

A GENETIC STUDY OF TRICOTYLY
IN HAPLOPAPPUS GRACILIS

by

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CHAPTER I

INTRODUCTION

Haplopappus gracilis (Nutt.) Gray, an annual species of the family Compositae found throughout the southwestern United States and northern Mexico, is normally dicotyledonous. However, both tricotyly and tetracotyly have been observed. These deviations from normal (also termed split cotyledon, schizocotyly, polycotyly, and pleiocotyly) may arise from a second cycle of cotyledon differentiation from the cotyledon primordia at early stages of development, giving schizocotyly (Palmer, 1968).

Tricotyly has been observed in a variety of plants including: petunias (Straub, 1948); tomatoes (Haskell, 1954, 1962; Palmer, 1957; Holtorp, 1944); carnations, carrots, and certain dicotyledonous weeds (Holtorp, 1944). **Several Russians, among them Litovchenko (1940), have studied the occurrence of tricotyly in food crops and concluded that tricotylyous plants were more productive.**

Straub (1948) reviewed the work of de Vries (1902) and concluded that one recessive gene with its expression modified by two dominant suppressors and the environment determined the expression of tricotyly. His conclusions were based on the fact that the highest percentages of tricots were realized after three generations of crosses. Straub

found that not only did his percentages increase by selection, but they were also higher in the fall than in the spring and early summer. Defoliation of plants in the spring produced a higher proportion of tricots, leading Straub to conclude that environment played an important role in expression.

Haskell (1954) and Palmer (1957) studied the effect of truss (position of a flower cluster) position on expression of tricotyly in tomato. Using the Clucas-99 variety, Haskell found that the percentage of tricots produced by fruits at the various truss positions, decreased from the first to third truss positions and rose at the fourth. He concluded that the trait was not completely genetically controlled. Palmer, on the other hand, working with the Harbinger variety, found that while tricot percentages varied from plant to plant and fruit to fruit, they did not vary significantly from truss to truss. He concluded that some factor which varied from time to time and from plant to plant, caused production of tricots.

Haskell (1949) found that experimental selection increased the frequency and range of pleiocotyly in outbreeding species of Cruciferae and Umbelliferae. Environmental influences also increased expression of the trait. He also found that selection for tricotyly in tomatoes produced correlated morphological and physiological disturbances in the normal balanced condition that they bore little resemblance to cultivated tomatoes.

Palmer (1960) tested the effect of certain growth substances on the heritability of split cotyledons in brassicas. Basing an experiment on the work of Haccius (1955), he tested the effect of 2,4-dichlorophenoxyacetic acid, indolacetic acid, and tri-iodobenzoic acid on tricotyly and found that growth substances did not significantly increase the number of tricots. The results indicated that tricotyly was inherited and the variation in expression was environmentally influenced.

Evidence that the influencing environmental factor might be temperature came from work by Harrison (1964) and Palmer (1968). Using varieties of snap-dragon (Antirrhinum majus), Harrison showed that tricotyly was not only heritable but that the phenotypic expression also increased when seed matured at 15° C as opposed to 25° C. Palmer experimented with two temperature regimes using Brassica napus. He found that phenotypic expression of the mutant increased for plants crossed at 16° - 21° C compared to those crossed at 21° - 26° C and concluded that temperature variations during seed development might be an important cause of seasonal and within plant variation of tricot frequency.

The purpose of this study was to determine whether temperature influences expression of tricotyly and to localize the split cotyledon (sc) gene.

CHAPTER II

MATERIALS AND METHODS

All seeds were germinated in flasks containing distilled water. The water was changed three times the first day, and once a day thereafter. The majority of the seeds germinated in 24 - 48 hours. Seedlings were transferred to expanded peat pots when about one cm long. The peat pots were then placed in pans covered with clear plastic and left under constant light for three days. After root tips emerged from the peat pots, the plants were transferred to soil in six-inch clay pots in the greenhouse.

