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Cytogenetic Studies in *Trifolium* Spp. Related to Berseem

II. Relationships within the *echinata* Group*

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Summary. In order to clarify the interspecific relationships between *T. carmeli* Boiss., *T. echinatum* M.B. and *T. latinum* Seb., numerous F_1, F_2 and BC_1 hybrids of these species were raised, and their growth, pollen fertility, cytology and seedset were determined.

T. carmeli was found to differ from both *T. echinatum* and *T. latinum* by at least three translocations, and their hybrids were semisterile as to pollen and seedset. *T. latinum* was more or less interfertile with *T. echinatum* and hybrids had normal meiosis, except for *T. echinatum* NYT 1401, whose hybrids with *T. latinum* were semisterile and evinced major chromosomal changes. Some implications of sterility and chromosomal heterogeneity are discussed. Back-crosses of *T. echinatum* × *T. latinum* F_1s to either parent resulted in partial breakdown of the self-incompatibility system found in both parental species and their F_1 hybrids.

Introduction

There has been some ambiguity in the taxonomy and nomenclature of East-Mediterranean annual clovers related to berseem. In a previous paper, the results of interspecific crossings among twelve related species were presented, and they were divided accordingly to five crossability groups. Hybrid seedset, or crossability, within groups was generally high, sometimes even equalling that of intraspecific cross combinations. However, crossability was low or very low in inter-group combinations (Putiyevsky and Katznelson, 1973).

The second crossability group comprises the following three species: 1. *T. echinatum* M.B., a native of the E. Mediterranean Basin, from Italy eastwards to Iran, and from S. Russia and the Caucasus in the north, to Israel in the south. It varies considerably in morphological traits (Katznelson, 1971b); 2. *T. latinum* Seb., native to Italy, S. Greece and Turkey; and 3. *T. carmeli* Boiss., indigenous to N. Israel and neighbouring areas. It is morphologically similar to *T. echinatum* and Zohary (1972) named it *T. echinatum* var. *carmeli*.

Biometric studies, however, suggest discontinuities between *T. echinatum* and *T. carmeli* (Katznelson, 1971b). They are backed by the crossability pattern, which indicates that *T. carmeli* belongs to two crossability groups: it sets hybrid seed easily not only with *T. echinatum* and *T. latinum*, but also with *T. scutatum* Boiss. and *T. plebium* Boiss. (Putiyevsky and Katznelson, 1973).

The present paper summarizes the results of extensive studies of F_1 and F_2 interspecific hybrids between the three species of the second crossability group — that may be termed the "echinata group" and clarifies the interrelationship between them.

Materials and Methods

The origin of lines used as parent plants as well as the two methods of obtaining hybrid seed, *i.e.* manually and by the natural hybridization programme (NHP) have been presented elsewhere (Putiyevsky and Katznelson, 1973; Katznelson, 1971a). Altogether, 394 intraspecific and 657 interspecific hybrid seeds of this group were germinated, each seed separately in a petri dish. The seedlings were planted in soil in 3 kg-pots and kept in a glasshouse.

The methods employed in the cytological and pollen fertility studies were described earlier (Putiyevsky and Katznelson, 1970). In each F_1 plant, several heads were bagged and triggered for self-pollination. Other heads were back-crossed, usually to the two parental lines (of the same accession number), and the number of flowers treated and the seeds produced was counted. All other heads were collected, threshed, the seed weight was determined, and calculated as % of total plant dry weight. Some F_2 and BC_1 seeds were germinated and their pollen fertility, cytology and seedset upon selfing and open pollination were determined, as in their parents.

Results

a. Germination and Development

As there were hardly any reciprocal differences, the data are summarized according to species combinations.

