In the latter case, however, farther evidences are required to reveal whether the breakage points of chromosomes in the four species are same or not. Now, if the breakage points differ from each other, these four species differ with respect to, what is called, interstitial translocation which do not involve chromosome ends. Because of the way in which chromosomes pair at meiosis, such differences would not be expected to give rise to multivalent rings or chains, but only to the frequent appearance of unpaired chromosomes and/or normal bivalents (STEBBINS 1970). It so, the hybrids within these four species would be partially sterile, since half of the resulting gametes would be deficient for one and duplicated for the other of the interchanged segments. The parental type (fertile) and translocation heterozygote (semisterile) would be expected to appear in the  $F_2$  generation in a 1:1 ratio. The pollen of all most possible  $F_1$  hybrids within these four species was highly fertile, excepted for the hybrid of *M. polonica* C 30 × *M. suaveolens* Redfield Yellow showed high pollen sterility because *M. polonica* C 30 was extremely highly pollen sterile. This indicates that the breakage points do not differ from each other and these interchanges of chromosomes observed in the interspecific  $F_1$  hybrids between *M. alba* and each of four species, *M. hirsuta*, *M. officinalis*, *M. polonica* and *M. suaveolens*, are of

the same origin.

On the other hand, the courses of meiosis in two hybrids, *M. taurica* × *M. wolgica* and *M. dentata* × *M. taurica*, were normal. It is apparent that among these three species there is no gross exchanges of chromosomes such as a reciprocal translocation found in the hybrid between *M. taurica* and *M. alba*. Therefore, the hybrids between *M. alba* and each of the two species, *M. dentata* and *M. wolgica*, which are not obtained yet, seem to be also heterozygous for a reciprocal translocation, agreeing with the result reported by BRINGHURST (1951). There are some difficulties to explain exactly whether three species, *M. dentata*, *M. taurica* and *M. wolgica*, differ from each other with respect to interstitial translocation, since the F<sub>1</sub> hybrids, *M. dentata* × *M. taurica* and *M. taurica* × *M. wolgica*, had high and partial sterility, respectively. However, it is likely to be concluded that structural differences by one reciprocal translocation between *M. alba* and each of the three species, *M. dentata*, *M. taurica* and *M. wolgica* are of the same origin, because the most of F<sub>2</sub> plants of *M. taurica* × *M. wolgica* were highly fertile and all of F<sub>2</sub> plants of *M. dentata* × *M. taurica*, though the number of F<sub>2</sub> plants were not sufficient due to poor seed setting and chlorophyll deficiency, had high pollen sterility. High pollen sterility of *M. dentata* × *M. taurica* F<sub>1</sub> may be caused by genic disharmony rather than chromosomal aberrances.

Cytological relationships among species of *Eumelilotus* obtained in this study are shown in Fig. 6. No hybrid seedling between *M. altissima* and

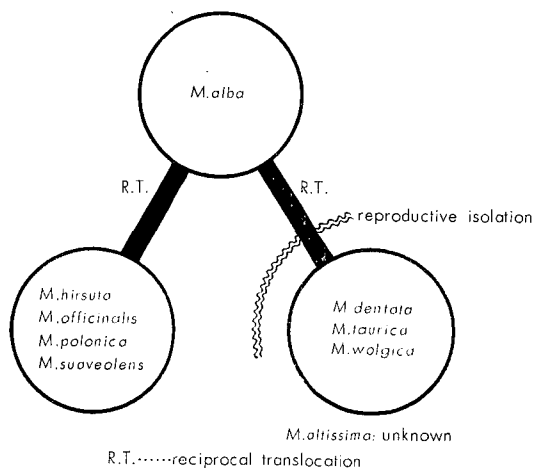


Fig. 6. Cytological relationships among species of the subgenus *Eumelilotus*.

any other species of *Eumelilotus* is able to survive to flowering stage due to heavy chlorophyll deficiency. Therefore, cytological relationships between *M. altissima* and the other species are not revealed at all. Moreover, it is not known whether chromosomal differences between *M. alba* and each of four species, *M. hirsuta*, *M. officinalis*, *M. polonica* and *M. suaveolens*, differ from those between *M. alba* and each of three species, *M. dentata*, *M. taurica*, and *M. wolgica*.

Interspecific differences in chromosome structure are common in many plant genera such as *Clarkia*, *Crepis*, *Elymus*, *Galeopsis*, *Gossypium*, and *Nicotiana*. KYHOS (1965) reported in *Chaenactis* (Compositae) that the two species having  $n=5$ , *C. fremontii* and *C. stevioides* have evolved from an ancestor similar to *C. glabriuscula* having  $n=6$  by a process which included a translocation followed by the loss of a centromere, the translocation being different from each species, as in similar ones found in *Crepis* and so on. In *Clarkia* (Onagraceae), there are several pairs of species which differ from each other with respect to several translocations but the morphological differences among the species are trivial or virtually undetectable (LEWIS 1966). He postulated that in *Clarkia* and perhaps other genera saltational speciation occurs through the simultaneous occurrence of numerous translocations. Also, differentiated sex chromosomes are associated with successive translocations in *Rumex* (B. W. SMITH 1964).