Before flowering the heads were covered with commercial tissues (Kimwipes) to prevent unwanted pollination. Crosses were made daily, starting with anthesis of the outer row of florets, by rubbing the heads together until all florets had opened and the stigmas had all withered. The seed were collected after a maturation period of about three weeks and allowed to dry at room temperature for two weeks. Seeds not used immediately were refrigerated to retain their viability.

Crosses were made among tetracots, tricots, incomplete tricots, and dicots. The first crosses were made in environmental chambers at temperatures of 65° F and 85° F and in the greenhouse at a temperature of approximately 75° F. (There

was a 14 hour light period at 65° F and a 12 hour light period at 85° F so that the flowers would open at about the same time.) The environmental chambers were used to determine the range of optimum temperatures for expression. Crosses between the 2n = 4 mutant and the 2n = 6 wild type were made in the greenhouse as the temperature was not expected to effect the heterozygote. The 2n = 4 mutants were crossed to the 2n = 5 heterozygous hybrid in environmental chambers at 85° F.

Seedlings to be karyotyped were placed in peat pots and the pots were wrapped in aluminum foil. After the roots had emerged approximately one cm from the peat, they were cut off and placed in a saturated solution of 1-bromonaphthalene for two hours prior to the time of peak mitotic activity and then fixed in a 4:1 solution of 95% ethyl alcohol-99% proprionic acid for a minimum of 2 days.

Before staining, the root tips were hydrolyzed in a 1:1 solution of 15% hydrochloric acid-95% ethyl alcohol for one minute. They were then placed in a working solution of 4:1 fixative for thirty seconds and then placed on slides in a drop of FLP orcein stain. The cells were separated by gently tapping with a pencil eraser on the cover glass before squashing (Jackson, 1974).

Haplopappus gracilis is chromosomally polymorphic with diploid chromosome numbers of 4, 5, and 6 (Jackson, 1965). The 2n = 4 race has a pair of metacentric chromosomes

designated A, and a pair of submetacentrics designated B. The $2n = 6$ race has a pair of chromosomes with submetacentric centromeres (B), a pair with subterminal centromeres (C_t), and a pair with submedian centromeres (D_t). The C_t and D_t chromosomes are about equal in length, and each is about half the length of an A.

The $2n = 5$ hybrid (Fig. 1) has one chromosome A, one C_t , one D_t , and a pair of B's. The B's are completely homologous and disjoin normally. The C_t and D_t pair with the A at pachytene and form a trivalent at metaphase I (Fig. 2). The chromosomes of the trivalent do not disjoin randomly but show preferential disjunction. Chromosome A goes to one pole while both C_t and D_t go to the opposite pole. This preferential disjunction allows one to determine linkage relationships if there is less than 50% crossing over when the mutant gene is on the A chromosome (Jackson, 1964).

The $2n = 4$ split cotyledon was crossed to the $2n = 6$ wild type. The resulting $2n = 5$ heterozygous hybrid was then backcrossed to the $2n = 4$ mutant. These seeds were then germinated and the phenotypes and karyotypes examined.

If the split cotyledon gene is on the A chromosome, and no crossovers occur between the gene and the centromere, two equal classes of progeny are expected: $2n = 4$ tricots and $2n = 5$ wild types. Crossover classes would give $2n = 4$ wild types and $2n = 5$ tricots. If the gene is on chromosome

Figure 1. The Karyotype of the $2n = 5$ hybrid ($2n = 4 \times 2n = 6$). The A chromosome can be seen at the top of the cell, the two B's in the middle, and the C_t and D_t at the bottom and right of the cell, respectively.

Figure 2. Metaphase I in the $2n = 4 \times 2n = 6$. The $B_g B_t$ bivalent is at the top of the cell. The $AC_t D_t$ trivalent is at the bottom, with the A or the middle chromosome.