The number of hybrid seeds that germinated, the number of albino seedlings and the number of plants

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that reached flowering stage are listed in Table 1. The % of germination in interspecific (BS) hybrid seeds was higher than in intraspecific (WS) hybrids, especially in the crosses of *T. echinatum.*

Altogether, there were six albino seedlings, two WS and four BS, out of 688 WS and BS seedlings. This value is small compared to the number of albinos obtained in an earlier study involving BS crosses of *T. carmeli* (Katznelson, 1971a). However, many of the plants died from other causes, but here again, the mortality rate in BS was lower: 28—40% up to flowering state, compared to more that 50% within *T. echinatum.*

This high mortality rate may be due to the small seed and seedling size of *T. echinatum*, or to lack of resistance to certain pathogens that attack young seedlings. The latter cause seems likely, as death rate was much higher in some specific cross combinations.

b. Pollen Fertility of F_1 Hybrids

Altogether, pollen fertility was examined in 142 interpopulation WS and 297 BS F_1 hybrids within the *echinata* group, and the results are presented in Table 2. Most of the intraspecific hybrids produced a high percentage of fertile pollen. Interspecific F_1 hybrid plants, however, manifested a wide range of pollen fertility. The majority of *T. carmeli* × *T. echinatum* and *T. carmeli* × *T. latinum* hybrids were in the 40—60% range, while the hybrids of *T. echinatum* × *T. latinum* were mostly fertile, except for a few semi-sterile plants. All the latter had *T. echinatum* NYT 1401, from N.E. Turkey, as a parent, while the other four *T. echinatum* lines from Thrace, Yugoslavia and S.W. Turkey produced fertile F_1 hybrids with the two *T. latinum* populations, both originating in N.E. Turkey.

At least some of the fertile, supposedly *T. carmeli* × × *T. echinatum* F_1 hybrids here were obtained by

Table 1. *Germination of F_1 hybrid seeds and seedling development*

Parental species combinations		No. of seeds exam.	No. of seeds germ.	Albino seedlings	No. of plants dead before flowering	Plants flowered		
						No.	% of all seeds	% of germinated seeds
T. ech.*	T. ech	130	73		39	34	25.2	45.9
T. lat	T. lat	5	5		2	3	60.0	60.0
T. car	T. car	259	149	2	42	105	40.5	70.5
Total intraspecific combinations		394	227	2	83	142	36.0	62.5
T. car	T. ech	286	170	3	66	101	35.3	59.4
T. car	T. lat	235	180		50	130	55.3	72.2
T. ech	T. lat	136	111	1	44	66	48.5	59.4
Total interspecific combinations		657	461	4	160	297	45.4	64.5

* Abbreviations: T. ech — *T. echinatum*; T. lat — *T. latinum*; T. car — *T. carmeli*

Table 2. *Frequency distribution of pollen fertility of intraspecific and interspecific F_1 hybrids*

Parental species combinations		No. of plants in each category of pollen fertility percent										Total plants exam.	Average pollen fertility %
		1—10	10—20	20—30	30—40	40—50	50—60	60—70	70—80	80—90	90—100		
T. ech	T. ech								1	23	10	34	87.8
T. lat	T. lat										3	3	91.0
T. car	T. car						1	4	9	26	65	105	89.1
Total intraspecific combinations							1	4	10	49	78	142	88.2
T. car	T. ech		1		9	36	29	8	6	4	8	101	55.0
T. car	T. lat			4	15	56	44	7	1	2	1	130	48.4
T. ech	T. lat			2	3		1		2	15	43	66	86.6
Total interspecific combinations			1	6	27	92	74	15	9	21	52	297	59.3