Based on the occurrence of the chromosome aberrations throughout the interspecific hybrids examined by the authors, it is considered that the structural differentiation of chromosomes such as reciprocal translocation plays a significant role during the process of speciation of *Eumelilotus*. It is obviously important to make clear the effects of chromosomal differences such as translocation in speciation. The interspecific hybrids, in the most of cases, show different  $F_1$  weakness, hybrid sterility, and hybrid breakdown, in the advanced generations, which play a role of isolation of species. Farther investigations are interested in making a whole picture of interspecific relationships of *Eumelilotus* from cytological and genic view points.

### Summary

Cross pollinations were made among eight species belonging to the subgenus *Eumelilotus* of the genus *Melilotus*. The interspecific  $F_1$  hybrids were examined in order to make clear the cytological relationships among these species systematically.

The results obtained are summarized as follows :

1. *M. officinalis* P.I. 178985 was crossed readily not only with *M. alba* but also with *M. hirsuta* and *M. polonica* and gave vigorous F<sub>1</sub> hybrids. Crossability of the strain of *M. officinalis* is interested in a convenient bridge for the interchange of genetic material among these four species.

2. In the F<sub>1</sub> hybrids, *M. alba* × *M. suaveolens*, *M. officinalis* × *M. alba*, *M. alba* × *M. hirsuta* and the reciprocal, *M. alba* × *M. polonica* and the reciprocal and *M. taurica* × *M. alba*, a cross-shaped configuration at pachytene and a ring or chain of four chromosomes at diakinesis and metaphase 1 were observed, which indicate that the F<sub>1</sub> hybrids are heterozygous for a reciprocal translocation.

3. In the F<sub>1</sub> hybrids, *M. officinalis* × *M. hirsuta*, *M. officinalis* × *M. polonica*, *M. polonica* × *M. suaveolens*, *M. hirsuta* × *M. suaveolens*, *M. hirsuta* × *M. polonica*, *M. dentata* × *M. taurica* and *M. taurica* × *M. wolgica*, the courses of meiosis were regular.

4. As far as examined, there was no evidence that intraspecific differences in chromosomes exist in any species.

5. Consequently it is considered that the F<sub>1</sub> hybrids between *M. alba* and each of two species, *M. dentata* and *M. wolgica*, though the hybrids were not examined, are heterozygous for a reciprocal translocation.

6. Cytological relationships obtained in this studies are as summarized in the figure 6.

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#### Literature cited

- BRINGHUST, R. S. 1951. Genetic analysis of chlorophyll deficiency in *Melilotus alba* × *M. dentata* hybrids with some observations on meiotic irregularities. Summaries of Doctoral Dissertations, Univ. Wis. 11: 96-67.
- 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. J. Bot. 48: 28-35.
- KITA, F. 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.
- . 1965. Studies on the genus *Melilotus* (Sweetclover) with special reference to interrelationships among species from a cytological point of view. J. Fac. Agri. Hokkaido Univ. 54: 25-122.
- , and M. NIIZEKI 1967. Studies of interspecific hybrids in the genus *Melilotus* from the plant breeding standpoint. VI. Cytological studies of the interspecific hybrids, *Melilotus polonica* × *M. alba* and *M. polonica* × *M. hirsuta*. Ann. Rep. Farm., Hokkaido Univ. 15: 38-43.
- KYHOS, D. W. 1965. The independent aneuploid origin of two species of *Chaenactis* (Compositae) from a common ancestor. Evolution 19: 26-43.
- LANG, R. C. and H. J. GORZ 1960. Factors affecting embryo development in crosses of *Melilotus officinalis* × *M. alba*. Agrono. J. 52: 71-74.
- LEWIS, H. 1966. Speciation in flowering plants. Science 152: 167-172.
- SHASTRY, S. V. S., W. K. SMITH and D. C. COOPER 1960. Chromosome differentiation in several species of *Melilotus*. Amer. J. Bot. 47: 613-621.
- SCHULZ, O. E. 1901. Monographie der Gattung *Melilotus*. Bot. Jahrb. 29: 660-735.
- SMITH, B. W. 1964. The evolving karyotype of *Rumex hastatulus*. Evolution 18: 93-104.
- SMITH, W. K. 1954. Viability of interspecific hybrids in *Melilotus*. Genetics 39: 266-279.
- STEBBINS, G. L. 1970. Chromosomal evolution in higher plants. Edward Arnold Ltd., London.
- STEVENSON, G. A. 1969. An agronomic and taxonomic review of the genus *Melilotus* Mill. Can. J. Plant Sci. 49: 1-20.
- WEBSTER, G. T. 1950. Fertility relationships and meiosis of interspecific hybrid in *Melilotus*. Agron. J. 42: 315-322.
- . 1955. Interspecific hybridization of *Melilotus alba* × *M. officinalis* using embryo culture. Agron. J. 47: 138-142.