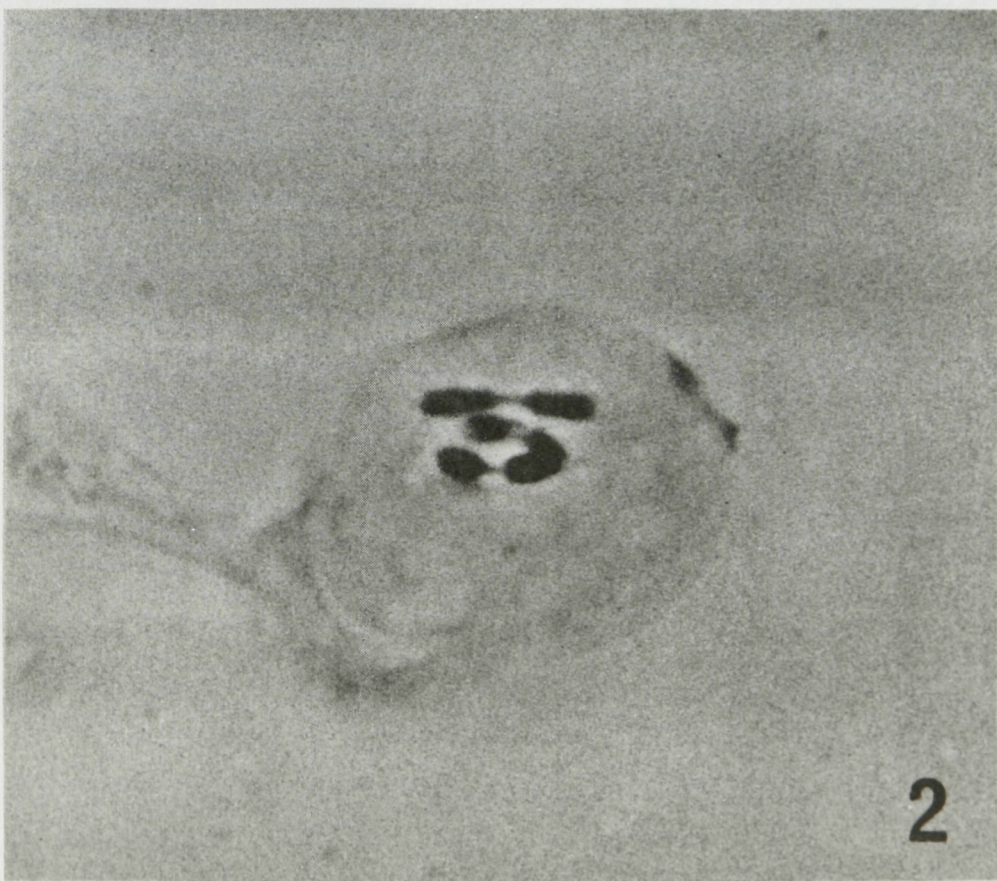
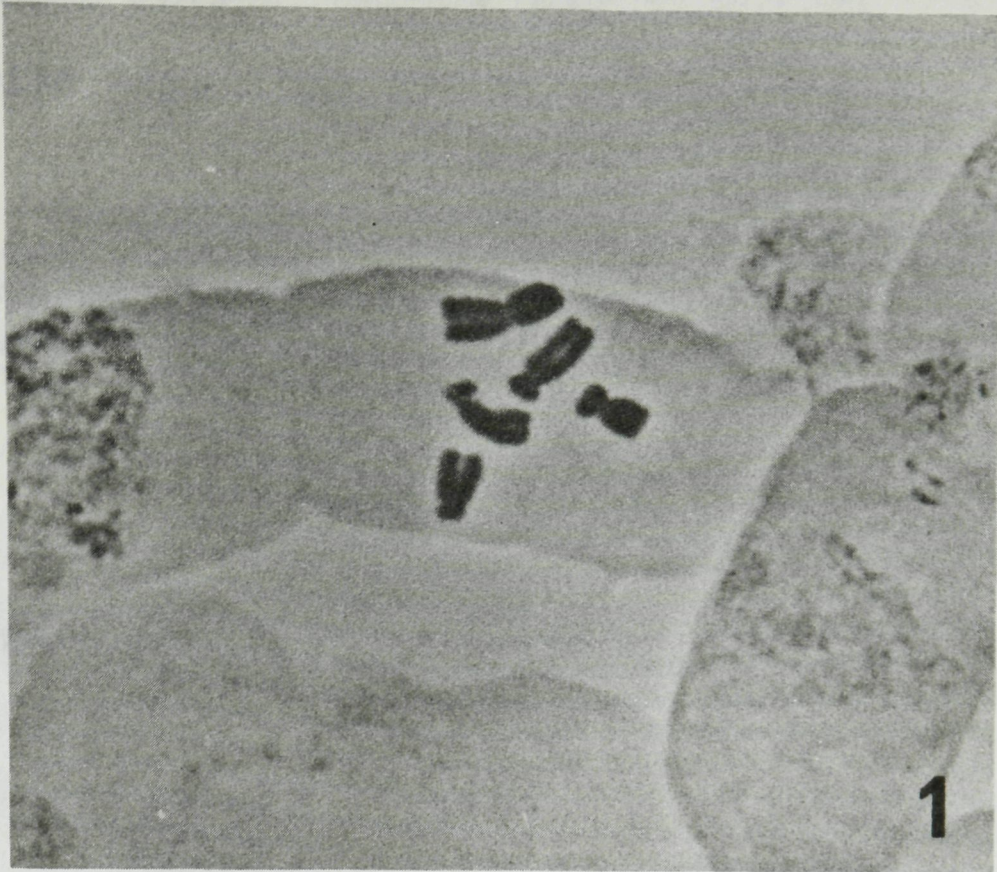
B, equal numbers of...