Table 3. *Chromosome configurations in meiosis of F_1 hybrids ($2n = 16$)*

Plant	Species		NYT		No. cells with the chromosome configuration:														No. cells exam.	Pollen fertility %
No.	♀	♂	♀	♂	2 I 7 II	4 I 6 II	8 II	1 I 6 II 1 III	2 I 4 II 2 III	3 I 5 II 1 III	5 II 2 III	6 II 1 IV	2 I 5 II 1 IV	4 I 4 II 1 IV	1 I 5 II 1 V	4 II 1 III	5 II 1 VI 1 V	2 I 4 II 1 VI		
1064/6	T. car	T. ech	251	220	1		10	8			3	2			1		5		30	36
396/5	T. car	T. ech	210	1401			3	8			2	2							15	50
1063/2	T. car	T. ech	210	220	3		3	7			2	6	2		4	1	2		30	48
971/19	T. ech	T. car	218	T. car*	2		9	9				2							22	44
974/7	T. ech	T. car	1401	T. car			13	6				1							20	58
1164/4	T. ech	T. car	1401	251	2		1	9		1	5	2			1				21	33
976/1	T. ech	T. car	1435	T. car	2		2	9			4	5			1		1		24	44
684/2	T. ech	T. car	1435	210	4		11	10			3	1					1		30	47
686/3	T. ech	T. car	1435	266	4		2	8			3	3			1				21	59
735/3	T. ech	T. car	1441	210	7		1	13		1	1	4			3				30	58
734/5	T. ech	T. car	1441	266	2		1	7			1	3					2		16	40
977/23	T. ech	T. car	1435	T. car	3	1	2	9			1	1			1				18	51
1163/3	T. ech	T. car	1425	T. car	4		3	11			2				3		1		24	38
687/1	T. ech	T. car	1435	266	2		5	7	1			2							17	38
115/2	T. car	T. lat	210	1451	8		5	7											20	37
372/1	T. car	T. lat	251	1451	19	1		10											30	36
543/1	T. car	T. lat	266	1451	1	1	9	11	1										23	51
375/1	T. car	T. lat	251	1497	6	1	5	2			1								15	82
1165/4	T. car	T. lat	210	1497	1		2	6			2	2	1	1					15	42
761/1	T. lat	T. car	1451	210	3	1	4	15	1		3	2					1		30	42
768/4	T. lat	T. car	1451	266	1		4	10											15	43
876/2	T. lat	T. car	1497	210	3		7	10		1									21	42
877/4	T. lat	T. car	1497	210			5	12				1		1			1		20	53
889/3	T. lat	T. car	1497	266	6	1	10	12			1								30	44
978/8	T. lat	T. car	1451	T. car	4	2	24												30	45
979/11	T. lat	T. car	1497	T. car	5	1	7	5											18	41
773/1	T. lat	T. car	1451	251	2		13	12			1	2							30	54
653/2	T. ech	T. lat	1401	1451	8	2	5	3											18	50
653/3	T. ech	T. lat	1401	1451	6		6												12	24
662/1	T. ech	T. lat	1401	1497	3		6	9			2	4			2			1	27	36
693/6	T. ech	T. lat	1435	1451	1		17												18	88
698/5	T. ech	T. lat	1435	1497	2		16												18	96
721/5	T. ech	T. lat	1441	1451			30												30	97
743/1	T. ech	T. lat	1441	1497	1		29												30	87
807/4	T. lat	T. ech	1451	218			30												30	86
809/1	T. lat	T. ech	1451	220	1		29												30	93
802/1	T. lat	T. ech	1451	1435	1		17												18	96
804/1	T. lat	T. ech	1451	1441	3		27												30	94
899/2	T. lat	T. ech	1497	218	1		29												30	80
896/2	T. lat	T. ech	1497	220			30												30	92
895/2	T. lat	T. ech	1497	1435	2		28												30	94
892/1	T. lat	T. ech	1497	1441	3		27												30	87
Total 42 plants	6 combinations				127	11	487	245	3	3	37	45	3	2	17	1	14	1	996	58

* Where NYT of pollen donor is not specified, byhrid seeds were produced by NHP.

NHP, and may in fact, be intraspecific hybrids or selfers.

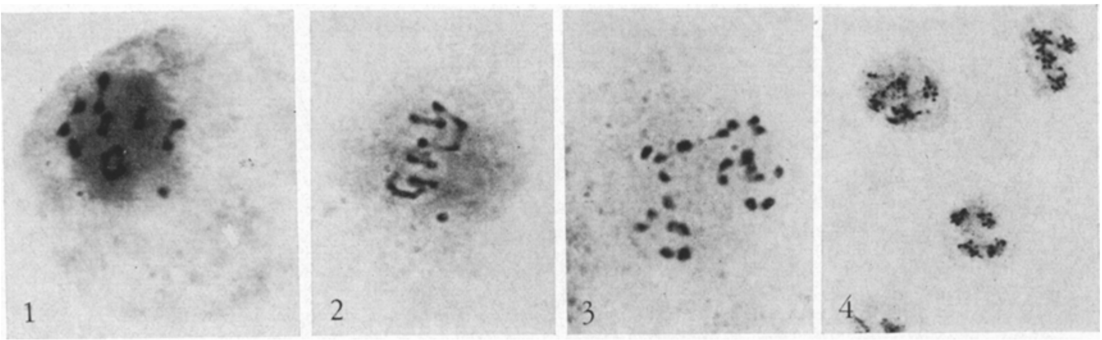
c. *Cytology of F_1 Hybrids*

While parental plants had normal meiosis, with eight bivalents (Putiyevsky and Katznelson, 1970) the interspecific hybrids with the *echinata* group show very clearly that the species concerned differ cytologically, and that a series of major chromosomal rearrangements occurred in this group. This is evident from Tables 3 and 4, where the results of the cytological observations are presented.

Table 3 lists F_1 hybrids with the regular 16 chromosome complement, while Table 4 analyzes two sib plants obtained by NHP (Katznelson, 1971a), one a trisomic, the other a triploid hybrid. Meiotic plates of some F_1 hybrids are presented in Plates 1—10.

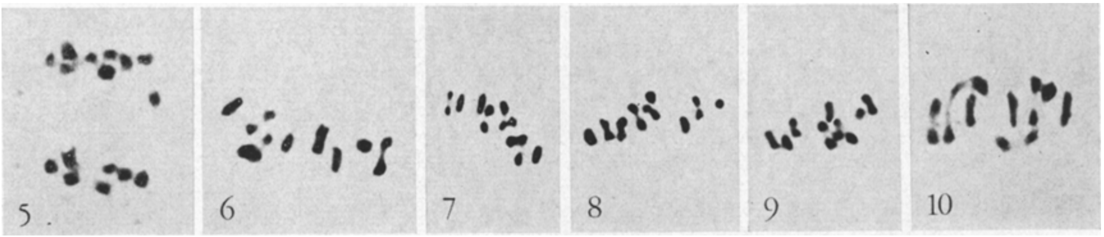
Table 4. *Chromosome configurations in meiosis of a trisomic and a triploid F_1 hybrid T. echinatum × T. carmeli*

	Valents	Chromosome configurations													Total valents	Average No. of chiasma
a. NHP 45-1	I		1	1	1	2	4	1	3	1	3	2	1	3	52	
2n = 17	II		3	4	3	4	3	5	4	6	5	6	8	7	151	
	III				2	1	1	2	2			1			20	
	IV		1	2	1	1	1			1	1				23	
	VI														1	12, 86
No. of cells analyzed			1	2	1	7	1	2	1	8	1	4	1	1		
b. NHP 45-2																
	I	3	1	1	2	4	4	5	1	5	2	4	6	8	118	
2n = 24	II	3	7	8	8	7	8	7	10	8	11	10	9	8	265	
	III	5	3	1	2	2			1	1					20	
	IV	1		1			1	1							3	14, 70
No. of cells analyzed		1	1	1	1	3	1	1	2	1	4	7	6	1		



Meiotic plates of *Trifolium* F_1 hybrids (×1800)

1. NHP 45—1 NYT 1469× *T. carmeli* M I: 2n = IV + 6 II + I;
2. NHP 45—1 NYT 1469× *T. carmeli* M I: 2n = VI + IV + 3 II + I;
3. NHP 45—2 NYT 1469× *T. carmeli* A I: 2n = 24;
4. NHP 45—2 NYT 1469× *T. carmeli* A II;



5—10. F_1 735/3 *T. echinatum* NYT 1441 × *T. carmeli* NYT 210 2n = 16

5. A I, unequal segregation 7:9;
6. M I: 1 III, 6 II, I;
7. M I: 2 III, 5 II;
8. M I: V, 5 II, I;
9. M I: V, 5 II, I;
10. M II: VI, 5 II.