overs, would...

$2n = 3$...

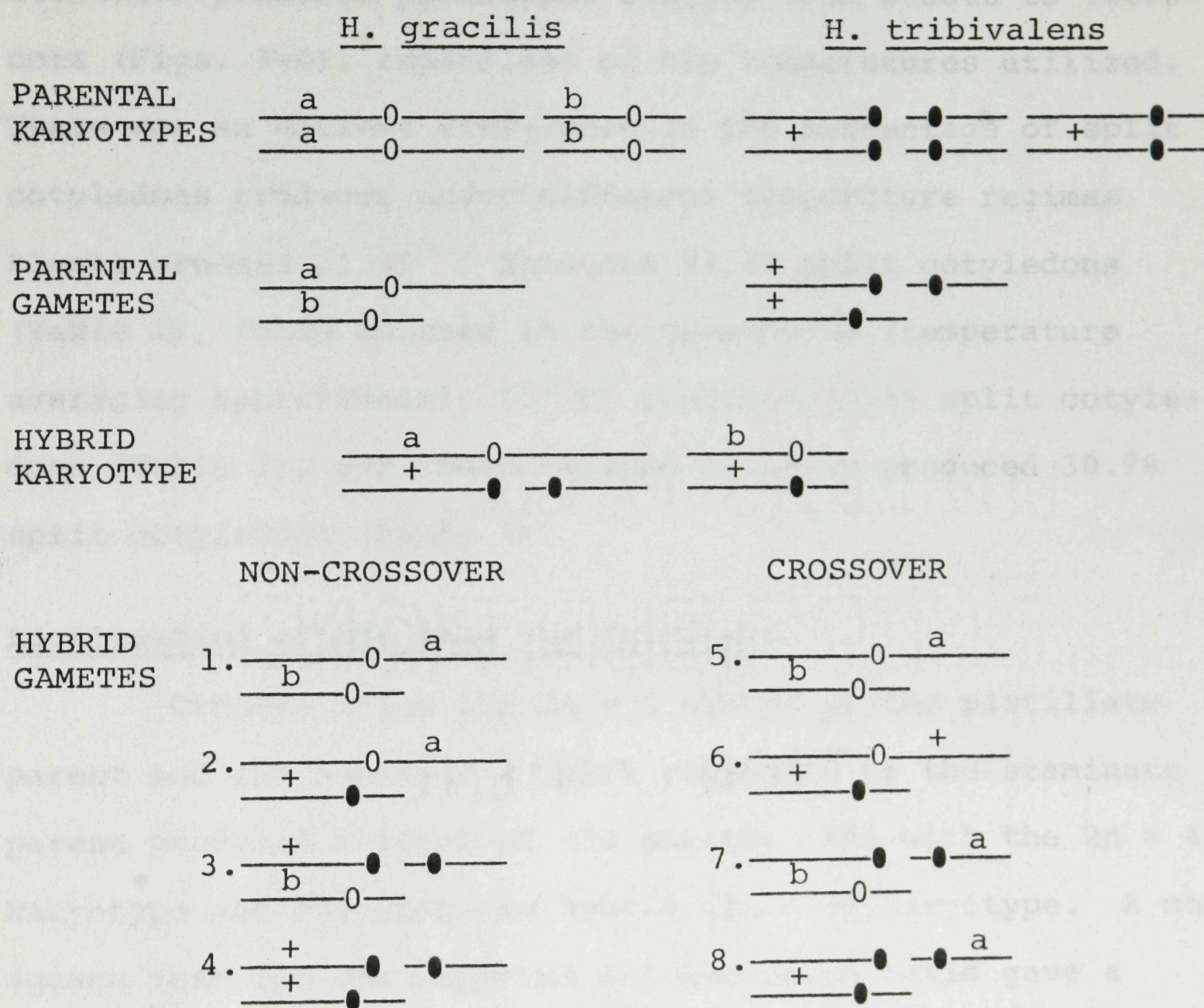
TABLE 1.