At least three translocations can be seen in combinations of *T. carmeli* × *T. echinatum*, and *T. carmeli* × × *T. latinum*. The existence of trivalents (Plates 5—7), quadrivalents (Plate 1), pentavalents and hexavalents (Plates 8—10) suggests that two translocations have a common pair of chromosomes. The existence of a third translocation is suggested by the high frequency of multivalent formations in the 2n = 16 plants, and is proven conclusively by the data on trisomic and trivalent plants, in Table 4. The trisomic hybrid NHP 45/1 had multivalent forms in twenty-eight out of thirty examined cells, and of these, fifteen cells had two or three multivalents each. Altogether, there were forty-four multivalents in the thirty examined cells. Univalents were also common, besides the expected solitary. In one cell we discerned a hexavalent and a quadrivalent (Plate 2), in another, two trivalents and a quadrivalent. This means that the parental plants differ by at least three translocations. The triploid NHP 45/2 had a much lower multivalent formation than its sib 45/1. Only twelve (out of thirty) cells had multivalents, and these were mostly trivalents, (up to five per cell), in contrast to a majority of quadrivalents in 45/1. However, triploidity itself proves that the structural difference between the parents is of at least three translocations, for there is no other explanation for the existence of four cells with eleven bivalents, or of two cells with ten bivalents and one trivalent. In fact, it is likely that the parents *T. echinatum* NYT 1469 and *T. carmeli* differ in more than three translocations, since in each of four cells only one univalent was found.

Gametic sterility in NHP 45/2 tends to be greater than in NHP 45/1, due to a much stronger, predictable, numerical imbalance. In spite of the large number of univalents, segregation was mostly "normal", that is, 12 chromosomes to each pole (Plates 3; 4). Nevertheless, the 15% fertile pollen in this plant is higher than could be expected in such a state of chromosomal and genetical chaos.

In contrast to the hybrids of *T. carmeli* with *T. echinatum* and *T. latinum*, those of *T. latinum* × *T. echinatum* were mostly normal cytologically. Thirteen out of fifteen F_1 hybrids of this combination (reciprocals included) had mainly bivalents, with occasional univalents. The two hybrids with multivalent formation had NYT 1401 as a parent.

It seems that there are some differencess in the frequencies of multivalent formation, according to cross combinations. The cells listed in Table 3 were grouped into three categories in Table 5, according to their chromosomal configurations: those with two translocations (3 III, 2 IV, V, VI), one translocation (III, IV) and no translocation (I, II).

The uniqueness of NYT 1401 among the *T. echinatum* lines is apparent, not only in the *T. echinatum* × × *T. latinum* hybrids, but in crossability, pollen fertility, and seedset among all its F_1 hybrids. The difference in the frequency of two-translocation cells between *T. carmeli* × *T. latinum* (4%) and *T. carmeli* × *T. echinatum* (16%) is apparent, and it does indicate some differences between *T. latinum* and *T. echinatum*.

d. Seedset of F_1 Hybrids

Four categories of seedset were distinguished: i. upon selfing; ii. and iii. backcrossing with pollen of ♀ and ♂ parental lines; and iv. seedset in other, unbagged heads, with very variable source of pollen, including many parents, F_1, and F_2 interspecific hybrids, not only of the *echinata* group, but of the other nine species as well (Putiyevsky and Katznelson, 1970).

As can be seen in Table 6, very few seeds were obtained from the bagged and selfed heads and the self-incompatibility of the parental species was retained. Except for the F_1 within *T. echinatum*, and *T. latinum*, where 1% of the selfed flowers set seed, the other combination had less than 0.1%.

In backcrosses to parental species, seedset percentages ranged from 11 to 57%, with distinctly higher values in intraspecific combinations and in *T. echinatum* × *T. latinum*, conforming to the data on pollen fertility and cytology, presented earlier. This may suggest that seedset is correlated with egg fertility in F_1 hybrids. While egg fertility has not been studied, it is assumed to be equal to the corresponding pollen fertility. However, whereas 40—50% pollen fertility may be enough to ensure the fertilization of a fertile egg and normal seed formation, a similar

Table 5. *The number of cells indicating none, one and two translocations in F_1 and F_2 interspecific hybrids*