7, gene
A and
stage



B, equal numbers of the following classes, including cross-overs, would occur: $2n = 4$ wild types; $2n = 4$ tricots; $2n = 5$ wild types; and $2n = 5$ tricots (Table 1).

TABLE 1.--Diagrammatic representation of crosses and possible F_1 gametes. The mutant genes are introduced on chromosomes A and B of standard $n = 2$ *H. gracilis*. Centromeres of standard chromosomes are white and those of the $n = 3$ race are black.



CHAPTER III

RESULTS AND DISCUSSION

Results

Determination of Temperature Influence

The crosses among tricots, incomplete tricots, and tetracots produced phenotypes ranging from dicots to tetracots (Figs. 3-6), regardless of the temperatures utilized. There was an obvious difference in the percentage of split cotyledons produced under different temperature regimes. Plants crossed at 85° F produced 93.3% split cotyledons (Table 2), those crossed in the greenhouse (temperature averaging approximately 75° F) produced 50.3% split cotyledons (Table 3), and those crossed at 65° F produced 30.7% split cotyledons (Table 4).

Localization of the Gene for Tricotyly

Crosses using the $2n = 5$ hybrid as the pistillate parent and the homozygous split cotyledon as the staminate parent produced a total of 382 plants: 188 with the $2n = 4$ karyotype and 194 with the hybrid ($2n = 5$) karyotype. A chi square test for the expected 1:1 karyotype ratio gave a probability greater than 0.7 ($\chi^2 = 0.094$).

From these plants, the following phenotypic classes were obtained: 97 $2n = 4$ dicots; 91 $2n = 4$ tricots; 101

Figures 3 - 6 show the different phenotypes of normal and

split cotyledons

Figure 3. A normal dicotyledon.

Figure 4. A normal dicotyledon with a split cotyledon

has split cotyledons

Figure 5. A tricotyledon. The cotyledon has split completely.

Figure 6. A tetracotyledon. Both cotyledons have split

completely.



Figures 3 - 6 show the different phenotypes of normal and split cotyledons.

Figure 3. A normal dicotyledon.

Figure 4. An incomplete tricotyledon. The top cotyledon has split partially.

Figure 5. A tricotyledon. The cotyledon has split completely.

Figure 6. A tetracotyledon. Both cotyledons have split completely.

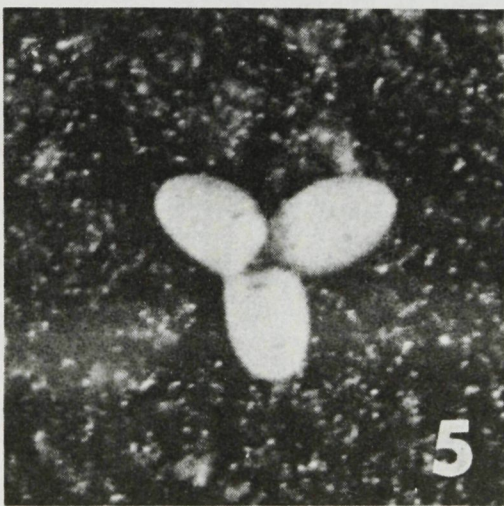
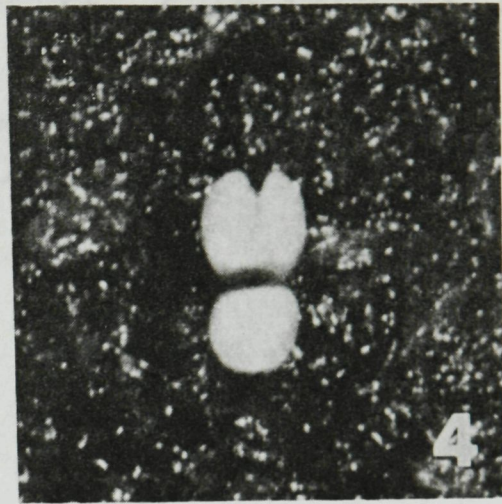


TABLE 2
RESULTS OF CROSSES AT 85° F
IN THE GROWTH CHAMBER

Crosses	Number Dicots	Number Split Cotyledons	% Split Cotyledons
Tetracot x Tetracot	2	26	92.8
Tetracot x Tricot	9	136	93.8
Tricot x Tricot	7	125	94.7
Tricot x Dicot*	4	47	92.2
Dicot* x Dicot*	5	42	89.4
Total Observed	27	376	93.3
Total Expected	0	403	100.0

*Derived from split cotyledon x split cotyled crosses

TABLE 3
RESULTS OF CROSSES IN THE GREENHOUSE

Crosses	Number Dicots	Number Split Cotyledons	% Split Cotyledons
Tetracot x Tetracot	--	--	--
Tetracot x Tricot	28	35	55.5
Tricot x Tricot	65	65	50.0
Tricot x Dicot*	39	33	45.8
Dicot* x Dicot*	35	36	50.7
Total Observed	167	169	50.3
Total Expected	0	336	100.0

*Derived from split cotyledon x split cotyled crosses

TABLE 4

RESULTS OF CROSSES AT 65° F
IN THE GROWTH CHAMBER

Crosses	Number Dicots	Number Split Cotyledons	% Split Cotyledons
Tetracot x Tetracot	19	9	32.1
Tetracot x Tricot	18	7	28.0
Tricot x Tricot	157	75	32.3
Tricot x Dicot*	68	24	26.1
Dicot* x Dicot*	22	11	33.3
Total Observed	284	126	30.7
Total Expected	0	410	100.0

*Derived from split cotyledon x split cotyled crosses

2n = 5 dicots; and 93 2n = 5 tricots. A chi square test for a 1:1:1:1 ratio gave a probability greater than 0.8 (Table 5).

TABLE 5

RESULTS OF CROSSES WITH THE 2n = 5 HYBRID
AS THE PISTILLATE PARENT

	2n = 4		2n = 5		X ²
	Dicots	Tricots	Dicots	Tricots	
Observed	97	91	101	93	0.618 (ns)
Expected	95.7	95.5	95.5	95.5	

Crosses using the $2n = 5$ hybrid as the staminate parent and the homozygous split cotyledon as the pistillate parent produced a total of 379 plants: 194 with the $2n = 4$ karyotype and 185 with the hybrid karyotype. A chi square test for the expected 1:1 karyotype ratio gave a probability greater than 0.5 ($\chi^2 = 0.214$).