Combination	Progeny	Total cells examined	0 translocation		1 translocation		2 translocations	
			No.	%	No.	%	No.	%
T. car × T. ech	F_1	340	125	37	159	47	56	16
	F_2	122	67	55	40	33	15	12
T. car × T. lat	F_1	297	162	55	123	41	12	4
	F_2	63	44	70	16	25	3	5
T. ech (exc. 1401) × T. lat	F_1	324	324	100	0	0	0	0
	F_2	40	39	97.5	1	2.5	0	0
NYT 1401 (T. ech) × T. lat	F_1	57	36	63	16	28	5	9

Table 6. *Seedset upon selfing, backcrosses and open pollination of F_1 hybrids*

Combination	No. of plants examined	Seedset upon:										
		Selfing			Backcrosses to the ♀ species			Backcrosses to the ♂ species			Open pollination	
		No. of Flowers	No. of seeds	%	No. of flowers	No. of seeds	%	No. of flowers	No. of seeds	%	Seed weight g.	$\frac{\text{Seed weight}}{\text{Plant weight}}$ %
T. ech × T. ech	34	6565	65	1.0	—	—	—	—	—	—	2.79	0.17
T. car × T. car	28	6945	1	0.01	281	93	33.1	127	61	48.1	9.35	1.75
T. lat × T. lat	3	700	7	1.0	—	—	—	—	—	—	0.05	0.07
Total intraspecific combinations	65	14210	73	0.5	281	93	33.1	127	61	48.1	12.19	0.57
T. car × T. ech	95	24345	20	0.1	280	30	10.7	224	36	16.1	17.33	0.53
T. car × T. lat	130	31037	29	0.1	553	89	16.1	555	93	16.7	18.88	0.82
T. ech × T. lat	64	11194	5	0.1	261	103	39.5	261	150	57.5	29.20	2.09
Total interspecific combinations	289	66576	54	0.1	1094	222	20.3	1040	279	26.8	65.4	0.94

level of fertility in eggs will reduce seed formation considerably.

There were no marked reciprocal differences between backcrosses to the male or female parental line.

The results of open pollination are expressed in seed weight as a percentage of plant weight. "Compatible" cross combinations, such as *T. carmeli* × *T. carmeli* or *T. echinatum* × *T. latinum* produced ample seed, 1.75 and 2%, of plant weight; hybrids of *T. carmeli* × *T. echinatum* and *T. carmeli* × *T. latinum* produced 0.53 and 0.82%, respectively. The corresponding values in normal plants of *T. echinatum* and *T. carmeli* growing in the field range from 3 to 15% (Katznelson, 1971b) and the less than 1% values obtained here are definitely the result of some sterility of eggs.

F_2 *Plants.*

Germination of F_2 and BC_1 seeds was excellent — 163 out of 164 seeds germinated. There were no albino seedlings, but fifty plants died before flowering. In the remaining 113 plants, pollen fertility and seedset were checked, while fourteen F_2 and one F_3 were analyzed cytologically. These F_2 were usually progenies of low fertility F_1 that manifested major chromosomal rearrangements. The results of pollen fertility appear in Table 7, and those of cytological observations in Table 8.

The average pollen fertility in BC_1, and especially in F_2 (i.e. seeds obtained by selfing F_1 plants) was much higher than in their parents, but also much more variable. In hybrids of *T. carmeli* × *T. echinatum* and *T. latinum*, pollen fertilities of F_1, BC_1 and F_2 were 51%, 70.5% and 81%, respectively.

Cytologically, some BC_1 plants stabilized on 8 II cells only (Table 8), while others still manifested the irregularities observed in their parents, i.e. multivalent formation. This trend is also apparent in Table 5.

Of specific interest was the appearance of 3 plants, two BC_1 (No. 134, 292) and one F_2 (No. 956) with up to ten B-chromosomes, originating from three F_1 hybrid parent plants that were studied cytologically and found to be devoid of B chromosomes. While the other parent plants used for backcrossing were not studied cytologically, other plants of the same source were found also to be devoid of Bs. This suggests that the B chromosomes in this case were formed in the F_2 plants. Plant 956, which resulted from selfing F_1 that did not have any B chromosomes, is much

Table 7. *Pollen fertility of F_2 and BC_1 interspecific hybrids*

Combination			No. of plants with the following pollen fertility percent										Total no. of plants exam.	Average pollen fertility
♀ = F_1	♂		0—10	10—20	20—30	30—40	40—50	50—60	60—70	70—80	80—90	90—100		
T.car	T.ech	B.C.				1		6	7	3	5	4	26	71.1
T.car	T.lat	B.C.	1	1		3	4	3	2	6	11	6	37	69.7
T.ech	T.lat	B.C.									5	38	43	93.2
T.car	T.ech	Selfing									1		1	81.0
T.car	T.lat	Selfing									2		2	81.0
T.lat	T.ech	Selfing					1					3	4	81.4

stronger evidence to the *de novo* appearance of B chromosomes.

As can be seen in Table 9, the F_2 progenies of *T. carmeli* × *T. echinatum* and *T. carmeli* × *T. latinum* cross combinations took after their parents in their almost complete self-incompatibility and low seedset in open pollination (except the one F_2 *T. carmeli* × *T. echinatum* obtained by selfing). The selfed *T. latinum* × *T. echinatum* were also self-incompatible. However, backcrosses of *T. latinum* × *T. echinatum* and their reciprocal crosses to both parental species gave strikingly different results. Of thirty F_2 plants of that combination that were selfed, eighteen set enough seed, up to 35% of their potential, to show conclusively that the self-incompatibility system of the parental species and of their F_1 progenies was broken. It should be noted that these F_2 plants resulted from many cross combinations, involving five populations of *T. echinatum* and two of *T. latinum*.

Discussion

The data presented in this paper show quite clearly that the three species differ cytologically. The chromosomal rearrangements between *T. carmeli* and the other two species involve at least three major chromosomal translocations, two of them shared by one pair of chromosomes. In addition, *T. echinatum* was shown to be heterogeneous, both cytologically and genetically. This is evinced by the different behaviour of NYT 1401, as compared to other *T. echinatum* lines, when crossed with *T. latinum*. However, while *T. latinum* forms completely fertile — cytologically normal — hybrids with the majority of *T. echinatum* lines, the cytological variance between *T. carmeli* × *T. latinum* hybrids on the one hand, and *T. carmeli* × *T. echinatum* (exct. NYT 1401) on the other, may suggest that the *T. latinum* studied here belongs in fact to the complex species *T. echinatum* and that the diagnostic character of three nerved calyx teeth (Zohary, 1969) may be of little value.

There are two other interesting points worth discussing in connection with the cytological evidence presented.

The first is the causative relationship between the three translocations and spore fertility. Pollen fertility in F_1 hybrids with two to three multivalents per cell was around 40–60%. This is a relatively low level, but it is much higher than the expected fertility even in cells with one translocation. According to previous findings (Katznelson and Morley, 1965), 33% balanced spores are expected in case of one translocation, if a quadrivalent is randomly segregated, and 50% if no quadrivalent is formed and the two heterologous bivalents segregate at random. The only explanation for the 40% pollen fertility in the *T. carmeli* × *T. echinatum* F_1 hybrid is that strong forces of selection exist in favor of an alternate distribution of multivalent components, and that there are

Table 8. *Multivalent formation in various BC_1, F_2 (and one F_3) progenies of interspecific hybrids within the echinata group*

F_2 plant number	Species F_1 = ♀ ♀	Species F_1 = ♀ ♂	Species ♂	NYT F_1 = ♀ ♀	NYT F_1 = ♀ ♂	NYT ♂	No. cells exam.	B chromosome per cell	No. cells in the chromosome configurations 2 I 7 II	4 I 6 II	8 II	1 I 6 II 1 III	3 I 5 II 1 III	6 II 1 IV	2 I 5 II 1 IV	5 II 2 III	1 I 5 II 1 V	Pollen fertility %
16	T. ech	T. car	T. car	1435	210	210	18		9			8	1					56
13	T. ech	T. car	T. ech	1435	210	1435	12		1		11							80
27	T. ech	T. car	T. ech	1435	266	1435	17		5	1	4	2	1	3	1			62
31	T. ech	T. car	T. car	1441	266	266	18		1		1	3	1	4		8		67
1089	T. car	T. ech	T. ech	210	220	220	24		3		8	8		2	1	1	1	53
1094	T. car	T. ech	T. ech	251	220	220	15				15							92
57	T. car	T. lat	T. car	210	1451	210	12		2		7	2				1		80
113	T. lat	T. car	T. car	1451	210	210	16		3		13							77
134	T. lat	T. car	T. car	1451	266	266	11	0:3:5	1		10							84
292	T. ech	T. lat	T. ech	1441	1451	1441	18	8:10			18							90
356	T. lat	T. ech	T. ech	1497	220	220	10				9			1				82
954	T. ech	T. car	Selfing	1441	210	Selfing	18		6		2	6		1		2	1	81
956	T. lat	T. car	Selfing	1451	T. car	Selfing	24	3	1		7	4		10		2		81
976	T. lat	T. ech	Selfing	1497	1441	Selfing	12				12							90
F_3 1338	T. lat × T. car	T. car	T. car	1451 × 266	266	266	10		1		2	5		2				28