From these plants, the following phenotypic classes were obtained: 103 $2n = 4$ dicots; 91 $2n = 4$ tricots; 98 $2n = 5$ dicots; and 87 $2n = 5$ tricots. A chi square test for a 1:1:1:1 ratio gave a probability greater than 0.5 (Table 6).

TABLE 6

RESULTS OF CROSSES WITH THE $2n = 5$ HYBRID
AS THE STAMINATE PARENT

	$2n = 4$		$2n = 5$		χ^2
	Dicots	Tricots	Dicots	Tricots	
Observed	103	91	98	87	1.611 (ns)
Expected	94.75	94.75	94.75	94.75	

Discussion

Determination of Temperature Influence

From these results there appears to be an environmental factor influencing expression. The plants were watered approximately the same, no growth regulators were used, and flowers used in the crosses were chosen at random. The only

environmental factor, other than temperature, that might have effected expression would have been the light period. This factor can be discounted on the basis of the greenhouse crosses. The light period there was much longer than the light period in either of the two chambers. If a longer light period tended to suppress expression, then the number of plants with split cotyledons from the crosses in the greenhouse should have been lower than the number observed at 65° F.

The question also arises as to whether the split cotyledon trait is polygenic, as has been suggested in other experiments (Straub, 1948). This possibility could explain the occurrence of incomplete tricots and tetracots. However, data from Tables 5 and 6 indicates the gene is located on chromosome B. These data indicate that if the trait is quantitative the genes controlling it are probably linked on the same chromosome. Further crosses using a second marker will be needed to answer this question.

With other environmental factors being equal and the data showing an increase in expression from 65° F to 85° F, it appears that temperature must be the influencing factor. A temperature of 85° F safely falls into the range of optimum temperatures, although it is not the optimum temperature.

It will take further experiments to determine the optimum temperature. The possibility exists that it is only a range of optimum temperatures which may be wide or narrow. Using the results of the crosses made in the greenhouse

(Table 3), it is reasonable to assume that the optimum temperature is higher than 85° F. This assumption is based on the fact that greenhouse temperatures may vary from the 60's to the 80's in a single day, depending on the time of day and the area in question. Crosses made at a narrower range of temperatures (for example 80° and 90° F) will be needed to completely resolve this problem.

The split cotyledon trait, or tricotyly, appears to be controlled by a single gene, which is influenced by higher temperatures. The different phenotypic expressions are apparently a result of development. Palmer (1960) concluded that the cotyledon splits early in development to produce tricots, earlier for tetracots, and later for the incomplete tricots.

Localization of the Gene for Tricotyly

Using the $2n = 5$ hybrid as both the pistillate and staminate parents did not produce any differences in the results. Thus, gametophyte selection was not occurring (Jordon, 1975; Jackson, 1975). The chi square tests for a 1:1:1:1 ratio did not give, in either case, a significant deviation from the expected. From this, it can be concluded that the split cotyledon gene is on chromosome B.

Had the gene been located on chromosome A, there would have been a significant deviation from the 1:1:1:1 ratio. This deviation would have been the result of the crossover classes: $2n = 4$ dicots and $2n = 5$ tricots. With such results,

one could determine the crossover frequency and the distance of the gene from the centromere of chromosome A.

However, with the gene located on chromosome B and only one marker, it is impossible to determine the crossover progeny. As a result one cannot determine the gene's location on the chromosome from the crossover frequency. If a second marker was introduced on the B chromosome, or the two B chromosomes were distinguishable (Jackson, 1964), it would be possible to determine the crossover classes, thus giving the gene's location.

From these results, it appears that the split cotyledon trait is recessive, influenced by higher temperatures, and located on the B chromosome.

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