Table 9. *Seedset in various progenies of interspecific F_1 hybrids*

Combinations		No. of plants exam.	Seedset upon selfing			Seedset upon open pollination	
♀ = F_1	♂		No. of flowers	No. of seeds	% seedset	seed weight g	$\frac{\text{seed weight}}{\text{plant weight}}$ %
T. car T. ech	B.C.	26	5755	11	0.2	1.34	0.09
T. car T. lat	B.C.	37	8108	27	0.3	4.21	0.13
T. ech T. lat	B.C.	43	6789	351	5.2	29.76	1.26
T. car T. ech	Selfing	1	245	0	0	0.86	1.01
T. car T. lat	Selfing	2	150	0	0	0.01	0.04
T. ech T. lat	Selfing	4	1223	9	0.7	0.06	0.05

relatively few heterologous bivalents. This kind of selection counterbalances the effect of major chromosomal rearrangement on spore sterility, and the prevalence of alternate distribution is quite obvious in our case (see the pentavalents in Plates 8, 9). This phenomenon is even more striking in the F_2 progeny, where plants with one or two multivalents had a pollen fertility of 80%, if we assume that stainability corresponds to fertility. The other point of interest is the possibility that a system of three translocations evolved gradually, and that there existed, or still exist, intermediate forms with one or two translocations. The validity of this assumption is corroborated by the cytological heterogeneity with the *T. echinatum-T. latinum* complex, stated earlier.

The partial breakdown of self-incompatibility in F_2 plants between *T. echinatum* and *T. latinum*, is of particular interest, because in cross combinations between each of them and *T. carmeli*, self-incompatibility is retained. The breakdown pattern appearing in combinations involving many different populations suggests the prevalence of the phenomenon among the species rather than individual plants, and one should note here that the same parent plants were also used for hybridization with *T. carmeli* and many other related clovers with no consequence of incompatibility breakdown.

A breakdown of incompatibility in interspecific *Trifolium* hybrids has been reported by Pandey (1957), in one F_1 hybrid between *T. repens* and *T. uniflorum*. The two species are incompatible, taxonomically removed, yet the breakdown already occurred in the F_1. This is in contrast to the case described here, where the taxa may be considered as one species, in view of the complete fertility in pollen and seed, and lack of other isolation mechanisms (Katznelson and Putiyevsky, in preparation). In addition, the breakdown was in 18 F_2 plants, progenies of eight different F_1 plants. Preliminary studies of these progenies suggest different degrees of breakdown of incompatibility, as in the example described by East (1934); the intervention of other genes, except the supposed S alleles (Lewis, 1954) may explain some of the results.

To sum up, the three species investigated here are in fact two species — *T. carmeli* and the complex of *T. echinatum*. The latter may tentatively be divided into at least three biosystematic units. The most common one would be the main stock of *T. echinatum*, found in the region between Yugoslavia and East Turkey; a second one is *T. echinatum* NYT 1401 and some other populations in East Turkey (Elazig — Bingol); and the third consists of the two populations of "*T. latinum*" from East Turkey, used in this study. Further studies, using Central-Mediterranean *T. latinum* and other *T. echinatum* lines, would help to clarify the nature of the *echinata* complex.

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